

The Bethesda
Handbook of

Clinical Hematology

FOURTH EDITION

Griffin P. Rodgers
Neal S. Young



Wolters Kluwer

THE BETHESDA HANDBOOK

OF CLINICAL HEMATOLOGY

FOURTH EDITION

EDITORS

GRiffin P. Rodgers, MD, MACP

Chief
Molecular and Clinical Hematology Branch
National Heart, Lung, and Blood Institute
Director
National Institute of Diabetes and Digestive and Kidney Disease
National Institutes of Health
Bethesda, Maryland

Neal S. Young, MD, MACP

Chief
Hematology Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland



Philadelphia • Baltimore • New York • London
Buenos Aires • Hong Kong • Sydney • Tokyo

Senior Acquisitions Editor: Ryan Shaw
Editorial Coordinator: Kerry McShane
Editorial Assistant: Amy Masgay
Marketing Manager: Rachel Mante Leung
Production Project Manager: David Saltzberg
Design Coordinator: Stephen Druding
Manufacturing Coordinator: Beth Welsh
Prepress Vendor: Newgen Knowledge Works Pvt. Ltd., Chennai, India

Fourth edition

Copyright © 2019 Wolters Kluwer.

Copyright © 2013, 2010 Lippincott Williams & Wilkins, a Wolters Kluwer business.
Copyright © 2005 by Lippincott Williams & Wilkins.

All rights reserved. This book is protected by copyright. No part of this book may be reproduced or transmitted in any form or by any means, including as photocopies or scanned-in or other electronic copies, or utilized by any information storage and retrieval system without written permission from the copyright owner, except for brief quotations embodied in critical articles and reviews. Materials appearing in this book prepared by individuals as part of their official duties as U.S. government employees are not covered by the above-mentioned copyright. To request permission, please contact Wolters Kluwer at Two Commerce Square, 2001 Market Street, Philadelphia, PA 19103, via email at permissions@lww.com, or via our website at lww.com (products and services).

9 8 7 6 5 4 3 2 1

Printed in China

9781496354037

2017057997

Library of Congress Cataloging-in-Publication Data
available upon request

This work is provided “as is,” and the publisher disclaims any and all warranties, express or implied, including any warranties as to accuracy, comprehensiveness, or currency of the content of this work.

This work is no substitute for individual patient assessment based upon health care professionals' examination of each patient and consideration of, among other things, age, weight, gender, current or prior medical conditions, medication history, laboratory data, and other factors unique to the patient. The publisher does not provide medical advice or guidance and this work is merely a reference tool. Health care professionals, and not the publisher, are solely responsible for the use of this work including all medical judgments and for any resulting diagnosis and treatments.

Given continuous, rapid advances in medical science and health information, independent professional verification of medical diagnoses, indications, appropriate pharmaceutical selections and dosages, and treatment options should be made and health care professionals should consult a variety of sources. When prescribing medication, health care professionals are advised to consult the product information sheet (the manufacturer's package insert) accompanying each drug to verify, among other things, conditions of use, warnings and side effects and identify any changes in dosage schedule or contraindications, particularly if the medication to be administered is new, infrequently used, or has a narrow therapeutic range. To the maximum extent permitted under applicable law, no responsibility is assumed by the publisher for any injury and/or damage to persons or property, as a matter of products liability, negligence law or otherwise, or from any reference to or use by any person of this work.

LWW.com

For our children, with love:
Chris and Gregory Rodgers
Andrea, Max, and Giorgio Young



PREFACE

Life is short, the art long
— Hippocrates (c. 460 to 357 BC)

The accessibility of blood and bone marrow has made hematology historically the engine of basic research in internal medicine. Hematology has thrived at the National Institutes of Health because of this close relationship with the research laboratory, and investigators from the various institutes in Bethesda have contributed to the knowledge of blood diseases from the study of individual patients with sometimes rare diseases and to the development of clinical protocols for the rigorous assessment of diagnostic criteria or treatments, both established and novel. Our hematology fellowship programs have fostered a scientific approach to hematology, not only to assess outcomes but also to advance the experimental basis of our understanding of blood diseases and the application of laboratory insights to their treatment in practice. The collegial relationships among local institutions and individuals in the greater Washington area who share training and patients have greatly furthered these efforts.

HANDBOOKS are intended to be highly accessible, both literally and figuratively, so as to be useful. Our The Bethesda Handbook of Clinical Hematology should be carried in the white coat pocket of the student, resident, and fellow on a hematology or oncology service and in the briefcase of the internist, hospitalist, family practitioner, and pediatrician, whose practice includes patients with blood diseases. We have purposely combined authors who are recognized experts in their fields with senior fellows who have had current experience of learning hematology and daily care of hematology patients, and encouraged a thoughtful approach to the presentation of the core knowledge, using tables, algorithms, meaningful figures, and bulleted text structures. The Bethesda Handbook of Clinical Hematology is organized according to disease categories and hematological problems of importance to the consulting and treating hematologist, and additional chapters are provided to acquaint the reader with familiar and new laboratory methodologies that underlie modern clinical approaches to diagnosis and treatment.

For the opportunity to publish a fourth edition of The Bethesda Handbook of Clinical Hematology, we thank our readers, who have supported our efforts with both kind comments and constructive criticisms and, very importantly, concrete purchases of the

book, more remarkable in an age of Internet-based medical information. Indeed, the plan is to have The Bethesda Handbook of Clinical Hematology available as a handheld application. The Bethesda Handbook of Clinical Hematology remains focused on providing the practitioner at every level of training practical, authoritative, and current guidance to the diagnosis and treatment of blood diseases and to consultative problems in hematology. Many aspects of hematology, as the field advances, have entered the domain of internal medicine but remain complex and challenging— from new anticoagulants to the mundane management of once fatal diseases like chronic myeloid leukemia and aplastic anemia.

All chapters have been revised and updated. We look forward to your responses to our authors' efforts.

Griffin P. Rodgers, MD, MACP
Neal S. Young, MD, MACP

Disclaimer: Dr. Rodgers's and Dr. Young's work as editors and authors was performed outside the scope of their employment as US government employees. Their work represents their personal and professional views and not necessarily those of the US government.

CONTRIBUTORS

Inhye E. Ahn, MD Staff Clinician, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Daisuke Araki, MD Hematology Fellow, Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland

A. John Barrett, MD Section Chief, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Minoo Battiwalla, MD, MS Staff Clinician, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Deepa Bhojwani, MD Associate Professor, Clinical Pediatrics, Pediatrics, Keck School of Medicine, University of Southern California; Director, Leukemia/ Lymphoma Program, Pediatrics, Children's Hospital Los Angeles, Los Angeles, California

Charles D. Bolan, MD Staff Clinician, Hematology Branch, National Heart, Lung, and Blood Institute, Clinical Center, National Institutes of Health, Bethesda, Maryland

Raul C. Braylan, MD Chief, Hematology Laboratory, Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland

Katherine R. Calvo, MD, PhD Hematology Section, Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland

Richard W. Childs, MD Chief, Transplantation Immunotherapy Section, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Bogdan Dumitriu, MD Staff Clinician, Hematology Oncology Department, Mid Atlantic Permanente Medical Group, Rockville, Maryland

Cynthia E. Dunbar, MD Senior Investigator and Section Head, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Thomas A. Fleisher, MD Scientist Emeritus, Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland

Patrick F. Fogarty, MD Medical Affairs, Hemophilia, Rare Disease, Pfizer, Collegeville, Pennsylvania

Peiman Hematti, MD Professor, Department of Medicine, University of Wisconsin-Madison, School of Medicine and Public Health, Madison, Wisconsin

Christopher S. Hourigan, BM BCh, DPhil Chief, Myeloid Malignancies Section, Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland

Matthew M. Hsieh, MD Staff Clinician, Molecular and Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Julie Jaffray, MD Assistant Professor, Department of Pediatrics, University of Southern California; Hematologist, Division of Hematology, Children's Hospital Los Angeles, Los Angeles, California

Dickran Kazandjian, MD Principal Investigator, Myeloma Program, Lymphoid Malignancies Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; Attending Physician, Office of Hematology and Oncology Products, Food and Drug Administration, Silver Spring, Maryland

Harvey G. Klein, MD Adjunct Professor, Medicine and Pathology, The Johns Hopkins School of Medicine; Chief, Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, Bethesda, Maryland

Neha Korde, MD Assistant Professor, Department of Medicine, Weill Cornell Medical College; Assistant Attending, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York

Ola Landgren, MD, PhD Professor, Department of Medicine, Weill Cornell Medical College; Chief of Myeloma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York

Susan F. Leitman, MD Director, Office of Clinical Research, Training and Medical Education, National Institutes of Health; Special Volunteer, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland

Vid Leko, MD Clinical Fellow, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Richard F. Little, MD, MPH Senior Investigator, Clinical Investigations Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health; Adjunct Investigator, HIV and AIDS Malignancy Branch, National Institutes of Health Clinical Center, Bethesda, Maryland

Johnson M. Liu, MD, FACP Les Nelkin Professor, Department of Pediatrics, Zucker School of Medicine at Hofstra/ Northwell, Hempstead, New York; Attending Physician, Departments of Medicine and Pediatrics, Cohen Children's Medical Center, New Hyde Park, New York

Jay N. Lozier, MD, PhD Senior Staff Clinician, Department of Laboratory Medicine, National Institutes of Health Clinical Center, Bethesda, Maryland

Sham Mailankody, MBBS Assistant Professor, Department of Medicine, Weill Cornell Medical College, New York, New York; Assistant Attending, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York

Harry L. Malech, MD Laboratory Chief and Senior Investigator, Laboratory of Host Defense, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Vera Malkovska, MD Director of Hematology, Washington Cancer Institute at the Washington Hospital Center, Washington, DC

Elisabet E. Manasanch, MD Assistant Professor, Department of Lymphoma/ Myeloma, University of Texas MD Anderson Cancer Center, Houston, Texas

Pierre Noel, MD Professor of Medicine, Mayo College of Medicine, Consultant, Hematology and Medical Oncology, Mayo Clinic, Phoenix, Arizona

Karolyn A. Oetjen, MD, PhD Clinical Fellow, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Matthew J. Olnes, MD, PhD Affiliate Professor, UAA-WWAMI School of Medical Education, University of Alaska, University of Washington; Medical Director, Hematology and Medical Oncology, Alaska Native Medical Center, Anchorage, Alaska

Patricia O'Neal, MD Medical Officer, Center for Drug Evaluation and Research, Office of Hematology and Oncology Products, Division of Hematology Products, U.S. Food and Drug Administration, Silver Spring, Maryland

Sandhya R. Panch, MD, MPH Medical Director, Cell Processing Section, Department of Transfusion Medicine, Clinical Center/ National Institutes of Health, Bethesda,

Maryland

Griffin P. Rodgers, MD, MACP Chief, Molecular and Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health; Director, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland

Mark Roschewski, MD Staff Clinician, Lymphoid Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Joseph Roswarski, MD Hematologist Oncologist, Department of Medicine, Tripler Army Medical Center, Honolulu, Hawaii

Geraldine P. Schechter, MD Professor Emeritus, Department of Medicine, George Washington University; Hematologist, Hematology/ Oncology Section, Medical Service, Veterans Affairs Medical Center, Washington, DC

Phillip Scheinberg, MD Head, Division of Hematology, Hospital A Beneficência Portuguesa, São Paulo, Brazil

Ramaprasad Srinivasan, MD, PhD Investigator and Head, Molecular Cancer Section, Urologic Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Clare Sun, MD Staff Clinician, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

John F. Tisdale, MD Senior Investigator, Molecular and Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Danielle M. Townsley, MD, MSc Staff Clinician, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Phuong T. Vo, MD Assistant Member, Clinical Research Division, Fred Hutchinson Cancer Research Center; Assistant Professor of Medicine, Medical Oncology, University of Washington, Seattle, Washington

Alan S. Wayne, MD Professor, Pediatrics and Medicine, Keck School of Medicine, University of Southern California; Director, Children's Center for Cancer and Blood Diseases; Head, Division of Hematology, Oncology and Blood & Marrow Transplantation, Children's Hospital Los Angeles, Los Angeles, California

Angela C. Weyand, MD Assistant Professor, Pediatrics and Communicable Diseases, University of Michigan Medical School; Pediatric Hematologist/ Oncologist, Pediatrics and Communicable Diseases, C.S. Mott Children's Hospital/ Michigan Medicine, Ann Arbor, Michigan

Adrian Wiestner, MD, PhD Chief, Lymphoid Malignancies Section, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Sri Lakshmi Hyndavi Yeruva, MD Attending Physician, Hematology and Oncology, Summit Health, Chambersburg, Pennsylvania

Agnes S. M. Yong, MB, BCh, PhD, FRCPA Associate Professor, Department of Hematology, Institute of Medical and Veterinary Science, University of Adelaide; Associate Professor, Department of Hematology, Institute of Medical and Veterinary Science, University of Adelaide, Adelaide, South Australia

Neal S. Young, MD, MACP Chief, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

CONTENTS

Preface

Contributors

Chapter 1 ■ Iron Deficiency

Bogdan Dumitriu and Griffin P. Rodgers

Chapter 2 ■ Deficiencies of Vitamin B₁₂ and Folate

Danielle M. Townsley and Griffin P. Rodgers

Chapter 3 ■ Hemolytic Anemia

Sri Lakshmi Hyndavi Yeruva and Patricia O'Neal

Chapter 4 ■ Sickle Cell Anemia and Thalassemia

Matthew M. Hsieh, John F. Tisdale, and Griffin P. Rodgers

Chapter 5 ■ Porphyrias

Peiman Hematti

Chapter 6 ■ Bone Marrow Failure Syndromes: Aplastic Anemia, Acquired and Constitutional; Paroxysmal Nocturnal Hemoglobinuria; Pure Red Blood Cell Aplasia; and Agranulocytosis

Phillip Scheinberg, Neal S. Young, and Johnson M. Liu

Chapter 7 ■ Myelodysplastic Syndromes

Danielle M. Townsley and Minoo Battiwalla

Chapter 8 ■ The Myeloproliferative Neoplasms: Polycythemia Vera, Essential Thrombocythemia, Primary Myelofibrosis, and Chronic Myelomonocytic Leukemia

Matthew J. Olnes

Chapter 9 ■ Neutrophil Disorders and Neutropenias

Matthew M. Hsieh and Harry L. Malech

Chapter 10 ■ Childhood Hematologic Diseases

Julie Jaffray and Alan S. Wayne

Chapter 11 ■ Acute Myeloid Leukemia

Christopher S. Hourigan and Vera Malkovska

Chapter 12 ■ Acute Lymphoblastic Leukemia

Deepa Bhojwani and Alan S. Wayne

Chapter 13 ■ Chronic Myelogenous Leukemia

Agnes S. M. Yong and A. John Barrett

Chapter 14 ■ Chronic Lymphocytic Leukemia

Inhye E. Ahn, Clare Sun, and Adrian Wiestner

Chapter 15 ■ Hodgkin Lymphoma

Joseph Roswarski and Mark Roschewski

Chapter 16 ■ Non-Hodgkin Lymphomas

Richard F. Little

Chapter 17 ■ Multiple Myeloma

Neha Korde, Sham Mailankody, Dickran Kazandjian, and Ola Landgren

Chapter 18 ■ Hematopoietic Stem Cell Transplantation

Phuong T. Vo, Ramaprasad Srinivasan, and Richard W. Childs

Chapter 19 ■ Thrombocytopenia

Karolyn A. Oetjen and Cynthia E. Dunbar

Chapter 20 ■ Disorders of Hemostasis I: Coagulation

Angela C. Weyand and Patrick F. Fogarty

Chapter 21 ■ Disorders of Hemostasis II

Angela C. Weyand and Patrick F. Fogarty

Chapter 22 ■ Venous Thromboembolism

Elisabet E. Manasanch and Jay N. Lozier

Chapter 23 ■ Consultations in Anticoagulation

Daisuke Araki and Jay N. Lozier

Chapter 24 ■ Blood Transfusion

Harvey G. Klein

Chapter 25 ■ Hemochromatosis

Sandhya R. Panch, Charles D. Bolan, and Susan F. Leitman

Chapter 26 ■ Consultative Hematology

Pierre Noel

Chapter 27 ■ Interpretation of Standard Hematologic Tests

Vid Leko, Katherine R. Calvo, and Geraldine P. Schechter

Chapter 28 ■ Basic Principles and Clinical Applications of Flow Cytometry

Thomas A. Fleisher and Raul C. Braylan

Index

Iron Deficiency

Bogdan Dumitriu and Griffin P. Rodgers

Iron deficiency is the most common cause of anemia throughout the world, with over 1 billion people being affected.¹ In the United States, 10% of women of childbearing age and young children have absolute iron deficiency.² Functional iron deficiency is more common in elderly patients, who have a high frequency of anemia resulting from inadequate utilization of iron stores.³ Clinical presentation includes fatigue, weakness, headaches, pallor, stomatitis, and glossitis. The presenting symptoms may also include appetite for non-nutritional or unusual food items (pica), restless leg syndrome,⁴ or beeturia.⁵ Although described in cases of severe iron deficiency, Plummer–Vinson syndrome (dysphagia, esophageal webs, atrophic glossitis with iron-deficient anemia), koilonychias (spoon nails), chlorosis (greenish color of skin), or blue sclerae are very rarely found at presentation in modern industrialized countries.

ABSOLUTE VERSUS FUNCTIONAL IRON DEFICIENCY

Iron-deficiency anemia is caused by:

- Decrease in total body iron (“absolute” iron deficiency) through either blood loss or poor intestinal iron absorption ([Table 1.1](#)).
- Inadequate utilization of iron stores (“functional” iron deficiency) either through increased demand or impaired bioavailability.

IRON METABOLISM

Approximately half of the 3 to 4g of total body iron is contained in the hemoglobin of circulating red cells (Figure 1.1). Non-erythroid iron is contained in reticuloendothelial system (RES), myoglobin, and the liver. Intracellular iron is stored in ferritin, and the circulating level of ferritin normally correlates closely with the intracellular iron stores.⁶

The average daily requirement of iron to support erythropoiesis is 20 mg. Most of the daily iron requirement is supplied by the recovery of erythroid iron through phagocytosis of senescent erythrocytes by RES. Every day, 1 to 2 mg of iron is obtained from dietary intake to compensate for losses in sweat, urine, and feces.⁷ For women during their childbearing years, there are additional losses due to menstruation (average of 0.3 to 0.5 mg iron/ day).⁸ To balance these losses:

- Adult males must absorb about 1 mg of iron each day from their diet, and menstruating females require about twice this much.
- During pregnancy and periods of rapid growth, iron balance must be positive in order to support increased production of hemoglobin and myoglobin.
- Negative iron balance results from increased loss of iron (nearly always due to bleeding), inadequate dietary intake, and increased utilization of iron ([Table 1.1](#)).

Table 1.1 Causes of Absolute Iron Deficiency

Increased Loss of Iron

Bleeding

- Menorrhagia
- Gastrointestinal
- Surgery
- Trauma
- Childbirth
- Excessive phlebotomy
- Blood donations
- Factitious
- Hemodialysis
- Hematuria

Chronic hemoglobinuria

- Mechanical heart valve hemolysis
- Paroxysmal nocturnal hemoglobinuria

Decreased Intake of Iron

Dietary deficiency

- Limited meat

Malabsorption

- Achlorhydria
- Gastric atrophy
- Partial gastrectomy
- Gastric bypass
- Proton pump inhibitors

Helicobacter pylori gastritis
Inflammatory bowel disease
Celiac disease

Increased Utilization of Iron

Pregnancy or lactation
Rapid growth
Recovery from anemia, erythropoietin-stimulating agent usage

Dietary iron is present in ferric (Fe^{+3}) salts in meat and vegetables and in heme in meat.⁷ Heme iron is the most bioavailable because it is soluble at the alkaline pH of the duodenum, where it is absorbed as an intact iron–porphyrin complex. In contrast, ferric iron is not soluble at alkaline pH and is not absorbable by the duodenal mucosa. To be absorbed, it must be solubilized in the acidic stomach where it is loosely complexed with small molecules such as amino acids. Ferric reductase in the duodenal mucosa reduces the iron to its divalent state, which can be transported into enterocytes.⁹ The enhancing effect of ascorbate on iron absorption results from the increased solubilization of ferric iron, as well as increased ferric reductase activity.¹⁰ In contrast, the absorption of ferric iron is impaired by achlorhydria and by foods containing iron chelators, such as tannins and phytates, which are prevalent in tea and cereals. Although medicinal iron is in its ferrous state, thus presumably unaffected by these factors, it is still recommended to have a 2-hour delay in taking anything that might impair absorption.

Ferrous iron is released from duodenal enterocytes via the exporter ferroportin, which is regulated by the hormone hepcidin, and becomes oxidized to ferric iron before binding to transferrin.⁹ Hepcidin binding to ferroportin induces internalization and degradation of ferroportin, thus decreasing cellular iron export.¹¹ The same export mechanism exists in macrophages and hepatocytes.¹²

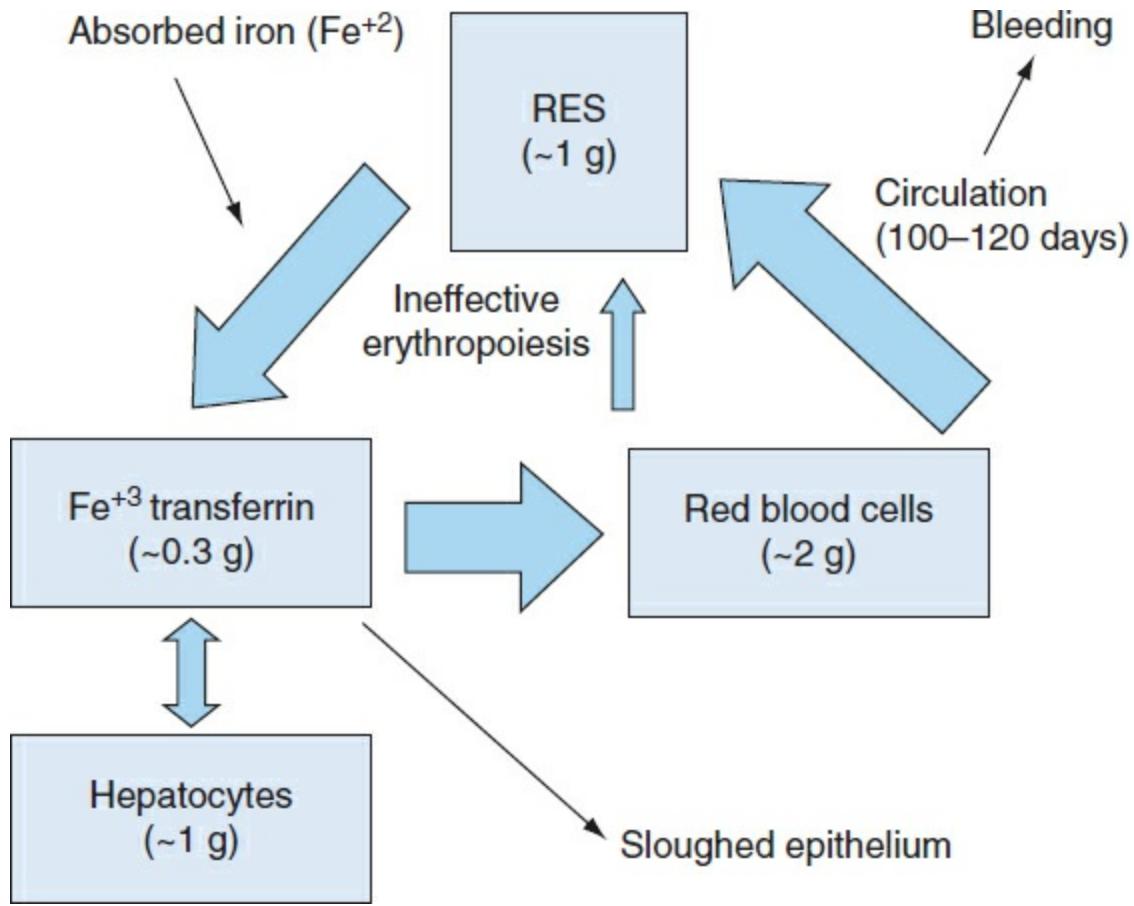


FIGURE 1.1 The iron cycle.

RES, reticuloendothelial system.

Once released in circulation, iron binds transferrin.⁹ Each transferrin molecule can bind one or two iron atoms. Diferric transferrin is taken up by developing red cells more easily than monoferric transferrin and delivers twice as much iron per molecule.¹³ Therefore, the concentration of diferric transferrin is critical to the support of erythropoiesis. Steady-state erythropoiesis requires a serum concentration of diferric transferrin that is achieved when the transferrin saturation is at least ~16%.¹⁴

ABSOLUTE IRON DEFICIENCY

A negative iron balance depletes body iron stores before iron-deficient erythropoiesis occurs.¹⁵ Multiple laboratory parameters associated with the iron-depletion state precede anemia (Table 1.2).

- Stainable marrow iron (RES hemosiderin) and serum ferritin are the primary markers of a negative iron balance. Serum ferritin accurately reflects body iron stores. Thus, a bone marrow biopsy is rarely needed. Serum ferritin below ~30 ng/dL is indicative of absolute iron deficiency, while in the presence of inflammation or liver disease, the

cutoff is higher (~100 ng/ mL).¹⁶

- | Reticulocyte hemoglobin is reported as part of the automated profile of reticulocytes. Due to the long half-life of mature erythrocytes in the circulation, reduced hemoglobin content in reticulocytes may be useful in cases of acute iron deficiency or for monitoring response to iron-repletion therapy. In the absence of thalassemia, reticulocyte hemoglobin values below 26 pg per reticulocyte indicate early iron deficiency.¹⁷
- | As storage iron becomes depleted and the iron supply to red cells becomes limiting, an increase in circulating transferrin receptors was reported.¹⁸ Elevated serum-soluble transferrin receptor concentration is not specific for iron deficiency and can be associated with erythroid hyperplasia. While a recent prospective multicenter trial suggested an added benefit of identifying absolute iron deficiency when anemia of chronic disease (ACD) was also present,¹⁹ it is not generally recommended for use in clinical practice.¹⁸
- | When the storage iron becomes depleted, serum iron and transferrin saturation begin to drop while the transferrin concentration usually rises.
- | When transferrin saturation reaches ~16%, the supply of iron to developing red cells becomes rate limiting, and the red cell count begins to decrease.
- | The new iron-deficient red cells are smaller than the older ones, and therefore the red cell distribution width (RDW) begins to increase.
- | When microcytes become more numerous, the mean cell volume (MCV) falls below the normal range, typically when the hemoglobin reaches ~10 g/ dL.

Table 1.2 Development of Laboratory Abnormalities During Negative Iron Balance

	Lab Test	Lab Finding
Early changes	Ferritin	<40 µg/ L
	Serum iron	<50 µg/ L
	Transferrin saturation	<15%
	Total iron-binding capacity	>450 µg/ dL
Late changes	Red cell count	<4 × 10 ⁶ / mm ³
	Red cell distribution width	>14.5%
	Mean corpuscular volume	<80 fL
	Hemoglobin	<13 g/ dL males <12 d/ dL menstruating females

Adapted with permission from Alleyne M, Horne MK, Miller JL. Individualized treatment for iron-deficiency anemia in adults. Am J Med. 2008;121(11):943–948.

FUNCTIONAL IRON DEFICIENCY

Hypoferremia despite seemingly adequate iron stores due to increased erythropoietic activity can be driven by endogenous erythropoietin stimulation as for patients recovering from absolute iron deficiency if the rate of supply of iron from their stores limits the rate of red cell production.²⁰ During pregnancy, iron requirements increase to 5 to 7 mg/ day, so iron supplementation is needed to prevent the depletion of iron stores.²¹ Erythropoietin-stimulating agent (ESA) administration in patients with chronic kidney disease (CKD) also causes increased erythroid iron demand, although iron sequestration also plays a role. These patients may have adequate iron stores, but their response to ESA is blunted until they are given iron supplementation.²² Thalassemia major leads to increased iron absorption and ultimately to iron overload due to high erythropoietic activity as well as pathological mechanisms caused by ineffective erythropoiesis.²³

Anemia of chronic inflammatory states or ACD accounts for most of the iron sequestration syndromes, but rare causes like hepcidin-producing adenomas, copper deficiency, and iron refractory iron deficiency anemia (IRIDA) have been described.²⁴ ACD develops in patients with chronic infectious, inflammatory, or neoplastic diseases. The anemia associated with functional iron deficiency is usually mild and asymptomatic.²⁵ Although usually normocytic, the MCV is often at the low end of normal and may be in the microcytic range. The serum iron concentration and transferrin saturation often suggest absolute iron deficiency, but the transferrin concentration is not elevated and may be low.²⁵ Furthermore, there is evidence of storage iron in the form of an elevated serum ferritin, as well as stainable iron in the bone marrow.

Patients with chronic illnesses can also have absolute iron deficiency, which can be particularly difficult to diagnose because of the effects of inflammation on the laboratory parameters of iron status. Chronic inflammation, for example, can suppress transferrin and elevate serum ferritin even in the true absence of storage iron.²⁶

Table 1.3 Serum Hepcidin Usage as Diagnostic Tool and Therapeutic Target

Disease	Iron	Ferritin	Transferrin Saturation	Hepcidin	Hepcidin Therapy ^a
IDA	Low	Low	Low	Low	—
ACD	Normal–high	Normal–high	Low	High–normal	Antagonist
ACD+IDA	Normal	Normal	Low	Normal	Antagonist
ESA resistance	Low–normal	Normal	Low	High	Antagonist
Ineffective erythropoiesis	Normal–high	High	High	Low–normal	Agonist
Transfused patient	High	High	High	Normal	Agonist

^aPredicted.

ACD, anemia of chronic disease; ESA, erythropoietin-stimulating agents; IDA, iron-deficiency anemia.

Data from Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood*. 2010;116(23):4754–4761.

Hepcidin biology is being aggressively developed for iron-related diseases. Diagnostic²⁷ and therapeutic^{28,29} applications have been proposed (Table 1.3). More robust assays are needed before hepcidin levels become generally available in the clinic.³⁰ However, research studies have already shown that serum hepcidin levels are very low or at undetectable levels in patients with absolute iron-deficient anemia.³¹ In contrast, iron administration upregulates hepcidin in healthy volunteers.³² Importantly, hepcidin is increased by the inflammatory cytokines such as interleukin-6.³³ Therefore, patients with increased inflammatory states possess a wide range of serum hepcidin levels (100 to 4,000 ng/ mL) when compared with healthy volunteers (5 to 350 ng/ mL).³¹

TREATMENT OF IRON-DEFICIENCY ANEMIA

Dietary Iron

Dietary review and counseling are needed for all patients evaluated for iron deficiency. Iron malabsorption or inhibition of absorption by other substances needs to be evaluated. Non-vegan patients should be encouraged to increase red meat or liver in their diet, as well as vitamin C, known to increase iron absorption. Because the heme of meat is so readily absorbed and without gastrointestinal side effects, it is an excellent source of iron. The presence of heme in the diet also increases the absorption of inorganic iron. Patients presenting with anemia usually require more than just diet supplementation.³⁴

Oral Iron Therapy

Several oral iron formulations are available, all containing iron sulfate, gluconate, or fumarate (Table 1.4). Most of them are tablets, non-enteric coated, enteric coated, or slow release, but they can also be elixirs, usually containing less elemental iron.

Slow release or enteric-coated formulations of iron are touted to cause fewer gastrointestinal side effects, but they also often contain less iron per dose and are considerably more expensive than the nonenteric salts (Table 1.4). Furthermore, they may release their iron below the duodenum, too distal for significant absorption.

A daily supplement of ~200 mg of elemental iron taken in a fasting state provides the marrow with enough iron to raise the blood hemoglobin concentration up to 0.25 g/ dL/ day in severely anemic patients.³⁵ Oral iron, however, causes nausea or constipation in

some patients. Because these symptoms correlate with the amount of iron ingested, the dose should be lowered until tolerable or the medication should be stopped altogether until the symptoms resolve and then restarted at a lower dose. Using an elixir of iron allows doses as low as 10 to 20 mg of elemental iron, and multivitamins often contain even smaller amounts. Low doses of iron can be therapeutic; the response is just slower.³⁶ Patients should be prescribed stool softeners as needed. Often they can avoid nausea by taking their iron with food. This practice reduces iron absorption, but it usually does not make patients refractory to iron. Alternatively, bedtime dosing may be used to increase the tolerability to oral formulations.

Table 1.4 Oral Iron Supplements

Iron Salt	Formulation	Dose (mg)	Elemental Iron (mg)	Price (\$) ^a
Iron sulfate	Tablets	325	65	2.80/ 100 tabs
	Elixir	220/ 5.0 mL	44	5.95/ 480 mL
	Solution	75/ 1.0 mL	15	8.29/ 50 mL
Iron gluconate	Tablets	324	38	6.00/ 100 tabs
Iron fumarate	Tablets	324	106	22.16/ 60 tabs

^aPer uptodate.com, December 2017.

Table 1.5 Oral Iron Supplement Absorption

	Inhibition of Iron Absorption	Facilitating Iron Absorption
Diet	Coffee, milk, tea, dietary fiber, phosphate-containing beverages/ meals	Vitamin C
	Over the counter supplements containing zinc, calcium, manganese, or copper	Acid food— tomatoes, citrus
Drugs	Antacids: H ₂ blockers, alkaline liquids, proton pump inhibitors	Nonenteric iron tablets
	Tetracyclines, quinolones	Fasting ingestion of iron tablets
	Pancreatic enzymes supplements, byphosphonates, cholestyramine	

A variety of medications can reduce oral iron absorption (Table 1.5) and should not be taken within several hours of iron tablets. Conversely, oral iron supplements can hinder the absorption of other medications (Table 1.6).

Oral iron absorption testing may be considered for patients suspected of malabsorption.³⁷ An 8- to 12-hour fasting serum iron is compared with the iron serum level 1 hour after the ingestion of 65 mg of elemental iron (325 mg tablet of ferrous sulfate). An increase in serum iron of more than 100 µg/ dL from the baseline demonstrates adequate absorption. In the case of malabsorption, gastrointestinal consultation should be sought to identify and treat reversible etiologies. Parenteral iron

treatment may be considered in cases of iron malabsorption (post-gastric bypass surgery, celiac disease, autoimmune gastritis, and Helicobacter pylori infection).³⁸

Iron supplements should be taken until the anemia resolves, which may require only a few weeks. Additional supplements are required to replenish iron stores. Several algorithms can be used to decide the length of the therapy (Table 1.7). The rate of iron absorption becomes slower once the patient is no longer anemic;³⁷ so the serum ferritin levels may be followed to determine when iron stores are replenished. Once the anemia is reversed, a serum ferritin of 40 to 50 µg/ L should be reached before the supplements are discontinued.³⁹

Table 1.6 Medications Malabsorbed When Coadministered With Iron

Quinolone Antibiotics

Thyroxine
Biphosphonates
Penicillamine
Cefdinir
Mycophenolate mofetil
Levodopa, carbidopa, and methyldopa
Zinc or copper salts

Table 1.7 Iron-Replacement Dose Estimates

A. Estimated total oral dose of elemental iron for anemia correction. Additional dosing cycle(s) of 5,000 mg may be required to replenish iron stores.

Hemoglobin (g/ dL)	Elemental Iron Total Dose (mg) ^a
>11	5,000
9–11	10,000
<9	15,000

B. Calculation based on total blood volume and hematocrit (Hct)

Total iron deficit = Iron stores deficit + Hemoglobin iron deficit

Iron stores deficit = 500–1,000 mg

Hemoglobin iron deficit = Body wt. (lb) × (Target Hb – Actual Hb)

Target Hb = 14 g/ dL.

Oral elemental iron replacement estimate (mg) = 10 × total iron deficit

^aAssuming 10% absorption, 60-kg patient.

Intravenous Iron Therapy

CKD and dialysis patients receiving ESA require intravenous (IV) iron therapy.⁴⁰ When compared with the oral iron treatment, the administration of 100 mg of IV elemental iron

twice weekly required 46% less erythropoietin to maintain the same hematocrit goal.⁴¹ Other inflammatory states associated with anemia benefit from the combination of ESA and IV iron, including inflammatory bowel disease (IBD), rheumatoid arthritis, and malignancy.²⁴ Other indications include patients not being able to tolerate an adequate dose of oral iron, such as during pregnancy, or when they have severe and recurrent gastrointestinal or uterine hemorrhage.

Multiple formulations of parenteral iron are currently marketed in the United States (Table 1.8). Until 1999 when the Food and Drug Administration (FDA) approved Ferrlecit for the treatment of anemia in renal failure patients, iron dextran formulations were the only available option. Iron dextran is now the only formulation that requires a test dose and premedication due to reported anaphylactic reactions. Because fewer adverse events were reported with the non-dextran formulations,⁴² iron dextran is being replaced in clinical practice. For all preparations, the infusion can be repeated weekly depending on the iron deficit magnitude. An increased amount of elemental iron per dose with newer formulations allows for more rapid correction of the iron deficit, with fewer hospital visits, as well as increased compliance.⁴³ The clinical effects of the oxidative stress and other inflammatory changes reported with parenteral iron treatment are not fully understood.⁴⁰

Table 1.8 Intravenous Iron Supplements

Trade Name	Ferric Salt	Elemental Iron (mg/ mL)	Maximum Amount/ Dose
Ferrlecit	Gluconate	12.5	125 mg/ 1 h
Venofer	Sucrose	20	100–400 mg/ 15 min –4 h
Feraheme	PSC	30	510 mg/ 1 min
Injectafer	Carboxymaltose	50	750 mg/ 1 min
Monofer (Europe only)	Isomaltoside	100	20 mg/ kg/ 60 min

PSC, polyglucose sorbitol carboxymethylether.

Red Blood Cell Transfusion

Red blood cell transfusion is reserved for acute presentation in hemodynamically unstable patients. The iron content of packed red blood cells (PRBCs) is around 1.0 mg of heme iron per 1.0 mL of packed erythrocytes. After 1 unit of PRBCs is transfused, the expected increase in hemoglobin and hematocrit is 1 g/ dL and 3%, respectively.⁴⁴ It may take 2 to 3 weeks for the effects of transfused PRBCs to be realized in the iron parameters.⁴⁵

RESPONSE TO IRON THERAPY

When iron is given orally in full doses or parenterally to otherwise healthy individuals,

- | Within 3 or 4 days, peripheral blood reticulocytes increase
- | Within the first week, the hemoglobin begins to rise

A failure to observe a rise in hemoglobin after 1 to 2 weeks can be due to an incorrect diagnosis of iron deficiency, continued bleeding (in which case reticulocytes will increase despite no improvement in the anemia), noncompliance with the therapy, malabsorption for oral iron therapy, or a combination of these factors.

TREATMENT OF FUNCTIONAL IRON DEFICIENCY

Increased iron requirements during stress erythropoiesis can be addressed with either oral or parenteral iron administration. In pregnancy, parenteral iron may be required due to intolerance to oral formulations. ESA treatment in patients with CKD or end-stage renal disease (ESRD) requires adequate iron stores. Iron supplementation for dialysis patients is usually parenteral.⁴⁰

In iron sequestration syndromes, the only truly satisfactory solution is adequate treatment of their underlying causes. Although anemia is typically mild, treatment with iron supplements should be attempted for those patients with more severe anemia who are being considered for transfusion therapy. Parenteral iron supplementation may be helpful due to decreased oral absorption.²⁵ Although giving both erythropoietin and iron can eliminate the need for red cell transfusions, the effect of this combination on a patient's distribution of iron is the same as that of transfusions: Iron accumulates in inaccessible stores. Long-term safety studies are needed to determine the iron-dosing schedules and limits in this clinical setting.

Treatment of Anemia in Patients With Advanced Malignancy

Advanced malignancies, as well as their treatment with chemotherapy, puts patients at a higher risk of anemia. In this setting, support with parenteral iron and ESA is the standard approach. Oral iron has been shown to be inferior to parenteral iron in patients receiving ESA for chemotherapy-induced anemia.⁴⁶ Multiple studies in recent years have shown a decreased survival in patients with different malignancies when receiving ESA.⁴⁷ Increased risks of thromboembolic disease, pro-angiogenic effects, as well as elevated blood pressure are proposed mechanisms.⁴⁷ The FDA recently issued a black box warning for cancer patients receiving ESA.

Treatment of Anemia Associated With Chronic Inflammatory Diseases

The anemia of rheumatoid arthritis has been reported to respond to parenteral iron alone, as well as to ESA alone, with elevations of hemoglobin from ~11.5 to ~12.5 g/

dL in both instances.^{48,49} An additive effect was reported when adding parenteral iron to ESA in one series.⁵⁰ In IBD, (1) absolute iron deficiency is common, (2) the anemia typically responds to iron alone, (3) parenteral iron is usually required because of gastrointestinal intolerance, and (4) erythropoietin can magnify the erythroid response.⁵¹ In contrast, anemic patients with chronic infections, including HIV, should receive iron only if they have absolute iron deficiency, because of the concern that an increased iron supply may promote the growth of certain microorganisms that are siderophoric, such as *Yersinia enterocolitica* or *Klebsiella pneumoniae*.^{52,53}

SUMMARY

Iron-deficiency anemia remains one of the most prevalent health problems in the United States and worldwide despite improved understanding of its pathophysiology and the availability of more oral and parenteral supplementation options. In addition to the assessment of the hematological status and iron parameters, effort should always be made to determine the cause of absolute or functional iron deficiency. In cases of hemorrhage or nutritional iron deficiency, the diagnosis and case management are usually accomplished in the primary care setting.

Specialized care is indicated when no cause is identified or the patient does not respond to oral therapy. In some cases, parenteral iron formulations may be required. Based on improved safety profiles, administration of parenteral iron may be provided in the outpatient setting. Iron-replacement regimens should be designed to correct the anemia and to additionally replenish iron stores. Rapid advances in iron and hepcidin biology are predicted to improve future diagnostic and therapeutic approaches to this disease.

References

1. Rastogi T, Mathers C. Global burden of iron deficiency anaemia in the year 2000. WHO Report. 2002;1–13.
2. Centers for Disease Control and Prevention. Recommendations to prevent and control iron deficiency in the United States. MMWR. 1998;47(RR3):1–29.
3. Vanasse GJ, Berliner N. Anemia in elderly patients: an emerging problem for the 21st century. Hematology Am Soc Hematol Educ Program. 2010;2010:271–275.
4. Silber MH, Richardson JW. Multiple blood donations associated with iron deficiency in patients with restless legs syndrome. Mayo Clin Proc. 2003;78(1):52.
5. Tunnessen WW, Smith C, Oski FA. Beeturia: a sign of iron deficiency. Am J Dis Child. 1969;117(4):424.
6. Heaney MM, Andrews NC. Iron homeostasis and inherited iron overload

- disorders: an overview. *Hematol Oncol Clin North Am*. 2004;18(6):1379–1403.
- 7. Bothwell TH, Baynes RD, MacFarlane BJ, et al. Nutritional iron requirements and food iron absorption. *J Intern Med*. 1989;226:357–365.
 - 8. Bothwell TH, Charlton RW. Iron Deficiency in Women. Washington, DC: The Nutrition Foundation; 1981.
 - 9. Andrews NC. Understanding heme transport. *N Engl J Med*. 2005;353:2508–2509.
 - 10. McKie AT, Barrow D, Latunde-Dada GO, et al. An iron-regulated ferric reductase associated with absorption of dietary iron. *Science*. 2001;291:1755–1759.
 - 11. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;306(5704):2090–2093.
 - 12. Donovan A, Lima CA, Pinkus JL, et al. The iron exporter ferroportin/ Slc40a1 is essential for iron homeostasis. *Cell Metab*. 2005;1(3):191–200.
 - 13. Huebers HA, Csiba E, Huebers E, et al. Competitive advantage of diferric transferrin in delivering iron to reticulocytes. *Proc Natl Acad Sci*. 1983;80:300–304.
 - 14. Bainton DF, Finch CA. The diagnosis of iron deficiency anemia. *Am J Med*. 1964;37:62–70.
 - 15. Alleyne M, Horne MK, Miller JL. Individualized treatment for iron-deficiency anemia in adults. *Am J Med*. 2008;121(11):943–948.
 - 16. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med*. 1974;290:1212–1216.
 - 17. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content. *Am J Hematol*. 2008;83(4):307–310.
 - 18. Mast AE, Blinder MA, Gronowski AM, et al. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. *Clin Chem*. 1998;44(1):45–51.
 - 19. Skikne BS, Punnonen K, Caldron PH, et al. Improved differential diagnosis of anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the sTfR/ log ferritin index. *Am J Hematol*. 2011;86(11):923–927.
 - 20. Hillman RS, Henderson PA. Control of marrow production by the level of iron supply. *J Clin Invest*. 1969;48:454–460.
 - 21. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. *Am J Clin Nutr*. 2000;72(1 suppl):257S–264S.
 - 22. Nurko S. Anemia in chronic kidney disease: causes, diagnosis, treatment. *Cleve Clin J Med*. 2006;73(3):289–297.
 - 23. Ginzburg Y, Rivella S. β -thalassemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. *Blood*. 2011;118(16):4321–4330.

24. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood*. 2010;116(23):4754–4761.
25. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. 2005;352:1011–1023.
26. Cash JM, Sears DA. The anemia of chronic disease: spectrum of associated diseases in a series of unselected hospitalized patients. *Am J Med*. 1989;87:638–644.
27. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood*. 2011;117(17):4425–4433.
28. Sasu BJ, Cooke KS, Arvedson TL, et al. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia. *Blood*. 2010;115(17):3616–3624.
29. Hashizume M, Uchiyama Y, Horai N, et al. Tocilizumab, a humanized anti-interleukin-6 receptor antibody, improved anemia in monkey arthritis by suppressing IL-6-induced hepcidin production. *Rheumatol Int*. 2010;30(7):917–923.
30. Young B, Zaritsky J. Hepcidin for clinicians. *Clin J Am Soc Nephrol*. 2009;4(8):1384–1387.
31. Ganz T, Olbina G, Girelli D, et al. Immunoassay for human serum hepcidin. *Blood*. 2008;112(10):4292–4297.
32. Pak M, Lopez MA, Gabayan V, et al. Suppression of hepcidin during anemia requires erythropoietic activity. *Blood*. 2006;108:3730–3735.
33. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004;113:1271–1276.
34. Crosby WH. The rationale for treating iron deficiency anemia. *Arch Intern Med*. 1984;144:471–472.
35. Pritchard JA. Hemoglobin regeneration in severe iron-deficiency anemia. *J Am Med Assoc*. 1966;195:97–100.
36. Radtke H, Tegtmeier J, Rocker L, et al. Daily doses of 20 mg of elemental iron compensate for iron loss in regular blood donors: a randomized, double-blind, placebo-controlled study. *Transfusion*. 2004;44:1427–1432.
37. Cook JD. Diagnosis and management of iron-deficiency anemia. *Best Pract Res Clin Haematol*. 2005;18:219–332.
38. Hershko C, Hoffbrand AV, Keret D, et al. Role of autoimmune gastritis, *Helicobacter pylori* and celiac disease in refractory or unexplained iron deficiency anemia. *Haematologica*. 2005;90(5):585–595.
39. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*. 1990;75(9):1870–1876.
40. National Kidney Foundation. KDOQI clinical practice guidelines and clinical

- practice recommendations for anemia in chronic kidney disease. *Am J Kidney Dis.* 2006;47:S11–S45.
41. Silverberg DS, Iaina A, Peer G, et al. Intravenous iron supplementation for the treatment of the anemia of moderate to severe chronic renal failure patients not receiving dialysis. *Am J Kidney Dis.* 1996;27:234–238.
 42. Chertow GM, Mason PD, Vaage-Nilsen O, et al. Update on adverse drug events associated with parenteral iron. *Nephrol Dial Transplant.* 2006;21:378–382.
 43. Gozzard D. When is high-dose intravenous iron repletion needed? Assessing new treatment options. *Drug Des Dev Ther.* 2011;5:51–60.
 44. Wiesen AR, Hospenthal DR, Byrd JC, et al. Equilibration of hemoglobin concentration after transfusion in medical inpatients not actively bleeding. *Ann Intern Med.* 1994;121(4):278–230.
 45. Hod EA, Brittenham GM, Billote GB, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. *Blood.* 2011;118(25):6675–6682.
 46. Auerbach M, Ballard H, Trout JR, et al. Intravenous iron optimizes the response to recombinant human erythropoietin in cancer patients with chemotherapy-related anemia: a multicenter, open-label, randomized trial. *J Clin Oncol.* 2004;22:1301–1307.
 47. United States Food and Drug Administration. FDA Drug Safety Communication: erythropoiesis-stimulating agents (ESAs): Procrit, EpoGen and Aranesp. <https://www.fda.gov/drugs/drugsafety/postmarketdrugsafetyinformationforpatientsa> Accessed December 2017.
 48. Bentley DP, Williams P. Parenteral iron therapy in the anaemia of rheumatoid arthritis. *Rheumatol Rehabil.* 1982;21:88–92.
 49. Pincus T, Olsen NJ, Russell IJ, et al. Multicenter study of recombinant human erythropoietin in correction of anemia in rheumatoid arthritis. *Am J Med.* 1990;89:161–168.
 50. Nordstrom D, Lindroth Y, Marsal L, et al. Availability of iron and degree of inflammation modifies the response to recombinant human erythropoietin when treating anemia of chronic disease in patients with rheumatoid arthritis. *1997;17:67–73.*
 51. Gasche C, Lomer MCE, Cavill I, et al. Iron, anaemia, and inflammatory bowel disease. *Gut.* 2004;53:1190–1197.
 52. Henry DH, Beall GN, Benson CA, et al. Recombinant human erythropoietin in the treatment of anemia associated with human immunodeficiency virus (HIV) infection and zidovudine. *Ann Intern Med.* 1992;117:739–748.
 53. Maynor L, Brophy DF. Risk of infection with intravenous iron therapy. *Ann Pharmacother.* 2007;41(9):1476–1480.

Deficiencies of Vitamin B₁₂ and Folate

Danielle M. Townsley and Griffin P. Rodgers

Besides iron deficiency, shortages of vitamin B₁₂ (cobalamin) and folate are the most common nutritional causes of anemia. The frequencies of these deficiencies are highly dependent on the population under study. Because vitamin B₁₂ deficiency usually develops as a consequence of insidious malabsorption that occurs over many years, it becomes more prevalent with advancing age. As folate deficiency is largely a consequence of inadequate dietary folate, it is most prevalent in populations at risk for malnutrition or areas where the food is unfortified.

VITAMIN REQUIREMENTS, SOURCES, AND STORES

To avoid clinically apparent ill effects, the daily adult requirement for vitamin B₁₂ is 1 to 3 µg and for folic acid ~200 µg (Table 2.1).^{1,2} Between 4 and 7 µg/ day of B₁₂ is necessary to prevent biochemical changes secondary to a limiting supply of the vitamin.³ This suggests that the current recommended dietary allowance (RDA) of 2.4 µg/ day may be insufficient. The daily requirement for folate has been more easily met since 1996, when the U.S. Food and Drug Administration (FDA) mandated that all grains be fortified with the vitamin to reduce the risk of neural tube defects in developing fetuses.⁴

Bacteria in the gut of herbivorous animals synthesize vitamin B₁₂ and supply it to their hosts, who in turn supply it to humans in the form of meat. There is no vitamin B₁₂ in plant products other than that attributable to bacterial contamination. Plants synthesize folic acid and provide it to man directly in fruits and vegetables and indirectly in meat from herbivores.

The human body normally stores 2 to 3 months' supply of folic acid, although

marginally nourished patients, such as chronic alcoholics, may have stores that can be depleted much sooner.⁵ In contrast, body stores of vitamin B₁₂ are normally sufficient for 5 to 10 years (Table 2.1).

METABOLIC ROLES OF FOLATE AND VITAMIN B₁₂

The metabolic roles of folate and B₁₂ are closely interrelated (Fig. 2.1). Folate derivatives are essential cofactors in thymidylate synthesis, which is a rate-limiting step in the synthesis of DNA. RNA synthesis, however, is not dependent on folate. Therefore, deficiency of folate limits gene transcription but not RNA translation, retarding cell division but not cytoplasmic protein synthesis. This leads to the typical cytonuclear dissociation of maturation characteristic of megaloblastic hematopoiesis. Because cobalamin supports the recycling of folate, vitamin B₁₂ deficiency causes megaloblastic changes by restricting the folate supply. This restriction can be at least partially overcome by increasing dietary folate, allowing the hematopoietic effects of cobalamin deficiency to be ameliorated by high doses of folic acid. In contrast, the hematopoietic effects of folate deficiency cannot be overcome by treatment with vitamin B₁₂.

Table 2.1 Vitamin B₁₂ and Folic Acid: Biology and Dosing

	Vitamin B ₁₂	Folic Acid
Source	Bacteria → Meat	Plants → Meat
Daily requirement	1–3 µg	~200 µg
Body store	2–5 mg	~20 mg
Time to deficiency	5–10 y	2–3 mo
Dosing		
Oral	2 mg/ d	
Parenteral	1 mg/ mo	1 mg/ d
Cost of dose per month ^a		
Oral	\$3.48	\$2.38
Parenteral	\$1.20	

^aAverage wholesale price as of December 2011, Pharmacy Department, W.G. Magnuson Clinical Center.

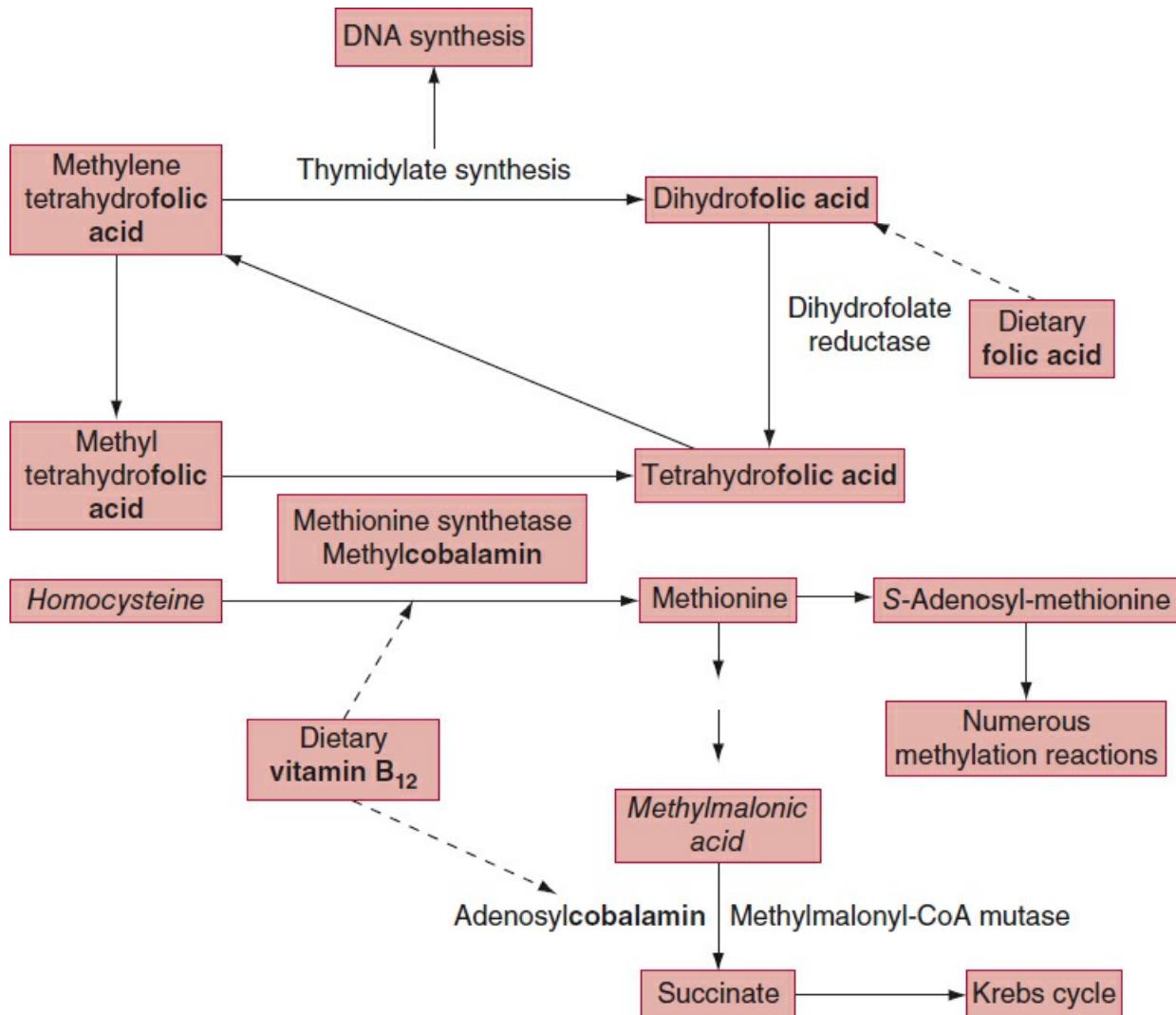


FIGURE 2.1 Metabolic pathways involving folic acid and vitamin B₁₂ (cobalamin).

Cobalamin is also necessary in the pathway leading to the synthesis of S-adenosylmethionine, which is the only donor of methyl groups for numerous reactions in the brain involving proteins, membrane phospholipids, and neurotransmitters.⁶ Presumably this explains the frequent neuropsychiatric signs and symptoms associated with vitamin B₁₂ deficiency.⁷ Folate is not involved in these reactions and cannot reverse the neuropsychiatric deficits caused by vitamin B₁₂ deficiency. However, methyl-tetrahydrofolate is the methyl donor for the synthesis of methionine, the precursor of S-adenosylmethionine. Therefore, folate deficiency may restrict the synthesis of S-adenosyl-methionine and produce some neuropsychiatric effects as well, although this is rare.⁸

DEVELOPMENT OF VITAMIN B₁₂ DEFICIENCY

Cobalamin deficiency is rarely caused by inadequate intake or increased use of the vitamin (Table 2.2). This is in part due to the pronounced enterohepatic circulation of cobalamin. Although strict vegetarians become depleted of vitamin B₁₂, vegetables often contain sufficient bacteria to provide a marginally adequate supply. A developing fetus shunts cobalamin from its mother, placing her at risk of deficiency, particularly if her baseline stores are low. Rarely, intestinal parasites can induce deficiency; for example the fish tapeworm, *Diphyllobothrium latum*, competes with the host for cobalamin.⁹ Acutely, cobalamin metabolism can be disrupted by nitrous oxide anesthesia and induce a rapid, usually transient, megaloblastic anemia.¹⁰ However, fatalities and severe neuropsychiatric damage have been associated with chronic administration in patients and recreational use of nitrous oxide.¹¹

Far more commonly, defects in any of the three levels of the gastrointestinal tract can lead to vitamin B₁₂ malabsorption: the fundus of the stomach, the pancreas, or the small bowel.¹² Obviously, surgical removal or bypass of any of these regions leads to B₁₂ malabsorption.¹³ Bariatric surgery is becoming more prevalent and subsequently an important risk factor for developing B₁₂ deficiency. Otherwise the etiology is inflammatory.

Table 2.2 Causes of Vitamin B₁₂ Deficiency

Gastrointestinal	Gastric atrophy: achlorhydria, achlorhydria + intrinsic factor deficiency Bariatric surgery Gastrectomy Gastric bypass Terminal ileal resection Extensive celiac disease Crohn's disease of the stomach Bacterial overgrowth in the small bowel (achlorhydria, anatomical defects, impaired motility) Zollinger-Ellison syndrome Pancreatic insufficiency HIV Intestinal parasites
Medications	Megadoses of vitamin C, metformin, proton pump inhibitors
Increased utilization	Pregnancy
Toxin	Nitrous oxide
Dietary	Strict vegetarianism
Rare congenital disorders	Congenital intrinsic factor deficiency Defective intrinsic factor-cobalamin receptors Abnormal plasma cobalamin transport Inborn errors of intracellular cobalamin metabolism

- Stomach: In the stomach, food (protein)-bound vitamin B₁₂ must be freed by digestion with pepsin and bound to “R-proteins,” which is a generic term for proteins that bind B₁₂.¹ The parietal cells in the fundus secrete both the acid necessary for this digestion and intrinsic factor, the protein to which cobalamin is later transferred in the alkaline duodenum. Therefore, any process that damages the parietal cells can lead to vitamin B₁₂ malabsorption and eventually deficiency. The most common cause is autoimmune atrophic gastritis, which increases in prevalence with age and is sometimes associated with other autoimmune diseases, such as thyroiditis. *Helicobacter pylori*, however, which typically causes antral gastritis, can occasionally also infect the fundus.^{14,15} Proton pump inhibitors induce chronic hypochlorhydria but rarely cause clinically significant B₁₂ malabsorption. Paradoxically, the hypersecretion of acid in the Zollinger–Ellison syndrome leads to B₁₂ malabsorption by acidifying the small bowel, which must remain alkaline for the transfer of B₁₂ from the R-binders to intrinsic factor. Antibodies to intrinsic factor, as in the case of pernicious anemia, also lead to a reduction of vitamin B₁₂-intrinsic factor complexes necessary for absorption in the small bowel.
- Pancreas: Deficiency of pancreatic enzymes impairs the digestion of R-binders in the small bowel and therefore the release of B₁₂ to intrinsic factor. Although pancreatic insufficiency causes cobalamin malabsorption, it rarely is significant enough to become clinically apparent.
- Small bowel: Vitamin B₁₂-intrinsic factor complexes are endocytosed by the mucosa of the terminal ileum. Inflammatory bowel disease or particularly extensive celiac or tropical sprue interfere with this process.¹⁶ Bacterial overgrowth in the small bowel, especially common in the elderly, competes for B₁₂ and makes it less available for absorption.¹⁷ Calcium dependent ileal membrane antagonism of metformin leads to diminished B₁₂ absorption in up to one-third of diabetic patients, but rarely leads to anemia, and is reversed with supplemental calcium.^{12,18,19} HIV infection is sometimes also associated with B₁₂ malabsorption, especially in the presence of chronic diarrhea.

DEVELOPMENT OF FOLIC ACID DEFICIENCY

A diet poor in fresh vegetables is a major cause of folic acid deficiency (Table 2.3). Cooked vegetables and meat are less satisfactory sources because cooking destroys much of the folate. (This is less of a problem for vitamin B₁₂.) The other major causes are gastrointestinal diseases that affect the jejunum, where folic acid is absorbed, and conditions such as pregnancy that increase folate requirements. Ethanol abuse and

several chronic medications ([Table 2.3](#)) lead to folate deficiency by interrupting folate metabolism or inhibiting its absorption.

Table 2.3 Causes of Folic Acid Deficiency

Diet	Lack of fresh vegetables
Gastrointestinal disease	Celiac disease (gluten-sensitive enteropathy) Dermatitis herpetiformis Tropical sprue Small bowel resection Crohn's disease Enteropathic diversion Extranodal lymphoma Amyloidosis
Medications	Cytotoxic agents: methotrexate Antibiotics: pyrimethamine, cycloserine, trimethoprim (pregnancy) Diuretics: triamterene Anticonvulsants: phenytoin, carbamazepine, phenobarbital, primidone
Ethanol	
Increased utilization/ loss	Pregnancy Chronic hemolysis (e.g., sickle cell anemia) Exfoliative dermatitis Chronic hemodialysis

PATIENT POPULATIONS AT RISK

Vitamin B₁₂ deficiency due to lack of intrinsic factor (“pernicious anemia”) is sometimes believed to be limited to elderly patients of European descent. In this population the median age at presentation is almost 70 years.

- Nevertheless, intrinsic factor deficiency may be almost as prevalent in African Americans and Latinos, who tend to present with vitamin B₁₂ deficiency a decade earlier.^{20,21}
- Older patients are actually more likely to become B₁₂ deficient from achlorhydria or small bowel bacterial overgrowth than from lack of intrinsic factor.^{15,22}

Although cobalamin deficiency is generally found in older age groups,²³ folic acid deficiency is likely to occur in any patient who has an inadequate diet or who has an increased need for the vitamin, for example, during pregnancy or hemolytic anemia ([Table 2.3](#)). Folate deficiency secondary to poor nutrition is generally rare in the United States since the introduction of mandatory folic acid fortification of cereal grains. A population study among centenarians indicates that less than 6% of the very old have low RBC folate levels.²⁴

CLINICAL PRESENTATION

The clinical presentation of vitamin B₁₂ and folic acid deficiency covers a wide range from asymptomatic, to life-threatening pancytopenia or myelopathy (Table 2.4). The investigation of any new neuropsychiatric changes should include an evaluation of vitamin B₁₂ (and folate status) even in the absence of hematologic signs of a deficiency.⁷ B₁₂ deficiency is commonly associated with neurologic changes, and only rarely with folate deficiency; hereditary folate malabsorption and/or metabolism are the exception and associates with progressive neurologic deterioration in childhood.²⁵ Classically, changes in the tongue mucosa and mouth angle stomatitis are the earliest signs of folate deficiency on physical examination.²⁶

Table 2.4 Clinical and Laboratory Presentations of Vitamin B₁₂ or Folic Acid Deficiency

Peripheral blood	Macrocytic red cells, oval macroovalocytes, fragmented misshapen cells, nucleated red cells, hypersegmented neutrophils (1% with 6 lobes or 5% with 5 lobes) Anemia, usually macrocytic but normo- or microcytic if accompanied by iron deficiency or thalassemia Leukopenia, thrombocytopenia, pancytopenia Elevated lactate dehydrogenase level, indirect bilirubin, decreased haptoglobin Bone marrow hypercellular, megaloblastic erythropoiesis with varied degrees of dysplasia and immature nuclear chromatin
Neuropsychiatric (B ₁₂ deficiency only)	Peripheral neuropathy (paresthesias, hyporeflexia) Spinal cord degeneration (weakness, hyperreflexia, reduced vibratory and position sense, incontinence, impotence) Memory loss, "Megaloblastic madness": altered mental status, disorientation, depression, delusions
Gastrointestinal	Malabsorption (weight loss, diarrhea, abdominal pain), glossitis
Reproductive	Infertility, fetal loss

LABORATORY EVALUATION

Hematologic Abnormalities

Macrocytosis develops before anemia when either folic acid or vitamin B₁₂ is limiting (Table 2.5).^{5,27} With the advent of automated blood cell analyzers, isolated macrocytosis has become a typical presentation of deficiencies of either vitamin, although other causes of macrocytosis are more common (Table 2.6).²⁸ An unexplained rise in mean cell volume (MCV) of 5 fL or more even within the normal range should also attract suspicion. However, the macrocytosis may be masked if the patient is also iron deficient or has a thalassemic trait. At hemoglobin concentrations less than ~10 g/dL, B₁₂ or folate deficiency leads to elevations of serum lactate dehydrogenase, which

can become quite high.^{5,27} This results from marked intramedullary death of developing red cells and a shortening of the circulating red cell life span but can mislead the clinician to suspect metastatic disease or a primary hemolytic anemia.

The earliest change in the peripheral blood caused by folate or vitamin B₁₂ deficiency is hypersegmentation of the neutrophils, which can be easily overlooked unless a blood smear is carefully examined. Finding even 5% of neutrophils with five lobes or just 1% with six lobes is highly suggestive of a deficiency, although this could also be seen with myelodysplasia. In advanced deficiencies characterized by severe anemia, pancytopenia can develop.

There is rarely if ever a need to perform a bone marrow examination in the evaluation of vitamin B₁₂ and folic acid status. The megaloblastic changes in the marrow are identical in both deficiencies and are variable in intensity. A marrow examination cannot rule out myelodysplasia or even a smoldering leukemic process until cobalamin and folate deficiencies have been excluded first.

Serum Vitamin Concentrations

When vitamin B₁₂ and folate deficiency is suspected, a common diagnostic starting point is measurement of the serum concentrations of the vitamins, which should be done after the patient has been fasting. The results, however, can be difficult to interpret (Table 2.7).

Serum folic acid does not reliably reflect the body's supply of the vitamin, unless it is consistently less than ~3 ng/ mL, and even then it does not distinguish between negative balance and actual tissue deficiency.²⁹ In general, the serum folate level reflects recent folate intake, and red cell folate is a better measurement of tissue folate stores. Because red cell folate is packaged at the time the cell is made and remains in the cell throughout its 3- to 4-month life span, the measured mean value may fail to reflect relatively recent reductions in dietary folate. Furthermore, the reproducibility of assays for red cell folate is relatively poor and hence borderline values can be misleading.^{29,30} To complicate matters further, red cell folate can be reduced by vitamin B₁₂ deficiency and lead to an erroneous diagnosis (Table 2.7).

Table 2.5 Progression of Laboratory Parameters

	Normal	Negative Balance	Depleted Stores	Tissue Deficiency	Anemia
Folic acid deficiency					
Serum folic acid (ng/ mL)	5–20	<3	<3	<3	>3
RBC folic acid (ng/ mL)	>200	>200	<200	<200	<200
Serum homocysteine (μmol/ L)	5–15	5–15	5–15	15–250	15–250

L)					
Hypersegmented neutrophils	0	0	0	+	++
MCV (fL)	80–95	80–95	80–95	90–110	100–130
Hemoglobin (g/ dL)	12–15	12–15	12–15	12–15	<12
Vitamin B₁₂ deficiency					
Serum cobalamin (pg/ mL)	200–900	150–500	100–300	50–250	50–250
Serum MMA (μmol/ L)	<0.4	<0.4	<0.4	0.4–20	1–20
Hypersegmented Neutrophils	0	0	0	+	++
MCV (fL)	80–95	80–95	80–95	90–110	100–130
Hemoglobin (g/ dL)	12–15	12–15	12–15	12–15	<12

MCV, mean cell volume; MMA, methylmalonic acid; RBC, red blood cell.

Table 2.6 Causes of Macrocytosis With or Without Anemia

Medications	Macrocytosis Alone	Macrocytic Anemia
Cytotoxic chemotherapy (methotrexate, hydroxyurea cytosine arabinoside, azathioprine, others)	+	+
Anticonvulsants (phenytoin, carbamazepine, primidone)	+	+
Antiretrovirals	+	+
Antibiotics (cotrimoxazole)	+	+
Liver disease	+	+
Alcoholism ± liver disease	+	+
Reticulocytosis	0	+
Folate deficiency	+	+
Vitamin B ₁₂ deficiency	+	+
Myelodysplasia	+	+
Hypothyroidism	+	+
>75 y old	+	+
Down's syndrome	+	0
Artifact (RBC agglutination)	+	0

RBC, red blood cell.

Table 2.7 Laboratory Tests for Folic Acid and Vitamin B₁₂ Deficiency

Folic Acid	Vitamin B ₁₂
------------	-------------------------

Serum folic acid	Nl to ↓	Nl to ↑
Red cell folic acid	Nl to ↓	Nl to ↓
Serum vitamin B ₁₂	Nl to ↓	Nl to ↓
Methylmalonic acid	Nl	↑↑
Homocysteine	↑	↑↑
Lactate dehydrogenase	↑	↑
Haptoglobin	Nl to ↓	Nl to ↓

Nl, normal.

Biochemical tests for serum vitamin B₁₂ are highly sensitive but specificity is rare.³¹ Pernicious anemia is the exception; presence of anti-intrinsic factor antibodies can lead to assay errors.³² In a patient with suspected cobalamin deficiency, it is recommended to verify at least two unrelated biochemical abnormalities, such as cobalamin and methylmalonic acid (MMA), and, always test for intrinsic factor antibodies.³³ Interpreting serum vitamin B₁₂ levels can be problematic because occasionally serum B₁₂ can be decreased in rare cases of folate deficiency.¹ A more frequent problem, similar to that with folic acid, relates to uncertainties in the physiologic levels of the vitamin. The “normal range” of serum cobalamin typically extends down to 200 pg/ mL, because healthy, non-anemic donors occasionally have B₁₂ concentrations that are this low. However, patients who are truly deficient in vitamin B₁₂ can have serum cobalamin levels as high as 300 pg/ mL and even higher.^{29,34} The reason for this discrepancy is that the total cobalamin is measured, rather than just the vitamin B₁₂ bound to transcobalamin, which is the metabolically available B₁₂ but represents only ~20% of the total serum vitamin. Therefore, individuals who have relatively low concentrations of transcobalamin 1, which binds the other ~80% of the serum vitamin B₁₂, can have alarmingly low B₁₂ levels (<100 pg/ mL) without any harmful effect because they have adequate amounts of B₁₂ bound to transcobalamin 2, which is necessary for transfer of B₁₂ to hematopoietic precursors. Occasionally, healthy patients have been described with low transcobalamin 1 levels.³⁵ Transcobalamin 1 can also be low in patients with multiple myeloma.³⁶ Such patients will be easier to identify when tests become available to measure B₁₂ specifically bound to transcobalamin rather than total serum B₁₂.³⁷

Table 2.8 Causes of Elevated Methylmalonic Acid and Homocysteine

	Methylmalonic Acid	Homocysteine
Vitamin deficiencies		Folic acid deficiency
	Vitamin B ₁₂ deficiency	Vitamin B ₁₂ deficiency, vitamin B ₆ (pyridoxine) deficiency

Genetic traits		Homozygous thermolabile tetrahydrofolate reductase
Renal disease	Renal insufficiency	Renal insufficiency
Endocrine	Pregnancy	Hypothyroidism
Drugs		Niacin, L-dopa
Metabolic	Volume contraction	Volume contraction

Serum Methylmalonic Acid and Homocysteine

Measurements of MMA and homocysteine (Hcy), although more expensive than the vitamin assays, answer the question of vitamin deficiency more reliably. Although these metabolites can be elevated for other reasons (Table 2.8), if these causes are excluded, these metabolites become specific reflections of vitamin B₁₂ and folate depletion at the tissue level.²¹ Usually MMA and Hcy both become elevated when B₁₂ is the limiting factor, whereas only Hcy becomes elevated when folate is limiting. However, in 1% to 2% of cases of vitamin B₁₂ deficiency, only Hcy will be elevated, whereas in ~10% of cases of folate deficiency, MMA will be high. Therefore, elevated Hcy alone does not always differentiate B₁₂ and folate deficiency but makes folate deficiency more likely.

Although normal levels of MMA and Hcy were reported to exclude a metabolic effect from B₁₂ deficiency, the negative predictive value of these assays has been questioned because of reports of patients with normal metabolites who clearly respond to vitamin B₁₂.²¹

Therapeutic Trial

If the laboratory evaluation is impossible because resources are lacking or evaluation is inconclusive, a therapeutic trial can be diagnostic if a single vitamin is given at a time. Vitamin B₁₂ should be given first because it will do nothing for folic acid deficiency, whereas replacement with folic acid will improve the anemia secondary to B₁₂ deficiency but not the neuropathic changes. The response to treatment can be judged by following the reticulocyte count and hemoglobin (see later). A more expensive way is to remeasure Hcy or MMA levels 2 to 5 days after one or the other of the vitamins has been administered. The metabolites fall only in response to replacement of the deficient vitamin.

DETERMINING THE CAUSE OF B₁₂ OR FOLATE DEFICIENCY

- The etiology of folate deficiency must always be determined because virtually all causes are either preventable or treatable. If the patient appears to have an adequate diet, a gastrointestinal evaluation is indicated to search for the underlying causes

shown in [Table 2.3](#).

In contrast, if vitamin B₁₂ is deficient and the patient is not a vegan and there are no symptoms of gastrointestinal disease, an argument can be made to take the evaluation no further and simply to treat the individual with the vitamin.

If the patient and/ or his physician feels compelled to confirm that the pathology lies in the stomach, however, a test for anti-intrinsic factor antibodies should be done.³⁸ A positive test is diagnostic of pernicious anemia and is found in half the cases. Anti-parietal cell antibodies are more common but are also found in a small percentage of normal individuals. Demonstrating an elevation in serum gastrin also strongly supports the diagnosis of gastric atrophy.³⁹

If the patient is unusually young to have achlorhydria or pernicious anemia (e.g., <50 years old or younger if African American), a gastrointestinal evaluation is indicated ([Table 2.2](#)).²⁰ In the past, the Schilling test was used in an attempt to detect vitamin B₁₂ malabsorption, but Schilling tests are no longer performed because of lack of a commercial source for radiolabeled cobalamin and the realization that this test also often gives misleading results. Because the incidence of many malignancies, especially gastric cancer, is slightly higher in patients with pernicious anemia than in age-and sex-matched controls, following the patient for any signs of gastrointestinal blood loss is wise, although more aggressive surveillance is not indicated.⁴⁰

TREATMENT/ RESPONSE

There are two treatment goals: replacement of the deficient vitamin and correction of the cause of the deficiency. The first goal is always achievable; the second may not be. Treatment should always be given if the clinical presentation is suspicious, even if the laboratory data are confusing, because the laboratory data are not totally sensitive and the consequences of undertreating can be devastating. The current evidence indicates that there is no benefit to further supplementation with vitamin B₁₂ or folate if a deficiency is nonexistent. For example, elevated levels of Hcy have been associated with an increased risk of vascular thrombosis; however, there is no role for supplementing vitamin B₁₂ or folate in a sole attempt to decrease Hcy levels and subsequent vascular events.^{41,42}

Vitamin B₁₂ repletion is traditionally given intramuscularly in North America, although the oral route is also efficacious and gaining in popularity.^{43–46} Oral supplementation is also effective in patients with pernicious anemia because approximately 1% of any oral dose of vitamin B₁₂ is absorbed by a simple diffusion across the mucosa. The recommended daily dose of 1 to 2 mg vitamin B₁₂ results in the absorption of ~10 to 20 µg, which is much more than the daily requirement. A caveat,

however, is that 1 mg tablets of vitamin B₁₂ are sometimes difficult to find in hospital pharmacies. In patients who are deficient in vitamin B₁₂, daily high-dose oral vitamin B₁₂ tablets (1,000 to 2,000 µg) are as effective as intramuscular injections and the tablets (500 to 1,500 µg) are available in the United States without a prescription.

Intramuscular B₁₂, of course, is perfectly acceptable if it is more practical for a patient, and this route would still be preferred by most physicians to treat a patient with neurologic symptoms or if compliance is a concern. Approximately 10% of the injected dose is retained. Although there are a variety of accepted regimens,^{33,47} daily injections of 50 to 100 µg should be given for a week, followed by weekly injections for a month, and then monthly injections of 1 mg. Patients with severe abnormalities should receive injections of 1,000 µg at least several times per week for 1 to 2 weeks, then weekly until clinically improving, followed by monthly injections. For most patients with vitamin B₁₂ deficiency, lifelong treatment is required because the underlying cause is not reversible.

The usual dose of oral folic acid is 1 mg/ day ([Table 2.1](#)). This is ample even during pregnancy or chronic hemolysis. If the deficiency is dietary, replacement with folic acid should continue until the diet has become adequate. A month of daily folic acid should be sufficient to replenish body stores. If the etiology of the deficiency is small bowel dysfunction, higher doses for longer periods may be necessary. It is important to rule out concomitant vitamin B₁₂ deficiency prior to replenishing folate because anemia may improve but the neurologic symptoms due to vitamin B₁₂ deficiency can progress and the diagnosis missed.

The response to correction of vitamin B₁₂ or folate deficiency is the same:

- | Mental changes and tongue soreness improve almost immediately after starting to replace the deficient vitamin.¹
- | After 4 to 5 days, reticulocytosis appears and may elevate the MCV even further.
- | Soon thereafter, the hemoglobin concentration begins to rise.
- | Neuropathic abnormalities, such as paresthesias, improve slowly, over several months, but may never disappear entirely if they have been long-standing.

If the hematologic response is blunted, additional etiologies for the anemia should be sought. It is not unusual for iron deficiency to accompany folate or vitamin B₁₂ deficiency. An underlying anemia of chronic disease is always a possibility.

References

1. Chanarin I. The Megaloblastic Anaemias. 3rd ed. Oxford: Blackwell; 1990.
2. Tighe P, Ward M, McNulty H, et al. A dose-finding trial of the effect of long-term folic acid intervention: implications for food fortification policy. *Am J Clin Nutr.*

2011;93(1):1–2.

3. Bor MV, von Castel-Roberts KM, Kauwell GP, et al. Daily intake of 4 to 7 microg dietary vitamin B-12-related biomarkers in a healthy young population. *Am J Clin Nutr.* 2010;91:571–577.
4. Jacques PF, Selhub J, Bostom AG, et al. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med.* 1999;340:1449–1454.
5. Lindenbaum J, Allen RH. Clinical spectrum and diagnosis of folate deficiency. In: Bailey LB, ed. *Folate in Health and Disease.* New York, NY: Marcel Dekker;1995:43–74.
6. Reynolds EH, Carney MWP, Toone BK. Methylation and mood. *Lancet.* 1984;2:196–198.
7. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Eng J Med.* 1988;318:1720–1728.
8. Carmel R, Green R, Rosenblatt DS, et al. Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program.* 2003:62–81.
9. Tanowitz HB, Weiss LM, Wittner M. Tapeworms. *Curr Infect Dis Rep.* 2001;3(1):77–84.
10. Hathout L, El-Saden S. Nitrous oxide-induced B-12 deficiency myopathy: perspectives on the clinical biochemistry of vitamin B-12. *J Neurol Sci.* 2011;301(1–2):1–8.
11. Flippo TS, Holder WD Jr. Neurologic degeneration associated with nitrous oxide anesthesia in patients with vitamin B12 deficiency. *Arch Surg.* 1993;128(12):1391–1395.
12. de Jager J, Kooy A, Lehert P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomized placebo controlled trial. *BMJ.* 2010;340:c2181.
13. Skroubis G, Sakellaropoulos G, Pouggouras K, et al. Comparison of nutritional deficiencies after Roux-en-Y gastric bypass and after biliopancreatic diversion with Roux-en-Y gastric bypass. *Obes Surg.* 2002;12:551–558.
14. Kaptan K, Beyan C, Ural AU, et al. Helicobacter pylori—is it a novel causative agent in vitamin B12 deficiency? *Arch Intern Med.* 2000;160:1349–1353.
15. Hershko C, Ronson A, Souroujon M, et al. Variable hematologic presentation of autoimmune gastritis: age-related progression from iron deficiency to cobalamin depletion. *Blood.* 2006;107:1673–1679.
16. Dahele A, Ghosh S. Vitamin B12 deficiency in untreated celiac disease. *Am J Gastroenterol.* 2001;96:745–750.
17. Haboubi NY, Montgomery RD. Small-bowel bacterial overgrowth in elderly people: clinical significance and response to treatment. *Age Aging.* 1992;21:13–

19.

18. Aroda VR, Edelstein SL, Goldberg RB, et al. Long-term metformin use and vitamin B12 deficiency in the Diabetes Prevention Program Outcomes Study. *J Clin Endocrinol Metab.* 2016;101(4):1754–1761.
19. Bauman WA, Shaw S, Jayatilleke E, et al. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care.* 2000;23(9):1227–1231.
20. Carmel R, Johnson CS. Racial patterns in pernicious anemia. *N Engl J Med.* 1978;298:647–650.
21. Savage DG, Lindenbaum J, Stabler SP, et al. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med.* 1994;96:239–246.
22. Dharmarajan TS, Adiga GU, Norkus EP. Vitamin B12 deficiency, recognizing subtle symptoms in older patients. *Geriatrics.* 2003;58:30–38.
23. Johnson MA, Hausman DB, Davey A, et al. Vitamin B12 deficiency in African American and white octogenarians and centenarians in Georgia. *J Nutr Health Aging.* 2010;14(5):339–345.
24. Hausman DB, Johnson MA, Davey A, et al. The oldest old: red blood cell and plasma folate in African American and white octogenarians and centenarians in Georgia. *J Nutr Health Aging.* 2011;15(9):744–750.
25. Geller J, Kromm D, Jayabose S, et al. Hereditary folate malabsorption: family report and review of the literature. *Medicine (Baltimore).* 2002;81(1):51–68.
26. Bjorkegren K, Svardsudd K. Reported symptoms and clinical findings in relation to serum cobalamin, folate, methylmalonic acid and total homocysteine among elderly Swedes: a population-based study. *J Intern Med.* 2003;254(4):343–352.
27. Stabler SP, Allen RH, Savage DG, et al. Clinical spectrum and diagnosis of cobalamin deficiency. *Blood.* 1990;76:871–881.
28. Savage DG, Ogundipe A, Allen RH, et al. Etiology and diagnostic evaluation of macrocytosis. *Am J Med Sci.* 2000;319:343–352.
29. Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. *Clin Chem.* 2000;46:1277–1283.
30. Pfeiffer CM, Zhang M, Lacher DA, et al. Comparison of serum and red blood cell folate microbiologic assays for national population surveys. *J Nutr.* 2011;141(7):1402–1409.
31. Carmel R. Diagnosis and management of clinical and subclinical cobalamin deficiencies: why controversies persist in the age of sensitive metabolic testing. *Biochimie.* 2013;95(5):1047–1055. doi: 10.1016/j.biochi.2013.02.008.
32. Carmel R, Agrawal YP. Failures of cobalamin assays in pernicious anemia. *N Engl J Med.* 2012;367(4):385–386. doi: 10.1056/NEJMc1204070.
33. Carmel R. How I treat cobalamin (vitamin B12) deficiency. *Blood.*

- 2008;112(6):2214–2221. doi: 10.1182/blood-2008-03-040253.
- 34. Tucker KL, Rich S, Rosenberg I, et al. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring Study. *Am J Clin Nutr.* 2000;71:514–522.
 - 35. Carmel R. A new case of deficiency of the R binder for cobalamin, with observations on minor cobalamin-binding proteins in serum and saliva. *Blood.* 1982;59:152–156.
 - 36. Hansen OP, Drivsholm A, Hippe E. Vitamin B12 metabolism in myelomatosis. *Scand. J Haematol.* 1977;18:395–402.
 - 37. Ulleland M, Eilertsen I, Quadros EV, et al. Direct assay for cobalamin bound to transcobalamin (holo-transcobalamin) in serum. *Clin Chem.* 2002;48:526–532.
 - 38. Fairbanks VF, Lennon VA, Kokmen E, et al. Tests for pernicious anemia: serum intrinsic factor blocking antibody. *Mayo Clin Proc.* 1983;58:203–204.
 - 39. Lindgren A, Lindstedt G, Kilander AF. Advantages of serum pepsinogen A combined with gastrin or pepsinogen C as first-line analytes in the evaluation of suspected cobalamin deficiency: a study in patients previously not subjected to gastrointestinal surgery. *J Intern Med.* 1998;244:341–349.
 - 40. Schafer LW, Larson DE, Melton LJ, et al. Risk of development of gastric carcinoma in patients with pernicious anemia: a population-based study in Rochester, Minnesota. *Mayo Clin Proc.* 1985;60:444–448.
 - 41. SEARCH Collaborative Group, Armitage JM, Bowman L, et al. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. *JAMA.* 2010;303(24):2486–2494.
 - 42. Løland KH, Bleie O, Blix AJ, et al. Effect of homocysteine-lowering B vitamin treatment on angiographic progression of coronary artery disease: a Western Norway B Vitamin Intervention Trial (WENBIT) substudy. *Am J Cardiol.* 2010;105(11):1577.
 - 43. Hvas A-M, Nexo E. Diagnosis and treatment of vitamin B12 deficiency. An update. *Haematologica.* 2006;91:1506–1512.
 - 44. Kuzminski AM, Giacco EJD, Allen RH, et al. Effective treatment of cobalamin deficiency with oral cobalamin. *Blood.* 1998;92:1191–1198.
 - 45. Bolaman Z, Kadikoylu G, Yukselen V, et al. Oral versus intramuscular cobalamin treatment in megaloblastic anemia: a single-center, prospective, randomized, open-label study. *Clin Ther.* 2003;25:3124–3134.
 - 46. Kim HI, Hyung WJ, Song KJ, et al. Oral vitamin B12 replacement: an effective treatment for vitamin B12 deficiency after total gastrectomy in gastric cancer patients. *Ann Surg Oncol.* 2011;18:3711–3717.
 - 47. Stabler SP. Clinical practice: vitamin B12 deficiency. *N Engl J Med.* 2013;368(2):149–160. doi: 10.1056/NEJMcp1113996.

Hemolytic Anemia

Sri Lakshmi Hyndavi Yeruva and Patricia O’Neal

Many diseases share the clinical feature of red blood cell (RBC) hemolysis. Hemoglobinopathies and immune-mediated hemolysis are the most common causes (see discussions in [Chapters 4](#) and [24](#), respectively). Very rare inherited or acquired diseases may also directly or indirectly result in increased red cell destruction.¹ Understanding the mechanisms that lead to hemolysis assists with the diagnosis, prognosis, and consideration of the most appropriate therapy. In this post-genomic era, correlations between genotype and phenotype are being pursued in cases of inherited hemolytic syndromes. Genetics-based discoveries are being translated into new clinical tools in anticipation of mechanism-specific therapies.

Normally, all circulating erythrocytes are subject to physiologic stresses such as turbulence in blood flow, endothelial damage, and age-related catabolic changes. These damaged erythrocytes are removed from the circulation by the reticuloendothelial system. Hemolytic anemia is caused by accelerated and premature destruction of erythrocytes and in hemolytic syndromes, erythrocyte clearance may be increased by the reticuloendothelial system (extravascular hemolysis) or the cells may be lysed within the circulation (intravascular hemolysis). Thus, RBC survival is generally shortened to less than 100 days (normal survival is approximately 120 days). When sufficient numbers of erythrocytes are destroyed, oxygen delivery to the tissues is impaired. Tissue hypoxia leads to an increased release of erythropoietin, which signals the bone marrow to produce more RBCs.

A hallmark of hemolytic anemia is an elevated number of immature erythrocytes (reticulocytes) in the peripheral blood. During low-level hemolysis, there is increased production of erythrocytes, as evidenced by reticulocytosis, which compensates for the hemolysis and minimizes the degree of anemia. Alternatively, patients with acute hemolysis or with underlying defects in hematopoiesis may present with pronounced

anemia without reticulocytosis. Hence, the evaluation of suspected hemolysis requires a consideration of the hemolysis itself as well as the marrow's ability to compensate. The diagnostic strategy usually begins with a search for common causes of hemolysis and proceeds toward rare etiologies. The extent of diagnostic studies should be guided by the magnitude of hemolysis and the available therapeutic options. With the information contained here, practicing clinicians should be able to develop a clinical approach, differential diagnosis, and a therapeutic plan for patients with suspected hemolysis.

ETIOLOGY AND DIFFERENTIAL DIAGNOSIS

Grouping the various causes of the disease generates a differential diagnosis for hemolysis. As shown in [Figure 3.1](#), hemolysis results from pathology intrinsic or extrinsic to the erythrocytes. Intrinsic hemolysis may be categorized further according to hemoglobin-, membrane-, or enzyme-based factors. Alternatively, the patient's immune status or infectious agents can lead to hemolysis in the absence of intrinsic defects. Other chemical or physical features of the erythrocyte environment can also cause hemolysis. A more complete differential organized according to these categories is shown in [Table 3.1](#).

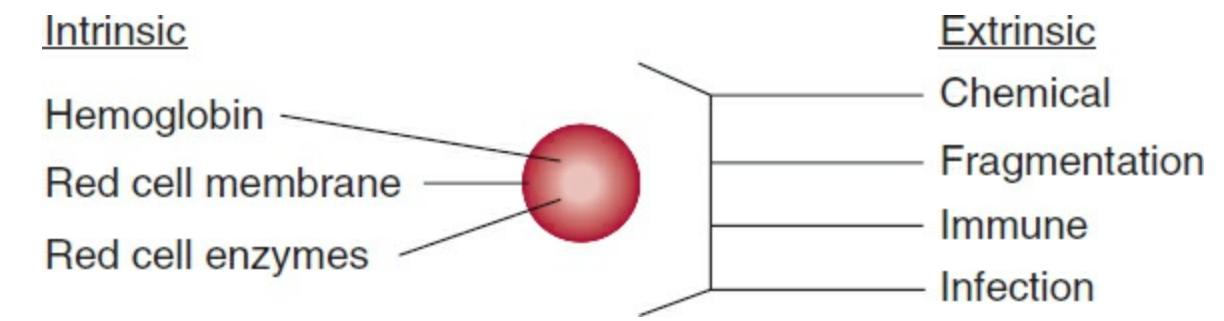


FIGURE 3.1 Intrinsic and extrinsic causes of hemolysis.

Most intrinsic causes of hemolysis are inherited, while the extrinsic causes of hemolysis are typically acquired. In some cases, such as paroxysmal nocturnal hemoglobinuria (PNH) or glucose-6-phosphate dehydrogenase (G6PD) deficiency, both intrinsic and extrinsic factors may contribute to the hemolytic picture. Consideration of the primary site of hemolysis (intravascular vs. extravascular) may also be helpful in determining the origin of erythrocyte destruction.⁶

To complete the differential diagnosis, underlying diseases or events that may in part mimic a typical hemolytic episode should be considered. The laboratory evaluation may be normal, with the exception of a single variable, such as hemoglobin, absolute reticulocyte count (ARC), or unconjugated bilirubin. For instance, the compensatory reticulocytosis that occurs after an acute hemorrhagic event may be mistaken for

evidence of hemolysis. In the absence of other clinical or laboratory abnormalities, artifactual reticulocytosis may be caused by a malfunction in the automated cell counter. Although hypersplenism may be associated with increased red cell clearance and anemia, the abnormal RBC morphology seen in patients with asplenia is usually not associated with hemolysis. Finally, patients with chronic idiopathic unconjugated hyperbilirubinemia (Gilbert's syndrome) are sometimes erroneously referred to a hematologist to rule out hemolysis.⁷

CLINICAL APPROACH TO PATIENTS WITH SUSPECTED HEMOLYSIS

As with most diseases, the approach to hemolysis involves a combination of clinical and laboratory investigations directed by the judgment and skills of the clinician (Fig. 3.2).

Low-level or chronic hemolysis should be suspected in all patients with unexplained anemia. A detailed history and physical examination should be the cornerstone of each patient's evaluation.

History

Onset/ duration (hereditary vs. acquired)

History of fatigue

History of jaundice

Abdominal pain/ cholelithiasis (chronic hemolysis)

Medications (may exacerbate enzyme deficiencies)

Travel (consider infection)

History of recent or current infection

Vascular/ cardiac surgery

Blood loss or sequestration (increases reticulocytes in the absence of hemolysis)

Discolored urine (intravascular hemolysis)

Complete family history (jaundice, gallbladder disease, splenectomy, hereditary anemia, or other inherited diseases)

Table 3.1 Differential Diagnosis of Hemolytic Anemia

Intrinsic Causes of Hemolysis	Extrinsic Causes of Hemolysis
<u>Hemoglobin</u> (see Chapter 4): Hb SS ^a Thalassemias Other hemoglobinopathies	<u>Immune</u> Transfusion-induced alloantibodies ^a Hemolytic disease of the newborn ^a Autoimmune syndromes ^a

Unstable hemoglobins

Membrane

Hereditary spherocytosis
Hereditary elliptocytosis
Hereditary stomatocytosis
Hereditary acanthocytosis
Hereditary pyropoikilocytosis
Hemolytic uremic syndrome^a
McLeod syndrome

Paroxysmal nocturnal hemoglobinuria^a

Abnormal Membrane Proteins

Ankyrin
Band 3
Band 4.1
Band 4.2
Band 4.5
Glycophorin C (Leach phenotype)
Spectrin
Stomatin

Enzymes

Glucose-6-phosphate dehydrogenase
Pyruvate kinase
Glucose phosphate isomerase
Pyrimidine 5' nucleotidase
Adenosine deaminase
Aldolase
2,3-Diphosphoglycerate mutase
Enolase
 γ -Glutamyl cysteine synthetase
Glutathione peroxidase
Glutathione reductase
Glutathione synthetase
Heme oxygenase-1
Hexokinase
Lecithin cholesterol acyltransferase
Phosphofructokinase
Phosphoglycerokinase
Triose phosphate isomerase

The more common causes are shown in italics.

^aDenotes an association with intravascular hemolysis.^{2–5}

Other Causes

Fragmentation/ physical damage
Heart valves (mechanical and infected)^a
Disseminated intravascular coagulopathy^a
Thrombotic thrombocytopenic purpura^a
Hemolytic uremic syndrome^a
Hemodialysis^a
Chlorine (in hemodialysate fluid)
Chloramine (in hemodialysate fluid)
Malignancy (metastatic disease)^a
Burns^a
Drowning^a
Marathon/ march hemoglobinuria^a
Vasculitis^a
Malignant hypertension^a
Arteriovenous malformation^a
Liver disease
Hypersplenism
Insect and snake venom^a

Infections

Malaria^a
Babesiosis^a
Bartonellosis (Oroya fever)^a
Clostridium perfringens^a

Chemical

Oxidants in presence of glucose-6-phosphate dehydrogenase deficiency^a
Arsine gas
Lead

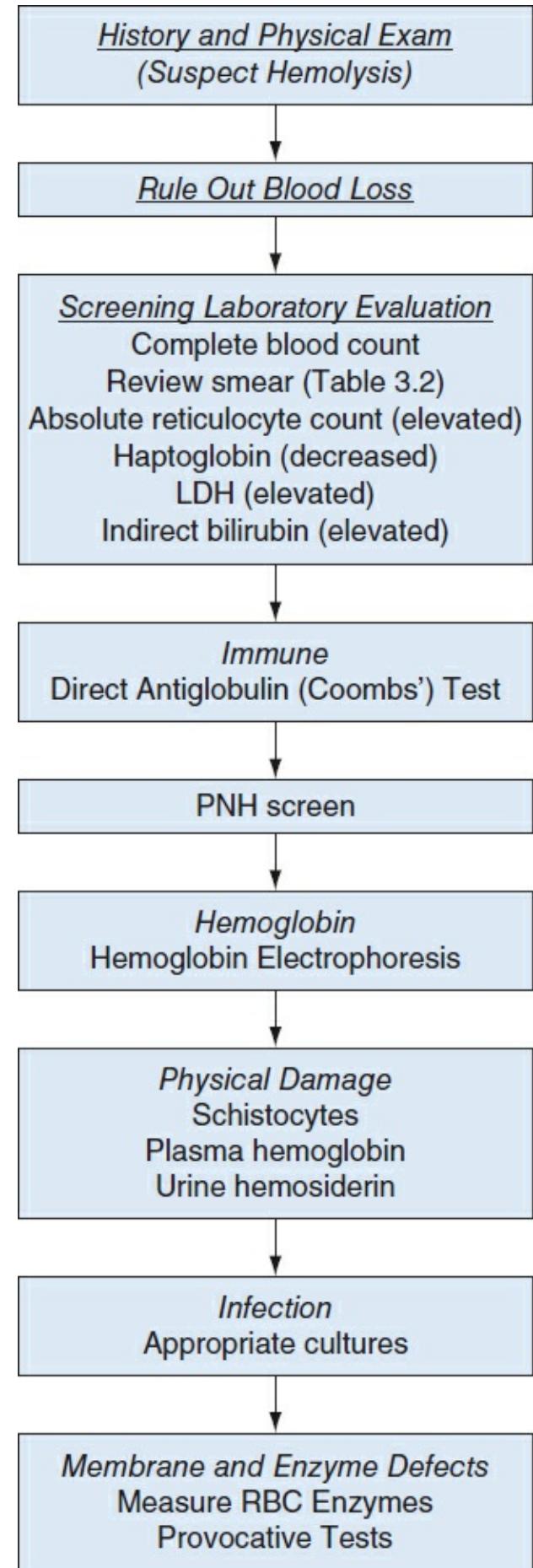


FIGURE 3.2 A clinical approach to hemolytic anemia.

LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell.

Physical Findings/ Examination

Increased temperature

Rapid pulse

Pallor

Jaundice (chronic hemolysis)

Mechanical click from heart valves

Splenomegaly

The laboratory evaluation is performed to confirm the suspected diagnosis, provide insights regarding the underlying mechanism, and gauge a therapeutic response. The complete blood count (CBC) usually confirms the diagnosis of anemia. Reticulocytosis increases the mean cell volume (MCV) and red cell distribution width (RDW). A critical test in the evaluation of all patients with suspected hemolysis is the reticulocyte count. An increased number of reticulocytes are present in hemolysis unless erythropoiesis is suppressed. Stressed erythropoiesis associated with acute hemolysis also causes the release of large polychromatic reticulocytes with a decreased area of central pallor into the circulation, called shift cells, which can be appreciated by evaluating the peripheral blood smear.⁸ Reticulocytes are also identified by their RNA content; so automated detection of RNA in the cells provides an accurate alternative to manual inspection. Normal values for reticulocytes in newborn infants range from 2.5% to 6.5% and fall to less than 2% by the second week of life. In adults, reticulocytes comprise 0.5% to 1.5% of circulating erythrocytes in the absence of anemia, consistent with the normal turnover of 1% of normal red cell mass per day in adults. Percentages above the normal range are usually detected in the setting of hemolysis due to increased erythropoiesis. However, in the setting of anemia, an uncorrected reticulocyte percentage may also reflect the prolonged survival of stress reticulocytes and the lower total number of circulating RBC. Therefore, an ARC more accurately measures the compensatory response than does the uncorrected reticulocyte percentage.⁹

$$\text{Absolute reticulocyte count (ARC)} = \text{Reticulocyte percentage}/100 \times \text{RBC count}/\mu\text{L}$$

The normal ARC ranges between 25,000 and 75,000/ μL . In patients with hemolysis, the ARC is usually elevated to levels greater than 100,000/ μL . If hemolysis is acute, a rise in reticulocytes may be delayed by 3 to 5 days.

Although a bone marrow examination is generally not required to determine the

etiology of uncomplicated hemolysis, the peripheral blood smear should never be overlooked. This simple test is rapid, inexpensive, and can provide important clues regarding the mechanism of hemolysis (Table 3.2).

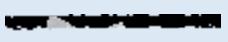
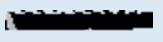
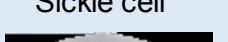
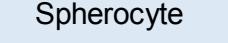
ACUTE INTRAVASCULAR HEMOLYSIS

The clinical syndrome associated with acute intravascular hemolysis deserves special attention because of its potential catastrophic consequences. Its recognition can lead to rapid institution of specific therapies and prevention of acute renal failure and death. Diagnosis and treatment of *Clostridium perfringens* sepsis or thrombotic thrombocytopenia purpura may be triggered by a hemolysis workup. Intravascular hemolysis is almost exclusively caused by extrinsic mechanisms, which may have the potential to be rapidly modified or reversed (Table 3.1).

Examination of several key laboratory values may also be done to assess the severity of intravascular hemolysis. Lactate dehydrogenase is released from hemolyzed RBCs. Small amounts of hemoglobin released into the circulation are metabolized in the liver after binding and clearance by haptoglobin. With robust intravascular hemolysis, a rapid decrease of serum haptoglobin to undetectable levels occurs. Free hemoglobin not bound to haptoglobin can be oxidized to methemoglobin or bound to transport proteins, such as hemopexin or albumin, which the liver then removes from the circulation. Free hemoglobin at levels of 100 to 200 mg/dL can be detected by visual examination of plasma or serum. The capacity of renal tubular cells to reabsorb free hemoglobin is limited, resulting in hemoglobinuria. Because renal tubular cells slough, iron staining can identify the tubular epithelium containing hemosiderin in the urine sediment. Cessation of hemolysis leads to a rapid recovery of the haptoglobin levels, but urine hemosiderin is detectable for longer periods (Fig. 3.3). Urine hemosiderin in the absence of urine hemoglobin provides clinical evidence for subacute or chronic intravascular hemolysis. In the absence of cirrhosis, reduced levels of haptoglobin (<28 mg/dL) provide 92% sensitivity and 98% specificity for predicting hemolysis.¹⁰

Table 3.2 Erythrocyte Morphology and Associated Pathology

Cell Type	Intrinsic	Extrinsic
Acanthocyte	Glutathione peroxidase deficiency, hereditary choroacanthocytosis, abetalipoproteinemia, McLeod syndrome, lecithin cholesterol acyltransferase deficiency	Liver disease (spur cell hemolytic anemia), asplenia
	Hemoglobinopathies, ineffective	Lead poisoning, 5'

	erythropoiesis	nucleotidase deficiency
	Basophilic stippling	
	Elliptocyte	Hereditary elliptocytosis, protein band 4.1 abnormalities, glycophorin C deficiency, pyropoikilocytosis
	Heinz bodies	G6PD deficiency, thalassemias, unstable hemoglobins Drug-induced oxidant injury
		Malaria (shown) Babesiosis Bartonellosis
	Parasites	Hereditary pyropoikilocytosis, α -spectrin mutation Burns
	Pyropoikilocytes	Microangiopathic hemolytic anemia (see Table 3.1)
	Schistocyte	Hemoglobin SS, hemoglobin SC, hemoglobin S, beta-thalassemia
	Sickle cell	Hereditary spherocytosis, ankyrin/spectrin deficiency, hemoglobin C disease, Band 3 defects, protein 4.2 defects Immune-mediated hemolysis infections, chemical injuries
	Spherocyte	Hereditary stomatocytosis Rh null disease Alcohol intoxication, liver disease

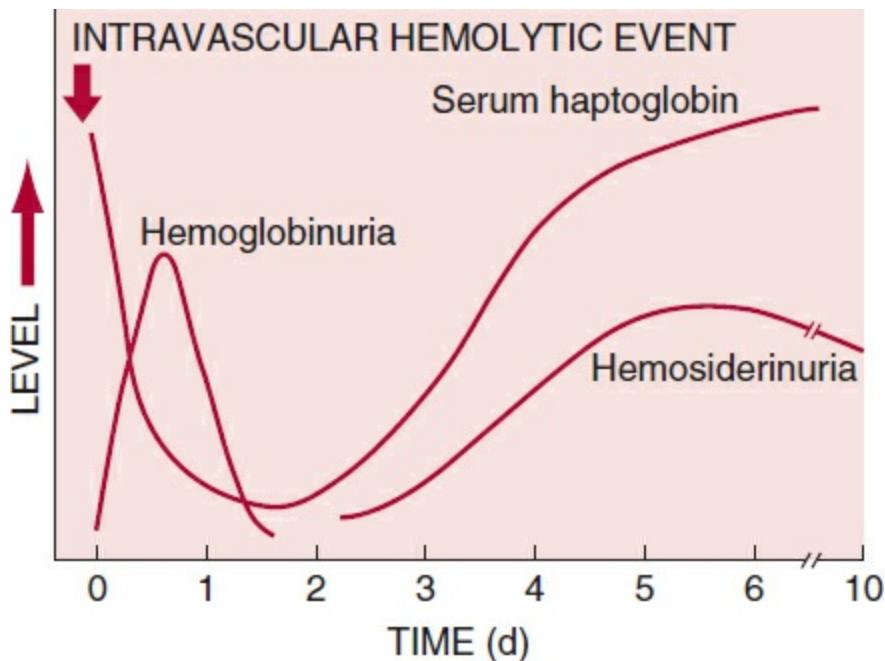
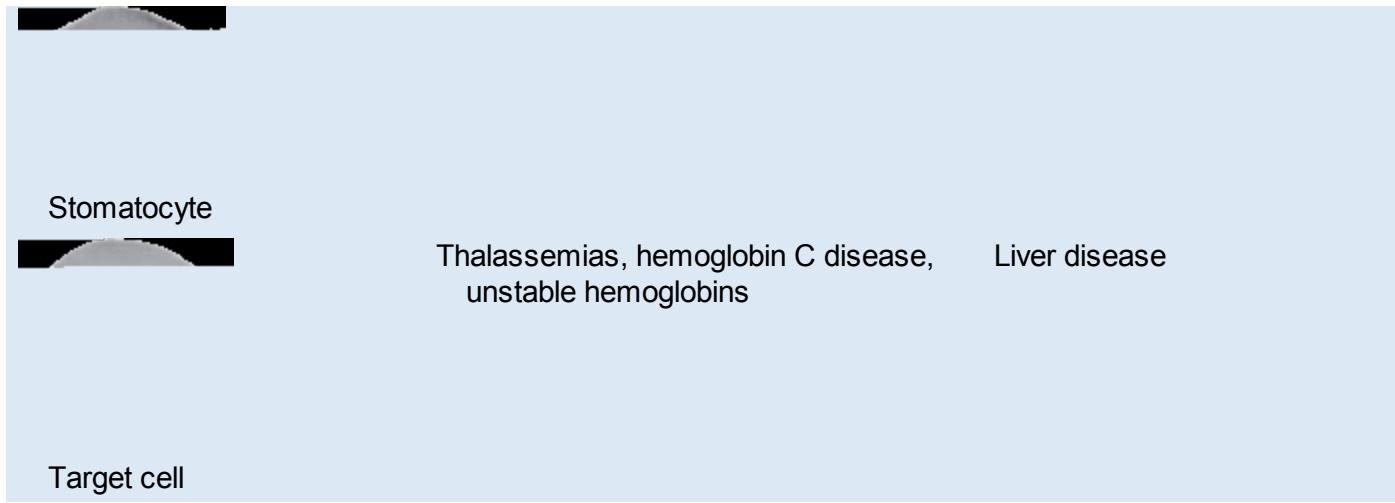


FIGURE 3.3 Indicators of acute intravascular hemolysis.

Adapted with permission from Hillman RS, Finch CA. Red Cell Manual. 7th ed. Philadelphia, PA: F. A. Davis; 1996.

SPECIAL CONSIDERATION OF ENZYME AND MEMBRANE DEFECTS

Once the more obvious causes of hemolysis are ruled out, the clinician must consider those etiologies less frequently encountered in daily practice, including enzyme or membrane defects. The laboratory evaluation can be confusing because of the numerous etiologies and the diversity of tests available. Therefore, the extent of diagnostic testing is dictated by the magnitude of hemolysis and the impact of a specific diagnosis on therapy. PNH is diagnosed by flow cytometry because of the associated absence of

glycosylphosphatidylinositol-anchored proteins (e.g., CD59) on the plasma membranes of hematopoietic cells (see [Chapter 6](#)). General evaluation of the erythroid cytoskeleton abnormalities can usually be assessed by peripheral blood smear evaluation. In the case of enzymopathies, specific functional assays are available from reference laboratories.

ERYTHROID ENZYMOGRAPHIES

Enzyme deficiencies are most often associated with congenital nonspherocytic hemolytic anemia. Inheritances of G6PD and phosphoglycerate kinase (PGK) deficiencies are chromosome X linked. Because the other red cell enzyme abnormalities exhibit an autosomal recessive mode of inheritance, they may be suspected in cases of unexplained hemolysis during infancy or childhood. While G6PD deficiency may be the most common enzyme deficiency in humans,¹¹ the other enzymopathies associated with hemolysis are rarely diagnosed. Based on their low incidence, a laboratory evaluation of suspected enzymopathies requires assays performed at specialized or research laboratories (e.g., Mayo Medical Laboratories, Rochester, MN), which measure the functional properties of each enzyme. In the setting of acute hemolysis, however, the magnitude of the functional deficit may be underestimated because of the generally higher levels of enzyme activity in reticulocytes and other “young” erythrocytes. As the clinical application of information contained in the human genome improves, genetic testing may become more practical for these enzymopathies. The success of clinical genotyping in this regard will depend on the number of mutations identified, as well as the strength of correlation between genotype and phenotype.

Enzyme deficiencies most commonly associated with hemolysis are linked to the prevention of oxidative damage or the generation of energy (adenosine triphosphate [ATP]) in RBCs. Glutathione reduction (hexose monophosphate shunt) is necessary for the prevention of oxidative damage from hydrogen peroxide to cellular proteins, including hemoglobin. Glycolysis (Embden–Meyerhof–Parnas pathway) provides the sole source of energy to the RBCs once they lose their mitochondria. The following section is a brief synopsis of the enzymopathies associated with hemolysis (organized according to the involved metabolic pathway).

Enzymes Involved in Glutathione Metabolism

G6PD deficiency is the most common RBC enzyme disorder associated with hemolysis. As an X-linked disorder, it is far more common in males, but females do present with the disease due to mosaicism of the X chromosome as well as compound heterozygosity of inheritance. It has been estimated that this disorder affects 400 million people throughout the world, with the highest frequencies occurring in populations from the Mediterranean region, Africa, and China.¹¹ Clinical classification is made according to

the magnitude of the enzyme deficiency and the severity of hemolysis.

Severe enzyme deficiency (less than 10% of normal activity) with chronic or intermittent hemolysis (Mediterranean and Asian populations).

Moderate enzyme deficiency (10% to 60% of normal) with intermittent hemolysis usually associated with infection or drugs. Approximately 10% to 15% of African American males are moderately deficient in G6PD activity.

Mild enzyme deficiency (>60% activity) without significant hemolysis.

Importantly, the severity of hemolysis among all G6PD-deficient patients depends on two major variables: G6PD protein and oxidative stress. G6PD deficiency is defined at the genetic level by mutations that cause either reduced synthesis of functional G6PD (quantitative defect) or production of abnormal G6PD (qualitative defect). The Johns Hopkins University (<http://omim.org/entry/305900>) has cataloged the known 400 G6PD variants. The neonatal hyperbilirubinemia in G6PD-deficient infants is caused by increased bilirubin production from erythrocyte breakdown and inadequate clearance by an immature liver. Neonates with severe G6PD deficiency are at the greatest risk of developing neonatal hyperbilirubinemia.

The second major factor in determining the level of hemolysis is the level of intracellular oxidative stress. G6PD acts to catalyze the conversion of glucose-6-phosphate to 6-phosphogluconate. This biochemical reaction is coupled to the production of NADPH, a key electron donor in the defense against oxidizing agents, and the subsequent reduction of glutathione. Erythrocytes that are exposed to oxidants or oxidative stresses become depleted of reduced glutathione (GSH). Once GSH is depleted, oxidation of other RBC sulphydryl-containing proteins (including hemoglobin) occurs. Oxidation of hemoglobin leads to the formation of sulfhemoglobin and hemoglobin precipitates called Heinz bodies. Heinz body inclusions are generated during acute, drug-induced hemolytic episodes. Patients with moderate G6PD deficiency are generally asymptomatic in the steady state. They present episodically with acute hemolytic anemia due to oxidative stress from infections, such as acute viral hepatitis and pneumonia. Hemolysis is also associated with the ingestion of fava beans, which contain pyrimidine aglycones (divicine and isouramil). Favism is most commonly associated with the G6PD variant in Mediterranean populations. Certain drugs and chemicals (Table 3.3) that increase the risk of hemolysis in G6PD-deficient patients should be avoided.¹² Hemolysis occurs 1 to 3 days after ingestion of these drugs or fava beans, with resolution usually within 1 week of cessation.

Testing for suspected G6PD deficiency can be performed by simple qualitative or

quantitative fluorescence tests that measure the production of NADPH. During episodes of acute hemolysis, measured levels of enzyme activity may be normal due to the loss of older erythrocytes with the least activity and due to increased number of younger erythrocytes with normal levels. So if a G6PD deficiency is suspected, enzyme activity should be repeated 2 to 3 months after the acute episode when cells of all ages are replenished. A more definitive diagnosis of G6PD deficiency requires genetic testing of the involved patient or family. Treatment of a G6PD-deficient individual depends on the degree of hemolysis. Potentially harmful foods and drugs should always be avoided by the patient, as well as nursing mothers of infants with G6PD deficiency. Patients with infection should be carefully monitored for early signs of increased hemolysis. Blood transfusion may be lifesaving during acute hemolytic episodes. Although controversial, splenectomy may be considered in cases of G6PD deficiency presenting with severe hemolysis that do not respond to other measures.

Table 3.3 Common Drugs and Chemicals to Avoid in Patients With Glucose-6-Phosphate Dehydrogenase Deficiency

Colchicine
Dapsone
Isoniazid
L-Dopa
Methylene blue
Nitrofurantoin
Phenazopyridine (Pyridium)
Primaquine
Rasburicase
Sulfamethoxazole
Toluidine blue
Trimethoprim
Quinine

γ -Glutamylcysteine synthetase is the rate-limiting enzyme in glutathione biosynthesis. Hemolytic anemia is associated with low activity of this enzyme and normal glutathione synthetase levels. These rare patients present with a history of lifelong anemia, intermittent jaundice, and spinocerebellar degeneration in adulthood.¹³

Glutathione peroxidase (GSH-Px) is primarily responsible for the elimination of hydrogen peroxide from erythrocytes. Production of this protein is dependent on adequate nutritional levels of selenium.¹⁴ Moderate deficiencies in GSH-Px activity may result in the formation of Heinz bodies and nonspherocytic hemolytic anemia in

infants. Similar to the case of G6PD deficiency, oxidizing agents should be avoided in these patients.

Glutathione reductase is the enzyme that reduces oxidized GSH in the presence of flavin adenine dinucleotide. Deficiency of glutathione reductase causes increased susceptibility to drug-induced hemolysis. Glutathione reductase activity increases with dietary supplementation of riboflavin, and a subset of these patients respond well to riboflavin dietary supplements. In some cases of glutathione reductase deficiency, the enzymatic activity cannot be restored by riboflavin supplementation due to a 2,246 base pair deletion found in the gene encoding for glutathione reductase.¹⁵

Glutathione synthetase deficiency is caused by autosomal recessive inheritance of mutations of the glutathione synthetase gene with subsequently low levels of glutathione in RBCs. The disease is marked by accumulation of the metabolite oxyproline in the urine.¹⁶ The patients present with the clinical triad of hemolysis, metabolic acidosis, and mental deterioration. Treatment includes vitamin C, vitamin E, bicarbonate, and avoidance of oxidative drugs.

Enzymes Involved in Glycolysis

Pyruvate kinase (PK) deficiency is associated with more than 150 genetic mutations.¹⁷ PK deficiency is the second most common enzymopathy associated with congenital nonspherocytic hemolytic anemia with a prevalence of approximately 1:20,000 in Caucasian populations. PK converts phosphoenolpyruvate to pyruvate, simultaneously generating ATP from adenosine diphosphate (ADP). PK activity decreases during RBC aging, as the enzyme is gradually denatured. The eventual result is failure of glycolysis as PK activity falls below a critical level. Because glycolysis is the sole source of ATP synthesis in the mature RBC, ATP depletion and hemolysis follow glycolytic failure.

Consistent with several other inherited causes of hemolysis, PK deficiency is postulated to provide some protection from malarial infection.¹⁸ Selection for variations of this gene may involve other factors as well, because PK deficiency is less common in Africa and other malarial-endemic regions. Most patients have compound heterozygotes for the two most common mutant forms of the enzyme. Approximately one-third of the cases present with jaundice during the newborn period, and one-third of those cases are severe enough to require transfusion. Death during the neonatal period may result from severe anemia. In individuals with milder forms of the enzymopathy, the anemia is less severe and the diagnosis may not be established until later in childhood. Unfortunately, poor correlation between PK activity and the severity of clinical hemolysis confounds the accuracy of prognosis.¹⁷ There is currently no reliable method to predict the success of splenectomy for individual cases.

Glucose phosphate isomerase (GPI) deficiency is the third most common glycolytic

enzyme deficiency associated with hemolytic anemia. GPI catalyzes the production of fructose-6-phosphate from glucose-6-phosphate. It is found in all ethnic groups, but is prevalent in individuals of European descent. More than two dozen genetic variants have been identified to date, with considerable variability in disease severity. In severe cases, anemia and hyperbilirubinemia are evident at birth. In addition to chronic hemolysis and hyperbilirubinemia, acute hemolytic crises can occur with viral and bacterial infections.¹⁹

Aldolase A deficiency has been found to cause moderately severe lifelong hemolytic anemia, sometimes requiring transfusions during acute hemolytic crises. Aldolase catalyzes the conversion of fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Abnormal expression of the aldolase variant causes hemolysis and myopathy.²⁰ Other congenital anomalies include short stature, mental retardation, delayed puberty, and a distinct facial appearance.

2,3-Diphosphoglycerate mutase (DPGM) deficiencies greater than 50% cause a compensated hemolytic anemia. DPGM converts 1,3-biphosphoglycerate to 2,3-diphosphoglycerate (2,3-DPG). Deficiencies of this enzyme may lead to polycythemia due to concomitant 2,3-DPG deficiency and G6PD deficiency without documented episodes of hemolysis.²¹

Enolase is the enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate. Case studies have shown a decrease in the enzyme activity in patients with a mild spherocytic hemolytic anemia.²²

Hexokinase deficiency causes a rare congenital hemolytic anemia, predominantly in persons of northern European ancestry. Hexokinase acts at the initial enzymatic step in glycolysis, catalyzing the conversion of glucose to glucose-6-phosphate. Hexokinase activity in reticulocytes is considerably higher than that in mature cells. Anemia is associated with a reduction of this enzyme activity to 25% that of normal erythrocytes.²³

Phosphofructokinase deficiency (also called Tarui disease) results in a glycogen storage disorder characterized by hemolysis and myopathy. Phosphofructokinase is an allosteric enzyme that catalyzes the irreversible conversion of fructose-6-phosphate to fructose-1,6-diphosphate. Most affected individuals have exhibited exertional myopathy resulting in weakness, easy fatigability, muscle cramps on exercise, and myoglobinuria. Hemolysis is caused by decreased erythrocyte deformability from leakage of calcium ions.²⁴

PGK deficiency results in a moderate-to-severe nonspherocytic hemolytic anemia. PGK converts 1,3 biphosphoglycerate to 3-phosphoglycerate. PGK deficiency is the only X-linked disorder involved in glycolysis. The disease phenotype is unusually pleomorphic and may include varying degrees of hemolytic anemia, mental retardation, and myopathy.²⁵

Triosephosphate isomerase (TPI) deficiency is a rare disorder characterized by severe hemolytic anemia and increased susceptibility to infection. In addition, progressive neurological deterioration is a hallmark of the associated disease. Deficiencies usually become evident during infancy, with spasticity, motor retardation, hypotonia, weakness, and seizures.²⁶

Other Enzymopathies Associated With Hemolysis

Pyrimidine 5' nucleotidase (uridine 5' monophosphate hydrolase) deficiency leads to accumulation of high concentrations of pyrimidine nucleotides within the erythrocytes that precipitate and cause basophilic stippling. Its diagnosis is confirmed by a decrease in the nucleotide OD260:OD280 ratio and measurement of enzyme activity. The disease severity is variable, but the patients typically manifest lifelong hemolysis with the expected sequelae.²⁷

Adenosine deaminase (ADA) is a purine catabolic enzyme that converts adenosine to inosine. ADA deficiency causes inherited severe combined immunodeficiency. In contrast, elevations in ADA cause hemolytic anemia. Studies show that ADA amplification in reticulocytes results from increased translation of ADA mRNA.²⁸

Heme oxygenase-1 is an enzyme involved in the conversion of heme to bilirubin. Heme oxygenase-1 further provides protection against certain oxidative stresses. Two pediatric patients were described with severe growth retardation, asplenia, an abnormal coagulation/ fibrinolysis system, and persistent hemolytic anemia. This enzyme deficiency may cause the unique findings of erythrocyte fragmentation and intravascular hemolysis in the absence of hyperbilirubinemia or decreased haptoglobin potentially due to a defect in the macrophage's ability to catabolize heme.²⁹

Lecithin cholesterol acyltransferase (LCAT) is an enzyme involved in lipoprotein metabolism. Deficiencies of LCAT result in erythroid membrane defects caused by excess unesterified cholesterol. The patients also develop renal disease with proteinuria and corneal opacifications, and their serum lipid profiles include decreased serum high-density lipoprotein (HDL) levels (55% to 10% normal).³⁰

ERYTHROID MEMBRANE DEFECTS

The RBC membrane comprises integral and peripheral proteins distributed in the context of a lipid bilayer. Integral membrane proteins interact to form a lattice-like structure (cytoskeleton) at the cytoplasmic surface of the lipid bilayer that is responsible for the strength and deformability of the RBC. Band 3, a protein that functions as an anion exchanger (AE1), is the major protein that physically links the lipid bilayer to the underlying membrane cytoskeleton. The cytoskeleton proteins include spectrin, ankyrin, actin, band 3, band 4.1, and band 4.2. Other red cell membrane proteins serve roles in

maintaining osmotic equilibrium or have adhesive properties. The exact functions of a number of erythroid membrane proteins remain vague.

Immune-mediated hemolysis results from antibodies directed toward erythrocyte membrane proteins. Approximately 24 proteins are largely responsible for transfusion-related alloimmunity (see [Chapter 24](#)). Nonimmune hemolysis may also be due to rare erythroid phenotypes involving those proteins. Nonimmune hemolysis due to the Rh-null phenotype is generally mild and well compensated with a reticulocyte count below 10%. The red cell morphology may be stomatocytic or spherocytic, and the RBC osmotic fragility is increased.³¹ Weak expression of the Kell blood group results in the so-called McLeod phenotype. This phenotype is X-linked and occurs with relatively high frequency in individuals who have chronic granulomatous disease (CGD). Neurodegeneration and acanthocytosis are the characteristic features of the McLeod phenotype. The associated hemolysis is mild with slightly elevated reticulocyte counts.³²

The first indication that a patient may have a membrane abnormality as a cause of hemolysis usually comes from microscopic examination of the peripheral blood smear. As shown in [Table 3.2](#), the presence of spherocytes, elliptocytes, stomatocytes, acanthocytes, or pyropoikilocytes may be the primary alert for an underlying membrane defect.

Hereditary spherocytosis (HS) is the most common hereditary anemia among people of northern European descent, occurring at a frequency of 1 in 5,000. It is commonly caused by mutations in the genes that encode the components of the erythroid cytoskeleton (α -or β -spectrin, ankyrin, band 3, protein 4.2). Autosomal recessive or dominant patterns of inheritance have been identified, and a positive family history is gathered in more than half of the cases. Patients are usually diagnosed in childhood with the clinical triad of anemia, jaundice, and splenomegaly. Spherocytes are identified by their small size, and absence of the central pallor seen in normal erythrocytes on a peripheral smear. Spherocytic RBCs are not specific to hereditary spherocytosis. Autoimmune hemolytic anemia may produce spherocytosis. This is excluded by negative direct antiglobulin (DAT) testing. Immune-based spherocytosis is ruled out by negative direct antiglobulin test (DAT) testing. Hematologic parameters also show a high mean corpuscular hemoglobin concentration (MCHC) due to cellular dehydration.

In most cases, the clinical presentation and hematological parameters are sufficient to make the diagnosis.³³ If the diagnosis is subtle or complicated, confirmation of the diagnosis using assays of membrane fragility, electrophoretic quantitation of membrane proteins, or genetic analyses may be considered. The osmotic fragility test for HS has been used to detect hemolysis by measuring the fraction of total hemoglobin released from red cells at progressively more dilute salt concentrations. As the hemolysis can

occur in any spherocyte at salt concentrations that do not affect normal RBC, this test is not specific for diagnosing HS. A cryohemolysis test may also be used to detect increased hemolysis in HS erythrocytes. Red cells are suspended in a hypertonic solution, briefly heated to 37°C, and then cooled to 4°C for 10 minutes. A widely separated degree of hemolysis between spherocytes and normal cells is seen with the cryohemolysis test, and asymptomatic disease carriers may also be identified.³⁴ Recently developed eosin-5'-maleimide-binding (EMA) test is a flow cytometric analysis of EMA-labeled intact RBCs with high specificity and sensitivity in diagnosing HS.³⁵ Hereditary elliptocytosis (HE) is endemic in areas of Africa and Asia. The disease also results from mutations in the α -spectrin, β -spectrin, and band 4.1 genes. In the homozygous state, hemolysis may be lifelong and is exacerbated by acute or chronic illnesses.³⁶ In the heterozygous state, people with HE have no clinical syndrome, with slight reticulocytosis and the characteristic abnormalities of the RBC morphology providing the only clues toward diagnosis.

Hereditary pyropoikilocytosis is a severe type of HE resulting from a mutation in either protein 4.1 or α -spectrin. The usual presentation involves mild-to-moderate hemolytic anemia with evidence of marked poikilocytosis. The spectrin in these abnormal cells has an increased sensitivity to thermal denaturation, and the cells exhibit mechanical fragility. As a result, the red cell volume distribution is broad, and a striking number of fragmented cells and microspherocytes are observed on the peripheral smear.³⁷

Hereditary stomatocytosis is identified by a pinched rather than circular area of central pallor in erythrocytes. Although all the patients share the common clinical feature of stomatocytes, research on defining the underlying cause of the morphological change has resulted in more specific clinical descriptions, including dehydrated hereditary stomatocytosis (xerocytosis), overhydrated hereditary stomatocytosis, and cryohydrocytosis. The common features include hemolysis and red cell cation leaks and variable severity of hemolysis.³⁸ Most importantly, splenectomy is not helpful and may result in a high risk of thromboembolic disease.³⁹

Acanthocytosis on the peripheral smear may be caused by abnormal lipids in hepatic cirrhosis, or by other abnormalities in lipids or band 3. Abetalipoproteinemia is a rare genetic disorder resulting in hypolipidemia, acanthocytosis, malabsorption of fat, retinitis pigmentosa, and ataxia. Infants with this autosomal recessive disorder are normal at birth but soon develop steatorrhea, abdominal distension, and growth failure. Retinitis pigmentosa and ataxia appear between ages 5 and 10 years and are progressive. Therefore, acanthocytosis seen on the peripheral smear of children in the absence of hemolysis or liver disease should alert the clinician to consider associated neurodegenerative conditions.⁴⁰

TREATMENT OPTIONS FOR CONFIRMED HEMOLYSIS

Therapeutic strategies for hemolytic anemia are determined by the underlying cause of red cell destruction, the magnitude of the anemia, and the cardiopulmonary status of the patient.

For extrinsic causes, the treatment plan usually becomes obvious at the time of diagnosis. Immune-mediated hemolysis may require immunoglobulin infusion, corticosteroids, or other immunosuppressive therapies. Transfusion therapy (packed RBCs) should be avoided unless necessary. However, RBC transfusion should not be withheld if a severely compromised cardiopulmonary status exists even when the selected RBC units are incompatible. In those rare cases, the department of transfusion medicine involved should be asked to identify the most compatible product available, and the transfusion should be closely monitored. Infections are treated with antimicrobials. For thrombotic thrombocytopenic purpura, p/Plasmapheresis is specifically indicated to reverse the depletion of the von Willebrand factor cleaving protease, ADAMTS-13, which would result in excessive high-molecular weight multimers of von Willebrand factor triggering disseminated platelet thrombi. Monoclonal antibody treatment (i.e., Rituximab) or immunosuppressive therapy have been used along with plasmapheresis for those refractory cases. Prevention of immune- or G6PD-mediated hemolysis involves discontinuation or avoidance of the associated medications. Monitoring urine hemoglobin and hemosiderin levels can determine the response to intravascular hemolysis therapy.

One acquired intrinsic red cell disorder, PNH, can be effectively treated by eculizumab, an anti-C5 antibody that can control the intravascular hemolysis by converting it to a milder extravascular hemolysis ([Chapter 6](#)). Eculizumab has also been shown to reduce the thrombosis risk that accompanies the intravascular hemolysis. Immunization with anti-meningococcal vaccination is required before eculizumab is prescribed.^{41,42} Eculizumab has also been reported to reverse thrombotic microangiopathy and renal failure in patients with atypical hemolytic uremic syndrome.⁴³

Despite the considerable advances in defining these genetic diseases at the molecular level, equally specific treatment regimens for other intrinsic causes of hemolysis are lacking. In general, intrinsic causes of hemolysis are inherited, and they are present during infancy or childhood. In those cases, prognosis and treatment are complex, as the hemolytic picture may change over time. The first question to ask is whether treatment is necessary. Chronic hemolysis may only require a yearly clinical evaluation of the CBC, ARC, and blood smear to determine if the patient can maintain adequate levels of erythropoiesis. Parvovirus infections in these patients may result in acute worsening of their anemia due to a sudden decrease in their erythropoiesis. Folic acid should be

given to all patients with chronic hemolysis (1 mg/ day) because this vitamin is consumed with the accelerated production of erythrocytes. Whether folic acid supplementation is still necessary in the United States, where the fortification of food folate has occurred since the mid-1990s, has not been evaluated. Transfusion regimens should be tailored for individual patients, and iron overload should be anticipated. Even in the absence of transfusion, iron overload may result from ineffective erythropoiesis. The increased metabolism of heme also leads to a significant increase in pigmented gallstone formation.

Treatments such as splenectomy or bone marrow transplantation should be reserved for marked hemolysis producing life-threatening anemia. As stated earlier, splenectomy should be discouraged for patients with hereditary stomatocytosis syndromes.³⁸ When severe hemolysis is caused by other membrane defects, splenectomy may be beneficial and indicated. In children, splenectomy should be delayed until the age of 6 years (if possible) due to the increased risk of sepsis. In general, splenectomy risks must be compared with those associated with lifelong transfusion. After a splenectomy is performed, special care must be taken to compensate for the loss of splenic function. The spleen is responsible for the clearance of encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Neisseria meningitidis*. The combined use of pneumococcal polysaccharide immunization and early empiric antibiotic therapy offers a high level of protection for postsplenectomy patients. It is estimated that sepsis is fatal in 40% to 50% of all splenectomized patients. Within that group, children with thalassemia and sickle cell syndromes have the highest risk of death.⁴⁴ In all cases, patients must be informed that the asplenic state carries a significant risk of overwhelming and life-threatening infection.

SUMMARY

A broad range of genetic and acquired diseases are manifested by hemolysis. The differential diagnosis is useful in developing diagnostic and therapeutic strategies and should be thought of in terms of intrinsic or extrinsic causes of erythrocyte damage. A careful search for the cause of hemolysis should be pursued because treatments are so different. When a common cause of hemolysis is not found, an underlying enzyme or membrane defect should be sought.

Clinical severity in all cases of hemolysis is determined by the rate of red cell destruction, and the host's ability to compensate by producing fresh erythrocytes. The disease can vary from a subtle and clinically silent syndrome to hemolysis of sufficient intensity to dominate the clinical picture and even cause death if left untreated. Every therapeutic plan should be designed for both the severity of disease as well as the cause of hemolysis.

HELPFUL INTERNET SITES

<https://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0063009/>

<https://rarediseases.org/rare-diseases/anemia-hemolytic-acquired-autoimmune/>

References

1. Lichtman MA, Beutler E, Kipps TJ, et al. *Williams Hematology*. 8th ed. New York, NY: McGraw-Hill Publishers; 2010.
2. Dacie JV. *The Haemolytic Anaemias: Secondary or Symptomatic Haemolytic Anaemias*. 3rd ed. Vols. 1–5. New York, NY: Churchill Livingstone; 1985–1999.
3. Nathan DG, Orkin SH, Oski FA. *Hematology of Infancy and Childhood*. 6th ed. Philadelphia, PA: WB Saunders; 2003.
4. Foerster J, Lee RG, Wintrobe MM, eds. *Wintrobe's Clinical Hematology*. 10th ed. Philadelphia, PA: Williams & Wilkins; 2008.
5. Hoffman R, Benz EJ, Shattil SJ, et al. *Hematology: Basic Principles and Practice*. 4th ed. Philadelphia, PA: Elsevier; 2005.
6. Beutler E, Luzzatto L. Hemolytic anemia. *Semin Hematol*. 1999;36:38–47.
7. Strassburg CP. Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, Rotor syndrome). *Best Pract Res Clin Gastroenterol*. 2010;5:555–571.
8. Finch CA, Hillman RS. *Red Cell Manual*. 7th ed. Philadelphia, PA: F.A. Davis; 1996.
9. Riley RS, Ben-Ezra JM, Tidwell A, et al. Reticulocytes and reticulocyte enumeration. *J Clin Lab Anal*. 2001;15:267–294.
10. Körmöczi GF, Säemann MD, Buchta C, et al. Influence of clinical factors on the haemolysis marker haptoglobin. *Eur J Clin Invest*. 2006;36:202–209.
11. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet*. 2008;371:64–74.
12. Youngster I, Arcavi L, Schechmaster R, et al. Medications and glucose-6-phosphate dehydrogenase deficiency: an evidence-based review. *Drug Saf*. 2010;33:713–726.
13. Hamilton D, Wu JH, Alaoui-Jamali M, et al. A novel missense mutation in the γ -glutamylcysteine synthetase catalytic subunit gene causes both decreased enzymatic activity and glutathione production. *Blood*. 2003;102:725–730.
14. Gondo H, Ideguchi H, Hayashi S, et al. Acute hemolysis in glutathione peroxidase deficiency. *Int J Hematol*. 1992;55:215–218.
15. Mojzikova R, Dolezel P, Pavlicek J, et al. Partial glutathione reductase deficiency as a cause of diverse clinical manifestations in a family with unstable hemoglobin (Hemoglobin Haná, β 63(E7) His-Asn). *Blood Cells Mol Dis*. 2010; 45:219–222.
16. Ristoff E, Larsson A. Inborn errors in the metabolism of glutathione. *Orphanet J*

Rare Dis. 2007;2:16–24.

17. Zanella A, Fermo E, Bianchi P, et al. Red cell pyruvate kinase deficiency: molecular and clinical aspects. *Br J Haematol*. 2005;130:11–25.
18. Wang C, Chiarelli LR, Bianchi P. Human erythrocyte pyruvate kinase: characterization of the recombinant enzyme and a mutant form (R510Q) causing nonspherocytic hemolytic anemia. *Blood*. 2001;98(10):3113–3120.
19. Kugler W, Lakomek M. Glucose-6-phosphate isomerase deficiency. *Baillière's Clin Haematol*. 2000;13:89–101.
20. Yao DC, Tolan DR, Murray MF, et al. Hemolytic anemia and severe rhabdomyolysis caused by compound heterozygous mutations of the gene for erythrocyte/muscle isozyme of aldolase, ALDOA. *Blood*. 2004;103:2401–2403.
21. Hoyer JD, Allen SL, Beutler E, et al. Erythrocytosis due to bisphosphoglycerate mutase deficiency with concurrent glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. *Am J Hematol*. 2004 Apr;75(4):205–258.
22. Boulard-Heitzmann P, Boulard M, Tallineau C. Decreased red cell enolase in 40 year old woman with compensated haemolysis. *Scand J Haemotol*. 1984;33:401–404.
23. Kanno H. Hexokinase: gene structure and mutations. *Baillière's Clin Haematol*. 2000;13:83–88.
24. Sabina RL, Waldenström A, Ronquist G. The contribution of Ca^{2+} -calmodulin activation of human erythrocyte AMP deaminase (isoform E) to the erythrocyte metabolic dysregulation of familial phosphofructokinase deficiency. *Haematologica*. 2006;91:652–655.
25. Beutler E. PGK deficiency. *Br J Haematol*. 2007;136:3–11.
26. Oláh J, Orosz F, Keserü GM, et al. Triosephosphate isomerase deficiency: a neurodegenerative misfolding disease. *Biochem Soc Trans*. 2002;30:30–38.
27. Zanella A, Bianchi P, Fermo E, et al. Hereditary pyrimidine 5'-nucleotidase deficiency: from genetics to clinical manifestations. *Br J Haematol*. 2006;133:113–123.
28. Chottiner EG, Cloft HJ, Tartaglia AP, et al. Elevated adenosine deaminase activity and hereditary hemolytic anemia: evidence for abnormal translational control of protein synthesis. *J Clin Invest*. 1987;79:1001–1005.
29. Fraser ST, Midwinter RG, Berger BS, et al. Heme oxygenase-1: a critical link between iron metabolism, erythropoiesis, and development. *Adv Hematol*. 2011;2011:473709.
30. Hovingh GK, de Groot E, van der Steeg W, et al. Inherited disorders of HDL metabolism and atherosclerosis. *Curr Opin Lipidol*. 2005;16:139–145.
31. Nash R, Shojania AM. Hematological aspect of Rh deficiency: a case report and review of literature. *Am J Hematol*. 1987;24:267–275.
32. Walker RH, Danek A, Uttner I, et al. McLeod phenotype without the McLeod

- syndrome. *Transfusion*. 2007;47:299–305.
- 33. Bolton-Maggs PH, Stevens RF, Dodd NJ, et al. Guidelines for the diagnosis and management of hereditary spherocytosis. *Br J Haematol*. 2004;126:455–474.
 - 34. Iglauer A, Reinhardt D, Schöter W, et al. Cryohemolysis test as a diagnostic tool for hereditary spherocytosis. *Ann Hematol*. 1999;78:555–557.
 - 35. Bianchi P1, Fermo E, Vercellati C, et al. Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. *Haematologica*. 2012;97(4):516–523.
 - 36. Delaunay J. The molecular basis of hereditary red cell membrane disorders. *Blood Rev*. 2007;21:1–20.
 - 37. Ramos MC, Schafernak KT, Peterson LC. Hereditary pyropoikilocytosis: a rare but potentially severe form of congenital hemolytic anemia. *J Pediatr Hematol Oncol*. 2007;29:128–129.
 - 38. Delaunay J. The hereditary stomatocytoses: genetic disorders of the red cell membrane permeability to monovalent cations. *Semin Hematol*. 2004;41:165–172.
 - 39. Stewart GW, Amess JA, Eber SW, et al. Thrombo-embolic disease after splenectomy for hereditary stomatocytosis. *Br J Haematol*. 1996;93:303–310.
 - 40. Jung HH, Danek A, Walker RH. Neuroacanthocytosis syndromes. *Orphanet J Rare Dis*. 2011;25:68.
 - 41. Brodsky RA, Young NS, Antonioli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2008;111:1840–1847.
 - 42. Parker CJ. Management of paroxysmal nocturnal hemoglobinuria in the era of complement inhibitory therapy. *Hematology Am Soc Hematol Educ Program*. 2011;2011:21–29.
 - 43. Nurnberger J, Witzke O, Opazo Saez A, et al. Eculizumab for atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;360:542–544.
 - 44. Bisharat N, Omari H, Lavi I, et al. Risk of infection and death among post-splenectomy patients. *J Infect*. 2001;43:182–186.

Sickle Cell Anemia and Thalassemia

**Matthew M. Hsieh, John F. Tisdale, and
Griffin P. Rodgers**

Normal hemoglobin within red blood cells is comprised of two α and two β chains with an α -to- β synthesis ratio of 1 : 1 (Table 4.1). Thalassemias are a group of quantitative disorders with insufficient production of α or β chains, leading to an imbalanced accumulation of β or α chains, respectively. In contrast, hemoglobinopathies (or abnormal hemoglobin structural variants) are a separate group of qualitative disorders, with abnormal β or α chains in normal quantity, of which sickle cell disease (SCD) is best recognized. Although these two disorders share features of variable degree of hemolytic anemia and transfusion-related complications, they differ in their pathophysiology, clinical manifestations, and management (Table 4.2). The thalassemias and hemoglobinopathies are commonly encountered in areas where malaria is endemic because abnormal genes offer protection against malaria.¹

PATHO PHYSIOLOGY

Thalassemias

Normally there are four copies of α -globin gene, two copies on each chromatid of chromosome 16 (Fig. 4.1). α -Globin chains are essential in the synthesis of both fetal and adult hemoglobin. α -Thalassemia syndromes result from deletions of a large α -globin gene segment from unequal crossover or recombination, and less frequently from mutations. The deleted segments of DNA vary in size and can involve one (+, same as α^+ or trans) or both (—, same as α^0 or cis) alleles on the same chromatid. A deletion of one gene (+/++) confers a silent carrier. A two-gene deletion (—/++ or +/--) is commonly referred to as α -thalassemia minor or trait with microcytosis, hypochromia, but little or no anemia. The deletion of three genes (—/--) leads to Hb H (β^4), which is an unstable form of hemoglobin. Hb H disease is manifested by hypochromia,

moderately severe hemolytic anemia, and splenomegaly. The absence of all four genes leads to hydrops fetalis with Hb Barts (γ 4). Hb Barts transports O₂ poorly, cause profound tissue hypoxia, leads to heart and liver failure, and is almost always incompatible with life without in utero red cell transfusion.

Both α -globin gene deletion haplotypes, (+) and (−), occur equally in Southeast Asians, whereas the (−) haplotype is much less common in Mediterraneans and rare in Africans. Hence all the α -thalassemic syndromes are seen in Southeast Asians, but hydrop fetalis is uncommon to rare in Mediterraneans and Africans. In addition to α -globin gene deletions, there are α -globin structural variants that may occur alone or in combination with α -gene deletions, and lead to further reduction of α -globin synthesis. The best characterized α -globin variant is Hb Constant Spring.

Table 4.1 Normal and Variant Hemoglobin

Name	Designation	Molecular Structure	Proportion in
Adult hemoglobin	A	$\alpha_2\beta_2$	Adults: 97% Newborns: 20%–25%
Adult hemoglobin	A ₂	$\alpha_2\delta_2$	Adults: 2.5% Newborns: 0.5%
Fetal hemoglobin	F	$\alpha_2\gamma_2$	Adults: <1% Newborns: 75%–80%
	HbH	β_4	Adults: 0% Newborns: 15%–25% in HbH disease
	Hb Barts	γ_4	Adults: 0% Newborns: 15%–25% in HbH disease, 100% in hydrop fetalis

HbH, hemoglobin H.

Table 4.2 General Features of Thalassemia and SCD

	α -Thalassemia	β -Thalassemia	SCD
Geographic	Equatorial Africa; Mediterranean Middle East; Arabian peninsula; Caribbean; India; Southeast Asia; South China		Africa; some Mediterranean regions, Middle East; parts of India
Pathophysiology	Quantitative Hb defect: Gene deletion(s) leading to reduced α -chain production and hemolytic anemia	Quantitative Hb defect: Mutations leading to reduced β -chain production and hemolytic anemia	Qualitative Hb defect: HbS polymerization leading to vaso-occlusion and hemolytic anemia
Therapy	Simple transfusions	Simple transfusions	Simple and exchange

Iron chelation	Iron chelation Hydroxyurea in selected individuals Transplantation	transfusions Analgesia Hydroxyurea Transplantation
Hb, hemoglobin; HbS, sickle hemoglobin; SCD, sickle cell disease.		

In contrast to α -globin genes, there are only two β -globin genes, one on each chromatid of chromosome 11. There are close to 200 mutations described, only about 20 mutations account for the majority of β -thalassemic individuals. Mutations are grouped by regional ethnic locations: Mediterranean basin, Southeast Asia, Africa, and Asian India. All disease-causing mutations alter β -globin gene mRNA transcription, processing, or translation. Some mutations decrease β -globin production by as little as 10%, and some by as much as 90%. Homozygosity or heterozygosity of variably affected alleles explains the wide range of β -thalassemic syndromes.

Patients with one abnormal allele have β -thalassemia minor or trait: the synthesis of the β chain is reduced by about one-half. Although normal Hb A ($\alpha_2\beta_2$) is mildly decreased, there is no accumulation of excess α -chains. There is hypochromia and microcytosis, but no clinically significant anemia, hemolysis, or ineffective erythropoiesis. Thalassemia intermedia refers to individuals with mild hemolytic anemia, secondary to compound heterozygosity of two mild β -thalassemia alleles, δ -and β -thalassemia, Hb E and β -thalassemia, β -thalassemia with hereditary persistence of fetal hemoglobin (HPFH), or coexistence of α -and β -thalassemia. The phenotype of two severe β -chain alleles is referred to as β -thalassemia major or Cooley's anemia, in which the synthesis of β -chains (and Hb A) is virtually absent with α -chains in great excess and consequently severe hemolytic anemia. The compensatory increase in Hb A₂ and F is inadequate to offset the lack of β -chain production.

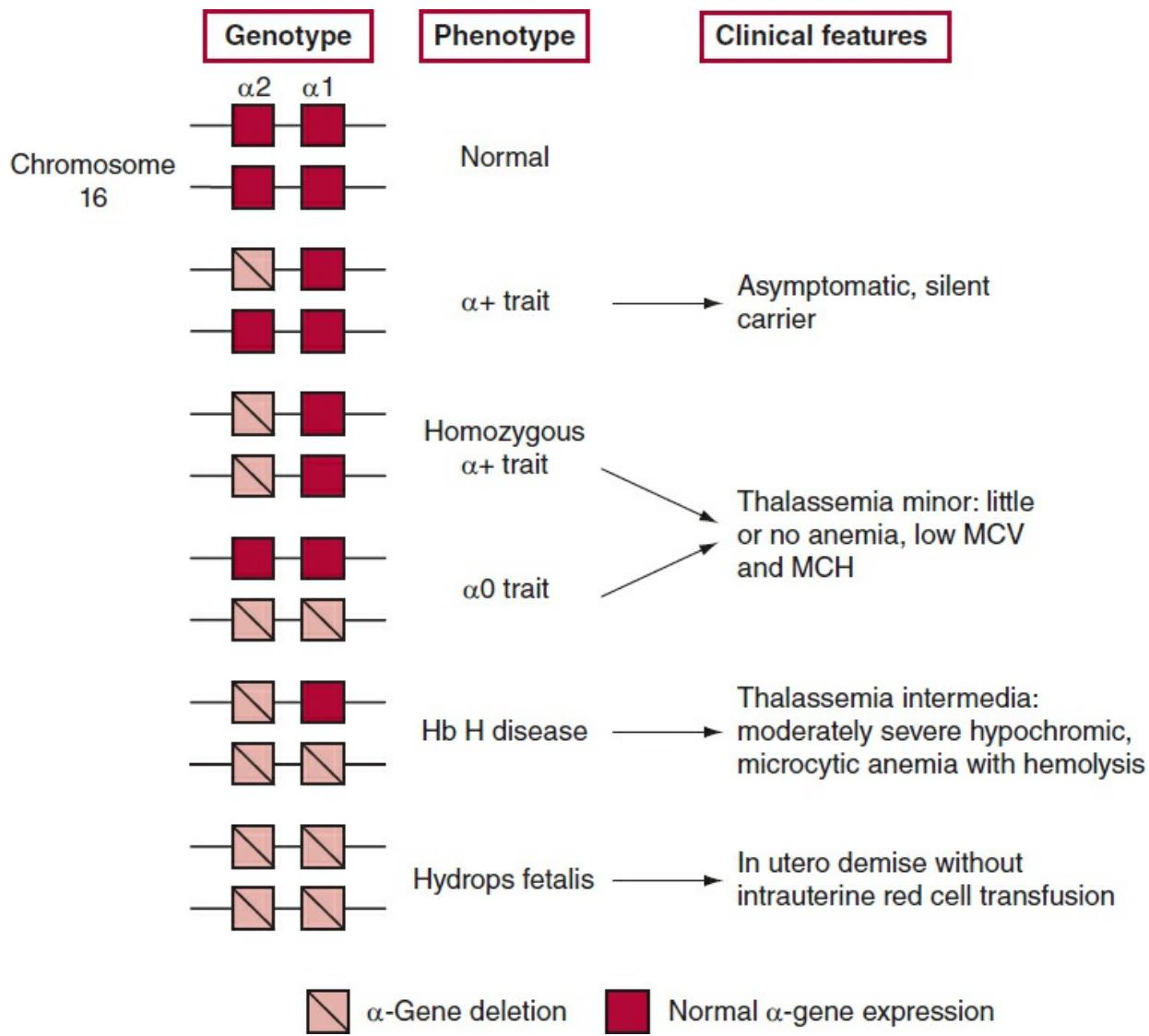


FIGURE 4.1 Diagram for α -thalassemia gene deletion and corresponding phenotypes.

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

Hemoglobinopathies: Hemoglobin Structural Variants

Hemoglobin variants of α or β chains, or the hemoglobinopathies, are most commonly caused by point mutations. The nomenclature of hemoglobinopathies employs alphabetic letters (S, C, or E), and sometimes with locations of first discovery (O^{Arab} or D^{Punjab}) or name of the index case (Lepore or Constant Spring), then followed by the chain, position, and amino acid substitution in that hemoglobin chain ($\beta 6 \text{ Glu} \rightarrow \text{Val}$).

Sickle Cell Disease

Sickle hemoglobin (HbS) is the best characterized hemoglobinopathy. SCD is an inherited disorder in which normal glutamic acid is substituted by valine in the sixth

codon of β -globin chain (β^6 Glu \rightarrow Val), which favors bonding of hemoglobin molecules. As a result, HbS is less soluble when deoxygenated (in the normal oxygenation–deoxygenation cycle), polymerizes and precipitates quickly in red cells, and causes a morphologic change to a crescent shape. These rigid sickle cells lead to hemolytic anemia and vaso-occlusion, which together cause all the complications of SCD.

The life span of sickle cells is about 10 to 20 days, compared to 120 days for normal red cells. In the absence of clinically significant pain episodes, there is a chronic hemolytic anemia with mean hemoglobin of 6 to 8 g/ dL, despite compensatory reticulocytosis greater than 5% or 150 k/ uL. Most sickle erythrocytes are removed in the spleen; some are destroyed intravascularly by mechanical forces or oxidative stress. Hemolysis has been implicated to activate inflammatory mediators, such as tumor necrosis factor-alpha (TNF- α), interleukin-2 (IL-2), thrombin, and platelet-activating factor.² Leukocytosis is common at baseline, and higher leukocyte counts are often associated with more frequent pain crises, stroke, and a shorter life expectancy in homozygous sickle disease (HbSS) patients. Free hemoglobin, released by hemolysis, can consume nitric oxide and participate in endothelial dysfunction to promote vasoconstriction.

Other Hemoglobinopathies (E, C, Lepore, D, O^{Arab}, Constant Spring)

HbE is a common hemoglobin variant present in about 15% to 30% of the individuals in southern China and Southeast Asia. HbE results from replacement of the normal glutamic acid to lysine in the 26th amino acid of the β -chain (β^{26} Glu \rightarrow Lys), and leads to only 50% of mRNAs being spliced normally. Individuals with heterozygous and homozygous HbE have mild anemia, hypochromia, and microcytosis. When HbE is combined with β -thalassemia, the clinical features resemble those of β -thalassemia intermedia.

HbC results from the substitution of the normal glutamic acid to lysine in the sixth amino acid of the β -chain. HbC is found mostly in individuals of African descent and is the second most common hemoglobinopathy in the United States and third most common worldwide. Carriers of HbC are asymptomatic; homozygous individuals (HbCC) exhibit mild hemolytic anemia but are largely asymptomatic. HbC combined with β -thalassemia produces mild to moderate hemolytic anemia with some features of β -thalassemia major. Compound heterozygosity with HbC and HbS (HbSC) leads to milder anemia with fewer leg ulcers, pain crises, and osteonecrosis than with homozygous SCD (HbSS); there is also a slightly lower risk of infection from encapsulated organisms. Retinal proliferative disease, avascular necrosis, and splenomegaly, however, manifest earlier and more frequently in HbSC disease.

Hb Lepore is a fused globin chain consisting of N-terminal half of δ -chain and C-

terminal half of β -chain, and is produced at very low levels (2.5%) compared to normal β -chains. Although typically seen in Greeks or Italians, this variant can occur in many ethnic groups of northern European descent. Hb Lepore can occur alone or in combination with other β -thalassemic mutations, leading to symptoms similar to β -thalassemia major. HbD (same as Hb Los Angeles or Hb Punjab) is another β -chain variant, and is seen in the Asian Indian population. When combined with β -thalassemia or SCD, the anemia is mild. HbO^{Arab}, a rare β -chain variant when combined with SCD (HbSO^{Arab}), behaves similar to severe SCD.

Hb Constant Spring, present in 5% to 10% of Southeast Asians, is caused by a point mutation in the stop codon of α -chain mRNA, leading to an elongated α -chain (α^{CS}). Because synthesis of α^{CS} is much reduced (to about 1%), Hb Constant Spring behaves like an α -chain deletion. When α^{CS} is combined with a cis α -thalassemic defect, it resembles hemoglobin H (HbH) disease (—/ α^{CS} α). Fortunately, α^{CS} is typically coupled with a normal α -chain gene (α^{CS} α) on the same allele, and hydrop fetalis has not been observed.

DIAGNOSIS AND SCREENING

The diagnosis of SCD and thalassemias is now accomplished by neonatal or prenatal testing. The goal of postnatal testing is to identify α -or β -thalassemia carriers, Hb S, C, E, and other clinically important hemoglobinopathies. The process typically begins with a complete blood count (CBC). When red indices are suggestive (Table 4.3, Figs. 4.2 and 4.3), peripheral blood smear and high-performance liquid chromatography (HPLC) provide a provisional diagnosis. HPLC has largely replaced traditional electrophoresis because it reliably quantitates the fraction of hemoglobin A, A₂, F, and S. Hemoglobinopathies are confirmed by isoelectric focusing or gel electrophoresis under alkaline (separates HbS from HbD/ G) or acidic (separates HbC, E, and O^{Arab}) conditions. Specific thalassemia mutations require polymerase chain reaction (PCR)-based DNA testing. Blood count indices vary widely and may deviate from typical values if there is concurrent iron deficiency or compound heterozygosity of other hemoglobinopathies. Any transfusion would also alter the hematologic parameters commonly found in each syndrome.

Table 4.3 Hematologic Characteristics of SCD and Thalassemia

Normal	Total RBC count: normal MCV > 78 fl (or cubic microliter) or MCH > 27 pg ^a HbA > 95%, F < 1%, A ₂ < 3%
α -Thalassemia minor (silent or trait)	MCV < 78 fl or MCH < 25 pg, elevated total RBC count ^a HbA 85%–90%, F 1%–7% ^b , A ₂ ~3.5% ^c

β -Thalassemia trait	MCV < 78 fl or MCH < 25 pg, elevated total RBC count ^a HbA 85%–90%, F 1%–7% ^b , A ₂ ~4–5% ^c
α -Thalassemia major (HbH disease)	MCV < 60 fl or MCH < 25 pg, elevated total RBC count ^a HbA variable, F 1%–40% ^b , A ₂ normal, HbH 0.8%–40% Severe microcytosis, anisocytosis, hypochromia, and HbH on peripheral smear
β -Thalassemia major	MCV < 70 fl or MCH < 25 pg, elevated total RBC count ^a HbA 85%–90% (transfused), HbF 1%–7% ^b , HbA ₂ >3.5% ^c Severe microcytosis, anisocytosis, hypochromia on peripheral smear
SCT	^a HbA near 50%, HbS 35%–40%
SCD	^a HbSS: HbS > 80%, HbA ₂ < 4%, and HbF variable %; sickle cells in peripheral smear HbSC: HbS and C each 30%–40%; less sickle cells but more target cells and spherocytes in peripheral smear
HbS/ β^+ thalassemia	MCV < 78 fl ^a HbS > 70%, HbA 10%–20%, HbA ₂ > 4%
HbS/ β^0 thalassemia	MCV < 78 fl ^a HbS > 70%, HbA < 10%, HbA ₂ > 4%

^aScreening hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) pattern.

^bHbF may be higher in individuals with concurrent $\delta\beta$ -thalassemia or hereditary persistence of fetal hemoglobin (HPFH).

^cHbA₂ may be <3.5% in individuals with concurrent iron deficiency, some α -thalassemia, $\delta\beta$ -thalassemia, or certain β -chain mutations.

Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; RBC, red blood cells; SC, heterozygous for hemoglobin S and C; SCD, sickle cell disease; SCT, sickle cell trait.

Work up for hemoglobinopathy: CBC (MCV), reticulocyte count, hemoglobin electrophoresis (HPLC), iron studies

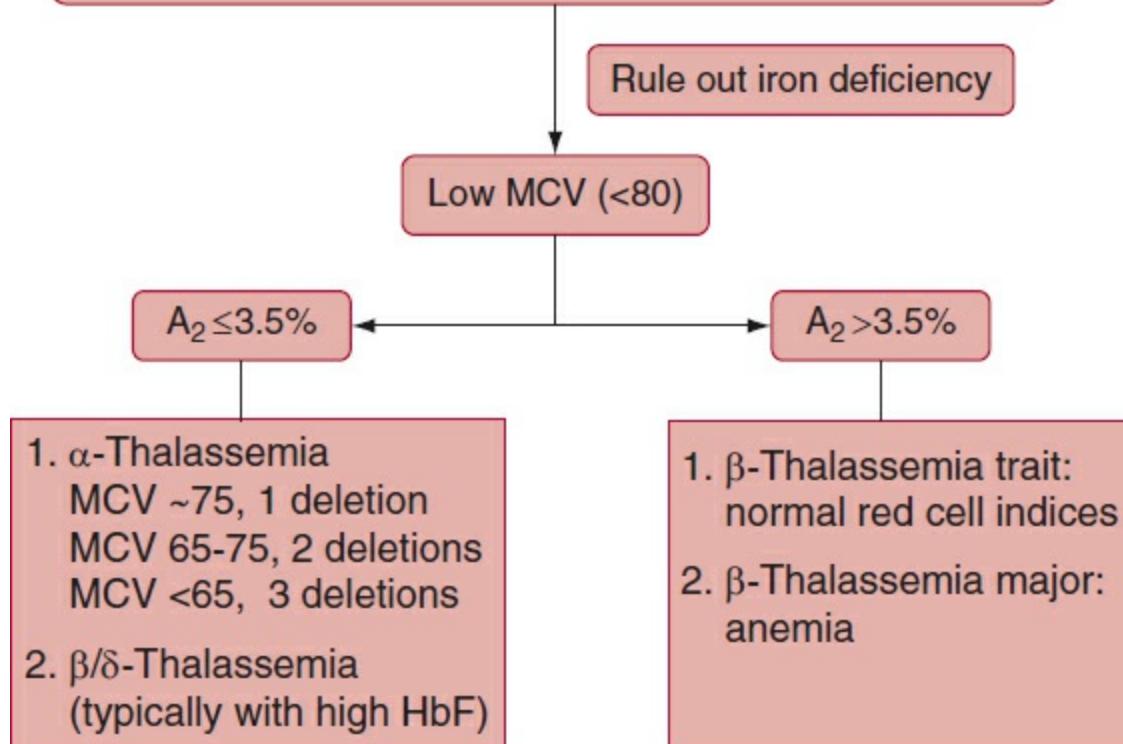


FIGURE 4.2 Diagnostic schema for thalassemia.

CBC, complete blood count; HbF, fetal hemoglobin; HPLC, high-performance liquid chromatography; MCV, mean corpuscular volume.

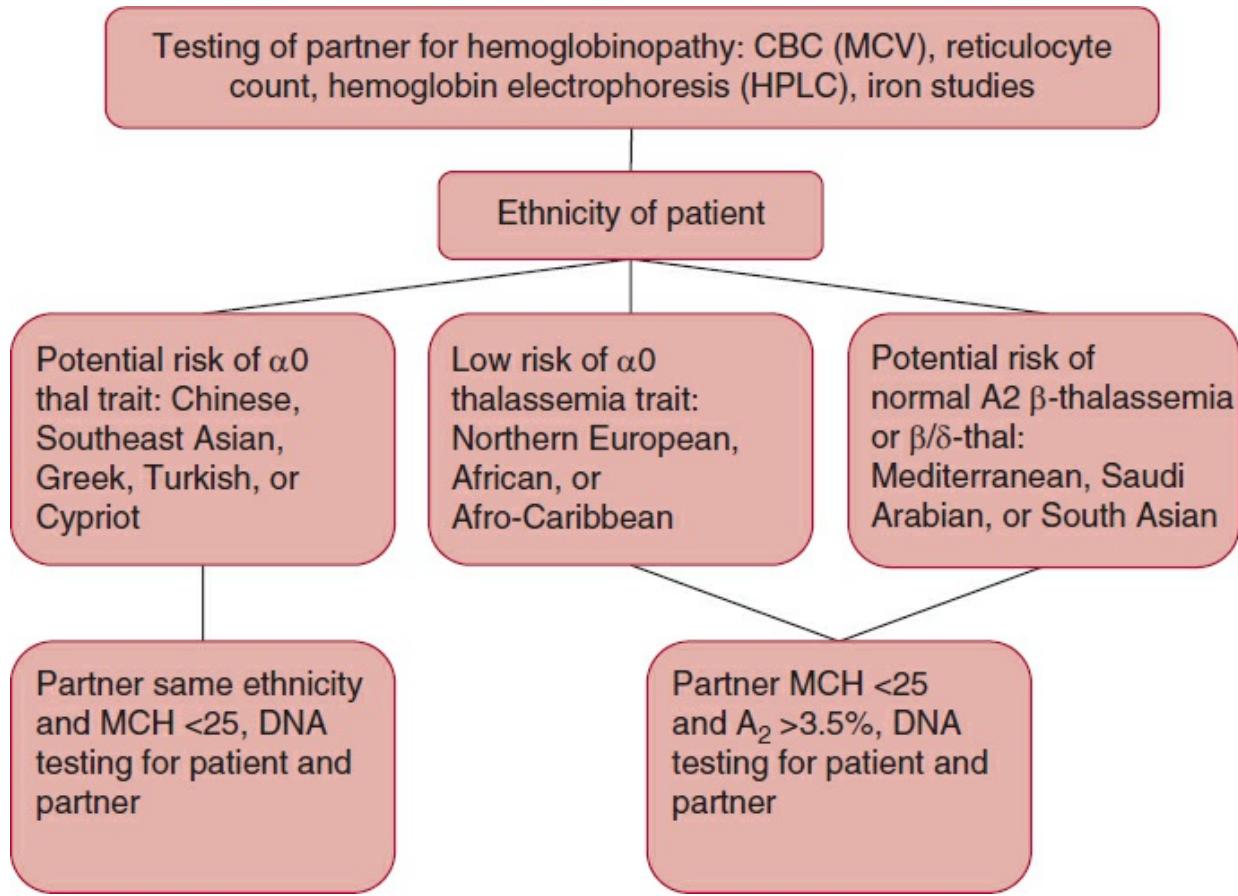


FIGURE 4.3 Prenatal diagnostic schema for thalassemias.

CBC, complete blood count; HPLC, high-performance liquid chromatography; MCV, mean corpuscular volume.

α-Thalassemia Trait and Disease

α-Thalassemia trait can be suspected with elevated total red blood cell number, normal or borderline HbA₂, mean corpuscular volume (MCV) less than 78 fl, and mean corpuscular hemoglobin (MCH) less than 25 pg. The peripheral blood smear in HbH disease can be stained with cresol blue to show HbH precipitates in erythrocytes and reticulocytes.

β-Thalassemia Trait and Major

β-Thalassemia trait can be suspected from an elevated total red blood cell number, MCV less than 78 fl, MCH less than 27 pg, and normal or slightly low hemoglobin. On HPLC, there is a characteristic elution pattern of variably elevated fetal hemoglobin (HbF) (higher in the Mediterranean variant, and lower in the African variant), normal HbA, and >3.5% HbA₂. However, HbA₂ may be normal (<3%) in individuals with concurrent iron deficiency or α-thalassemia, compound heterozygous δ-and β-thalassemia, or those with certain β-chain mutations. With β-thalassemia major, the

MCV is usually less than 70 fl, MCH less than 25 pg, and hemoglobin levels and HbA on HPLC reflect the transfused RBCs.

Sickle Cell Trait and Disease

Sickle cell trait (SCT) or disease can be diagnosed by the combination of CBC, HPLC, and the sickle solubility test. SCT has near-normal red cell indices; hemoglobin may be slightly low to normal and the MCH and red cell distribution width (RDW) may be slightly elevated. HbS will comprise 35% to 40% on HPLC. On the other hand in SCD, hemoglobin will range from 6 to 8 g/ dL, and sickle erythrocytes will be seen on peripheral blood smear. HbS will comprise 80% to 90% of total hemoglobin and slightly elevated HbA₂ on HPLC (due to co-elution of HbS with HbA₂). Trait or SCD is then confirmed by sickle solubility test, and acidic or alkaline gel electrophoresis to screen for other concurrent hemoglobinopathies, such as HbD or G. HbSC disease is easily distinguished by HPLC. Additionally, the peripheral smear in HbSC disease shows fewer sickle cells, more spherocytes and target cells, and an uneven distribution of hemoglobin among red blood cells.

CLINICAL SYNDROMES AND TREATMENT OF SICKLE CELL DISEASE

Vaso-Occlusive Episodes (Pain Crises)

VOE is the most frequent clinical manifestation of SCD, and can occur spontaneously or precipitated by infection, stress, dehydration, or changes in weather or temperature. Frequent assessment and adjustment of pain therapy, involvement of a pain management service, and other consultations are important to address the complex etiology of pain in SCD. Evaluation begins by obtaining a full history of current and prior VOEs. Physical examination and vital signs identify any signs or symptoms related to a pain episode. Acute pain can affect multiple sites: bones, joints, the cardiopulmonary system, central nervous system (CNS), or abdominal visceral organs. Chronic pain is typically confined to leg ulcers and the skeletal system.

Mild acute pains are often managed in the outpatient setting with a combination of nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and/ or an oral opioid. Moderate to severe acute pain typically requires intervention in a day hospital or emergency department, which begins with rapid assessment of the pain, hydration using 5% dextrose in half-normal saline (D5 ½ NS) and 20 mEq KCl, not exceeding 1.5 times maintenance, and an opioid analgesic (typically morphine, hydromorphone, or fentanyl). The choice, dose, and frequency of medication depend on the patient's outpatient drug regimen and prior responses. Severe pain is managed by inpatient bolus and continuous

infusion of an opioid analgesic, often through patient-controlled analgesia (PCA) pumps. In those with poor intravenous access, subcutaneous injection is an acceptable short-term alternative; however, intramuscular injection should be avoided because the absorption varies. Parenteral meperidine should not be used as first-line treatment because the metabolite, normeperidine, has a long half-life and increases the risks of mood disturbances and seizures. Opioid agonists are metabolized by the liver and excreted variably by the kidney, and dose reduction may be necessary in those with hepatic impairment (Table 4.4). Meperidine and morphine have active metabolites, should be used with great caution in patients with renal impairment. Common side effects of all opioids, nausea, vomiting, pruritus, constipation and respiratory depression, should be monitored and treated accordingly.

Non-opioid analgesics such as acetaminophen and NSAIDs have a ceiling effect and are often used with an oral opioid agonist. The total acetaminophen dose should not exceed 4g daily in adults with normal hepatic function. Gastrointestinal, renal, and hematologic toxicities should be monitored. Benzodiazepines, antidepressants, antiemetics, and opioid agonist–antagonists (such as pentazocine, nalbuphine, and butorphanol) are useful adjuncts to opioid agonists and potentiate their analgesic effects. Gabapentin and tricyclic antidepressants can be useful for neuropathic pain.

Red cell transfusions are not routinely administered during VOEs, but are important for concurrent complications, such as acute chest syndrome (ACS), stroke, or other organ ischemia and damage.

Infections

Because of functional asplenia, SCD patients are at an increased risk of infection with encapsulated organisms: *Streptococcus pneumoniae*, *Hemophilus influenza*, and *Neisseria meningitidis*. Thus fever should be evaluated and managed promptly as a potential sepsis event, and empiric antibiotics administered while awaiting blood or urine culture and chest radiograph results. Neonatal diagnosis enables prompt initiation of penicillin prophylaxis and family education about vigilant monitoring for infections. In a placebo-controlled clinical trial,³ prophylactic penicillin prevented 84% of life-threatening *S. pneumoniae* infections. Vaccinations against *S. pneumoniae* should begin concurrently with penicillin prophylaxis. Penicillin may be discontinued in those older than 5 years of age who have completed vaccination, because there was no statistically significant additional benefit compared to placebo. Patients allergic to penicillin can receive azithromycin (10 mg/ kg, up to 250 mg/ day).

Table 4.4 Comparative Profiles of Common Opioid Agonists for Acute Pain

Dose

Narcotic	Equivalent Analgesic Dosing	Site of Metabolism and Excretion ^a	Adjustment in Hepatic Impairment	Dose Adjustment in Renal Impairment
Fentanyl	IV or transdermal: 25 mcg/ h	Liver: inactive metabolites Kidney: 75% of metabolites	No	
Hydromorphone	IV: 1.5 mg PO: 7.5 mg	Liver: inactive metabolites Kidney: little excretion	Consider	GFR 20–50 mL/ min: 50%–75% of normal dosing
Oxymorphone	IV: 1 mg PO: 10 mg	Liver: active and inactive metabolites Kidney: 30–40% excretion	Consider	GFR < 20 mL/ min: 25%–50% of normal dosing
Meperidine	IV: 75 mg PO: 300 mg	Liver: several active metabolites Kidney: >70% excretion	Yes	Use of meperidine with renal impairment should be avoided
Morphine	IV: 10 mg PO: 30 mg	Liver: active and inactive metabolites Kidney: >90% excretion	Yes	GFR 20–50 mL/ min: 50%–75% normal dosing GFR < 20 mL/ min: avoid morphine or 25%–50% of normal dosing with caution
Oxycodone	IV: not available PO: 30 mg	Liver: active metabolites Kidney: variable excretion of metabolites	Yes, start one-third to half of normal dose	GFR 20–50 mL/ min: 33% to 50% of normal dosing GFR < 20 mL/ min: 33% of normal dosing
Hydrocodone	IV: not available PO: 20–30 mg	Liver: active metabolites Kidney: 30% excretion	Consider	GFR 20%–50 mL/ min: 50 to 75% of normal dosing

^aOpioid agonists are typically metabolized in the liver into active or inactive products, and are variably excreted by the kidneys.

GFR, glomerular filtration rate; IV, intravenous; PO, per oral.

Human parvovirus B19 is commonly spread among school-age children. B19 infects erythroid progenitors and causes transient red cell aplasia. Although there is a wide range of clinical severity, influenza-like symptoms, fever, pain, and splenic sequestration can accompany an acute infection. Laboratory testing may reveal acute anemia, reticulocytopenia, and immunoglobulin M (IgM) antibody to parvovirus. Milder forms of SCD, for example, HbSC or HbS/ β⁺ thalassemia, hydroxyurea (HU) treatment, or chronic transfusions, do not protect SCD individuals from developing severe

complications related to B19.⁴ Parvovirus infection is also known as Fifth Disease, but in patients suffering transient aplastic crisis, the characteristic facial rash is absent. B19 can cross the placenta and cause hydrop fetalis and stillbirths; thus, pregnant staff should be strictly isolated.

Central Nervous System and Eye Disease

CNS (Stroke)

Stroke is a major complication more frequently observed in HbSS than in HbSC. Children tend to have thrombotic strokes, and adults hemorrhagic strokes. Because the incidence of stroke is 11% up to 20 years of age, children with HbSS should be screened with transcranial Doppler (TCD) every 6 to 12 months from age 2 to 18 years. For primary stroke prevention, the Stroke Prevention (STOP) trial showed that children (age 2 to 16 years) who had a TCD velocity greater than 200 cm/s in the internal carotid or middle cerebral artery had improvement in the TCD velocity and a much lower incidence of brain infarction, when managed on long-term transfusions to maintain HbS <30%, compared with supportive care (penicillin prophylaxis, vaccinations, folic acid supplementation, treatment of acute crises, and transfusions as needed).⁵ A follow-up study to the original STOP trial showed that when long-term transfusions were discontinued, TCD velocity quickly became abnormal and a few children developed acute strokes.⁶ Individuals 18 years and older tend to have lower transcranial velocities; thus, TCD is not performed.

Children with suspected stroke or TIAs are evaluated promptly, and hydration, therapy for hypoxia or hyperthermia, and blood pressure stabilization should follow immediately. Tissue plasminogen activator (t-PA) has not been extensively used in children and therefore is not routinely recommended. The use of antiplatelet agents, aspirin, or clopidogrel, is uncertain, but may be appropriate in selected circumstances. If a thrombotic stroke is present, exchange transfusions are initiated to reduce the HbS level to <30%. If a hemorrhagic stroke is present, the source and the extent of bleeding are identified and the treatments are individualized; exchange transfusions may be indicated to reduce the HbS level to <30%. If imaging studies do not identify any abnormality, the next steps may involve observation, simple transfusions, and/or participation in clinical trials.

As the recurrence rate for thrombotic strokes is high, long-term transfusion therapy to maintain HbS <30% until 16 to 18 years of age should be planned. Long-term transfusions can also be considered in hemorrhagic stroke or vasculopathy (aneurysm, arterial stenosis, or Moya-Moya). Allogeneic stem cell transplantation should be encouraged in those with HLA-matched siblings. A multicenter Stroke With Transfusion Changing to Hydroxyurea (SWiTCH) trial was stopped early that compares long-term

transfusions + iron chelation with HU + phlebotomy for secondary stroke prevention. The results showed a nonstatistically significant difference: no stroke in 66 patients in the transfusion arm, and 7 strokes in 60 patients in the HU arm.⁷ A recent international multicenter trial showed that in children (age 5 to 15 years) who had silent cerebral infarct on magnetic resonance imaging (MRI) with normal TCD velocities, long-term transfusion was better than observation in preventing new overt or silent infarcts.⁸

Adults with acute strokes or TIAs may be managed similarly to children.⁹ If a thrombotic stroke is identified, t-PA, antiplatelet therapy, and/ or exchange transfusions can be considered. If a hemorrhagic stroke is identified, treatment is based on the source and the extent of bleeding; exchange transfusion to reduce the HbS level <30% may be indicated. For long-term therapy or secondary prophylaxis, antiplatelet therapy may be continued, with or without chronic transfusions to maintain the HbS level <30%. For secondary stroke prevention in adults, blood transfusions should be continued; switching to HU should be done ideally in a clinical trial. Those with HLA-matched siblings should be encouraged to pursue allogeneic stem cell transplantation.

There is currently no single best screening method to identify adults who are at high risk for stroke. MRI/ magnetic resonance angiography (MRA) of brain can be considered in those who have general risk factors for thrombotic strokes (age, prior transient ischemic attacks, and systemic hypertension), risk factors specific to SCD (prior history of ACSs, dactylitis, severe anemia, and leukocytosis), or recurring symptoms such as headaches or memory deficit.

Eye Disease

Neovascularization results from repetitive VOEs within the eye and leads to visual impairment. These proliferative changes are often asymptomatic early in the disease process; clinically detectable retinal changes are typically discovered between 15 and 30 years of age. Patients with HbSC and sickle-thalassemia are disproportionately more prone to develop clinically significant ophthalmologic problems. Annual eye examinations starting in adolescence, carefully evaluating visual acuity, papillary reactivity, and anterior and posterior structures are important. There is peripheral arteriolar occlusion in stage I eye disease, vascular remodeling and neovascularization in stages II and III, vitreous hemorrhage in stage IV, and retinal detachment in stage V.

In patients with SCD or SCT, direct eye trauma that causes bleeding into anterior chamber may require urgent evaluation, as RBCs can occlude the trabecular channels, increase intraocular pressure, and cause acute glaucoma.

Cardiovascular Manifestations

Individuals with SCD have lower blood pressures compared to individuals with other

types of chronic anemia. Renal sodium wasting and anemia are postulated as possible causes although other mechanisms may be present. Blood pressures in SCD correlate with age, hemoglobin, and body-mass index. The risk of stroke and mortality increases when systolic or diastolic blood pressures approach those of age-, sex-, and race-matched normal individuals.

Other cardiac manifestations include frequent systolic flow murmurs, typically related to the degree of anemia. A loud P₂ may suggest elevated right-sided pressure. On echocardiograms, small amount of pericardial effusions are found in approximately 10% of all studies; cardiac output, cardiac chamber size, and myocardial wall thickness are increased to improve the stroke volume without increasing the heart rate. With long-term and consistent increases in cardiac output, the ability to perform physical work is reduced by half in adults and by one-third in children. Right-sided heart failure is increasingly recognized in those with other sickle-related complications. Individuals with suboptimally treated transfusional iron overload may progress to dilated cardiomyopathy. Myocardial infarction due to coronary arterial occlusion is rare, but damage from small vessel diseases may occur. Sudden death due to unexplained arrhythmia or autonomic dysfunction also has been described in adults with SCD, presumably from excess iron interfering with the cardiac conduction system.

Pulmonary Complications

Acute Complications

ACS typically presents with cough, chest pain, and other respiratory symptoms, and confirmed by a temperature higher than 38.5°C, a new pulmonary infiltrate affecting at least one lung segment on chest imaging, and rales on auscultation (indicating multilobar involvement).¹⁰ Children tend to have more respiratory symptoms (wheezing, cough, and fever), whereas adults report musculoskeletal pain and dyspnea, and have a more severe course. Risk factors for ACS are HbSS, low HbF, high baseline hemoglobin (11 g/ dL or greater), high white blood cell count (greater than 15 k/ uL), and prior episodes of ACS. ACS is a frequent cause of death in both children and adults with SCD, the second leading cause of hospitalization, and the most frequent complication following surgery and anesthesia. Complications from ACS include CNS injury (anoxia, infarct, or hemorrhage), seizure, or respiratory compromise with or without multiorgan failure. Frequent ACS episodes are associated with shortened survival.

Only less than half of ACS events have identifiable causes. These include pneumonia, pulmonary infarction from vaso-occlusion within pulmonary vasculature, fat embolism, or pulmonary thromboembolism. Microbiologic culture of sputum may reveal a variety of atypical organism (chlamydia or mycoplasma), viruses (respiratory syncytial virus), and bacteria (*Staphylococcus aureus*, *S. pneumoniae*, or *H. influenza*);

up to 30% of microbiologic cultures are negative.¹¹ Systemic fat embolism syndrome is rare, and occurs when the infarcted/ necrotic bone marrow and fat being released and lodged in the pulmonary vasculature. Fat embolization can precipitate or develop concurrently with ACS; multiorgan failure may result. Risk factors for developing systemic fat embolism syndrome are HbSC, pregnancy, and prior corticosteroid treatment.

Treatment for ACS includes oxygen, antibiotics coverage for atypical organisms, simple or exchange transfusions to improve oxygen saturation, bronchodilators (as airway hyperreactivity often accompanies ACS), and analgesia for pain. All these efforts are aimed at reducing the percentage of sickle erythrocytes and minimizing sickle polymerization. After an episode of ACS is successfully managed, strategies to prevent future episodes may include vaccinations (especially against *S. pneumoniae*), HU, continuing transfusions, or allogeneic stem cell transplantation.

Chronic Complications

Pulmonary hypertension is multifactorial in its pathogenesis, including sickle cell-related vasculopathy, pulmonary damage from recurrent ACS, high blood flow from anemia, chronic thromboembolic disease, and chronic hemolysis. Clinically, pulmonary hypertension can manifest as dyspnea, clubbing, loud second heart sound (P_2), an enlarged right side of the heart on chest radiograph, and 95% or less oxygen saturation on room air at rest. Pulmonary hypertension can initially be screened with an echocardiogram showing a tricuspid regurgitation jet velocity (TRV) of ≥ 2.9 m/s and subsequently confirmed by right heart catheterization with a mean pulmonary arterial pressure of ≥ 25 mmHg.¹²

There is currently no single preferred treatment for patients with pulmonary hypertension: optimizing medical care by emphasizing HU therapy, improving anemia, ruling out left-sided heart failure, and continuous or nocturnal oxygen as indicated. Single or combination therapy using phosphodiesterase inhibitors, prostacyclin analogs, endothelin receptor antagonists, soluble guanylate cyclase stimulator, calcium channel blockers, anticoagulation with warfarin (target INR 2-3), and nitric oxide are often used.

Gall Bladder, Hepatic, and Splenic Manifestations

SCD can affect the hepatobiliary and splenic systems in multiple ways (Table 4.5). Hyperbilirubinemia (typically <4 mg/ dL of unconjugated bilirubin) from chronic hemolysis is common. Other factors that increase total bilirubin level include cholesterol intake, presence of Gilbert's syndrome, and cephalosporin antibiotic use. Biliary sludge and cholelithiasis can occur as early as 2 to 4 years of age, has similar

clinical manifestations, and are managed similarly as those without SCD. Elevation in the direct or conjugated bilirubin from baseline of 0.1 to 0.4 mg/ dL can indicate sickle-related liver disease.

Table 4.5 SCD Manifestations in Gall Bladder, Liver, and Spleen

Gall bladder	1. Biliary sludge 2. Acute and chronic cholelithiasis
Liver	1. Hemosiderosis: from transfusional iron overload 2. Hepatic crisis (RUQ syndrome): may occur with pain crisis and presents clinically with fever, jaundice, RUQ pain with hepatic enlargement, and transient elevation in AST or ALT. 3. Viral hepatitis: mostly from transfusions. AST and ALT typically increase slowly over months. 4. Hepatic sequestration: typically with elevated bilirubin and alkaline phosphatase above baseline, with or without hepatomegaly. This is typically treated with simple transfusions.
Spleen	1. Splenic infarction: gradually occurs over years 2. Acute splenic sequestration: acute drop of >2 g/ dL of hemoglobin, splenomegaly; more often in children. This is treated with simple transfusions; chronic transfusion may be considered.

ALT, alanine transferase; AST, aspartate transferase; RUQ, right upper quadrant.

Acute splenic sequestration is caused by trapping of erythrocytes, and presents with weakness, pallor, tachypnea, an acute drop in hemoglobin (typically 2 g/ dL or greater than 20% from baseline), or abdominal fullness from acute splenomegaly (2-cm increase in palpated spleen size). This syndrome can be precipitated by infections, and is typically seen in children younger than 5 years with HbSS, older children with HbSC, or in a few adults with HbS/ β^+ thalassemia. Children tend to have recurrent and severe episodes that may require transfusion; rarely do they need a long-term plan of chronic transfusion therapy and/ or splenectomy. Although splenic sequestration in children tends to be more severe, it is uncommon in adults and is managed with supportive care and observation.

Renal Abnormalities

There are several sickle-related manifestations in the kidneys. There is supranormal creatinine secretion in the proximal tubules, which explains lower serum creatinine (near 0.5 mg/ dL). A serum creatinine approaching 1.0 mg/ dL can indicate subtle renal insufficiency. The renal medulla is composed of tubules and blood vessels collectively call vasa recta; this region is chronically in acidic, hypoxic, and hypertonic environment, and is thus very susceptible to HbS polymerization. Over time, gradual loss of vasa recta leads to an inability to concentrate urine. Hyposthenuria develops early in childhood, is frequently associated with nocturia, and is the chief reason why

SCD individuals are so prone to dehydration. HbS sickling can also lead to papillary necrosis and hematuria. Other renal manifestations include proteinuria from chronic glomerular damage, which in some individuals can progress to renal insufficiency or failure; angiotensin-converting enzyme (ACE) inhibitors can ameliorate this progression. A decrease in hemoglobin from baseline due to renal insufficiency can be treated by erythropoietin injection (overlapped with HU) and/ or transfusion. Although serum erythropoietin levels are already elevated in renal failure, they are relatively lower when corrected for the degree of anemia.

Priapism

Priapism is a sustained painful erection that is stuttering (duration <3 hours and spontaneous resolution) or prolonged (>3 hours). Priapism begins early in puberty, and as many as 80% of men with SCD will have experienced at least one episode by 20 years of age. Priapism is caused by vaso-occlusion of the venous drainage of the penis, and physical examination will reveal a hard penis with a soft glans. Oral hydration, activities such as exercise to divert blood flow away, pseudoephedrine, and analgesia should be instituted at the onset of priapism. A prolonged priapism represents an urologic emergency and requires urgent evaluation, intravenous hydration, and analgesia. If not improved within 1 hour, blood aspiration and/ or injection with dilute epinephrine or phenylephrine to corpus cavernosum may be needed.¹³ Simple or exchange transfusions are sometimes used.

Recurrent priapism can lead to impotence and fibrosis. No treatment has been well studied. Shunting procedure between the glans and distal corpus cavernosum (Winter's procedure), α -adrenergics (e.g., pseudoephedrine), and medications reducing the frequency of erection, tricyclic antidepressants, β -blockers, or leuprolide, have all shown variable success. Simple or exchange transfusions can be considered. Recent studies have grouped patients into two potentially distinct phenotypic presentations: one group who has priapism and leg ulcers and the other who has frequent vaso-occlusive crises and osteonecrosis.

Skeletal Complications and Leg Ulcers

Repetitive vaso-occlusion in marrow sinusoids eventually cause bone infarction. Osteonecrosis ensues when ischemic necrosis of juxta-articular bone in femur, humerus, or tibia. In children prior to bone maturation, osteonecrosis is treated conservatively with analgesia, NSAIDs, and protected weight bearing. In adults, secondary degenerative arthritis can compound osteonecrosis, and the usual conservative treatment is ineffective. Core decompression and osteotomy with aggressive physical therapy have been reported to offer temporary relief of pain and increased joint mobility;¹⁴ joint

replacement is reserved for those with severe symptoms or advanced disease.

Dactylitis or “hand–foot” syndrome in infants and young children presents with pain or swelling in one or more extremities (hands or feet). Plain radiograph may show periosteal elevation and moth-eaten appearance. This syndrome usually requires hydration and analgesia, typically transfusions or antibiotics are not necessary.

Bacteremia can lead to osteomyelitis or septic arthritis. Both presents with warmth, tenderness, and edema caused by vaso-occlusion in bones; fever in the acute phase, increase in white blood cell count, and positive blood cultures help to distinguish infection from pain crisis. Positive microbial culture from aspiration of the bone or joint is diagnostic. Both these infections are treated with surgical drainage and short-term (2 to 6 weeks) intravenous antibiotics. Additional temporary joint mobility with exercises to improve range of motion in the convalescent phase may be indicated for septic arthritis.

Leg ulcers are seen in 10% to 20% of individuals with HbSS, and much less in individuals with HbSC or HbS/ β^+ -thalassemia. Frequency of leg ulcers increases with age and is associated with lower mean hemoglobin (6 g/ dL or less) and higher lactate dehydrogenase (LDH). Their exact etiology is unclear, but trauma, chronic vaso-occlusion, edema, hemolysis, and venous thrombosis have all been implicated.¹⁵ Ulcers tend to locate in the dorsum of the feet, ankles, or tibia; other sites are rare. They begin as small hyperpigmented area with edema, pain, and dysesthesia, subsequently appearing as denuded and “punched out” ulcer. Ulcers are usually infected locally, and osteomyelitis is rare. Two principles are important in promoting wound healing: reduction of local edema by elevation and/ or pressure dressing, and debridement of ulcers by frequent wet-to-dry dressing changes to maximize granulation. There are many topical treatments available, but no single therapy works uniformly. Topical or systemic antibiotics are generally not helpful and eventually select for antibiotic-resistant organisms. Ulcers smaller than 4 cm usually heal in weeks; larger ones may require consultation with wound care service and plastic surgery for skin flaps. Transfusions can be considered for recurrent or persistent ulcers. The use of HU in leg ulcer is controversial. There are reports of HU causing leg ulcers in individuals with SCD and myeloproliferative disease. But in the Multicenter Study of Hydroxyurea (MSH), HU did not appear to change the incidence of leg ulcers.¹⁶ Other reports, however, suggest that HbF elevation is associated with reduced rates of leg ulcers.

THERAPY

Transfusions

Transfusions are an important therapy for SCD (Table 4.6), and can be separated into simple episodic, simple chronic, or exchange. It is important to notify the blood bank of

the type (simple or exchange), indication, and duration (episodic or chronic). A detailed transfusion history should be maintained that includes the total number of prior red cells units, presence of any red cell antibodies, percent HbS, and the target hemoglobin or hematocrit.

Red cell antigen difference between sickle cell patients and blood donors (mostly Caucasians) is one reason for the high rate of alloimmunization. Alloimmunization can be minimized by typing for other Rh (D, E/ e, and C/ c), and Kell (K) antigens, in addition to the usual ABO typing. In developing countries, nonroutine use of leukocyte filters is another reason for high rate of alloimmunization. Prestorage leukocyte depletion is now commonly employed in blood banks. When a patient has a prior transfusion history, other minor antigens (Kidd, S, and Duffy) should also be typed. Other potential complications include the usual transfusion reactions that can occur in non-SCD patients: volume overload, acute/ delayed hemolytic reactions, transfusion transmitted infections, and iron overload.

HbF Induction

The beneficial effect of HbF was first recognized from the observations that neonates with HbSS do not develop SCD-related symptoms in the first 6 months, and patients with co-inheritance of SCD and HPFH, such as in Saudi Arabia and India, have milder symptoms. Currently HU is the only Food and Drug Administration (FDA)-approved drug for HbF induction. HU is a cell cycle (S-phase)-specific agent that blocks the conversion of ribonucleotides to deoxyribonucleotides. Its primary clinical impact is HbF induction, which inhibits HbS polymerization, but other benefits may include reduced leukocyte and platelet counts, less hemolysis, decreased bone marrow cellularity, and generation of nitric oxide.

Table 4.6 Indications for Transfusions in SCD

Indications for Simple Episodic Transfusions	Consider in
<ol style="list-style-type: none">1. ACS (mild to moderate)2. Severe anemia (hgb <5 g/ dL) or a decrease of >20% from baseline3. Preoperatively for major surgery with general anesthesia (target hgb 9 g/ dL and HbS ≤ 60%)¹⁷4. Symptomatic patients with heart failure, dyspnea, hypotension, or other organ failure5. Infection-related anemia (parvovirus B19) or hemolysis-related anemia (concomitant G6PD deficiency)6. Hepatic sequestration7. Splenic sequestration (more in children, drop in Hb by 2 g/ dL, acute splenomegaly, with thrombocytopenia)	<ol style="list-style-type: none">1. Prolonged priapism2. Nonhealing leg ulcers

Indications for Simple Chronic Transfusions	Consider in
<ol style="list-style-type: none"> Primary or secondary stroke prevention⁵ Complicated pregnancy (by progressive anemia, preeclampsia, increased pain episodes, prior pregnancy loss, multiple gestations)— starting at 20 weeks¹⁸ 	<p>Children following acute chest syndrome¹⁹</p> <ol style="list-style-type: none"> Pulmonary hypertension, moderate or severe Silent, hemorrhagic, or vasculopathic stroke Chronic heart failure Splenic sequestration in children; transfuse until 5–6 years of age Chronic debilitating pain Renal failure-related anemia Recurrent priapism

Indications for Exchange Transfusions

- Acute chest syndrome (moderate or severe)
- Stroke (thrombotic, consider in hemorrhagic)
- One or multiple organ failure
- HbSC patients with any of following:
 - Preoperatively for major surgery requiring general anesthesia²⁰
 - Hepatic sequestration

ACS, acute chest syndrome; SCD, sickle cell disease.

The MSH was a randomized placebo-controlled clinical trial that confirmed the beneficial effects and the safety of HU. The 150 HU treated patients had fewer pain episodes and ACS, required fewer transfusions, and experienced minimal toxicity.¹⁶ Approximately 70% of SCD patients are likely HU-responsive: steady-state HbF should increase twofold from baseline or to 10% to 15%, total hemoglobin should increase by 1 to 2 g/ dL, or subjective reduction in the severity and frequency of pain (Table 4.7).

HU can be started at 10 to 15 mg/ kg daily, and adjusted by 5 mg/ kg/ day increment every 6 to 8 weeks, to a target of approximately 25 mg/ kg daily. Compromised hepatic or renal function may require lower dosing (5 to 7.5 mg/ kg). Within a week of therapy, HbF-containing reticulocytes will rise; at the end of 2 to 3 weeks, HbF-containing red cells will increase. Other hematologic effects include an increase in MCV (to >100) and decrease in leukocytes (mostly neutrophils), platelets, and reticulocytes. Two to 3 months are usually required before the effects on HbF and blood counts are stabilized; a trial of 6 months is preferred to assess clinical benefit. HU in several cohorts of children has shown to be safe and efficacious in reducing pain and sickle-related complications.²¹ Higher doses of HU are associated with higher levels of HbF, and may be associated with less organ injury and improved survival.²²

Table 4.7 Clinical Use and Monitoring of Hydroxyurea

Indications	<ul style="list-style-type: none"> Adults, adolescent, or children HbSS or HbS/ β⁰-thalassemia with frequent pain, history of ACS, severe vaso-occlusive events requiring hospital admissions (≥3 per
-------------	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

	year), severe anemia
Dosing	<ul style="list-style-type: none"> ■ Start with 10–15 mg/ kg/ day ■ Adjust by 5 mg/ kg/ day increment every 6–8 weeks, target 25–35 mg/ kg/ day ■ Duration: 6–12 months trial
Monitoring	<ul style="list-style-type: none"> ■ Initially: CBC every 2 weeks, chemistry every 2–4 weeks, HbF every 6–8 weeks ■ At stable dose of hydroxyurea, CBC and chemistry every 4–8 weeks, HbF every 8–12 weeks ■ Keep ANC > 2,000/ μL, reticulocytes > 100 K/ μL, and platelet >100 k/ μL ■ If marrow toxicity occurs, stop for 2 weeks, and start at a lower dose when blood counts recover
Treatment Endpoint	<ul style="list-style-type: none"> ■ Less severe or frequent pain ■ Increased HbF to 10%–20% or 2- to 2.5-fold increase from baseline ■ Increased hemoglobin if severely anemic, typically 1–2 g/ dL ■ Improved well-being, weight gain
Cautions	<ul style="list-style-type: none"> ■ Dose reduction in hepatic or renal insufficiency ■ Contraception for men and women

ACS, acute chest syndrome; ANC, absolute neutrophil count; CBC, complete blood count; Hb, hemoglobin.

There are other inducers of fetal hemoglobin available in research studies, 5-azacytidine, decitabine, and histone deacetylase (HDAC) inhibitors (dimethylbutyrate). These drugs aim to “reactivate” gamma globin expression that has been silenced in the postnatal hemoglobin switching process.

Other Drugs

There are agents under active investigation that modify different aspects of SCD pathophysiology. These include HbS modifiers (urea, organic compounds), inhibitors of Gardos channel (clotrimazole, ICA 17403), chloride and cation channel blocker (dipyridamole, magnesium pidolate, NS-3623), anti-HbS polymerization agents (5-hydroxymethyl furfural, GBT440), blockers of endothelial adhesion (P-and/ or E-selectin inhibitors, GMI-1070, Sel-G1), and antiplatelet agents (prasugrel).

SPECIAL TOPICS

Contraception and Pregnancy

HU is a teratogen in animal models; therefore, both male and female patients on HU should use contraception, and discontinue the drug if pregnancy is planned. Recent long-term updates from the MSH investigators indicated that neonates delivered from women receiving HU therapy throughout pregnancy or from men taking HU did not have any teratogenic effects,²³ but how to proceed with any unplanned pregnancy should be

discussed. HU is also secreted into breast milk, and breastfeeding should be avoided.

HU is considered as an oral chemotherapeutic drug, thus it is not surprising that there is a perceived risk of developing malignancy. Although there are case reports of SCD patients who have developed leukemia while receiving HU, this rate is probably similar to the general population. No definitive risk increase has been directly attributed to HU for individuals with myeloproliferative diseases or cyanotic heart disease.

Table 4.8 Perioperative Considerations in SCD

Preoperative

1. Simple transfusions to achieve hemoglobin of 10 g/ dL in HbSS and HbS/ β^0 thalassemia. Individuals with HbSC may require exchange transfusion, especially prior to abdominal surgery.
2. Blood typing for additional antigens, such as C, E, Kell, Kidd (Jk), S, and Duffy (Fy), to minimize alloimmunization.
3. Hydration

Postoperative

1. Hydration, oxygen, and monitoring respiration and peripheral perfusion.
2. Monitoring for acute chest syndrome, infection, pain crisis, or stroke.

Pregnant women with SCD are at an increased risk of miscarriage, preeclampsia, sickle pain crises, acute anemia or hemolysis, or infections.^{24,25} Maternal mortality rate is low and the overall outcome of the pregnancies is favorable, but infants tend to be born prematurely (average at 34 to 37 weeks) and small for gestation age (less than tenth percentile). HbSS women tend to have more frequent or severe complications than HbSC women. Transfusions are usually reserved for pregnancies complicated by progressive anemia, increased pain episodes, preeclampsia, prior pregnancy loss, or multiple gestations; prophylactic transfusions are generally discouraged.

Anesthesia and Surgery

Surgery and general anesthesia have higher morbidity and mortality in SCD compared to the general population. The risk is higher in those with HbSS or HbS/ β^0 thalassemia than those with HbSC or HbS/ β^+ thalassemia.¹⁷ Complications may be as frequent as in those receiving regional anesthesia. ACS and postoperative infection are the most common, followed by pain crisis and stroke (Table 4.8).

SICKLE CELL TRAIT

Approximately 8% of African Americans have SCT. Under physiologic conditions, vaso-occlusion does not occur. Carriers have normal life expectancy and many participate successfully in competitive sports or rigorous military training. There is exercise-related mortality from rhabdomyolysis, which can be minimized by avoiding

heat stress, dehydration, and sleep deprivation. Current controversy exists whether to implement universal sickle cell screening among competitive collegiate sports. However, most medical societies (including hematologists and pediatricians) agree that proper education, efforts to prevent dehydration, and vigilant monitoring for rhabdomyolysis as currently employed by the U.S. military services, are better than universal screening of athletes to prevent morbidity and mortality in trait carriers.

Compared to the general population, persons with SCT have normal risk of developing heart disease, stroke, leg ulcers, or arthritis. They are not more likely to develop complications from anesthetic agents. However, SCT is associated with increased risk for traumatic eye injury, hyposthenuria, hematuria, splenic infarction, pulmonary embolism, and proteinuria.²⁶ If traumatic eye injury occurs with hemorrhage into anterior chamber (hyphema), erythrocytes may clog the trabecular outflow channels, increase intraocular pressure, and lead to acute glaucoma, requiring urgent evaluation and treatment. Pregnant women with SCT are at an increased risk for urinary tract infections.

CLINICAL SYNDROMES AND TREATMENT OF THALASSEMIAS

Although the thalassemic syndromes are highly variable, severity is directly related to the imbalance of α -to β -chain ratio: higher the imbalance, more severe the phenotype where excess unpaired α or β chains precipitate in erythrocyte precursors, resulting in their early death and ineffective erythropoiesis. Excess unpaired α or β chains also denature intracellular hemoglobin, promoting splenic sequestration and hemolysis, and eventual splenomegaly and anemia. In β -thalassemia major, any genetic conditions that reduce α -chain excess (co-inheritance of α -thalassemia, or increase in δ -or γ -chain production) or preserve some β -chain synthesis (a mild or silent β -thalassemic allele) ameliorate the severity of β -thalassemia.

Without regular red cell transfusions, chronic hemolytic anemia and tissue hypoxia stimulate bone marrow expansion, and produce skeletal and metabolic derangements: bony deformities, fractures, extramedullary hematopoiesis, and increased gastrointestinal iron absorption. In addition, red cell membrane damage, activation of platelets and endothelium, and abnormal levels of coagulation inhibitors (antithrombin III, protein C and S) all contribute to an increased risk of thromboembolism.

Other complications of thalassemia include leg ulcers, gallstones, and folate deficiency. There are also rare forms of α -thalassemia with mental retardation and developmental abnormalities (mutations in the ATRX gene), and β -thalassemia minor with thrombocytopenia (mutation in GATA1).

Transfusions and Splenomegaly

Transfusions and iron chelation are the mainstay of therapy, and have improved the quality of life and extended life expectancy in thalassemia individuals. After the diagnosis in infancy or childhood, the decision to start transfusion depends on the degree of impact anemia has on the child: fatigue, reduced growth velocity, skeletal dysmorphism, poor weight gain, or organomegaly. Once started, the target hemoglobin of 9 to 10 g/ dL is reasonable, although others have used a higher target. Transfusions are maintained every 2 to 4 weeks and continued through adulthood. Improvements in the clinical signs and symptoms can be seen in adequately transfused individuals. Red cell alloimmunization can be minimized as in SCD by typing for major and minor blood group antigens (ABO, Rh, Kell, Kidd, and Duffy) and prestorage leukocyte depletion.

Splenomegaly can be seen in those with inadequate transfusions or red cell alloimmunization, and is associated with worsening anemia, leukopenia, and thrombocytopenia. Hemoglobin will typically drop about 1.5 g/ dL/ week in non-splenectomized individuals; in hypersplenism, this rate of decline will be higher and eventually there will be an inadequate rise in posttransfusion hemoglobin. Splenectomy will improve these hematologic parameters and transfusion effectiveness, but should be performed after vaccination for encapsulated organisms (*S. pneumoniae*, *H. influenza*, and *Neisseria meningitidis*), and in children older than 5 years. Postsplenectomy transfusions should produce a 1 g/ dL/ week decrease in hemoglobin. Penicillin prophylaxis is appropriate. Postsplenectomy thrombocytosis can be variable, but generally does not require antiplatelet therapy.

Iron Overload and Chelation

Chronic transfusions and early iron chelation have shifted the major thalassemic complications from those secondary to the hemolytic anemia to the sequelae of iron overload. Iron chelation should start when ferritin is approaching 1,000 ng/ L, approaching 20 units of red cells, or within 18 months from the start of chronic red cell transfusion. Excess iron from cumulative transfusions overwhelms the transferrin system and accumulates in the liver, heart, and various endocrine organs. The most serious result is nonuniform iron deposition in cardiac myocytes that eventually leads to heart failure and sudden unpredictable arrhythmia, which previously accounted for the majority of deaths in thalassemic individuals. MRI with T2* sequences is quickly becoming the standard method of cardiac evaluation: 5 to 10 ms is seen in severe overload, 10 to 20 ms in moderate overload, and >20 ms in normal nonoverload states.²⁷ Excess iron also accumulates in the liver, producing hepatic inflammation, dysfunction, and fibrosis. Furthermore, iron overload also affects endocrine organs and can cause reduced growth velocity in children, hypothyroidism, hypogonadism with pubertal delay or arrest, hypoparathyroidism leading to hypocalcemia and osteoporosis,

and diabetes.

The toxic effects of excess iron can be minimized by long-term maintenance of iron chelation. Deferoxamine (Desferal) can be administered at as early as 2.5 years of age at 20 to 60 mg/ kg (or 1.5 to 4g per adolescent or adult) per day, delivered by subcutaneous or intravenous injection over 8 to 12 or 24 hours, and typically for at least 5 days a week.²⁸ Twice a day subcutaneous bolus injection may also be efficacious.²⁹ Side effects of deferoxamine are infrequent; they include impaired vision or hearing, motor-sensory neuropathy, changes in renal or pulmonary function, joint pain, metaphyseal dysplasia, or growth retardation. Oral deferasirox (Exjade) should be titrated to a target dose of 30 mg/ kg/ day, and 40 mg/ kg/ day for those with cardiac iron deposition. Side effects include abdominal pain, diarrhea, rash, arthralgia, and mild increases in liver enzymes and serum creatinine. A newer formulation of deferasirox (Jadenu) has less side effects than Exjade and is more convenient to take. The dosing is about two-thirds of Exjade doses. Oral deferiprone has been approved and has side effects of gastrointestinal discomfort, joint pain, and agranulocytosis. These three chelators individually have been shown to reduce iron burden in the liver or heart; many hematologists prefer combination therapy for those with severe cardiac iron to quickly remove iron.³⁰ Yearly eye and audiology evaluations should be performed prior to iron chelation and while on transfusions and chelation.

Fetal Hemoglobin Induction

The major end point for HbF induction in thalassemia is an increase in total hemoglobin. Unfortunately, HU has not been able to achieve this goal in most thalassemic major patients receiving chronic transfusions, possibly due to a loss of HbF response with transfusion or to certain mutations that are resistant to HbF induction. HU, however, has had some effect in Hb Lepore/ β-thalassemia, Hb E/ β-thalassemia, and β-thalassemia intermedia. Erythropoietin can also be used with HU but the response is variable. Other inducers of HbF (such as decitabine or HDAC inhibitors) can be considered.

THERAPY WITH CURATIVE INTENT

Hematopoietic Stem Cell Transplantation

Myeloablative hematopoietic stem cell transplantation (HSCT) is currently the only cure for SCD and thalassemia,^{31,32} with the best outcome from HLA-matched sibling donors. In SCD, HSCT is typically recommended for patients younger than 17 years, nonresponsive to HU, or had prior SCD-related organ damage (e.g., stroke, ACS, frequent pain crises, and multiple sites of osteonecrosis). For thalassemia, HSCT is also typically recommended for those younger than 17 years with signs of liver

dysfunction or fibrosis from iron damage (Pesaro class II or III). The disease-free survival can be as high as 90% to 95% with 10% risk of graft versus host disease. Often the GvHD is easily treated and most of the children can gradually discontinue immunosuppression. Posttransplant they enjoy improved quality of life and growth velocity. The preexisting organ damage from the underlying disease and the long-term effects from HSCT (small increase in secondary cancer, reduction in gonadal hormones/sterility, changes in thyroid function) are closely monitored periodically.

Recent encouraging clinical data showed that nonmyeloablative HSCT can achieve mixed donor and host hematopoiesis with successes approaching those of myeloablative HSCT.³³ This nonablative approach, with less toxicity from conditioning regimen, is a reasonable alternative for young or older adults who otherwise meet criteria for a standard myeloablative HSCT or for those with severe organ dysfunction. For patients without matched sibling donors, umbilical cord blood transplantation can be considered for pediatric patients. Approaches using matched unrelated donors for Caucasians and haploidentical donors for African Americans are currently being tested and optimized.

Gene Therapy

Autologous transplantation following the insertion of a normal or therapeutic globin gene into hematopoietic stem cells is continually being refined for SCD and thalassemia.³⁴ Significant advances have been made toward this goal using lentiviral vector based on the HIV. Therapeutic correction of murine models of both β-thalassemia and SCD has been achieved using this approach. There is additional progress in achieving moderate levels of engraftment of genetically modified cells in the nonhuman primate autologous transplant model. Clinical human gene therapy trials have begun and the results of the first reported patient with β-thalassemia/ HbE demonstrate the therapeutic potential of this approach.³⁵ Further progress is clearly necessary as the therapeutic effect in this patient was derived from an equal contribution of endogenous HbF, HbE, and the therapeutic transgene. Suggested health maintenance schedule for SCD and thalassemias is provided in [Table 4.9](#).

Table 4.9 Suggested Health Maintenance Schedule

	Routine Health Maintenance	Supplement	Blood Tests	Special Studies
SCD				
Age 0–2 y	Penicillin prophylaxis Prevnar (13 valent), routine vaccinations	Folate, multivitamin, iron if appropriate	CBC every 3–6 mo	

Age 2–18 y	Penicillin prophylaxis until age 5 Pneumovax (23 valent), routine vaccinations, influenza vaccination yearly Eye exam yearly	Folate; iron if appropriate	CBC every 6 mo, renal and hepatic function and iron studies yearly	TCD and O ₂ saturation every 6–12 mo
Age >18 y	Pneumovax, influenza vaccination yearly; hepatitis A and B vaccination; Eye exam every 1–2 years Audiology exam yearly if on iron chelation	Folate; iron if appropriate	CBC, HbF (if on hydroxyurea) every 3–6 mo Screen for proteinuria and iron overload. Renal and hepatic function every 6 mo	When clinically indicated: abdominal ultrasound for biliary sludge and kidney changes, echo, and PFTs

Thalassemia

Age 0–2 y	Routine vaccinations Possible iron chelation Growth velocity curve	Folate, multivitamin	CBC every 3–6 mo	
Age 2–18 y	Routine vaccinations; vaccinations for possible splenectomy in those >5 y; Possible iron chelation; Growth velocity curve	Folate, multivitamin	CBC, hepatic, renal functions, iron studies every 3–6 mo	Evaluate for iron overload Endocrine, eye, and audiology evaluation yearly
Age >18 y	Vaccinations for possible splenectomy	Folate, multivitamin	CBC, hepatic, renal functions (including evaluation for proteinuria), iron studies every 6–12 mo	Evaluate for iron overload Endocrine, eye, audiology, follow-up yearly DXA

CBC, complete blood count; DXA, bone density x-ray absorptiometry; PFTs, pulmonary function tests; SC, heterozygous hemoglobin S and C; SCD, sickle cell disease; TCD, transcranial Doppler.

References

1. Gong L, Maiteki-Sebuguzi C, Rosenthal PJ, et al. Evidence for both innate and

- acquired mechanisms of protection from *Plasmodium falciparum* in children with sickle cell trait. *Blood*. 2012;119(16):3808–3814.
2. Ataga KI, Cappellini MD, Rachmilewitz EA. Beta-thalassaemia and sickle cell anaemia as paradigms of hypercoagulability. *Br J Haematol*. 2007;139:3–13.
 3. Gaston MH, Verter JI, Woods G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. *N Engl J Med*. 1986;314:1593–1599.
 4. Smith-Whitley K, Zhao H, Hodinka RL, et al. Epidemiology of human parvovirus B19 in children with sickle cell disease. *Blood*. 2004;103:422–427.
 5. Adams RJ, McKie VC, Hsu L, et al. Prevention of a First stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial doppler ultrasonography. *N Engl J Med*. 1998;339:5–11.
 6. Adams RJ, Brambilla D. Discontinuing prophylactic transfusions used to prevent stroke in sickle cell disease. *N Engl J Med*. 2005;353:2769–2778.
 7. Ware RE, Helms RW. Stroke with transfusions changing to hydroxyurea (SWiTCH). *Blood*. 2012;119(17):3925–3932.
 8. DeBaun MR, Casella JF. Transfusions for silent cerebral infarcts in sickle cell anemia. *New Engl J Med*. 2014;371:1841–1842.
 9. The Management of Sickle Cell Disease, 4th ed.: National Institutes of Health: National Heart, Lung, and Blood Institute. 2002, NHLBI NIH pub 02-2117.
 10. Platt OS. The acute chest syndrome of sickle cell disease. *N Engl J Med*. 2000; 342:1904–1907.
 11. Vichinsky EP, Neumayr LD, Earles AN, et al. Causes and outcomes of the acute chest syndrome in sickle cell disease. *N Engl J Med*. 2000;342:1855–1865.
 12. Parent F, Bachir D, Inamo J, et al. A hemodynamic study of pulmonary hypertension in sickle cell disease. *N Engl J Med*. 2011; 365:44–53.
 13. Mantadakis E, Ewalt DH, Cavender JD, et al. Outpatient penile aspiration and epinephrine irrigation for young patients with sickle cell anemia and prolonged priapism. *Blood*. 2000; 95:78–82.
 14. Mukisi-Mukaza M, Manicom O, Alexis C, et al. Treatment of sickle cell disease's hip necrosis by core decompression: a prospective case-control study. *Orthop Traumatol Surg Res*. 2009; 95:498–504.
 15. Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood*. 2006;107:2279–2285.
 16. Charache S, Terrin ML, Moore RD, et al. Effect of Hydroxyurea on the frequency of painful crises in sickle cell anemia. *N Engl J Med*. 1995;332:1317–1322.
 17. Vichinsky EP, Haberkern CM, Neumayr L, et al. A comparison of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. The Preoperative Transfusion in Sickle Cell Disease Study Group. *N*

- Engl J Med. 1995;333(4):206–213.
- 18. Koshy M, Burd L, Wallace D, et al. Prophylactic red-cell transfusions in pregnant patients with sickle cell disease. A randomized cooperative study. N Engl J Med. 1988;319:1447–1452.
 - 19. Miller ST, Wright E, Abboud M, et al. Impact of chronic transfusion on incidence of pain and acute chest syndrome during the Stroke Prevention Trial (STOP) in sickle-cell anemia. J Pediatr. 2001;139:785–789.
 - 20. Neumayr L, Koshy M, Haberkern C, et al. Surgery in patients with hemoglobin SC disease. Preoperative Transfusion in Sickle Cell Disease Study Group. Am J Hematol. 1998;57:101–108.
 - 21. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet. 2011;377:1663–1672.
 - 22. Fitzhugh CD, Hsieh MM, Allen D, et al. Hydroxyurea-increased fetal hemoglobin is associated with less organ damage and longer survival in adults with sickle cell anemia. PloS One. 2015;10:e0141706.
 - 23. Ballas SK, McCarthy WF, Guo N, et al. Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. J Natl Med Assoc. 2009;101:1046–1051.
 - 24. Villers MS, Jamison MG, De Castro LM, et al. Morbidity associated with sickle cell disease in pregnancy. Am J Obstet Gynecol. 2008;199:125 e121–125.
 - 25. Yu CK, Stasiowska E, Stephens A, et al. Outcome of pregnancy in sickle cell disease patients attending a combined obstetric and haematology clinic. J Obstet Gynaecol. 2009;29:512–516.
 - 26. Key NS, Derebail VK. Sickle-cell trait: novel clinical significance. Hematology Am Soc Hematol Educ Program. 2010: 418–422.
 - 27. Pennell DJ, Porter JB, Cappellini MD, et al. Continued improvement in myocardial T2* over two years of deferasirox therapy in beta-thalassemia major patients with cardiac iron overload. Haematologica. 2011;96:48–54.
 - 28. Davis BA, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. Blood. 2000;95:1229–1236.
 - 29. Franchini M, Gandini G, de Gironcoli M, et al. Safety and efficacy of subcutaneous bolus injection of deferoxamine in adult patients with iron overload. Blood. 2000; 95:2776–2779.
 - 30. Aydinok Y, Kattamis A, Cappellini MD, et al. Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. Blood. 2015;125:3868–3877.
 - 31. Angelucci E. Hematopoietic stem cell transplantation in thalassemia. Hematology Am Soc Hematol Educ Program. 2010;2010:456–462.

32. Hsieh MM, Fitzhugh CD, Tisdale JF. Allogeneic hematopoietic stem cell transplantation for sickle cell disease: the time is now. *Blood*. 2011; 118: 1197–1207.
33. Hsieh MM, Fitzhugh CD, Weitzel RP, et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. *JAMA*. 2014;312:48–56.
34. Arumugam P, Malik P. Genetic therapy for beta-thalassemia: from the bench to the bedside. *Hematology Am Soc Hematol Educ Program*. 2010;2010:445–450.
35. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature*. 2010; 467:318–322.

Porphyrias

Peiman Hematti

The porphyrias are a diverse group of uncommon metabolic disorders caused by inherited deficiencies of the enzymes involved in the heme biosynthetic pathway,¹ except for one recently described porphyria syndrome due to a gain-of-function mutation. Mutations in the genes of all these heme-synthesizing enzymes have been identified at the molecular level. An exception to the genetic origin for these disorders is porphyria cutanea tarda (PCT), in which the enzyme deficiency in most cases is acquired. In all these ecogenic disorders, it is the interaction of genetic, physiologic, and environmental factors that causes disease in affected individuals. Each defective enzyme results in a characteristic clinical phenotype of porphyria, although the disease mechanisms are not fully understood. Any patient with a long history of undiagnosed abdominal pain and/ or atypical neuropsychiatric symptoms should have porphyria in his or her differential diagnosis.² Usually, biochemical testing is recommended as the first step because quantifying precursor and porphyrin levels functionally characterize the porphyria. However, after a biochemical diagnosis of porphyria, the responsible mutation should be determined by DNA testing. After the specific mutation has been identified, further DNA analysis can be performed more easily in other family members. Screening of family members is important especially for acute porphyrias, as carriers of the mutation should be informed to avoid certain medications and other environmental factors that are potentially porphyrogenic. Roles of the hematologist in the diagnosis and/ or management of porphyria disorders include supervising phlebotomies in PCT to decrease hepatic iron overload and ameliorate skin lesions; diagnosis of erythropoietic porphyrias and their treatment by red blood cell transfusion and, if indicated, by hematopoietic cell transplantation; and management of acute attacks of hepatic porphyrias by infusion of heme.

EPIDEMIOLOGY

PCT is the most prevalent of the porphyrias, both genetic and acquired combined, but acute intermittent porphyria (AIP) is the most common of the genetic porphyrias. AIP has an estimated incidence of 5 in 100,000 in the United States and northern European countries. Approximately 90% of patients with this inherited enzyme deficiency remain symptom free throughout their lives. In contrast, only several cases of δ-aminolevulinic acid dehydratase (ALAD) deficiency have been thus far reported.

PATHOPHYSIOLOGY

Heme is a complex of an iron atom and protoporphyrin IX. It is produced in a multistep biosynthetic pathway that functions mostly in the erythroid bone marrow and hepatocytes. Approximately 85% of the heme produced in the body is synthesized in erythroid cells to provide for hemoglobin formation; most of the remainder is produced in the liver to provide heme for cytochrome P-450 and other enzymes. Eight enzymes are involved in a tightly regulated biosynthetic pathway that sequentially converts glycine and succinyl CoA into heme ([Fig. 5.1](#)). In eukaryotic cells, the first and the last three steps of this pathway localize in mitochondria, while the others are cytoplasmic. Sequences of the genes for all these enzymes and their molecular defects have been well characterized.

The first enzyme active in the pathway, δ-aminolevulinic acid synthase (ALAS), is encoded by two genes: ALAS1, which is expressed ubiquitously in all cells, and ALAS2, which is expressed only in erythroid cells. To date, no mutation has been identified in ALAS1, and until very recently all reported pathogenic mutations of ALAS2 were loss of function and resulted in recessive X-linked sideroblastic anemia, the only non-porphyria syndrome due to abnormalities in the heme biosynthetic pathway. Recently, gain-of-function mutations in the ALAS2 gene were found in eight families, causing X-linked dominant protoporphyria (XLDPP). In general, mutations of these enzymes result in porphyria syndromes because of overproduction of metabolic precursors and intermediates and/ or their accumulation in tissues. All of these intermediate products are potentially toxic, and their overproduction causes the neurovisceral and/ or photocutaneous symptoms characteristic of porphyria syndromes.

Despite the characterization of these disorders genetically and molecularly, the exact pathophysiologic mechanisms responsible for specific organ manifestations are not fully understood.³ Porphyrias are heterogeneous, with numerous mutations found for each gene. There is a significant interaction between specific inherited genetic defects and acquired or environmental factors that result in a spectrum of clinical manifestations in affected patients. Patients with gene mutations for the acute hepatic forms of porphyrias may remain asymptomatic unless exposed to certain medications ([Table 5.1](#)) or hormones, or they are stressed by starvation, infection, surgery, or other intercurrent

disorders. Under these environmental circumstances, affected patients develop characteristic neurologic disturbances. Photocutaneous hypersensitivity and skin damage occurs after exposure to ultraviolet light. When porphyrins absorb light of this wavelength, they produce free radicals that can induce oxidant tissue damage. Consequently, avoidance of precipitating factors is key in the therapy of porphyrias.⁴

CLASSIFICATION AND CLINICAL MANIFESTATIONS

For clinical purposes, porphyrias can be classified into hepatic and erythropoietic types depending on the major tissue site of production and accumulation of the heme precursors. The major manifestations of the hepatic porphyrias are neurovisceral symptoms, including abdominal pain, neurologic symptoms, and psychiatric disorders, whereas the erythropoietic porphyrias usually present primarily with cutaneous photosensitivity and hemolytic anemia. Porphyrias can be also classified according to their clinical presentations into (1) acute porphyrias presenting with life-threatening neurovisceral manifestations and (2) nonacute (or cutaneous) porphyrias characterized by photosensitivity syndromes, but there can be some overlap in clinical manifestations. However, because the porphyrias are well characterized at the molecular genetic level, they are better specifically classified by their unique enzyme deficiencies.^{5,6}

DIAGNOSIS

Many symptoms of the porphyrias are nonspecific, and diagnosis requires a high index of suspicion. However, although the porphyrias are often suspected in a patient with vague and unexplained complaints, actual diagnosis is rare. A useful first step is to determine which one of the three major manifestations of the porphyrias—neurovisceral symptoms, photosensitivity, or hemolytic anemia—is present.^{7,8}

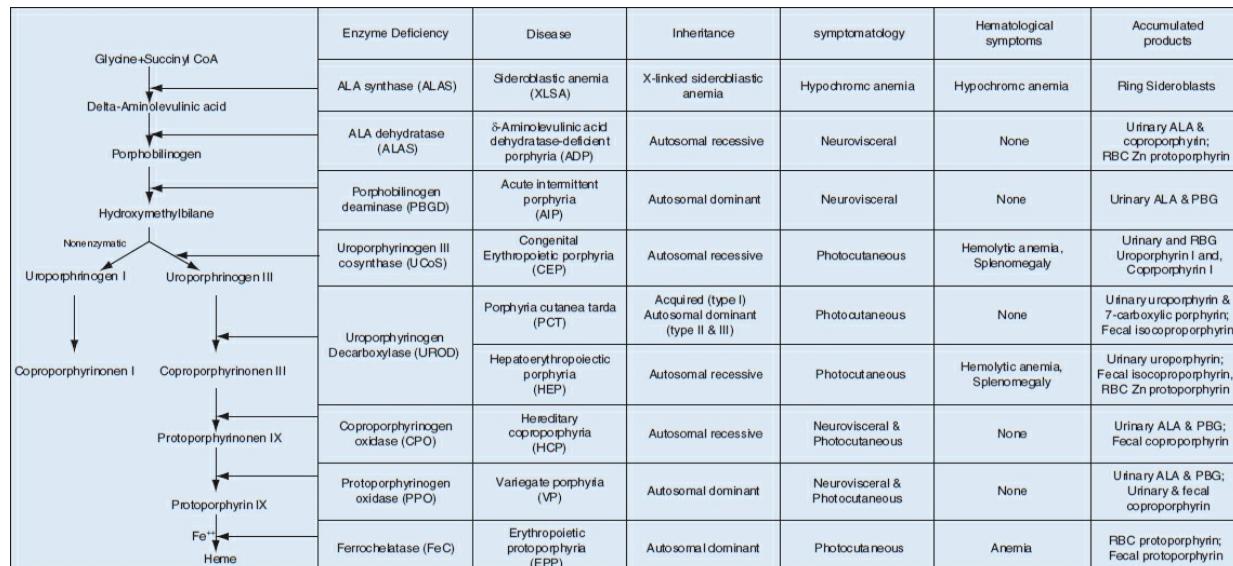


FIGURE 5.1 Classification of porphyrias based on their corresponding enzymatic deficiencies, mode of inheritance, major symptoms, and biochemical abnormalities.

ALA, δ-aminolevulinic acid; RBC, red blood cell; PBG, porphobilinogen; PCT, porphyria cutanea tarda.

Table 5.1 Drugs Considered Unsafe or Safe for Acute Porphyrias

Unsafe Drugs	Safe Drugs
Alcohol	Acetaminophen
Barbiturates	Allopurinol
Calcium channel blockers	Aspirin
Carbamazepine	Atropine
Danazol	Cimetidine
Diclofenac	Corticosteroids
Erythromycin	Warfarin
Metoclopramide	Gabapentin
Isoniazid	Gentamycin
Phenytoin	Insulin
Progesterone	Narcotic analgesics
Rifampin	Penicillin and derivatives
Sulfonamide antibiotics	Phenothiazines
Valproic acid	Propanalol

This list is not comprehensive and does not reflect all information and opinions. Please refer to available texts and websites for a more extensive list of drugs and their updated status for use in porphyrias.

- | Neurovisceral symptoms are present in ALAD deficiency porphyria (ADP), AIP, hereditary coproporphyria (HCP), and variegate porphyria (VP).
- | Photosensitivity is present in congenital erythropoietic porphyria (CEP), PCT, hepatoerythropoietic porphyria (HEP), HCP, VP, erythropoietic protoporphyrina (EPP), and XLDPP.
- | Neurovisceral symptoms and photosensitivity are present in HCP and VP.
- | Hemolytic anemia is present in CEP, HEP, and EPP.

Laboratory testing is then required to confirm or exclude the various types of porphyrias. The diagnosis is made initially by detection of the metabolite(s) produced and/ or excreted in excess in red blood cells, plasma, urine, and/ or feces.⁷ Porphyrin precursors in urine and total porphyrins in plasma are the initial diagnostic tests for acute and cutaneous porphyrias, respectively. Today the diagnosis of many of the

porphyrias can be confirmed by measuring the enzymatic activity in the appropriate tissue directly or by specific molecular genetic testing. Family screening is advisable to prevent acute attacks in presymptomatic stages, with DNA analysis for the identification of the mutations now standard.

SPECIFIC TYPES OF PORPHYRIAS

X-Linked Dominant Protoporphyria

This most recently described syndrome is an extremely rare and the only type of porphyria that is not caused by enzyme deficiency but by gain-of-function deletions in ALAS2.⁹ ALAS2 gain of function leads to excessive production of protoporphyrin in red blood cells and leads to a clinical presentation similar to EPP (hence also known as EPP variant form). While affected males often develop a severe form of the disease females could remain asymptomatic or can develop a severe form of the disease. Photosensitivity is the characteristic finding, but many of these patients developed liver disease, which range from mild liver abnormalities to overt liver failure, and some had mild anemia. Supportive and preventive interventions are similar as those for EPP.

δ -Aminolevulinic Acid Dehydratase Deficiency Porphyria

ADP is an autosomal-recessive porphyria caused by markedly deficient activity of ALA dehydratase, the second enzyme in the heme biosynthetic pathway. The diagnosis has been unequivocally confirmed only in a few cases. Clinical manifestations are primarily neurovisceral and their treatment and prevention are as for other acute porphyrias. Lead poisoning should be excluded, because it also diminishes the activity of ALA dehydratase, may present as a clinical phenocopy, and is far more common.

Acute Intermittent Porphyria

AIP is inherited as an autosomal dominant condition resulting from a partial deficiency of porphobilinogen deaminase (PBGD) activity, the third enzyme of the pathway. Approximately 90% of heterozygotes remain biochemically normal and clinically asymptomatic throughout their lives. Clinical expression of the disease is usually the result of exposure to factors such as endogenous and exogenous corticosteroid hormones, a low-calorie diet, certain drugs (barbiturates and sulfonamide antibiotics are the most commonly implicated), alcohol ingestion, and stresses such as intercurrent illnesses, infection, and surgery. Symptoms usually develop after puberty and are more frequent in women. The pathophysiologic hallmark of the disease is neurologic dysfunction affecting peripheral, autonomic, and/or central nervous systems occurring as intermittent acute attacks. The most common symptom is acute abdominal pain (in 90% of cases), which may be generalized or localized, but tenderness, fever, and

leukocytosis are absent because the symptoms are neurologic in origin, from the visceral autonomic nervous system involvement. Gastrointestinal manifestations also include abdominal distention, nausea, vomiting, diarrhea, or constipation. Peripheral sensory or motor neuropathy is another common feature of AIP. Psychiatric symptoms, including hysteria, anxiety, apathy, depression, phobia, psychosis, agitation, disorientation, hallucinations, and schizophrenic-type behaviors, can be the only manifestations of the disease. Acute attacks may be accompanied by seizures, either a manifestation of the porphyria itself or caused by hyponatremia (from inappropriate secretion of antidiuretic hormone), which also commonly occur during attacks. Sympathetic hyperactivity results in tachycardia (in 80% of cases), hypertension, tremors, and sweating. Because of the nonspecific nature of symptoms and signs, the use of highly sensitive and specific laboratory tests is essential to the diagnosis.

During acute attacks, symptomatic treatment may include narcotic analgesics, phenothiazines, low-dose benzodiazepines, and propranolol for hypertension and tachycardia. Although intravenous glucose (at least 300 g/day) can be effective in acute attacks of porphyria, intravenous heme is now considered the treatment of choice to reduce the excretion of porphyrins. Infusion of heme should be initiated as soon as possible after the onset of an attack, but the rate of recovery depends on the degree of neuronal damage and may take days to months. Human heme solution stabilized with arginine (Normosang) is widely available except in the United States, where the lyophilized form (Panhematin) is Food and Drug Administration (FDA) approved.⁶ Any intercurrent infection or disease should also be treated immediately. Identification and avoidance of precipitating factors are also essential for prevention. Cyclical attacks in some women are associated with fluctuations in estrogen and progestins and can be prevented by administering a long-acting gonadotropin-releasing hormone analogue.

Congenital Erythropoietic Porphyria

CEP, an autosomal-recessive disorder also known as Gunther's disease, is caused by the deficient activity of uroporphyrinogen III cosynthase (the fourth enzyme of the pathway) and is associated with hemolytic anemia and cutaneous lesions. Severe cutaneous photosensitivity usually begins in early infancy as blistering of sun-exposed areas of the skin. Recurrent vesicles, bullae, and secondary infection can lead to cutaneous scarring and deformities. Porphyrin deposition may also occur in bones, leading to a brownish discoloration of teeth. Protecting the skin from sunlight is essential.

Mild to severe hemolytic anemia and secondary splenomegaly are features of CEP, and anemia can be severe. Transfusion is effective but results in iron overload if chronic. Splenectomy may reduce hemolysis and decrease the transfusion requirement.

In transfusion-dependent children, allogeneic hematopoietic stem cell transplantation can be considered.

Porphyria Cutanea Tarda

PCT, the most common of the porphyrias, is caused by an acquired or inherited deficiency of uroporphyrinogen decarboxylase (the fifth enzyme of the pathway). The disease occurs worldwide but its exact incidence is not known. The disease can be sporadic (noninherited or type I, most common) or familial (types II and III), although these subtypes are not distinguishable clinically. The frequency of disease varies in relation to risk factors such as alcohol use, smoking, and hepatitis C and HIV infection. The hallmark of PCT is cutaneous photosensitivity presenting as chronic blistering lesions on sun-exposed areas of skin without neurologic manifestations.⁸ Chronic changes, including cutaneous thickening, scarring, and calcification can mimic systemic sclerosis. Facial hypertrichosis and hyperpigmentation are also common. PCT is almost always associated with abnormalities in liver function tests, and the risk of developing hepatocellular carcinoma is significantly increased in this disease.

Alcohol ingestion; estrogens; iron supplements and, if possible, any other drugs that may exacerbate the disease; and sun exposure should be avoided. A complete response can usually be achieved by repeated phlebotomy to reduce hepatic iron and is still considered standard treatment. Low-dose chloroquine or hydroxychloroquine are also effective, especially when phlebotomy is not indicated. Chloroquine slowly mobilizes the porphyrins from the liver and increases their excretion into the urine. In contrast, similar skin lesions in VP, HCP, CEP, and HEP are unresponsive to these therapeutic interventions.

Hepatoerythropoietic Porphyria

This rare form of porphyria has been recently described. HEP is clinically indistinguishable from CEP and is caused by homozygous or compound heterozygous defects of the same enzyme involved in PCT. Patients usually present after birth with dark urine in the diapers followed by severe photosensitivity with blistering skin lesions and scleroderma-like scarring. Hemolytic anemia is often present with splenomegaly. The avoidance of sunlight is essential.

Hereditary Coproporphyria

HCP is an autosomal dominant porphyria resulting from the deficiency of coproporphyrinogen oxidase (the sixth enzyme of the pathway). The neurovisceral symptoms and other manifestations as well as the precipitating factors are virtually identical to those of AIP but photosensitivity similar to PCT may also occur in one-third

of the patients. Avoidance of precipitating factors as in AIP is important. Neurologic symptoms are treated as in AIP but in contrast to PCT, phlebotomy or chloroquine is not effective for cutaneous lesions.

Variegate Porphyria

This hepatic porphyria, the result of a mutation of the protoporphyrin oxidase gene (the seventh enzyme in the pathway), is transmitted as an autosomal dominant disorder and is particularly common in South African whites (prevalence of 3 in 1,000) because of a genetic founder effect from a couple who emigrated from Holland to South Africa in the late 1600s. The disease was termed variegate because it can present with either neurovisceral symptoms, cutaneous photosensitivity, or both. Neurovisceral symptoms are very similar to those of AIP and are provoked by the same precipitates. Acute attacks are treated with glucose and heme infusion as in AIP. Occurrence of skin manifestations is usually separate from the neurovisceral symptoms, and avoiding sun exposure is the only effective preventative measure for cutaneous photosensitivity.

Erythropoietic Protoporphyria

EPP, also known as protoporphyrin or EPP-classic form, results from the deficiency of ferrochelatase activity, the last enzyme in the heme biosynthetic pathway. EPP is the most common erythropoietic porphyria and the third most common porphyria in general. Skin photosensitivity beginning in childhood is typical of the disease but the skin lesions are different from other porphyrias. Erythema, burning, and itching accompanied by swelling can develop within minutes of sun exposure, but sparse vesicles and bullae are seen in only a minority of the cases. Chronic skin changes may occur but severe scarring is rare. Treatment involves avoidance of sun exposure and the use of topical sun screens. Oral β -carotene (120 to 180 mg/ day) can be effective in many patients with EPP, in contrast to those with photosensitivity from other forms of porphyria. The mechanism of action of β -carotene is not clear but is attributed to its antioxidant effect. A recent multicenter, randomized, double-blind placebo control trial of an alpha-melanocyte stimulating hormone analog, afamelanotide, showed increased tolerance to sunlight exposure and improved quality of life in patients with EPP.¹⁰ In some patients, accumulation of protoporphyrin causes chronic liver disease that can progress to hepatic failure and death. Neurovisceral symptoms are seen only in patients with severe hepatic complications. Protoporphyrin-rich gallstones may occur. Mild anemia is sometimes seen in patients with EPP, but hemolysis is either infrequent or very mild. Splenectomy may be helpful when the disease is accompanied by hemolysis and significant splenomegaly. Caloric restriction, drugs, and exogenous sex hormones should be avoided. Intravenous heme therapy is sometimes beneficial. Liver

transplantation has been performed but the protoporphyrin-induced damage can recur in the donor liver.¹¹

References

1. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. *Blood*. 2012;120(23):4496–4504.
2. Sassa S. Modern diagnosis and management of the porphyrias. *Br J Haematol*. 2006;135(3):281–292.
3. Foran SE, Abel G. Guide to porphyrias. A historical and clinical perspective. *Am J Clin Pathol*. 2003;(119 suppl):S86–S93.
4. Dombeck TA, Satonik RC. The porphyrias. *Emerg Med Clin North Am*. 2005;23(3):885–899.
5. Kauppinen R. Porphyrias. *Lancet*. 2005;365(9455):241–252.
6. Puy H, Gouya L, Deybach JC. Porphyrias. *Lancet*. 2010;375(9718):924–937.
7. Anderson KE, Bloomer JR, Bonkovsky HL, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. *Ann Intern Med*. 2005;142(6):439–450.
8. Poblete-Gutierrez P, Wiederholt T, Merk HF, et al. The porphyrias: clinical presentation, diagnosis and treatment. *Eur J Dermatol*. 2006;16(3): 230–240.
9. Whatley SD, Ducamp S, Gouya L, et al. C-terminal deletions in the ALAS2 gene lead to gain of function and cause X-linked dominant protoporphyrria without anemia or iron overload. *Am J Hum Genet*. 2008;83(3):408–414.
10. Langendonk JG, Balwani M, Anderson KE, et al. Afamelanotide for Erythropoietic Protoporphyrria. *N Engl J Med*. 2015;373(1):48–59.
11. Singal AK, Parker C, Bowden C, et al. Hepatology. Liver transplantation in the management of porphyria. *2014;60(3):1082–1089*.

Bone Marrow Failure Syndromes: Aplastic Anemia, Acquired and Constitutional; Paroxysmal Nocturnal Hemoglobinuria; Pure Red Blood Cell Aplasia; and Agranulocytosis

**Phillip Scheinberg, Neal S. Young, and
Johnson M. Liu**

ACQUIRED BONE MARROW FAILURE SYNDROMES

The bone marrow (BM) failure syndromes are characterized by inadequate blood cell production leading to low red blood cell, white blood cell, and/ or platelet counts in the peripheral blood. Marrow failure can be acquired or constitutional and may affect all three blood cell lines, resulting in pancytopenia, or only a single lineage. In most cases, the BM shows a simple deficiency of the related precursor cells, but marrow failure can also occur with relatively cellular marrows, presumably due to ineffective hematopoiesis, and could be associated with cytogenetic abnormalities (see [Chapter 7](#)) or a genetically altered cell, as in paroxysmal nocturnal hemoglobinuria (PNH), discussed in this chapter because of its intimate relationship with aplastic anemia (AA). Even the paradigmatic syndrome of AA clinically and pathophysiologically shows overlap with related diseases ([Fig. 6.1](#)).

Constitutional AA (due, e.g., to TERT and TERC mutations in dyskeratosis congenita [DKC]) can present later in life and without classical physical stigmata; generally, adult patients have chronic and moderate blood count depression. Measurement of telomere length (TL) is useful in patients before making treatment decisions: TL when short (less

than first percentile) points to an underlying telomeropathy, and mutations in genes of the telomerase complex (TERC, TERT, DKC1, TINF2, RTEL1) can be established by genomic screening using commercial panels. In telomere disease, accelerated erosion of the ends of the chromosomes at each cell division leads to a syndrome of marrow failure, pulmonary fibrosis, and cirrhosis (early graying of the hair is a useful clue).¹ The clinical phenotype is variable, even within a pedigree, in organs affected and the severity of dysfunction. Germ-line GATA2 gene mutations can also occur in patients with AA, sometimes associated with warts and mycobacterial infections. Markedly reduced monocytes, and B and natural killer (NK) cells on simple blood counts and on flow cytometry are a consequence, and again, mutations can be identified on genomic screens.² GATA2 haploinsufficiency is a spectrum of disorders, which include the monoMAC syndrome (mycobacterial infections and monocytopenia), lymphedema familial myelodysplastic syndrome and acute myeloid leukemia (AML), and chronic neutropenia.² In all constitutional BM failure syndromes, a careful, directed history of the patient and the family is highly desirable and can reveal the diagnosis in the examination room.

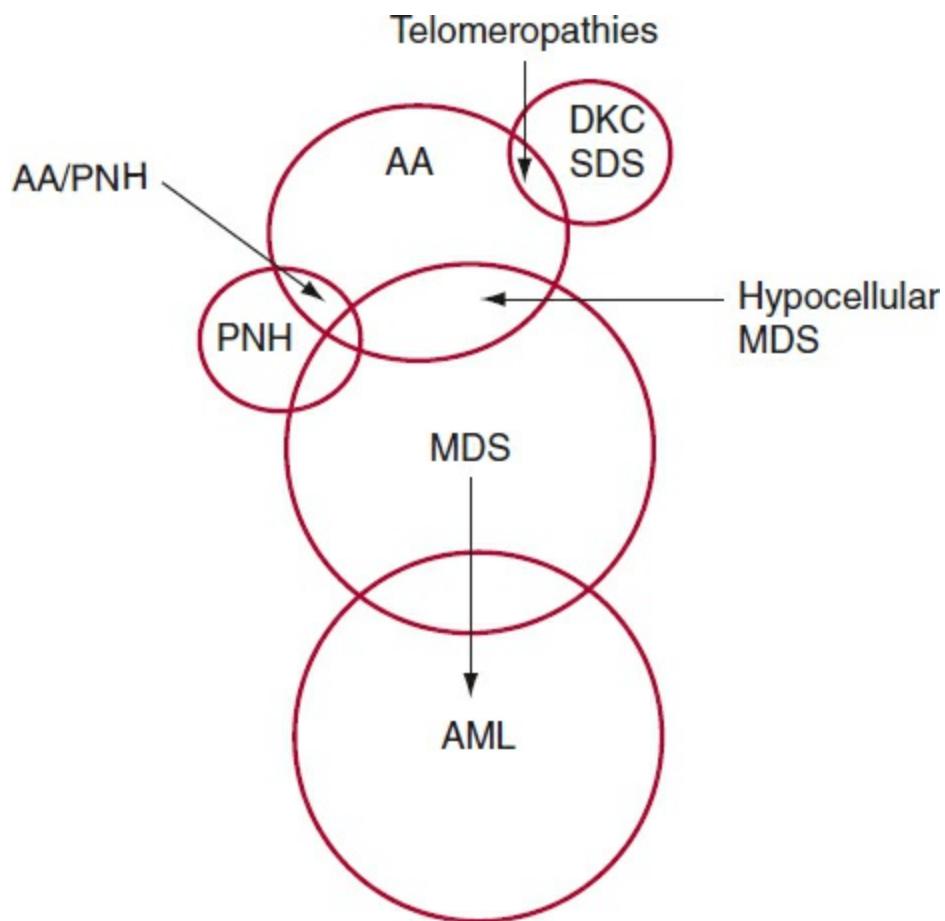


FIGURE 6.1 Venn diagram of the relationship among bone marrow failure syndromes.

AA, aplastic anemia; AML, acute myeloid leukemia; DKC, dyskeratosis congenita; MDS, myelodysplasia; PNH, paroxysmal nocturnal hemoglobinuria; SDS, Shwachman–Diamond syndrome.

ACQUIRED APLASTIC ANEMIA

Acquired AA is characterized by pancytopenia with a hypocellular, often empty, BM. AA is uncommon in the West: Its incidence in Europe is about two new cases per million of population. However, the disease is two-to threefold more frequent in East Asia and probably elsewhere in the developing world. In most series, patients are young, with the majority presenting between 15 and 25 years of age. Historically, chemicals (benzene) and medical drugs (chloramphenicol) were implicated as causative, but without satisfactory mechanisms of pathogenesis. The most important current associations are with nonsteroidal anti-inflammatory drugs, antithyroid drugs, penicillamine, allopurinol, and gold (Table 6.1).³ Nonetheless, most AA is idiopathic, and it is usually not possible to assign an environmental cause in an individual patient. One objective association is with prior seronegative hepatitis, present in 5% to 10% of patients in most case series.

Etiology and Pathophysiology

- Hematopoiesis is severely reduced in all AA, as observed in BM specimens, CD34 cell counts, magnetic resonance imaging, or in colony culture assays of progenitors.
- Clinical and laboratory studies suggest that most acquired AA is secondary to immunologically mediated destruction of hematopoietic cells by cytotoxic lymphocytes (CTLs) and their cytokine products, especially interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α).
- Marrow failure rarely can follow infectious mononucleosis (Epstein–Barr virus [EBV] infection) and is a component of the stereotypical post–hepatitis AA syndrome. EBV and the putative agent of seronegative hepatitis likely behave as triggers for immune system activity. In contrast, parvovirus B19 directly infects and kills erythroid progenitor cells and causes transient red cell aplasia and occasionally chronic pure red cell aplasia (PRCA), but not AA.
- Direct killing of marrow cells by cytotoxic agents occurs following cancer chemotherapy, producing transient marrow aplasia, but is probably unusual as a mechanism of idiosyncratic drug-associated AA.

Table 6.1 Drugs Associated With Aplastic Anemia in the International Aplastic Anemia and Agranulocytosis Study

Allopurinol

Antibiotics

Antithyroid drugs
Butazones
Cardiovascular drugs
Corticosteroids
Diclofenac
Furosemide
Gold
Indomethacin
Nonsteroidal analgesics
Penicillamine
Phenothiazines
Piroxicam
Psychotropic drugs
Sulfonamides^a

^aOther than trimethoprim–sulfonamide combination.

Clinical Features

- | Anemia leads to fatigue, weakness, lassitude, headaches, and in older patients dyspnea and chest pain, and these symptoms are most commonly responsible for the clinical presentation.
- | Thrombocytopenia produces mainly mucosal bleeding: petechiae of the skin and mucous membranes, epistaxis, and gum bleeding are frequent and early complaints. Bleeding is not brisk from low platelets unless in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage.
- | Infection is unusual at presentation.
- | Dark urine suggests PNH.
- | Occasionally, moderate cytopenias are identified serendipitously by routine blood work or at preoperative evaluation.
- | Constitutional symptoms (malaise, anorexia, and weight loss) should be absent.

Physical findings range from a well-appearing patient with minimal findings to an acutely ill patient with signs of systemic toxicity. Cachexia, lymphadenopathy, and splenomegaly are not seen and suggest an alternative diagnosis.

- | Thrombocytopenia results in petechiae, ecchymoses, gingival oozing, epistaxis, and subconjunctival and retinal hemorrhage.
- | Anemia is reflected in pallor of the skin, mucous membranes, and nail beds.
- | Constitutional AA is suggested by areas of hyper- or hypopigmentation of the skin, abnormal hands and thumbs, short stature (Fanconi anemia [FA]), and nail dystrophy

and oral leukoplakia (DKC).

Diagnosis and Differential Diagnosis

At diagnosis:

- Marked pancytopenia or reduction in two of three or, less commonly, one of three cell lines.
- Peripheral blood smear shows reduced platelets and neutrophils, and normal red cells.
- Microspherocytes and giant platelets suggestive of peripheral destruction are not present.
- BM markedly hypocellular on biopsy (1 cm core); mainly residual lymphocytes, plasma cells, and mast cells on an aspirate smear.
- Overall, marrow biopsy cellularity is low (<30%, excluding lymphocytes), but there may be pockets of cellularity, so-called hot-spots.
- Myeloblasts should not be increased.
- Megakaryocytes are almost always absent.
- Marrow cytogenetics should be normal, but some authorities accept cytogenetic abnormalities, such as trisomy 6 or 8, loss of Y or del20q, as consistent with AA in the absence of significant dysplastic marrow findings.

Table 6.2 Differential Diagnosis of Pancytopenia

Pancytopenia With Hypocellular Bone Marrow

- Acquired aplastic anemia
- Inherited aplastic anemia
- Some myelodysplastic syndromes
- Rare aleukemic leukemia
- Some acute lymphoblastic leukemia
- Some lymphomas of the bone marrow

Pancytopenia With Cellular Bone Marrow

- Primary bone marrow diseases
 - Myelodysplasia syndromes
 - Paroxysmal nocturnal hemoglobinuria
 - Myelofibrosis
 - Hairy cell leukemia
 - Some aleukemic leukemia
 - Myelophthysis
 - Bone marrow lymphoma
- Secondary to systemic diseases
 - Systemic lupus erythematosus, Sjögren's syndrome

Hypersplenism
Vitamin B₁₂, folate deficiency (familial defect)
Overwhelming infection
Alcoholism
Brucellosis
Ehrlichiosis
Sarcoidosis
Tuberculosis and atypical mycobacteria

Hypocellular Bone Marrow With or Without Cytopenia

Q fever
Legionnaire's disease
Toxoplasmosis
Mycobacteria
Tuberculosis
Anorexia nervosa, starvation
Hypothyroidism

Table 6.3 Diseases Easily Confused With Aplastic Anemia

Disease	Distinguishing Characteristics	Diagnostic Test
Constitutional ^a		
Fanconi anemia	Younger patients; family history and physical anomalies (short stature, café au lait spots, anomalies of the upper limb or thumb)	Chromosome analysis of stressed blood lymphocyte cultures
Dyskeratosis congenita	Younger patients; family history and physical anomalies (nail changes, leukoplakia)	Short telomeres mutations in TERC, TERT, TINF2, RTEL, DKC1
Acquired		
Myelodysplasia	Older patients, insidious onset, marrow usually normo- or hypercellular	Marrow morphology Marrow cytogenetics
Aleukemic leukemia	Very young or very old patients	Blasts in buffy coat and spicules
PNH	Hemolysis (high LDH, low haptoglobin, hemoglobinuria), venous thrombosis	Deficient GPI-anchored proteins on flow cytometry
Myelofibrosis	Hepatosplenomegaly Leukoerythroblastic blood smear	Fibrosis on marrow biopsy
Large granular lymphocytosis	Older age, insidious, neutropenia	Large granular lymphocytes in peripheral smear Flow cytometry T cell receptor gene rearrangement

^aPhenotypic abnormalities may be subtle or absent.

GPI, glycosylphosphoinositol anchor; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria.

In secondary marrow failure, the degree of pancytopenia is usually moderate, and the underlying illness is usually obvious from history and physical examination (e.g., stigmata of alcohol liver disease, presence of other autoimmune disease or infection). However, pancytopenia has many causes, of which AA is not the most common ([Table 6.2](#)).

It is very important to distinguish among primary marrow diseases ([Table 6.3](#), [Fig. 6.2](#)):

- | Constitutional AA presenting in adults. A family history is highly suggestive. FA pedigrees often have instances of leukemias and myelodysplasia (MDS); telomeropathy families often include not only malignant hematologic diseases but also pulmonary fibrosis and hepatic cirrhosis. There may be no or only subtle physical stigmata. Patients younger than 40 years (or older if the history or examination is suggestive) should be tested for FA. Although phenotypic abnormalities have been classically described in both FA and DKC, patients with adult-onset constitutional AA may have subtle signs on routine physical examination or no characteristic findings at all.¹
- | MDS is hypocellular in about 20% of cases. Dysplastic changes in AA when present are mild and limited to erythrocytes. In MDS, megaloblastic changes are more extreme; megakaryocytes are preserved and can be aberrantly small and mononuclear; and myeloid precursors may be increased, left shifted, and poorly granulated. Chromosomal analysis of BM cells is almost always normal in AA, while MDS is often associated with cytogenetic abnormalities. Nonetheless, the distinction may be so difficult that some patients are best labeled AA/ MDS.
- | PNH/ AA: Small PNH expanded clones are common—in as many as 50% of cases at presentation—in the setting of marrow failure now that flow cytometry has replaced the Ham test. Growth of clone size over time may lead to clinical hemolysis. Thrombosis is rare.
- | Acute lymphocytic leukemia in children and AML in the elderly can occasionally present with pancytopenia and marrow hypocellularity.
- | Myelofibrosis has a characteristic leukoerythroblastic blood picture, marrow is dry tap (rather than watery, as in AA), and hepatosplenomegaly is common.
- | Large granular lymphocytosis is characterized by prolonged neutropenia, less frequently anemia or thrombocytopenia, and increased numbers of large granular lymphocytes in the peripheral blood. Marrow is usually cellular; diagnosis rests on flow cytometry or molecular evidence of rearrangement of the T cell receptor.

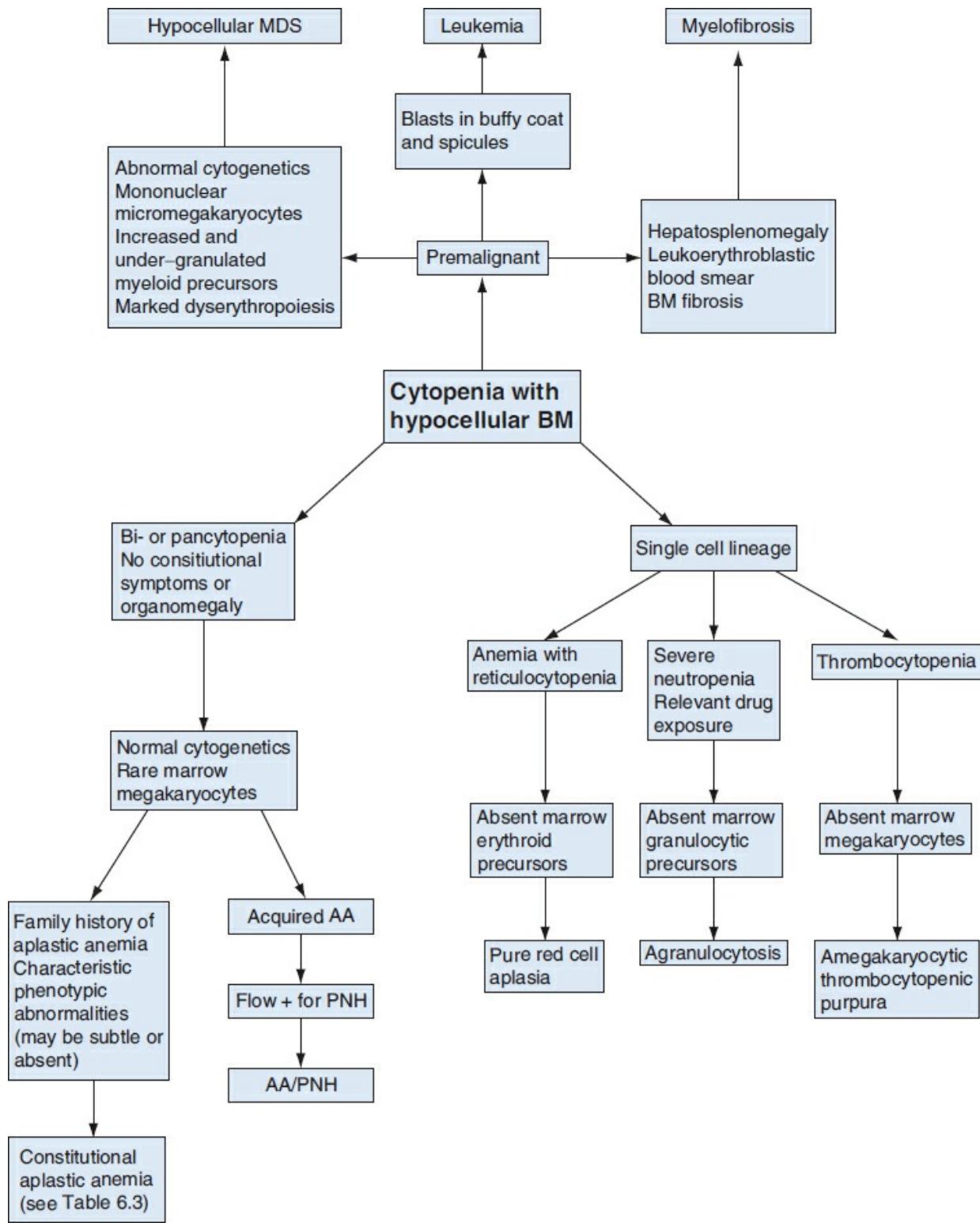


FIGURE 6.2 Differential diagnosis of cytopenias.

BM, bone marrow; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria.

Table 6.4 Bone Marrow Transplantation Versus Immunosuppression

Limitations	Hematopoietic Stem Cell Transplantation	Immunosuppression
Applicability	HLA-matched sibling	All
Cost	High	Moderate
Age limits	Best in children Adults <40 y	All ages
Outcome	65%–90% long-term survival	70% hematologic response
Short-term toxicity	10%–30% die of GVHD, infection, pneumonitis, veno-occlusive hepatic disease, or graft failure	Rare anaphylaxis
Long-term effects	Hematologic cure; modest increase in risk of solid tumors Delayed secondary organ dysfunction (endocrine, bone)	Incomplete cure with possibility of development of relapse and clonal evolution (MDS, AML)

AML, acute myeloid leukemia; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria.

Severe AA is defined by two of the following three peripheral blood count (Camitta) criteria

- | Absolute neutrophil count (ANC) < 500/ μL
- | Platelet count < 20,000/ μL
- | Reticulocyte count (automated) < 60,000/ μL

Definitive Treatments

Definitive therapy of AA consists of allogeneic hematopoietic stem cell transplantation (HSCT) or immunosuppression; both have dramatically changed the natural course of this illness, with a 5-year survival of 80% in patients undergoing either treatment.⁴ Support with growth factors alone or in combination with transfusion is of unproven long-term benefit and is unlikely to address the underlying pathophysiology of the disease. The main distinctions between immunosuppression and HSCT are shown in Table 6.4.

- | HSCT cures AA. Most transplants are performed using a histocompatible sibling donor, and most recipients are young. Overall, long-term survival is about 70% to 80%, with better results observed in children.^{5,6} HSCT is preferred in children till about age 20 who have an appropriate donor. With current cyclophosphamide-based conditioning, major toxicities are related to graft-versus-host disease (GVHD) and infection (not always easily separable). GVHD and mortality risk increase with recipient age (>40 years of age).^{5,7} The source of donor cells may be important: in retrospective studies, granulocyte colony-stimulating factor (G-CSF)-mobilized stem cells produced a higher rate of chronic GVHD and mortality in younger patients than

did BM cells.^{8–10} Modest numbers of erythrocyte and platelet transfusions do not appear to increase the risk of graft rejection, especially with leukocyte-depleted products.¹¹

Seventy percent of patients will lack a suitably matched sibling donor. Transplantation from matched unrelated donors (MUD) is now more feasible with the development of huge donor registries and an effective network. Overall, results have been about half as good as with human leukocyte antigen (HLA)-matched family members, but outcomes are improving with modifications of conditioning regimens and high-resolution molecular typing for donor selection^{12,13}; in more recent studies, outcomes with alternative donor sources approximate standard transplant outcomes especially in younger patients.^{14–16} Donor searches should be initiated early for younger patients who might be eligible later for a MUD in case of failure of immunosuppression. Of note, the success rate for identifying a suitable MUD in the United States decreases in non-Caucasian patients such as in the African American and Hispanic ethnic groups. For unclear reasons, umbilical cord blood transplantation has been associated with poor results in marrow failure states.¹⁷ In current practice, unrelated transplant is offered for children who have failed a single course of immunosuppression and to adults who are refractory to multiple courses of antithymocyte globulin (ATG) and other therapies such as eltrombopag and/ or androgens. Haploididential donor HSCT with cyclophosphamide on days +3 and +4 (as GVHD prophylaxis) has been performed in small case series in eligible patients who lack a histocompatible unrelated donor and remain severely pancytopenic and transfusion dependent after one or more courses of immunosuppression. Preliminary results appear encouraging, however, until more mature data including more patients with longer follow-up is available, this HSCT platform should be considered experimental.¹⁸

Immunosuppression using regimens combining ATG and cyclosporine A (CsA) is standard therapy. About two-third of patients improve to transfusion-independence and, overall, survival rates at 5 years are comparable to HSCT. Immunosuppression is almost always preferred in older patients, especially if the neutrophil count is not severely decreased. One frequently used protocol for horse ATG is 40 mg/ kg/ day for 4 days. Rabbit ATG is administered at 3.5 mg/ kg/ day for 5 days. Corticosteroids, such as methylprednisolone at 1 mg/ kg, are administered during the first 2 weeks to ameliorate serum sickness. In a recent randomized study, hematologic response rate at 6 months was inferior with rabbit ATG (37%) compared to horse ATG (68%) when given as first therapy.¹⁹ This large difference in response translated in worst survival at 3 years after rabbit ATG (70%) as compared to horse ATG (94%).¹⁹ A European prospective study showed similar results.²⁰ Thus, horse ATG is the preferred initial immunosuppressive treatment in severe aplastic anemia (SAA).

In patients who are refractory or relapse after initial horse ATG, hematologic

recovery can be achieved with repeated immunosuppression. A second course of rabbit ATG or alemtuzumab can be effective in refractory patients, with hematologic response rates of about 30% to 40% reported with either regimen.^{21–23} In relapse, alemtuzumab (without cyclosporine) is also active and produces hematologic response in 55% of cases, which is comparable to the reported rates of rabbit ATG in this setting.²² As first therapy, alemtuzumab fared poorly, with response rate of only 19% in a prospective randomized study.²² Thus, a repeat course of immunosuppression with rabbit ATG or alemtuzumab may be an option in relapsed and refractory SAA.

Recently, a non-immunosuppression regimen has emerged as an attractive alternative to immunosuppressants as salvage therapy in patients refractory to an initial course of horse ATG. The thrombopoietin receptor agonist eltrombopag showed activity as single agent in horse ATG-refractory patients. At a daily dose of 150 mg, eltrombopag produced hematologic response in 40% to 50% of patients, in most in two or three hematopoietic lineages.^{24,25} This well-tolerated outpatient regimen was continued until patients showed either no response, relapse, or had a robust recovery in counts in which case the drug was quickly tapered to off (possible in five patients).²⁴ Clonal evolution in this primarily refractory cohort occurred in 15% to 20% of cases.^{24,25} Eltrombopag has been approved as single agent in several countries in patients who had insufficient response to an initial course of immunosuppression. When eltrombopag was added to horse ATG and cyclosporine as first-line therapy, the combination resulted in a high overall hematologic (80% to 90%) and complete response rate (40% to 60%), with best results when all three drugs were administered beginning on day 1.²⁶ BM cellularity often increases in responding patients, and exploratory laboratory analyses show increased progenitor cells, suggesting that eltrombopag stimulates a primitive hematopoietic cell compartment.

Another non-immunosuppression option in the refractory setting includes male hormones such as danazol. A recent report suggested that danazol can be particularly effective in patients with an underlying telomeropathy; in vitro sex hormones increase expression of the telomerase gene. In phase I/II prospective study, danazol was administered to patients with telomeropathy who had associated marrow failure and/or pulmonary fibrosis.²⁷ Danazol appeared to increase TL with most who enrolled experiencing hematologic improvement.

Major toxicities of ATG include immediate allergic reaction, serum sickness, and transient blood count depression. Anaphylaxis is rare, but has been fatal and may be predictable by skin testing. Treatment of ATG allergy is mainly symptomatic: intravenous hydration, antihistamines (for urticaria) and meperidine (for rigors), and increased doses of corticosteroids (for symptomatic serum sickness). CsA is begun at 10 mg/ kg in adults and 12/ mg/ kg in children, with dose adjustments to maintain blood

levels about 200 to 400 ng/ mL. We administer CsA for 6 months following ATG. It is common for CsA to be tapered after 6 to 12 months with limited data to support this practice. A retrospective Italian study suggested that a CsA taper may be of benefit in preventing relapse, but in our experience, in about 70 responders who had their CsA tapered prospectively from 2003 to 2010, we did observe a delay in the occurrence of relapse but did not ultimately prevent it when compared to historical control.^{28,29} Renal and liver function monitoring is required to avoid nephrotoxicity; hypertension, gingival hypertrophy, and tremulousness, which are common side effects. Alemtuzumab is usually well tolerated with infusion-related toxicities more manageable than with ATG. In AA, while immunosuppression (ATG, alemtuzumab) reactivates latent herpes virus infection and increases EBV and cytomegalovirus (CMV) in the circulation, disease is rare and routine prophylactic or preemptive antiviral therapy is not required.^{22,30}

Prognosis is strongly correlated to hematologic response at 3 months, especially the robustness of blood count recovery defined by absolute reticulocyte and platelet.³¹ Pre-therapy, the absolute reticulocyte has also been correlated with a better response rate and survival.^{32,33} In a retrospective analysis, the neutrophil count did not correlate with hematologic response outcomes but associated to short-term mortality.³⁴ Despite the lack of progress in developing more efficacious immunosuppressive regimens, survival in SAA has improved over the years, especially among nonresponders to an initial course of horse ATG, with survival in 5 years increasing nearly threefold among this group (from 23% in the 1990s to 57% in recent years).³⁴ This improvement has been attributed to better supportive care (with antifungals) and more effective salvage therapies with repeat immunosuppression and salvage HSCT. Rates of graft rejection and survival have also improved over time with better transplantation and supportive care protocols, less immunogenic blood products, and closer HLA matching between unrelated donors and recipients with high-resolution tissue typing.^{16,35–37} However, rates of acute and chronic GVHD have remained steady over the years. A BM source of stem cells (compared to mobilized peripheral blood CD34+ cells) and conditioning with alemtuzumab have been associated with lower rates of GVHD.^{9,10,38}

Even after hematologic response to ATG, blood counts may fall, especially on withdrawal of CsA. Reinstitution of CsA usually suffices, but retreatment with ATG may be necessary and relapse is sometimes irreversible and fatal. Evolution to a clonal hematologic disease occurs in about 15% of patients over the decade after initial therapy, manifesting as a dysplastic BM or cytogenetic abnormalities, especially monosomy 7. Pretreatment TL may identify increased risk for clonal evolution long term. In reports from the National Institutes of Health (NIH), patients with the shortest TL (adjusted for age) had a threefold higher risk of acquiring a new cytogenetic abnormality, and five-to sixfold higher for monosomy 7 and complex cytogenetics, when

compared to those with longer pretreatment TL.^{39,40} These differences translated into a survival disadvantage associated with short telomeres (66%) compared to long (84% in 6 years).

Other therapies that are occasionally successful include growth factor combinations (erythropoietin and G-CSF), androgens, and high-dose cyclophosphamide (prohibitively toxic due to the prolonged neutropenia that it induces).^{41,42} Our approach to treatment is shown in Figure 6.3.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

PNH is a rare clonal disease of the BM, which can produce a clinical triad of (1) hemolysis, (2) venous (and rarely arterial) thrombosis, and (3) AA.⁴³

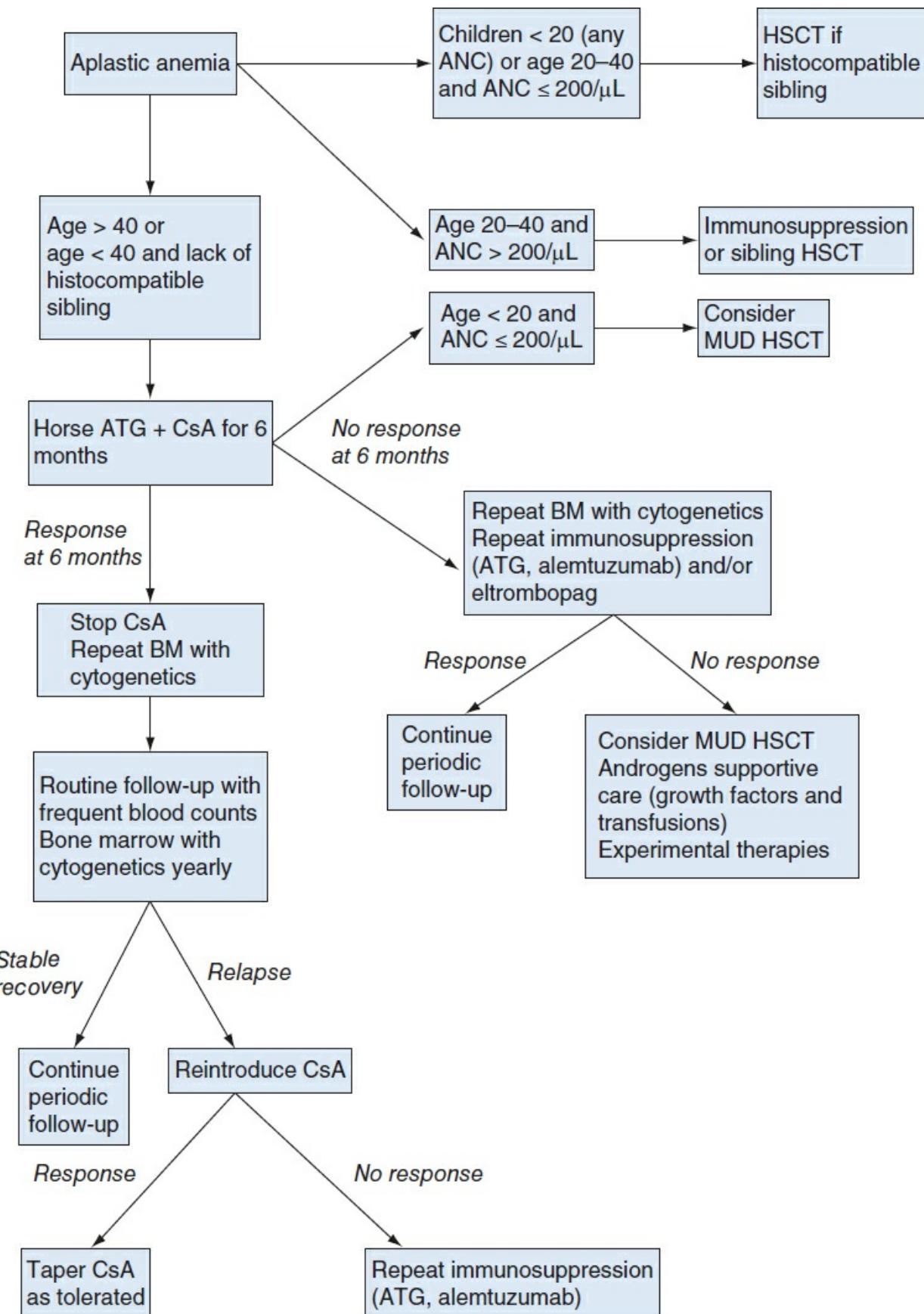


FIGURE 6.3 Treatment of severe aplastic anemia.

ANC, absolute neutrophil count; ATG, antithymocyte globulin; BM, bone marrow; CsA,

cyclosporine A; HSCT, hematopoietic stem cell transplantation; MUD, matched unrelated donor.

Etiology and Pathophysiology

- A somatic mutation in a gene called PIG-A occurs in a hematopoietic stem cell.
- Leads to deficient synthesis of a glycolipid moiety called the glycosylphosphoinositol anchor (GPI).
- Lack of cell surface presentation of a large family of GPI-linked proteins.
- Absence of one of these proteins, CD59, on the cell surface of erythrocytes leads to their susceptibility to complement and to intravascular hemolysis.
- PIG-A mutant cells are probably present in normal adult marrow, but clonal expansion of these cells is unusual— except especially in AA (about 50% of cases) and in MDS, where they may range in size from small to large.
- Which GPI-anchored proteins are important in permitting clonal expansion in AA and MDS and in the thrombotic proclivity is unknown.

Clinical Features

- Intravascular hemolysis, classically as periodic bouts of dark urine in the morning but also as continuous red cell destruction and without hemoglobinuria.
- Venous thrombosis in unusual sites, especially hepatic, mesenteric, and portal veins and intracranial veins.
- Marrow failure, frank AA or poor marrow function despite a relatively cellular histology.

Diagnosis

In general, intravascular hemolysis is unusual (see [Chapter 3](#)), and PNH should be considered in the setting of a suggestive history, hemoglobinuria, and elevated lactate dehydrogenase (LDH). There may be accompanying iron deficiency and neutropenia/thrombocytopenia.

PNH patients can present with abdominal pain due to Budd–Chiari syndrome or symptoms of stroke.

PNH clonal expansion should be sought in patients with AA and MDS. Peripheral blood flow cytometry provides evidence of a PNH clonal expansion through quantification of GPI-anchored proteins on erythrocytes and granulocytes (especially the latter in the transfused patient). However, severe hemolysis and thrombosis typically occur only in patients with large clones (>50%).

Treatment

The course is highly variable, where clone size may range from small and

inconsequential clinically to large and associated with clinical hemolysis. AA patients post-immunosuppressive therapy with expanded clones may be asymptomatic; modest and intermittent hemolysis managed with transfusions alone is consistent with long survival; conversely, PNH can be associated with catastrophic thrombotic events. Clones may spontaneously disappear in some patients.

- | Transfusion to maintain hemoglobin levels consistent with full activity. Use of washed erythrocytes is not necessary.
- | Iron supplementation may be required; loss of hemoglobin as a result of intravascular destruction prevents secondary hemochromatosis.
- | Corticosteroids, usually in moderate doses (30 to 50 mg prednisone on alternate days), have been employed to control hemolysis but never rigorously tested. A short trial in a patient with continuous red cell destruction may be warranted.
- | Marrow failure presenting as frank AA with associated PNH should be treated with HSCT or immunosuppression (see earlier discussion).
- | Most patients in Western series die of thrombotic complications, and thromboses, once they occur, may be refractory to anticoagulation. An uncontrolled trial has suggested that coumadin prophylaxis is effective, but the relative risk of hemorrhage secondary to chronic anticoagulation for years, even decades, in this population remains unclear.⁴⁴
- | HSCT is the only curative therapy, but may carry a higher risk in PNH due to comorbid conditions; non-myeloablative conditioning regimens may offer improved survival.^{11,45} Transplant can be considered in younger patients with severe marrow failure or thrombotic complications. However, the survival is worst in those who had a prior thrombotic event compared to those with only the hemolytic form of the disease (without prior thrombosis).⁴⁶
- | Eculizumab (a monoclonal antibody directed to the active component of C5) has been approved by the Food and Drug Administration (FDA) for patients with PNH and is marketed as Soliris. In a large prospective multicenter trial, the drug blocked intravascular hemolysis, which translated to a clinically significant improvement in anemia, transfusion requirements, and in quality of life measures.^{47,48} Eculizumab also appeared to dramatically reduce the risk of clinical thromboses in patients with PNH, which is the major cause of morbidity and mortality in this disease.⁴⁹ Meningococcal vaccine is mandatory at least 14 days prior to starting eculizumab, and patients should also be maintained on an appropriate prophylactic antibiotic regimen. Long-term results indicate that eculizumab changes the natural history of the disease, and the life expectancy of PNH patients may be similar to that of the general population.⁵⁰ In some cases, anemia may persist and/ or hemolysis may not be entirely blocked with eculizumab.^{51,52} In these cases, the dose of eculizumab can be increased to 1,200 mg every 2 weeks and/ or the interval between the infusion shortened from 14 to 10 days.

The effectiveness of corticosteroids in this circumstance is variable and unproven.

PURE RED CELL APLASIA

PRCA is defined as anemia with absent reticulocytes and marrow erythroid precursor cells.⁵³ This rare aregenerative anemia has a number of clinical associations and is also usually responsive to treatment.

Etiology and Pathophysiology

- Constitutional PRCA is Diamond–Blackfan anemia (DBA) and is secondary to inherited mutations in ribosomal protein genes (see the following).
- Acquired PRCA often behaves as an immunologically mediated disease. Clinical associations include thymoma (but probably <10% of PRCA cases), collagen-vascular syndromes, myasthenia gravis, chronic lymphocytic leukemia, and large granular lymphocytic leukemia.
- PRCA may also be seen in MDS, especially with 5q-syndrome. The phenotype is due to acquired loss of a ribosomal protein gene (RPS14) on chromosome 5, as occurring in the germline in DBA.
- Parvovirus B19 infection causes erythema infectiosum (fifth disease) in children and transient aplastic crisis in patients with underlying hemolysis. Virus infection is ordinarily terminated by production of neutralizing antibodies. Persistence of parvovirus results from failure to mount a neutralizing antibody response, leading to chronic erythroid precursor destruction and anemia. Persistence of parvovirus B19 can occur in an immunodeficient host: in congenital immunodeficiencies (Nezelof's syndrome), iatrogenic (immunosuppressive drugs and cytotoxic chemotherapy), and HIV infection-induced immunodeficiency.

Clinical Features and Diagnosis

Reticulocytes are very low or absent; erythroid precursor cells are usually absent but a few normoblasts may be present in the marrow. There are morphologic clues: giant pronormoblasts signal parvovirus; uninuclear micromegakaryocytes, 5q-syndrome. Other blood counts are normal, as are cytogenetics (except for PRCA associated with MDS).

Thymoma should be excluded by computed tomography (CT) scan.

In persistent parvovirus infection and PRCA, antibodies to virus are usually absent, or only immunoglobulin M (IgM) may be observed; virus can be detected in the blood by DNA hybridization.

Treatment

- For DBA, corticosteroids are standard; patients may be dependent on exquisitely low doses, and relapse may not always be responsive to reinstitution of treatment.
- For acquired PRCA, corticosteroids in moderate doses are usually first therapy, followed by either other immunosuppressants such as CsA, ATG, or cytotoxic drugs such as moderate doses of azathioprine or cyclophosphamide, administered orally. In a few case reports, the monoclonal antibodies to CD20 (rituximab) and CD25 (daclizumab) have been shown effective.⁵⁴
- PRCA associated with a large granular lymphocytic leukemia clone can be highly responsive to alemtuzumab monotherapy.⁵⁵
- Thymomas should be excised as they are locally invasive, but surgery does not necessarily resolve the anemia.
- Persistent parvovirus B19 infection responds to intravenous immunoglobulins at 0.4 g/kg daily for 5 to 10 days. Patients with large viral loads, especially in the acquired immunodeficiency syndrome, may relapse and require periodic retreatment.

AGRANULOCYTOSIS

Severe neutropenia with either complete or partial absence of myeloid precursor cells is agranulocytosis.

Etiology and Pathophysiology

- Most agranulocytosis is drug associated (Table 6.5). Idiopathic pure white cell aplasia (without exposure to a suspicious drug) is exceedingly rare (and like PRCA may also be associated with thymoma).^{56,57}
- Mechanisms of drug destruction of granulocyte precursors include direct effects (as with thorazine) and immune (antibody)-mediated effects (as with dipyrone) (Table 6.6).

Diagnosis and Treatment

- The patient is usually older with a history of clear exposure to an incriminated agent, usually with the introduction of the drug in the preceding 6 months. Absent neutrophils on smear should lead to a confirmatory BM examination.
- Classic presentation is fever and sore throat.
- Recovery occurs spontaneously but over a highly variable period, from a few days to several weeks. G-CSF or granulocyte colony-stimulating factor (GM-CSF) is almost always administered without clear evidence of efficacy.
- Fever and signs of infection require prompt administration of broad-spectrum antibiotics by a parenteral route.
- Mortality remains substantial (about 10%) due to the combination of patient age, comorbid conditions, and lethal sepsis.

CONSTITUTIONAL BONE MARROW FAILURE SYNDROMES

Among the constitutional disorders that present with AA, it is important to consider FA and DKC (Table 6.7). Genes mutated in FA and in DKC have been identified and are important in the functions in the cell; they play a key role in genomic stability and the maintenance of telomeres, respectively. An algorithm for laboratory testing to exclude FA and DC is presented subsequently.

Table 6.5 Drugs Associated With Agranulocytosis

Heavy metals
Gold
Arsenic compounds
Analgesics
Aminopyrine, dipyrone
Butazones
Indomethacin
Ibuprofen
Acetaminophen
Para-aminosalicylic acid
Sulindac
Antipsychotics, sedatives, antidepressants
Phenothiazines
Tricyclics
Chlordiazepoxide
Barbiturates
Serotonin reuptake inhibitors
Anticonvulsants
Phenytoin
Ethosuximide
Carbamazepine
Antithyroid drugs
Propylthiouracil
Methimazole
Cardiovascular drugs
Procainamide
Captopril
Nifedipine
Quinidine

Propranolol
Methyldopa

Propafenone

Aprinidine

Sulfa drugs

Thiazide diuretics like spironolactone and acetazolamide

Oral hypoglycemics

Sulfasalazine

Dapsone

Sulfa antibiotics

Antibiotics

Sulfa antibiotics

Pyrimethamine

Penicillins

Cephalosporins

Macrolides

Vancomycin

Clindamycin

Aminoglycosides

Antituberculosis agents

Levamisole

Antimalarials

Mebendazole

Antifungals

Fluconazole

Antiviral

Zidovudine

Antihistamines

Cimetidine

Ranitidine

Chlorpheniramine

Miscellaneous

Isotretinoin

Omeprazole

Colchicine

Allopurinol

Aminoglutethimide

Metoclopramide

Ticlopidine

- Tamoxifen
- Penicillamine
- Insecticides
- Hair dye
- Chinese herbal medicines

FANCONI ANEMIA

- Autosomal recessive (most FA genes) or X-linked (FANCB) inheritance; most common of the constitutional syndromes, seen in all races; diagnosed on the basis of positive chromosome breakage test (see subsequently)
- Mutations in any of the at least 20 genes: FANCA, FANCB, FANCC, FANCD1/ BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/ BACH1/ BRIP1, FANCL, FANCM, FANCN/ PALB2, FANCO/ RAD51C, FANCP/ SLX4, FANCQ/ ERCC4, FANCR/ RAD51, FANCS/ BRCA1, FANCT/ UBE2T, FANCU/ XRCC2, and MAD2L2/ REV7
- Chief criteria of pancytopenia, hyperpigmentation, malformation of the skeleton, small stature, and hypogonadism
- Malformations of the eye, ear, genitourinary and gastrointestinal tracts, and cardiopulmonary and central nervous systems can occur
- FA is notoriously heterogeneous in the degree and number of clinical manifestations, and patients presenting solely with either congenital malformations or hematologic abnormalities may either be misdiagnosed or go unrecognized entirely.

Table 6.6 Immune Versus Toxic Agranulocytosis

Parameters	Immunologic	Toxic
Paradigm drug	Aminopyrine	Phenothiazine
Time to onset	Days to weeks	Weeks to months
Clinical	Acute, often explosive symptoms	Often asymptomatic or insidious onset
Rechallenge	Prompt recurrence with small test dose	Latent period, high dose required
Laboratory	Leucoagglutinins	Evidence of direct or metabolite mediated toxicity to cells

Clinical Features

- The diagnosis is suggested when a child presents with hyper- or hypopigmented skin lesions; short stature (poor growth); anomalies of the upper limb or thumb; male

hypogonadism; microcephaly; characteristic facial features, including a broadened nasal base, epicanthal folds, and micrognathia; and structural renal abnormalities. When this constellation of physical anomalies is accompanied by BM failure (which may often trigger initial medical evaluation), confirmation of the diagnosis can be made by standard diepoxybutane (DEB) or mitomycin C (MMC) chromosome breakage analysis (see the following).

- | The mean age at diagnosis is 8 to 9 years.

Diagnostic Tests

- | Chromosome breakage test with DEB or MMC, performed on peripheral blood cells.
- | Based solely on definition by the DEB test, nearly 40% of patients may be free of major physical anomalies. These FA patients with normal appearances may go unrecognized unless there is a high index of suspicion for familial disease.
- | A challenge is the diagnosis of FA in older patients. Although the mean age of diagnosis is in the first decade of life, FA has been described in the fifth and sixth decades of life.

Hematologic Presentations and Cancer Predisposition

- | The symptoms and signs of FA typically relate to the hematologic presentation of cytopenias from marrow failure. Often, thrombocytopenia or leukopenia is noted before full pancytopenia; furthermore, the pancytopenia typically worsens with time. Almost all FA patients will develop hematologic abnormalities in their lifetime. Erythropoiesis is usually macrocytic.
- | Classically, the BM is hypocellular and fatty, indistinguishable from that of acquired AA. Microscopic examination of the marrow may show dyserythropoiesis and dysplasia. Some patients may develop or even present with a morphologically defined MDS or frank AML.
- | The crude risk of leukemia (exclusive of MDS) is 5% to 10%, whereas the cumulative incidence of leukemia is about 10% by age 25 years. Less commonly recognized is the probability of developing MDS, approximately 5%, which appears also to correlate with a poor prognosis for FA patients. Clonal karyotypic abnormalities, identical to those seen in non-FA MDS and secondary AML, are frequently found in FA patients, whether or not they meet marrow morphologic criteria for a defined MDS. The prognostic significance of these clonal chromosomal abnormalities in FA patients is not entirely clear, however, since cytogenetic changes can fluctuate over time. Certain clonal abnormalities may be associated with poor prognoses, such as gains of chromosome 3q.
- | Solid organ malignancies occur frequently, with a crude risk of 5% to 10% of patients overall (the risk increases with age, as those patients who have survived into

adulthood develop solid tumors). Particularly common are vulvar, esophageal, and head and neck cancers. In addition to these (presumed) de novo tumors, a subset of long-term survivors of SCT will develop secondary malignancies, typically head and neck.

Patients with biallelic mutations in FANCD1/ BRCA2, FANCJ/ BACH1/ BRIP1, and FANCN/ PALB2 , as has been demonstrated thus far, present with an FA phenotype associated with childhood cancers and leukemia. Heterozygotes for FANCD1/ BRCA2, FANCJ/ BACH1/ BRIP1, FANCN/ PALB2 , and FANCS/ BRCA1 are at increased risk for breast and other cancers.

Table 6.7 Constitutional Bone Marrow Failure Syndromes and Associated Genes

Clinical Syndrome	Clinical Features and Associated Cancers	Gene	Inheritance Pattern
Fanconi anemia	Skin pigmentation lesions; short stature; anomalies of the upper limb or thumb; male hypogonadism; microcephaly; characteristic facial features; structural renal abnormalities Pancytopenia Associated MDS, AML, squamous cell carcinoma of head, neck, vulva Heterozygotes for FANCD1/ BRCA2, FANCJ/ BACH1/ BRIP1, FANCN/ PALB2, and FANCS/ BRCA1: increased risk for breast and other cancers	FANCA FANCC FANCD1/ BRCA2 FANCD2 FANCE FANCF FANCG FANCI FANCJ/ BACH1/ BRIP1 FANCL FANCM FANCN/ PALB2 FANCO/ RAD51C FANCP/ SLX4 FANCQ/ ERCC4 FANCR/ RAD51 FANCS/ BRCA1 FANCT/ UBE2T FANCU/ XRCC2 MAD2L2/ REV7 FANCB	AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR AR XLR
Dyskeratosis congenita	Abnormal skin pigmentation, nail dystrophy, mucosal leukoplakia, pulmonary fibrosis, cirrhosis, early graying of the hair Pancytopenia Associated MDS, AML, squamous cell carcinoma of skin, head, neck, vulva	DKC1 TERC TINF2 CTC1 NHP2 NOP10 PARN WRAP53 TERT RTEL1 ACD	XLR AD AD AR AR AR AR AR AR AD or AR AD or AR AD or AR
Diamond Blackfan	Thumb, upper limb and craniofacial	RPS19	AD

anemia	abnormalities are common. Others: cardiac septal defects, urogenital anomalies, growth retardation	RPS6 RPS7 RPS10 RPS17 RPS24	AD AD AD AD AD
	Anemia	RPS26 RPS28	AD AD
	Moderately increased risk of MDS, AML, and solid organ malignancies including osteosarcomas	RPS29 RPL5 RPL11 RPL15 RPL17 RPL19 RPL26 RPL31 RPL35A GATA1 TSR2	AD AD AD AD AD AD AD AD AD XLR XLR
	Exocrine pancreatic insufficiency, short stature, skeletal, neurodevelopmental abnormalities	SBDS	AR
	Neutropenia		
	Associated MDS, AML		
	Severe neutropenia and early stage maturation arrest of myelopoiesis, leading to bacterial infections from early infancy	ELANE/ ELA2 GFI1 G6PC3 HAX1	AD AR AR AR
	Neutropenia		
	Associated MDS, AML		
	Severe thrombocytopenia due to a lack of megakaryocytes in the bone marrow from birth	MPL	AR
Congenital amegakaryocytic thrombocytopenia	Thrombocytopenia		
	Case report of ALL, MDS		

AD, autosomal dominant; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AR: autosomal recessive; MDS, myelodysplastic syndrome; XLR, X-linked recessive.

Stem Cell Transplantation and Supportive Care

- Allogeneic SCT from an HLA-matched sibling donor is the only curative therapy for the hematologic manifestations of FA (aplasia or MDS). Typically, decreased doses

of cyclophosphamide and irradiation must be used in order to avoid severe toxicity due to the chemo-and radiosensitivity of FA patients. Transplantation centers, which generally adopted this modified conditioning regimen with or without thoracoabdominal irradiation, have reported good results for FA patients that did not present with leukemia or preleukemic transformation.

- Umbilical cord blood transplantation from related donors has also been successfully applied to a small number of FA patients. A few FA patients have also undergone successful HSCT with cord blood from unrelated donors.
- Clearly, young patients with an HLA-compatible sibling should be treated by HSCT at the earliest stages of marrow failure in preference to other therapies. However, most patients do not have an HLA-identical donor and are dependent on the identification of a suitably matched nonsibling relative or unrelated donor. A smaller number of FA patients have undergone HSCT from such alternative sources (matched unrelated and haploidentical family donors) to treat either aplasia or MDS, with or without clonal chromosomal abnormalities. The results from these alternative donor transplants have generally been inferior to those from matched sibling donor transplants, but are improving.

Patients lacking a suitable HLA-compatible donor (either sibling or matched unrelated) may benefit from chronic administration of androgens or hematopoietic growth factors, which may serve as temporizing measures.

Androgens

- Androgens have been shown to induce hematologic responses in approximately 50% of FA patients although their effectiveness in raising blood counts may be neither durable nor complete in all lineages. Typically, androgen therapy is initiated when the platelet count is consistently below 30,000/ μL and/ or the hemoglobin less than 7 gm/dL. Orally administered oxymetholone, at a dose of 2 to 5 mg/ kg/ day, is usually combined with prednisone, 5 to 10 mg every other day, in order to counterbalance the anabolic properties of oxymetholone with catabolic actions of corticosteroids.
- Androgen therapy is associated with liver toxicities including transaminase enzyme elevation, cholestasis, peliosis hepatitis, and hepatic tumors.

Human Papillomavirus Vaccination

Human papillomavirus (HPV) vaccination should be considered at age 9 years to reduce the risk of gynecologic cancers in females and oral cancers in all individuals.

Hematopoietic Growth Factors

- Levels of most growth factors are markedly increased in FA as they are in acquired AA, likely as a compensatory physiologic response.

- One worrisome aspect of chronic growth factor administration is the theoretical risk of stimulating a leukemic clone, particularly in patients prone to developing MDS or AML or speeding the process of stem cell exhaustion.
- Chronic administration of G-CSF may have transient beneficial effects on multiple hematopoietic lineages in some patients.

DYSKERATOSIS CONGENITA

- Classical DKC is an inherited BM failure syndrome characterized by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and mucosal leukoplakia.
- It has been observed in many races and estimated prevalence of DKC is approximately 1 per 1,000,000 persons.
- X-linked recessive (mutations in DKC1), autosomal dominant (heterozygous mutations in the RNA component of telomerase, TERC, or in TINF2), and autosomal recessive (mutations in CTC1, NHP2, NOP10, PARN, and WRAP53) forms of the disease are recognized. Heterozygous mutations in the enzymatic component of telomerase (TERT) as well as in ACD or RTEL1 can lead to variable phenotypes.
- A variety of other (dental, gastrointestinal, genitourinary, hair graying/ loss, immunological, neurological, ophthalmic, pulmonary, and skeletal) abnormalities have also been reported.
- BM failure is the principal cause of early mortality with an additional predisposition to malignancy and fatal pulmonary complications.
- Clinical manifestations in DKC often appear during childhood although there is a wide age range. The skin pigmentation and nail changes typically appear first, usually by the age of 10 years. BM failure usually develops below the age of 20 years; 80% to 90% of patients will have developed BM abnormalities by the age of 30 years. In some patients, the BM abnormalities may appear before the mucocutaneous manifestations and can lead to an initial diagnosis of idiopathic AA.
- The clinical features of these disorders are very heterogeneous and this makes diagnosis based on clinical criteria alone difficult and unreliable. In many pedigrees, the disease only manifests in adulthood, hematologic manifestations can range from severe AA and AML to mild macrocytosis, and affected members may only have lung or liver disease.
- Oxymetholone can produce durable hematological responses in more than >50% of DKC and FA patients, but patients have to be monitored carefully for side effects. Androgens may function by increasing TERT transcription.²⁷
- The current definitive treatment is allogeneic HSCT. In both DKC and FA patients, low-intensity transplant protocols are producing prompt engraftment, reduced toxicity, and have the potential to reduce the risk of secondary malignancies.

Diagnostic Testing for Aplastic Anemia With Short Telomeres

Telomeres are the tips of chromosomes and are maintained by a complex that includes the enzyme telomerase reverse transcriptase (TERT), its RNA component (TERC), the protein dyskerin, and other associated proteins (NHP2, NOP10, and GAR1). TL measurement is now commercially available and should be a screening test in most cases of severe AA, especially with a typical clinical history (moderate, slowly progressive AA) or if the family history suggests an inherited syndrome.

AA with short telomeres is typical in DKC patients. Because the genes mutated in the X-linked recessive (DKC1) and other (TERC and others) DKC subtypes are now known, it is possible to substantiate the diagnosis in a significant proportion of DKC patients. In particular, it is appropriate to screen for the DKC1 gene if patients are male and have two out of the following: abnormal skin pigmentation, nail dystrophy, leukoplakia, or BM failure.

Besides patients with a typical DKC pedigree, however, there are also patients with acquired AA with short telomeres who can carry mutations in TERT and TER C and not have the physical abnormalities observed in DKC or have a family history suggestive for telomeropathy. Overall, mutations in telomerase genes (TERC or TERT but not DKC1) appear to explain the short telomeres detected in about 10% of patients with AA with some responding to conventional immunosuppressive therapy as is applied in acquired AA cases. The failure of organs other than the BM, including the liver and the lung, may also be associated with TERT mutations.

MARROW FAILURE INVOLVING A SINGLE LINEAGE

Diamond–Blackfan Anemia

- | This is probably the second most common constitutional marrow failure syndrome after FA.
- | Most patients present with anemia in the neonatal period or in infancy.
- | Approximately 30% of affected children present with a variety of associated physical anomalies. Thumb and upper limb malformations and craniofacial abnormalities are common. Other defects: atrial or ventricular septal defects, urogenital anomalies, and prenatal or postnatal growth retardation.
- | A moderately increased risk of developing MDS and solid organ malignancies
- | Cases are sporadic, with an equal sex ratio, but 10% to 25% of patients have a positive family history for the disorder.
- | Heterozygous mutations in the ribosomal protein genes RPS19, RPS24, RPS17, RPS6, RPS10, RPS26, RPL5, RPL11, and RPL35A account for most cases, whereas GATA1-and TSR2-related DBA are inherited in an X-linked manner.

Hematologic Findings

- | Minimal diagnostic criteria for DBA: normochromic anemia in infancy (<2 years), low reticulocyte counts, absent or decreased BM red cell precursors (<5% of nucleated cells), and a normal chromosome breakage test (to rule out FA).
- | Additional features: presence of malformations, macrocytosis, elevated fetal hemoglobin (HbF), and elevated erythrocyte adenosine deaminase (eADA) level.
- | Some patients identified after the age of 2 years after a more severely affected family member is first diagnosed.
- | Anemia usually severe at the time of diagnosis (usually macrocytic).
- | The BM aspirate is usually normocellular, but erythroblasts are markedly decreased or absent. The other cell lines are normal but mild to moderate neutropenia, thrombocytopenia, or both may occur later in the course.
- | Progression of the single-lineage erythroid deficiency of DBA into pancytopenia and AA is rare but may occur.
- | Differential diagnosis includes transient erythroblastopenia of childhood (TEC). Both TEC and DBA show similar marrow morphology, but TEC is self-limited, with a recovery within 5 to 10 weeks.

Treatment

- | Initial treatment in DBA is transfusions, but long-term administration of red cells may cause secondary hemochromatosis.
- | Corticosteroids are the mainstay of treatment, and at least 50% of patients respond. There is no known predictor of steroid responsiveness, and later relapses occur. During treatment, some patients may recover sensitivity to corticosteroids or even proceed to a spontaneous remission.
- | Allogeneic bone marrow transplantation (BMT) is a treatment option for DBA in steroid-resistant patients.
- | Hematopoietic growth factor therapy with interleukin-3 (IL-3) or erythropoietin (EPO) has been attempted for DBA.

Shwachman–Diamond Syndrome

- | It is probably the third most common constitutional marrow failure syndrome after FA.
- | Autosomal recessive disorder usually manifests in infancy and characterized by exocrine pancreatic insufficiency, short stature, and BM dysfunction.
- | Mutations in the SBDS gene account for about 90% of cases.
- | The SBDS protein appears to be involved in multiple functions, including ribosome maturation.
- | Additional clinical features include metaphyseal dysostosis, epiphyseal dysplasia, immune dysfunction, liver disease, growth failure, renal tubular defects, insulin-

- dependent diabetes mellitus, and psychomotor retardation.
- Hematological manifestations: neutropenia, raised HbF levels, anemia, thrombocytopenia, impaired neutrophil chemotaxis.
- Predilection for malignant myeloid transformation and MDS.

Clinical Diagnostic Criteria

Clinical Diagnosis Fulfill the combined presence of hematological cytopenia of any given lineage (most often neutropenia) and exocrine pancreas dysfunction

Hematologic abnormalities may include:

- Neutropenia $< 1.5 \times 10^9/\text{L}$ on at least two occasions over at least 3 months
- Hypoprotective cytopenia detected on two occasions over at least 3 months
- Pancreatic dysfunction may be diagnosed by the following: reduced levels of pancreatic enzymes adjusted to age (fecal elastase, serum trypsinogen, serum (iso)amylase, serum lipase).

Tests that support the diagnosis but require corroboration:

- Abnormal 72 hours fecal fat analysis
- Reduced levels of at least two fat-soluble vitamins (A, D, E, and K)
- Evidence of pancreatic lipomatosis (e.g., ultrasound, CT, magnetic resonance imaging (MRI), or pathological examination of the pancreas by autopsy)

Molecular Diagnosis: Biallelic SBDS Gene Mutation Positive genetic testing for SBDS mutations known or predicted to be deleterious, for example, from protein modeling or expression systems for mutant SBDS.

Treatment

- Treatment of hematologic manifestations (neutropenia, BM failure) may involve hematopoietic growth factor therapy with G-CSF. For neutropenia unresponsive to G-CSF, SAA, MDS, or leukemia, hematopoietic HSCT may be considered.

Severe Congenital Neutropenia and Cyclic Neutropenia

- Originally described as an autosomal recessive disorder (Kostmann syndrome) but majority of severe congenital neutropenia (SCN) cases due to dominant-acting point mutations in the neutrophil elastase (ELANE or ELA2) gene. Mutations in the protooncogene GFI1 that target and repress ELANE have also been implicated. Autosomal recessive mutations in G6PC3 or HAX1 can also cause SCN.
- Characterized by severe neutropenia and an early-stage maturation arrest of myelopoiesis, leading to bacterial infections from early infancy.
- Greater than 90% of these patients respond to G-CSF (filgrastim, lenograstim) with

ANC that can be maintained around $1.0 \times 10^9/\text{L}$. Adverse events include mild splenomegaly, moderate thrombocytopenia, osteoporosis, and malignant transformation into MDS/ leukemia. Development of additional genetic aberrations (G-CSF-receptor or RAS gene mutations, monosomy 7) during the course of the disease indicates an underlying genetic instability.

- | Hematopoietic SCT is still the only available treatment for patients refractory to G-CSF.
- | Cyclic neutropenia (CN), which is also caused by mutations in ELANE, is an autosomal dominant disorder (sporadic or inherited) characterized by regular oscillations of neutrophils from near normal to severely low levels, generally with a 21-day periodicity. ANC nadirs are associated with fever, mouth ulcers, pharyngitis, sinusitis, or more serious infections. CN usually presents early in childhood but be asymptomatic, and transformation to MDS and AML has not been reported. Symptomatic CN is responsive to G-CSF, which typically shortens nadir duration and increases ANC but usually does not ablate cycling.

Congenital Amegakaryocytic Thrombocytopenia

- | Characterized by severe thrombocytopenia due to a lack of megakaryocytes in the BM from birth.
- | Diagnosis is based mainly on the exclusion of other forms of congenital thrombocytopenia with ineffective megakaryopoiesis such as FA.
- | Molecular basis for this autosomal recessive disorder may be homozygous or compound heterozygous mutations in the MPL gene coding for the thrombopoietin receptor.
- | At the time of diagnosis, the BM of congenital amegakaryocytic thrombocytopenia (CAMT) patients is normocellular with a normal representation of all hematopoietic lines except for megakaryocytes. During the course of CAMT, the disease usually evolves into AA.
- | SCT has been shown to be the only curative therapy.

References

1. Calado RT, Young NS. Telomere diseases. *N Engl J Med.* 2009;361(24):2353–2365.
2. Ganapathi KA, Townsley DM, Hsu AP, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. *Blood.* 2015;125(1):56–70.
3. Kaufman DW, Kelly JP, Issaragrisil S, et al. Relative incidence of agranulocytosis and aplastic anemia. *Am J Hematol.* 2006;81(1):65–67.
4. Bacigalupo A, Brand R, Oneto R, et al. Treatment of acquired severe aplastic

- anemia: bone marrow transplantation compared with immunosuppressive therapy — The European Group for Blood and Marrow Transplantation experience. *Semin Hematol.* 2000;37:69–80.
5. Gupta V, Eapen M, Brazauskas R, et al. Impact of age on outcomes after transplantation for acquired aplastic anemia using HLA-identical sibling donors. *Haematologica.* 2010;95:2119.
 6. Locasciulli A, Oneto R, Bacigalupo A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica.* 2007;92(1):11–18.
 7. Bacigalupo A, Giannarco S, Sica S. Bone marrow transplantation versus immunosuppressive therapy in patients with acquired severe aplastic anemia. *Int J Hematol.* 2016;104(2):168–174.
 8. Bacigalupo A, Socie G, Schrezenmeier H, et al. Bone marrow versus peripheral blood as the stem cell source for sibling transplants in acquired aplastic anemia: survival advantage for bone marrow in all age groups. *Haematologica.* 2012;97(8):1142–1148.
 9. Eapen M, Le Rademacher J, Antin JH, et al. Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia. *Blood.* 2011;118(9):2618–2621.
 10. Schrezenmeier H, Passweg JR, Marsh JC, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. *Blood.* 2007;110(4):1397–1400.
 11. Srinivasan R, Takahashi Y, McCoy JP, et al. Overcoming graft rejection in heavily transfused and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation. *Br J Haematol.* 2006;133:305–314.
 12. Margolis DA, Casper JT. Alternative-donor hematopoietic stem-cell transplantation for severe aplastic anemia. *Semin Hematol.* 2000;37:43–55.
 13. Passweg JR, Perez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transpl.* 2006;37(7):641–649.
 14. Devillier R, Dalle JH, Kulasekararaj A, et al. Unrelated alternative donor transplantation for severe acquired aplastic anemia: a study from the French Society of Bone Marrow Transplantation and Cell Therapies and the EBMT Severe Aplastic Anemia Working Party. *Haematologica.* 2016;101(7):884–890.
 15. Kennedy-Nasser AA, Leung KS, Mahajan A, et al. Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric

- severe aplastic anemia. *Biol Blood Marrow Transplant.* 2006;12(12):1277–1284.
- 16. Maury S, Balere-Appert ML, Chir Z, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. *Haematologica.* 2007;92(5):589–596.
 - 17. Peffault de Latour R, Purtill D, Ruggeri A, et al. Influence of nucleated cell dose on overall survival of unrelated cord blood transplantation for patients with severe acquired aplastic anemia: a study by eurocord and the aplastic anemia working party of the European group for blood and marrow transplantation. *Biol Blood Marrow Transplant.* 2011;17(1):78–85.
 - 18. Bacigalupo A, Sica S. Alternative donor transplants for severe aplastic anemia: current experience. *Semin Hematol.* 2016;53(2):115–119.
 - 19. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med.* 2011;365(5):430–438.
 - 20. Marsh JC, Bacigalupo A, Schrezenmeier H, et al. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party. *Blood.* 2012;119(23):5391–5396.
 - 21. DiBona E, Coser P, Camba L, et al. Rabbit ATG (r-ATG) plus cyclosporin (CYA) and granulocyte colony stimulating factor (G-CSF) is an effective treatment for aplastic anemia (AA) patients (pts) unresponsive to a first course with horse ALG (h-ALG) therapy. *Bone Marrow Transplant.* 1999;23(suppl 1):S28–S28.
 - 22. Scheinberg P, Nunez O, Weinstein B, et al. Activity of alemtuzumab monotherapy in treatment-naïve, relapsed, and refractory severe acquired aplastic anemia. *Blood.* 2012;119(2):345–354.
 - 23. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit anti-thymocyte globulin and cyclosporine for patients with relapsed or refractory severe aplastic anemia. *Brit J Haem.* 2006;133:622–627.
 - 24. Desmond R, Townsley DM, Dumitriu B, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. *Blood.* 2014;123(12):1818–1825.
 - 25. Olnes MJ, Scheinberg P, Calvo KR, et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. *N Engl J Med.* 2012;367(1):11–19.
 - 26. Dumitriu B, Scheinberg P, Desmond R, et al. Eltrombopag added to standard immunosuppression for aplastic anemia accelerates count recovery and increases response rates. *Blood.* 2015;126(23):LBA-2.
 - 27. Townsley DM, Dumitriu B, Liu D, et al. Danazol treatment for telomere diseases. *N Engl J Med.* 2016;374(20): 1922–1931.
 - 28. Saracco P, Quarello P, Iori AP, et al. Cyclosporin A response and dependence in children with acquired aplastic anaemia: a multicentre retrospective study with long-term observation follow-up. *Br J Haematol.* 2008;140(2):197–205.
 - 29. Scheinberg P, Rios O, Scheinberg P, et al. Prolonged cyclosporine administration

- after antithymocyte globulin delays but does not prevent relapse in severe aplastic anemia. *Am J Hematol.* 2014;89(6):571–574.
30. Scheinberg P, Fischer SH, Li L, et al. Distinct EBV and CMV reactivation patterns following antibody-based immunosuppressive regimens in patients with severe aplastic anemia. *Blood.* 2007;109(8):3219–3224.
 31. Rosenfeld S, Follman D, Nuñez O, et al. Antithymocyte globulin and cyclosporine for severe aplastic anemia. Association between hematologic response and long-term outcome. *J Am Med Assoc.* 2003;289:1130–1135.
 32. Afable MG, II, Shaik M, Sugimoto Y, et al. Efficacy of rabbit anti-thymocyte globulin in severe aplastic anemia. *Haematologica.* 2011;96(9):1269–1275.
 33. Scheinberg P, Wu CO, Nunez O, et al. Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. *Br J Haematol.* 2009;144(2):206–216.
 34. Valdez JM, Scheinberg P, Nunez O, et al. Decreased infection-related mortality and improved survival in severe aplastic anemia in the past two decades. *Clin Infect Dis.* 2011;52(6):726–735.
 35. Bacigalupo A, Socie' G, Lanino E, et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. *Haematologica.* 2010;95(6):976–982.
 36. Perez-Albuerne ED, Eapen M, Klein J, et al. Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. *Br J Haematol.* 2008;141(2):216–223.
 37. Viollier R, Socie G, Tichelli A, et al. Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. *Bone Marrow Transplant.* 2008;41(1):45–50.
 38. Marsh JC, Gupta V, Lim Z, et al. Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft-versus-host disease after allogeneic stem cell transplantation for acquired aplastic anemia. *Blood.* 2011;118(8):2351–2357.
 39. Dumitriu B, Feng X, Townsley DM, et al. Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia. *Blood.* 2015;125(4):706–709.
 40. Scheinberg P, Cooper JN, Sloand EM, et al. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. *JAMA.* 2010;304(12):1358–1364.
 41. Scheinberg P, Townsley D, Dumitriu B, et al. Moderate-dose cyclophosphamide for severe aplastic anemia has significant toxicity and does not prevent relapse and clonal evolution. *Blood.* 2014;124(18):2820–2823.
 42. Tisdale JF, Dunn DE, Geller NL, et al. High-dose cyclophosphamide in severe aplastic anemia: a randomized trial. *Lancet.* 2000;356:1554–1559.

43. Rosse WF, Nishimura J. Clinical manifestations of paroxysmal nocturnal hemoglobinuria: present state and future problems. *Int J Hematol.* 2003;77:113–120.
44. Hall C, Richards S, Hillmen P. Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH). *Blood.* 2003;102:3587–3591.
45. Pantin J, Tian X, Geller N, et al. Long-term outcome of fludarabine-based reduced-intensity allogeneic hematopoietic cell transplantation for debilitating paroxysmal nocturnal hemoglobinuria. *Biol Blood Marrow Transplant.* 2014;20(9):1435–1439.
46. Peffault de Latour R, Schrezenmeier H, Bacigalupo A, et al. Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria. *Haematologica.* 2012;97(11):1666–1673.
47. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2004;350:552–559.
48. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2006;355(12):1233–1243.
49. Hillmen P, Muus P, Duhrsen U, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood.* 2007;110(12):4123–4128.
50. Kelly RJ, Hill A, Arnold LM, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood.* 2011;117(25):6786–6792.
51. Risitano AM, Notaro R, Marando L, et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. *Blood.* 2009;113(17):4094–4100.
52. Lin Z, Schmidt CQ, Koutsogiannaki S, et al. Complement C3dg-mediated erytrophagocytosis: implications for paroxysmal nocturnal hemoglobinuria. *Blood.* 2015;126(7):891–894.
53. Kang EM, Tisdale J. Pure red cell aplasia. In: Young NS, ed. *The Bone Marrow Failure Syndromes.* Philadelphia, PA: W.B. Saunders, 2000.
54. Sloand EM, Scheinberg P, Maciejewski JP, et al. Brief communication: successful treatment of pure red-cell aplasia with an anti-interleukin-2 receptor antibody (Daclizumab). *Ann Int Med.* 2006;144:181–185.
55. Dumitriu B, Ito S, Feng X, et al. Alemtuzumab in T-cell large granular lymphocytic leukaemia: interim results from a single-arm, open-label, phase 2 study. *Lancet Haematol.* 2016;3(1):e22–e29.
56. Andersohn F, Konzen C, Garbe E. Systematic review: agranulocytosis induced by nonchemotherapy drugs. *Ann Intern Med.* 2007;146(9):657–665.

57. Young NS. Agranulocytosis. In: Young NS, ed. The Bone Marrow Failure Syndromes. Philadelphia, PA: W.B. Saunders; 2000:156–182.

Myelodysplastic Syndromes

Danielle M. Townsley and Minoo
Battiwalla

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterized by ineffective hematopoiesis and a variable tendency to progress to acute myelogenous leukemia (AML). Increasingly, MDS is diagnosed incidentally when modestly abnormal blood counts trigger a bone marrow examination.

MDS is a disease of older adults; the median age is the mid-60s. Estimates of its incidence range from 4 to 160 per 100,000 people, and in the elderly, the rate may be 10-fold higher, making MDS a relatively common hematologic disease.^{1–3} In a well-characterized population study that included bone marrow biopsies for all subjects, the incidence of MDS in males 80 years or older was 35 per 100,000 people.⁴ Death due to MDS occurs from the complications of cytopenias and/ or progression to AML, but many patients succumb first to comorbidities of the elderly.

Myelodysplasia of the marrow can also be seen in aplastic anemia, especially as a late event after immunosuppressive treatment, in the course of Fanconi anemia, and with paroxysmal nocturnal hemoglobinuria (PNH) and T-large granular lymphocytosis (T-LGL) lymphoproliferative disorders, and preceding AML (Fig. 7.1). The recent availability of myeloid mutation analysis allows an unprecedented ability to establish the cardinal feature of clonality that drives the cytopenias associated with MDS and is a more reliable way to distinguish MDS from phenotypic mimics (such as micronutrient-deficient states).

ETIOLOGY

MDS is a clonal myeloid neoplasm induced by the acquisition of somatic mutations and distinguished from AML by <20% myeloblasts in the bone marrow. In the majority of cases (85%), MDS is a de novo phenomenon with no definitive antecedent cause. In

secondary MDS (15%), prior chemotherapy (alkylating agents and topoisomerase inhibitors) and ionizing radiation are clearly etiologic; the latency period between exposure and the development of secondary MDS is typically 2 to 10 years. Radiation has been implicated in the marrow failure syndromes; it has been historically reported in occupationally and accidentally exposed individuals and in atomic bomb victims; and solvents and smoking are also associated. Childhood MDS is exceedingly rare (incidence rate = 0.01/ 100,000); it can be seen de novo or in patients with a history of acquired or constitutional aplastic anemia, especially Fanconi anemia. MDS in early adulthood, age <40 years, is also rare and should prompt investigation of an inherited etiology.

The bone marrow is typically hypercellular, implying that ineffective hematopoiesis rather than the absence of stem cells causes the cytopenias. In general, early MDS (refractory anemia) is characterized by a clonal expansion of myelodysplastic stem cells with defective differentiation and increased apoptosis of myeloid and erythroid precursors. Late MDS (in transition to leukemia) is associated with reduced apoptosis. Although the principal defect is in the hematopoietic stem cells, immunological factors and the bone marrow microenvironment contribute to the bone marrow failure. There are significant abnormalities in apoptosis, cytokine profiles, angiogenesis, and the T-cell repertoire. Specific genetic abnormalities, in particular, abnormalities in chromosome 7 and a complex karyotype, predispose to leukemic transformation. In contrast, 5q-, del 20q, and Y are recurrent chromosomal abnormalities not associated with a high risk of transformation.

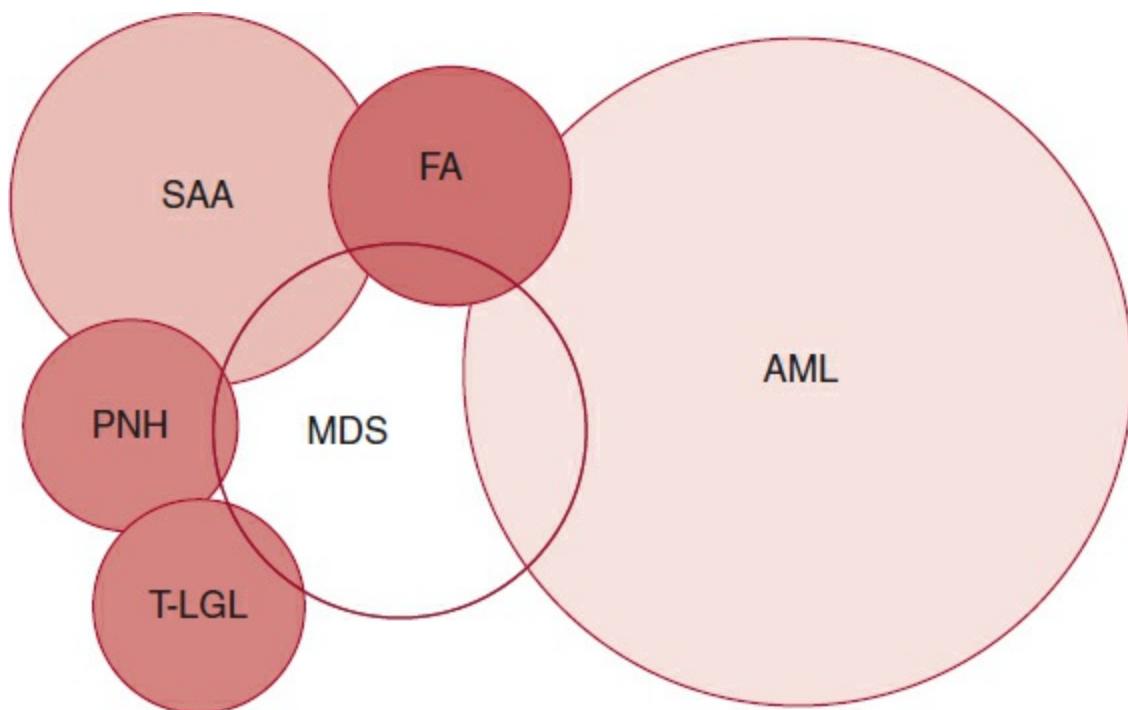


FIGURE 7.1 Clonal hematopoiesis and myelodysplastic syndrome. Clonal and non-clonal hematopoietic states and their corresponding relationship with MDS are represented as a Venn diagram. Examples of non-clonal states that mimic the cytopenias and morphologic dysplasia seen in MDS are shown on the left, whereas in the middle diagram, the relationship between MDS and other bone marrow failure disorders is shown. Patients with unexplained cytopenias that do not meet criteria for MDS are designated as having ICUS or CCUS if clonality is identified with an unknown risk of developing MDS.

AML, acute myelogenous leukemia; CCUS, clonal cytophenias of undetermined significance; CHIP, clonal hematopoiesis of indeterminate significance; EBV, Epstein–Barr virus; IBMF, inherited bone marrow failure; ICUS, idiopathic cytophenias of undetermined significance; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria; SAA, severe aplastic anemia; T-LGL, T-cell large granular lymphocytosis.

Adapted from Bejar R. Myelodysplastic syndromes diagnosis: what is the role of molecular testing? *Curr Hematol Malig Rep*. 2015;10:282–291.

Advances continue to be made in refining the understanding of the molecular mechanisms underlying specific MDS subtypes. Insights include the identification of haploinsufficiency of the RPS14 gene for the phenotype of del(5q), the high frequency of uniparental disomy in patients with normal metaphase cytogenetics, spliceosome protein mutations (e.g., SF3B1, U2AF1, SRSF2), epigenetic regulators (DNMT3A, ASXL1, TET2, EZH2, IDH 1/ 2), cohesins and structural proteins (STAG2), proliferation factors (JAK2, NRAS), differentiation factors (RUNX1, ETV6, SETBP1), and the importance of cyclin D1 in trisomy 8.^{5–11}

CLONALITY AND MOLECULAR FEATURES

Clonal hematopoiesis is a universal feature of MDS, supported by cytogenetic and molecular genetic data.^{12,13} Aside from T cells, most of the cells constituting peripheral blood arise from the abnormal clone. The acquisition of genetic abnormalities in the clone can be due to chromosomal imbalances (approximately half of the cases)¹⁴ or somatic mutations in myeloid cancer genes.¹³ Recurrently mutated MDS-associated genes are identified in 80% to 90% of patients and impact fundamental cellular biology pathways (Table 7.1); the majority encodes messenger RNA splicing machinery or epigenetic modifications. Most mutations result in global changes in gene expression patterns, and may affect genomic stability, clonal dominance, and disease progression. Only a few genes, when mutated, clearly determine the phenotype: SF3B1 with MDS with ring sideroblasts (MDS-RS),¹⁵ ATRX with acquired alpha-thalassemia, and SRSF2 with myeloproliferative/ MDS overlap syndromes.¹⁶ Spliceosome gene

mutations (e.g., SF3B1) are fairly specific to MDS-RS; impaired gene splicing leads to iron retention in the mitochondria of erythroid precursors and ringed sideroblasts.¹⁷

Table 7.1 Recurrent Somatic Gene Mutations in MDS

	Gene	Frequency in MDS (%)
Splicing factors	SF3B1 ^a	15–30
	SRSF2	5–10
	U2AF1	5–10
	ZRS2	5
Epigenetic regulators	TET2	15–25
	DNMT3A	12–18
	IDH1/ IDH2	5
	ASXL1	10–30
	EZH2	5–10
Transcription factors	RUNX1	5–15
	GATA2	<5
	ETV6	<5
	TP53	5–10
Growth factor signaling	NRAS/ KRAS	5–10
	JAK2	<5

^aPatients harboring mutations in **bold** have sufficient data to indicate worse prognosis independent of IPSS. Whereas SF3B1 mutations identify a subgroup, MDS-RS and MDS/ MPN, which fare better than IPSS-R would suggest. Some genes when mutated are more commonly found in other myeloid malignancies: SRSF2, NRAS/ KRAS, EXH2, and ASXL1 in CMML; JAK2 in MPN. Germline mutations in GATA2, RUNX1, and ETV6 are also possible in MDS.

CMML, chronic myelomonocytic leukemia; IPSS-R, Revised International Prognostic Scoring System; MDS, myelodysplastic syndromes; MDS-RS, MDS with ring sideroblasts; MPN, myeloproliferative neoplasm.

Current evidence does not support using somatic mutations alone for the diagnosis of MDS.¹⁸ No single gene is observed in the majority of MDS cases, and many genes are mutated in other disorders with different clinical implications. As an example, SF3B1 mutations occur in other hematologic malignancies like chronic lymphocytic leukemia (CLL).¹⁹ Occasionally, somatic myeloid mutations may be identified in healthy older individuals in the absence of cytopenias or dysplastic morphology, typically in DNMT3A or TET2 genes.²⁰ Termed clonal hematopoiesis of indeterminate potential (CHIP), these individuals have an increased risk of myeloid or lymphoid neoplasia and increased all-cause mortality.²¹ Mutation analysis can help identify clonal hematopoiesis and, depending on the context, provide clinically relevant information. Yet the role of somatic mutations in determining progression or stability of disease, as

well as response to therapy, is the subject of active investigation.

CLINICAL FEATURES

Anemia is the most common cytopenia at presentation; however, 80% have varying degrees of other cytopenias, namely thrombocytopenia and neutropenia.²² Approximately 17% of patients aged 65 years or older with unexplained anemia (“anemia of the elderly”) have peripheral blood count abnormalities consistent with MDS.^{23,24} Bleeding out of proportion to the severity level of thrombocytopenia is common due to platelet dysfunction. Similarly, neutrophil dysfunction can lead to bacterial and fungal infections without severe neutropenia.²⁵ Leukocytosis and splenomegaly are rare in the absence of MDS/ myeloproliferative neoplasm (MPN) overlap disorder. Comorbid immune disorders are also more frequently diagnosed simultaneously with MDS for unclear reasons; examples include relapsing polychondritis, vasculitis, and inflammatory bowel diseases.^{26,27} The clinical course of MDS is variable: Patients may be asymptomatic or have mild anemia progressing to transfusion dependence over many years, while others have an aggressive course with progressive pancytopenia and rapid evolution to acute leukemia. Even in the most advanced cases, death from cytopenias is likely to occur prior to leukemic transformation.²²

DIAGNOSTIC STUDIES AND MORPHOLOGIC CLASSIFICATION

Minimum diagnostic criteria for MDS require unexplained persistent cytopenia(s), and evidence of either clonality (such as the case with an MDS-defining cytogenetic abnormality) or unambiguous dysplastic marrow morphology (dysplasia in at least 10% of the cells of one of the myeloid lineages or excess blasts).^{28,29}

- Peripheral blood smear typically shows macrocytosis although rarely it can be normocytic due to mutations in the ATRX gene leading to an acquired α-thalassemia phenotype;³⁰ hypogranular neutrophils sometimes with Pelger–Huët nuclei and other abnormal nuclear patterns; and circulating micro-megakaryocytes. Significant numbers of large granular lymphocytes should raise suspicion of a T-LGL/ MDS overlap syndrome.
- Bone marrow biopsy is frequently hypercellular but may be frankly hypocellular or fibrotic in about 20% of MDS. Moderate to severe bone marrow fibrosis, although variably characterized histologically, is an important adverse prognostic feature.^{31,32} Abnormal localization of immature precursors (ALIPs) near bony trabeculae is characteristic of MDS. Increase in myeloblasts and dysplastic morphology in the white cell and/ or the megakaryocytic lineages can be seen on the aspirate smear.

Mononuclear, small, or dysplastic megakaryocytes are evidence of MDS. Erythroid dysplasia alone is less specific, but large numbers of ringed sideroblasts identify a specific MDS subtype.

- | Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS) are characterized by meaningful cytopenias and dysplasia, respectively, which do not meet the minimum diagnostic criteria for MDS.³³ A subset of patients with ICUS/ IDUS show evidence of clonal hematopoiesis as indicated by the presence of somatic mutations in MDS-related genes, termed clonal cytopenias of undetermined significance (CCUS). Although the natural history of these conditions is not yet well characterized, in some cases, they may precede a myeloid malignancy and the utility of serial follow-up is under investigation.
- | Chromosome analysis of marrow cells is critical; abnormal cytogenetics strongly influence prognosis. Even in the absence of conclusive morphologic dysplasia, a presumptive diagnosis of MDS can be made in the presence of specific recurring chromosomal abnormalities, including -5 and -7.²⁹ However, approximately 50% of MDS patients will have a normal karyotype as determined by routine metaphase cytogenetics.³⁴ Karyotyping should be repeated periodically as chromosome patterns can evolve. Fluorescent in situ hybridization (FISH) analysis may provide more subtle information than karyotyping alone. A chromosome breakage test for Fanconi anemia is recommended for younger patients even if physical examination is normal. SNP array karyotyping and somatic mutation testing, although currently used only in the research setting, may become more widely available when the prognostic significance of specific molecular abnormalities is clarified.
- | Flow cytometry has limited utility; blast enumeration, critical to prognosis, can be assessed by routine morphology. Nevertheless, expert flow cytometry can be highly specific in diagnosing MDS, and may offer useful phenotypic information such as PNH or LGL.³⁵
- | Human leukocyte antigen (HLA) typing is needed to evaluate younger patients for allogeneic transplantation and may provide predictive information for responsiveness to immunosuppression.
- | Morphologic classification of MDS uses the World Health Organization (WHO) schema ([Table 7.2](#)) that incorporates dysplastic morphology, including blast count, cytopenias, and select mutation information.²⁹

Table 7.2 World Health Organization (WHO) Classification of Myelodysplastic Syndromes/ Neoplasms

Name	Ring Sideroblasts	Myeloblasts
MDS with single lineage dysplasia (MDS-SLD)	<15% (<5%) ^a	BM < 5%, PB < 1%, no Auer rods

MDS with multilineage dysplasia (MDS-MLD)	<15% (<5%) ^a	BM < 5%, PB < 1%, no Auer rods
MDS with ring sideroblasts (MDS-RS)		
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	≥15% / ≥5% ^a	BM < 5%, PB < 1%, no Auer rods
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	≥15% / ≥5% ^a	BM < 5%, PB < 1%, no Auer rods
MDS with isolated del(5q) ^b	None or any	BM < 5%, PB < 1%, no Auer rods
MDS with excess blasts (MDS-EB)		
MDS-EB-1	None or any	BM 5%–9% or PB 2%–4%, no Auer rods
MDS-EB-2	None or any	BM 10%–19% or PB 5%–19% or Auer rods
MDS, unclassifiable (MDS-U)	None or any	BM < 5%, PB = 1%, no Auer rods
■ With 1 % blood blasts	None or any	BM < 5%, PB = 1%, no Auer rods
■ With single lineage dysplasia and pancytopenia	15% ^d	BM < 5%, PB = 1%, no Auer rods
■ Based on defining cytogenetic abnormality ^c		BM < 5%, PB = 1%, no Auer rods
Refractory cytopenia of childhood	None	BM < 5%, PB < 2%

^aIf SF3B1 mutation is present.

^bdel(5q) alone or with one additional abnormality except -7 or del(7q).

^cComplex karyotype (three or more abnormalities), unbalanced abnormalities such as -7/ del(7q), del(5q)/5t(5q), i(17q)t(17p), -13/ del(13q), and del(11q) and balanced abnormalities, such as t(11;16)(q23.3;p13.3), t(3;21)(q26.2;q22.1), and 5(1;3)(p36.3;q21.2).

^dCases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD.

BM, bone marrow; MDS, myelodysplastic syndromes; PB, peripheral blood.

From Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127:2391–2405.

PROGNOSTIC CRITERIA AND CLINICALLY RELEVANT SUBTYPES

- Accurate classification and prognosis for this highly heterogeneous disorder is necessary to individualize therapy. Several prognostic models have been developed to risk stratify patients with MDS: The Revised International Prognostic Scoring System (IPSS-R) is the most functional (Tables 7.3 and 7.4). Derived from a large series of treatment-naïve MDS patients, information is combined from cytogenetics, cytopenias, and blast count to generate a prognostic score that places patients in five clinical risk groups with differing risks of progression to acute leukemia and survival.³⁶ See

<http://www.mds-foundation.org/ipss-r-calculator>

- Age, bone marrow fibrosis, and somatic mutations (Table 7.1) are not included in the IPSS-R model but have independent prognostic significance.⁸
- 5q-syndrome is one of the several specific MDS syndromes. Deletion of 5q, between bands q31 and q33, is separate in the WHO classification. 5q-usually manifests as anemia, with or without mild neutropenia and platelet counts either preserved or elevated. The prognosis is relatively good. Several cytokines, growth factors, and their receptors are found at the 5q locus; haploinsufficiency of ribosomal gene RPS14 has been identified as potentially causative. Lenalidomide (Celgene Corporation, Summit, NJ), a thalidomide analog, is especially efficacious in 5q-syndrome.
- Hypocellular MDS, although not categorized in any schema, may be easily confused with aplastic anemia, and patients may respond more favorably to immunosuppression with antithymocyte globulin (ATG) or alemtuzumab.
- Chronic myelomonocytic leukemia (CMML) is biologically distinct, now classified as a myelodysplastic neoplasm/ MPN by the WHO, which is discussed in other chapters.
- Therapy-related (or secondary) MDS is an important subtype, constituting about 15% of cases in most series. This subtype has the highest rate of progression (75%) to acute leukemia, is difficult to treat, and is rapidly fatal. Almost all patients have recurrent chromosomal abnormalities: deletions in chromosomes 5 and/ or 7 occur at a mean interval of 4 to 5 years after exposure to alkylating agents, and 11q23 abnormalities follow in a shorter period after topoisomerase II inhibitors. A very high frequency of therapy-related MDS is seen in patients who have undergone high-dose chemotherapy with autologous stem cell rescue (up to 19% at 10 years), more likely due to the cumulative prior therapy, especially with alkylating agents, rather than the autologous transplant itself. Overall, the median survival period is only 9 months.
- MDS can be associated with LGL. Significant numbers of circulating T-LGLs should prompt suspicion of this overlap syndrome; the diagnosis is confirmed by a clonal pattern of T-cell receptor gene rearrangement. Cases of T-LGL/ MDS may have hematological responses to therapy directed against the T-LGL component, such as cyclosporine (CsA) or alemtuzumab.
- MDS presenting in childhood or early adulthood (<40 years) is unusual and should lead to evaluation for inherited/ germline mutations indicative of a familial MDS: Fanconi anemia, GATA2-deficiency, telomere disease (dyskeratosis congenita), Li Fraumeni (TP53), and mutations in RUNX1, CEBPA, ANKRD26, ETV6, SRP72, and DDX41.³⁷

Table 7.3 Revised International Prognostic Scoring System (IPSS-R) Values

	0	0.5	1.0	1.5	2.0	3.0	4.0
Karyotype ^a	Very good	—	Good	—	Intermediate	Poor	Very poor

Blasts (%)	≤ 2	—	$>2 \text{ to } <5\%$	≥ 8	$5\% \text{--} 10\%$	$>10\%$	—
Hemoglobin (g/dL)	≥ 10	—	$8 \text{ to } <10$	—	—	—	—
Platelet (K/uL)	≥ 100	50 to <100	<50	—	—	—	—

^aVery good, -Y, del(11q); Good, normal, del(5q), del(12q), del(20q), double including del(5q); Intermediate, del(7q), +8, +19, i(17q), any other single or double independent clones; Poor, -7, inv(3)/t(3q)/del(3q), double including -7/ del(7q), complex: 3 abnormalities; and Very poor, complex: >3 abnormalities.

Scores: Low, 0; Int-1, 0.5–1.0; Int-2, 1.5–2.0; and High, >2.5.

Data from <http://www.mds-foundation.org/ipss-r-calculator>

Table 7.4 IPSS-R Prognostic Risk Category Clinical Outcomes

	Very Low	Low	Intermediate	High	Very High
Risk score	≤ 1.5	$>1.5\text{--}3$	$>3.0\text{--}4.5$	$>4.5\text{--}6$	>6
Proportion of patients	19%	38%	20%	13%	10%
Median survival (y)	8.8	5.3	3.0	1.6	0.8
Time to 25% evolution to AML (years)	NR	10.8	3.2	1.4	0.73

AML, acute myeloid leukemia; IPSS-R, Revised International Prognostic Scoring System; NR, not reached.

STANDARD TREATMENTS

Therapeutic strategies combine supportive care, suppression of the MDS clone and its leukemic progeny, efforts to improve bone marrow function, and curative attempts with allogeneic stem cell transplantation (SCT). Cytotoxic chemotherapy, including leukemia induction, has never been shown to improve survival despite patients achieving remission and is generally considered futile. Optimum management often requires the application of some or all of these approaches, preferably in the context of a research protocol (Table 7.5). Diagnostic precision and risk stratification is of pivotal importance in therapeutic decision-making for the MDSs. Grouping patients into lower risk versus higher risk strata according to IPSS-R is an essential step before considering therapy.

Supportive Care

Cytopenias are the single most important contributor to mortality. Supportive care to maintain adequate peripheral counts and to prevent or treat infections is critical to the patient with MDS. Even moderate degrees of anemia may not be well tolerated by the

elderly, especially in the presence of cardiopulmonary disease, and maintenance of higher hemoglobin levels (>9 g/ dL) can improve the quality of life without altering transfusion frequency.

- | Leukodepletion of blood products and single-donor platelet transfusions reduce the risk of eventual alloimmunization to platelets. If a prophylactic regimen is adopted, 10,000/ μ L is usually an adequate platelet transfusion threshold. Aminocaproic acid may be a useful adjunct in patients who are refractory to platelet transfusions although this has not been studied in clinical trials.
- | Neutrophils may be dysfunctional in MDS. Infections in the setting of neutropenia must be treated promptly and aggressively.
- | Growth factors are frequently used in MDS and are used at the lowest doses that maintain a response.³⁸ Combinations of erythropoietin and granulocyte colony-stimulating factor (G-CSF) are synergistic, with hematologic improvements in 40% of low-grade MDS patients.³⁹ Growth factor combinations can be effective even when individual factors fail to improve blood counts. Patients requiring fewer than 2 units of red blood cells per month and a serum erythropoietin level less than 500 U/ L have a higher probability of response ($>70\%$) to erythropoietin plus G-CSF according to an established predictive model.^{39–41} Erythropoietin and G-CSF therapy does not appear to hasten leukemic progression, but there is also little evidence for a positive impact on survival. The thrombopoietin receptor agonists (eltrombopag and romiplostim) have shown some activity in clinical trials for MDS but their use remains entirely investigational.⁴² Thrombopoietin is active in stimulating hematopoietic stem and progenitor cells, and theoretically leukemic cells. Therefore, off-trial use of these drugs is cautioned until safety can be established. Recently, single-agent eltrombopag was not associated with an increased risk of leukemic progression when studied in patients with higher-risk MDS,⁴³ and preliminary data on lower-risk MDS are promising.
- | Iron overload is inevitable in patients with prolonged transfusion dependence of red cells, and hyperferritinemia (levels $>2,500$ ng/ mL) is associated with inferior survival, suggesting that patients may benefit from chelation.⁴⁴ However, chelation therapy for MDS is controversial because definitive studies proving a benefit have not been performed. Iron chelation should be considered in patients who are younger, without serious comorbidities, and who are in favorable diagnostic categories.

Table 7.5 Investigational Therapeutic Strategies for Myelodysplastic Syndrome

Supportive/ growth factors

- Thrombopoietin receptor agonists (eltrombopag, romiplostim)
- TGF- β target (luspatercept, galunisertib)

Newer HMAs

- Oral HMA (SGI-110, CC-486, ASTX727)
- Class 1 HDAC inhibitor (mocetinostat, panobinostat)

Combination therapies

- HMA + ESAs, HMA + eltrombopag
- Lenalidomide + eltrombopag, lenalidomide + ESAs

Epigenetic targets

- IDH1/ IDH2 inhibitors
- BET inhibitors

Immune checkpoint inhibitors

- PD1, PD-L1 inhibitors (nivolumab, durvalumab)
- CTLA4 inhibitor (ipilimumab)

Signal transduction inhibitors

- PLK-1 inhibitor (volasertib)
- Multikinase (rigosertib)
- EGFR inhibitor (erlotinib)

BET, bromodomain and extraterminal; EGFR, epidermal growth factor receptor; ESA, erythropoietic stimulating agents; HDAC, histone deacetylase; HMA, hypomethylating agents; IDH, isocitrate dehydrogenase; LSD, lysine-specific demethylase 1; PD1, programmed death 1; PD-L1, programmed death-ligand 1; PLK-1, polo-like kinase 1; TGF- β , transforming growth factor β .

Stem Cell Transplantation

Allogeneic SCT is the only curative therapy but has been particularly underutilized in MDS because of older recipient age, comorbidities, and nonavailability of HLA-identical sibling donors. In recent years, SCT options for MDS have evolved rapidly, catalyzed by careful patient selection through better risk stratification, improved outcomes with unrelated and alternate donors, along with general advancements in technology and supportive care. Reduced intensity conditioning (RIC) has greatly extended the safety of transplantation to older recipients with comorbidities. However, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1102 phase III trial has shown that the rates of relapse are higher with RIC, and it should be reserved for those unable to receive full conditioning intensity.⁴⁵

Favorable transplant outcomes are more likely in younger patients, those with a short interval between diagnosis and transplant, and patients with HLA-identical siblings.⁴⁶ Generally, patients with IPSS risk of Int-2 or high would benefit from an allogeneic transplant as soon as a donor is identified, whereas those with IPSS risk of low or Int-1 would benefit from waiting till progression.⁴⁷ Survival outcomes after SCT from HLA-matched unrelated donors have been similar to those from matched siblings; the improvement is in part attributed to the use of high-resolution HLA typing to screen for HLA disparity at the allele level.⁴⁶ Data from the Center for International Blood and

Marrow Transplant Research (CIBMTR) document that survival rates decrease precipitously in advanced-stage MDS; survival is approximately 30% for both HLA-matched related and unrelated transplants, which is comparable or slightly worse than the transplant outcomes for a similar age group with AML. The IPSS score also predicts relapse and survival; patients with low-risk disease (low risk/ Int-1 by IPSS) have significantly lower relapse rates (13% vs. 43%) and better disease-free survival (55% vs. 28%) than those of patients with high-risk MDS.⁴⁸ Therefore, the decision for SCT requires balancing the probability of disease progression with procedural morbidity and mortality.

Disease-Modifying Hypomethylating Agents (DNA Methyltransferase Inhibitors)

The DNA methyltransferases function to hypermethylate the CpG promoter regions of many tumor suppressor genes and decrease their gene expression. Hypermethylation is one of the many epigenetic modifications that can influence gene expression without changing the DNA sequence. In malignancy (such as in MDS), acquired hypermethylation of tumor suppressor genes downregulates expression, increasing the potential for dysplastic growth. Hypomethylating agents (HMAs) 5-azacitidine (Vidaza, Celgene Corporation, Summit, NJ) and its active metabolite 5-aza-2'-deoxyazacitidine (decitabine or Dacogen, Otsuka Inc, Rockville, MD) are inhibitors of DNA methyltransferase 1. At low doses, they induce cellular differentiation via effects on gene expression, whereas at higher doses, these analogs of cytidine can be incorporated into DNA (decitabine) or both RNA and DNA (azacitidine) to exert a direct cytostatic effect.

Azacitidine is currently approved for use for patients with all MDS subtypes. A definitive phase III trial (Aza-001) compared azacitidine (75 mg/ m²/ day × 7 days q4 weeks) versus a choice of three conventional regimens (best supportive care, low-dose Ara-C, or Ara-C + daunorubicin) for Int-2 to high IPSS-risk patients.⁴⁹ Patients were treated until disease progression. A significant survival benefit of azacitidine treatment was seen over conventional care regimens (24.5 months vs. 15 months median overall survival). Clinical trials exploring the efficacy of oral azacitidine in MDS are ongoing.⁵⁰

Decitabine is Food and Drug Administration (FDA) approved for the treatment of IPSS-scored Int-1 or higher-risk patients. At a dose of 15 mg/ m² given intravenously to the inpatient every 8 hours for 3 days every 6 weeks, a phase III trial demonstrated a statistically significant overall response rate (17% vs. 0%) and improvement in quality of life compared with supportive care alone, but only a statistically insignificant trend toward improved overall survival or time to leukemogenesis.⁵¹ An alternative

outpatient dosing schedule of 20 mg/ m² given intravenously once daily for 5 consecutive days every 4 weeks demonstrated similar efficacy.⁵² A subsequent European Organisation for Research and Treatment of Cancer (EORTC) trial comparing the 3-day dosing schedule versus best supportive care in intermediate or high-risk MDS demonstrated improvements in progression-free survival and AML transformation but no impact on overall survival.⁵³

Dose and schedule optimization of the demethylating agents is ongoing. If tolerated, patients should receive an extended course of therapy (e.g., up to six cycles) before deeming it ineffective. Maintenance therapy is important, and hematological responses are not a precondition for a survival benefit. Demethylating agents should now be considered the first line of treatment in Int-2 and high-IPSS patients who are not transplant candidates or as a bridge to allogeneic BMT.⁵⁴

Although the DNA methyltransferase inhibitors (azacitidine and decitabine) represent the standard of care for transplant-ineligible high-risk MDS patients, not all patients will respond and most responders will experience disease progression within 2 years of response.⁴⁹ In this situation, the prognosis is poor with no definitive standard salvage option.⁵⁵ Switching HMAs may be considered if there is no response or disease progression after the initial response, but this approach has not yet been validated in significant patient numbers.⁵⁶

Immunomodulatory Agents and Immunosuppression

Lenalidomide is an oral analog of thalidomide with far greater potency, superior safety, and established efficacy in treating MDS. Lenalidomide is approved for use in patients with transfusion-dependent anemia and low-or Int-1-risk MDS with deletion 5q with or without other cytogenetic abnormalities. A landmark clinical trial in MDS patients demonstrated rapid responses (median time to response: 4.6 weeks), including cytogenetic response and complete transfusion independence in 67% of patients with isolated deletion 5q.⁵⁷ A confirmatory randomized phase III study comparing lenalidomide with best supportive care in low-or Int-1-risk MDS with deletion 5q demonstrated similar results.⁵⁸ Approximately 50% of patients with deletion 5q experienced grade 3 or 4 neutropenia or thrombocytopenia early in the course of the treatment. Those patients who had a greater platelet and neutrophil decline while on therapy had an increased transfusion-independent response, which suggests a direct cytotoxic effect of lenalidomide specific to the deletion 5q clone.⁵⁹ Fifty percent of patients will have a clinical and cytogenetic relapse after 2 to 3 years of treatment. A recent study suggests that there may be a small deletion 5q stem cell population that persists despite treatment with lenalidomide, accounting for the relapse rate.⁶⁰ Lenalidomide is indicated in patients with lower-risk MDS with deletion 5q and

transfusion dependence. Patients who lack deletion 5q demonstrated a 26% transfusion-independent response rate.⁶¹

Immunosuppression with horse ATG (hATG) at 40 mg/ kg/ day × 4 days produces hematological responses in about one-third of patients with low-risk MDS.⁶² Subjects who are younger than 50 years, with a shorter duration of red cell transfusion dependence and who are HLA-DR15+ are most likely to respond to immunosuppression.⁶³ In a retrospective analysis of 129 MDS patients treated with ATG and/ or CsA, younger age was the most significant factor favoring response to therapy.⁶⁴ Other favorable factors affecting the response were HLA-DR15 positivity and combination ATG plus CsA treatment. Alemtuzumab (Campath) is an alternative choice for immunosuppressive therapy, and has also been shown to improve blood counts and induce cytogenetic remissions in selected patients with Int-1 MDS.⁶⁵

Investigational Treatments

Many patients with MDS fail standard treatments and should be referred for clinical trials. Areas of active investigation include oral HMAs or novel histone deacetylase (HDAC) inhibitors, combination therapy, optimizing dosing and sequence of therapy, mutation-directed therapy, or immune checkpoint inhibitors (Table 7.5).⁶⁶ Some investigational agents may benefit specific subsets of MDS. Luspatercept and galunisertib are drugs capable of blocking transforming growth factor β activation, a pathway that impairs erythropoiesis, and preliminary data are promising for lower-risk MDS with ringed sideroblasts.^{67,68}

SUMMARY

With the advent of high-throughput genomic sequencing, we have gained new insights into the biology of MDS. When evaluating a patient, many factors need to be considered in the decision to treat, including age, comorbidities, karyotype, HLA status, prognostic-risk assessment, and availability of sibling and unrelated donor matches (Fig. 7.2). Growth factors should be tried on low-risk patients with MDS if their erythropoietin level is low. Azacytidine, decitabine, and lenalidomide remain the only three drugs with FDA approval—but a wide variety of newer drugs are under clinical trials. Lenalidomide can be used in patients with deletion 5q, and HMAs should be reserved for patients with higher-risk MDS or as a bridge to SCT. Hematopoietic SCT remains the only option for cure, and with improvements in care for older patients undergoing transplantation, the age limit has been progressively raised. When possible, patients should always be referred to a clinical trial to further define the heterogeneous nature of MDS and improve the therapeutic options for disease subsets.

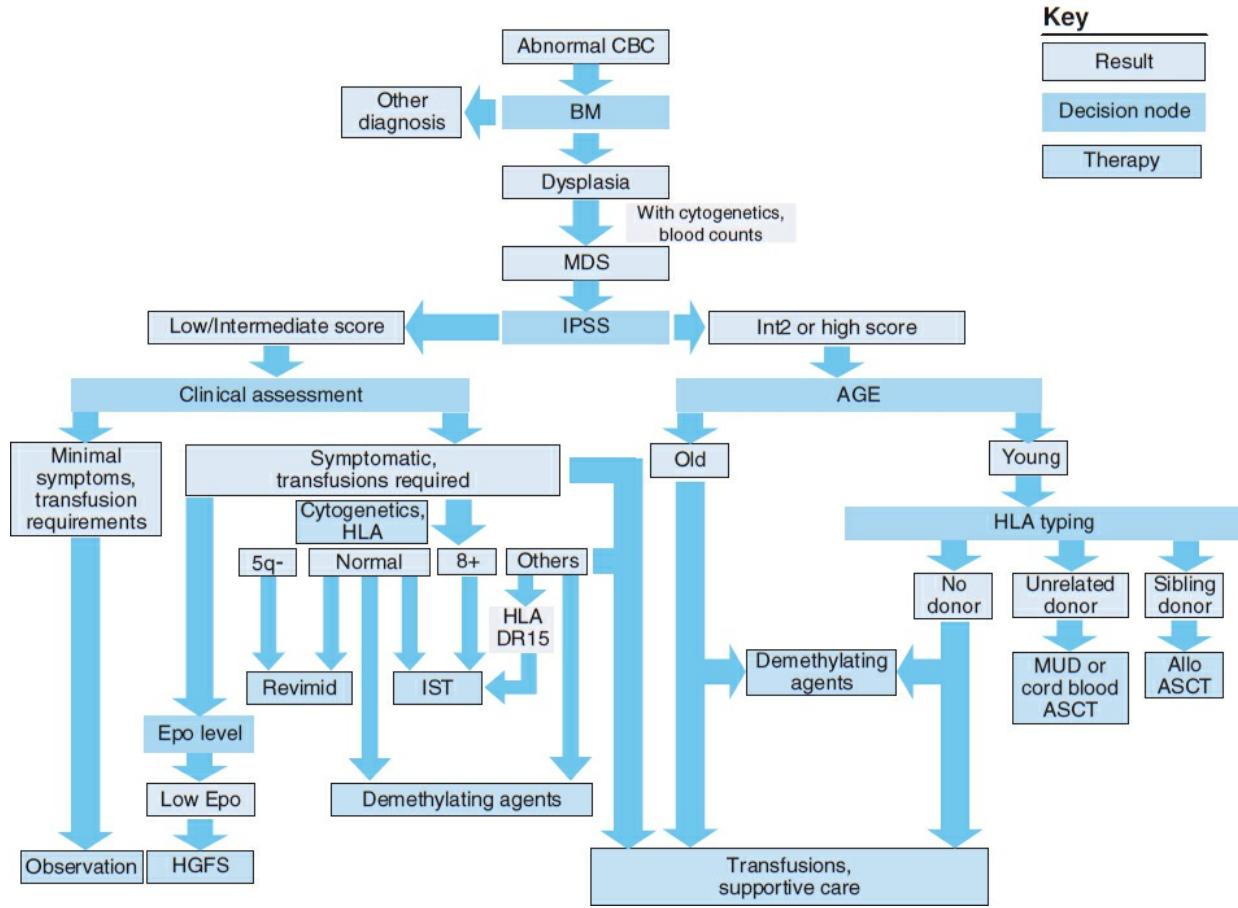


FIGURE 7.2 Risk-adjusted schema for management of MDS. When deciding on the treatment for MDS, a careful prognostic assessment is essential. Age and comorbidities also influence treatment options that are realistic. Note that 5-azacitidine is approved for use in patients with all MDS classifications and IPSS scores. Decitabine is approved for patients with IPSS scores that are intermediate-1 or higher.

IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndrome.

References

1. Rollison DE, Howlader N, Smith MT, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001–2004, using data from the NAACCR and SEER programs. *Blood*. 2008;112:45–52.
2. Cogle CR, Craig BM, Rollison DE, et al. Incidence of the myelodysplastic syndromes using a novel claims-based algorithm: high number of uncaptured cases by cancer registries. *Blood*. 2011;117:7121–7125.
3. Goldberg SL, Chen E, Corral M, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. *J Clin*

- Oncol. 2010;28:2847–2852.
4. Neukirchen J, Schoonen WM, Strupp C, et al. Incidence and prevalence of myelodysplastic syndromes: data from the Dusseldorf MDS-registry. *Leuk Res.* 2011;35:1591–1596.
 5. Ebert BL, Pretz J, Bosco J, et al. Identification of RPS14 as a 5q-syndrome gene by RNA interference screen. *Nature.* 2008;451:335–339.
 6. Maciejewski JP, Mufti GJ. Whole genome scanning as a cytogenetic tool in hematologic malignancies. *Blood.* 2008;112:965–974.
 7. Sloand EM, Pfannes L, Chen G, et al. CD34 cells from patients with trisomy 8 myelodysplastic syndrome (MDS) express early apoptotic markers but avoid programmed cell death by up-regulation of antiapoptotic proteins. *Blood.* 2007;109:2399–2405.
 8. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med.* 2011;364:2496–506.
 9. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med.* 2009;360:2289–2301.
 10. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med.* 2011;365:1384–1395.
 11. Walter MJ, Ding L, Shen D, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia.* 2011;25:1153–1158.
 12. Walter MJ, Shen D, Ding L, et al. Clonal architecture of secondary acute myeloid leukemia. *N Engl J Med.* 2012;366:1090–1098.
 13. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. *Nat Rev Cancer.* 2017;17(1):5–19.
 14. Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood.* 2007;110:4385–4395.
 15. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature.* 2011;478:64–69.
 16. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood.* 2013;122:3616–3627; quiz 99.
 17. Inoue D, Bradley RK, Abdel-Wahab O. Spliceosomal gene mutations in myelodysplasia: molecular links to clonal abnormalities of hematopoiesis. *Genes Dev.* 2016;30:989–1001.
 18. Bejar R. Myelodysplastic syndromes diagnosis: What is the role of molecular testing? *Curr Hematol Malig Rep.* 2015;10:282–291.
 19. Damm F, Mylonas E, Cosson A, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. *Cancer Discov.* 2014;4:1088–1101.

20. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med.* 2014;371:2477–2487.
21. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood.* 2015;126:9–16.
22. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood.* 1997;89:2079–2088.
23. Guralnik JM, Eisenstaedt RS, Ferrucci L, et al. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood.* 2004;104:2263–2268.
24. Steensma DP, Tefferi A. Anemia in the elderly: how should we define it, when does it matter, and what can be done? *Mayo Clin Proc.* 2007;82:958–966.
25. Toma A, Fenaux P, Dreyfus F, et al. Infections in myelodysplastic syndromes. *Haematologica.* 2012;97:1459–1470.
26. Hebbar M, Kozlowski D, Wattel E, et al. Association between myelodysplastic syndromes and inflammatory bowel diseases. Report of seven new cases and review of the literature. *Leukemia.* 1997;11:2188–2191.
27. Hebbar M, Brouillard M, Wattel E, et al. Association of myelodysplastic syndrome and relapsing polychondritis: further evidence. *Leukemia.* 1995;9:731–733.
28. Valent P, Horny HP, Bennett JM, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: consensus statements and report from a working conference. *Leuk Res.* 2007;31:727–736.
29. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127:2391–2405.
30. Gibbons RJ, Pellagatti A, Garrick D, et al. Identification of acquired somatic mutations in the gene encoding chromatin-remodeling factor ATRX in the alpha-thalassemia myelodysplasia syndrome (ATMDS). *Nat Genet.* 2003;34:446–449.
31. Della Porta MG, Malcovati L. Myelodysplastic syndromes with bone marrow fibrosis. *Haematologica.* 2012;96:180–183.
32. Della Porta MG, Malcovati L, Boveri E, et al. Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. *J Clin Oncol.* 2009;27:754–762.
33. Valent P, Bain BJ, Bennett JM, et al. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. *Leuk Res.* 2012;36:1–5.
34. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. *J Clin Oncol.* 2011;29:504–515.

35. Westers TM, Ireland R, Kern W, et al. Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European leukemianet working group. *Leukemia*. 2012;26(7):1730–1741.
36. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood*. 2012;120:2454–2465.
37. Feuerstein S, Drazer MW, Godley LA. Genetic predisposition to leukemia and other hematologic malignancies. *Semin Oncol*. 2016;43:598–608.
38. Rizzo JD, Brouwers M, Hurley P, et al. American Society of Clinical Oncology/American Society of Hematology Clinical Practice guideline update on the use of epoetin and darbepoetin in adult patients with cancer. *J Oncol Pract*. 2010;6:317–320.
39. Jadersten M, Malcovati L, Dybedal I, et al. Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. *J Clin Oncol*. 2008;26:3607–3613.
40. Hellstrom-Lindberg E, Gulbrandsen N, Lindberg G, et al. A validated decision model for treating the anaemia of myelodysplastic syndromes with erythropoietin + granulocyte colony-stimulating factor: significant effects on quality of life. *Br J Haematol*. 2003;120:1037–1046.
41. Greenberg PL, Attar E, Bennett JM, et al. NCCN clinical practice guidelines in oncology: myelodysplastic syndromes. *J Natl Compr Canc Netw*. 2011;9:30–56.
42. Li W, Morrone K, Kambhampati S, et al. Thrombocytopenia in MDS: epidemiology, mechanisms, clinical consequences and novel therapeutic strategies. *Leukemia*. 2016;30:536–544.
43. Platzbecker U, Wong RS, Verma A, et al. Safety and tolerability of eltrombopag versus placebo for treatment of thrombocytopenia in patients with advanced myelodysplastic syndromes or acute myeloid leukaemia: a multicentre, randomised, placebo-controlled, double-blind, phase 1/2 trial. *Lancet Haematol*. 2015;2:e417–e426.
44. Malcovati L. Impact of transfusion dependency and secondary iron overload on the survival of patients with myelodysplastic syndromes. *Leuk Res*. 2007;31 Suppl 3:S2–S6.
45. Pasquini MC, Logan B, Wu J, et al. Results of a phase III randomized, multi-center study of allogeneic stem cell transplantation after high versus reduced intensity conditioning in patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML): Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0901. *Blood*. 2015;126:LBA-8.
46. Runde V, de Witte T, Arnold R, et al. Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. *Chronic Leukemia Working Party of the European Group for Blood and Marrow*

- Transplantation. Bone Marrow Transplant. 1998;21:255–261.
47. Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. *Blood*. 2004;104:579–585.
 48. de Witte T, Hermans J, Vossen J, et al. Haematopoietic stem cell transplantation for patients with myelo-dysplastic syndromes and secondary acute myeloid leukaemias: a report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol*. 2000;110:620–630.
 49. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol*. 2009;10:223–232.
 50. Garcia-Manero G, Gore SD, Cogle C, et al. Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. *J Clin Oncol*. 2011;29:2521–2527.
 51. Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer*. 2006;106:1794–1803.
 52. Steensma DP, Baer MR, Slack JL, et al. Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: the Alternative Dosing for Outpatient Treatment (ADOPT) trial. *J Clin Oncol*. 2009;27:3842–3848.
 53. Lubbert M, Suciu S, Baila L, et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate-or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. *J Clin Oncol*. 2011;29:1987–1996.
 54. Greenberg PL, Stone RM, Bejar R, et al. Myelodysplastic syndromes, version 2.2015. *J Natl Compr Canc Netw*. 2015;13:261–272.
 55. Prebet T, Gore SD, Esterni B, et al. Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. *J Clin Oncol*. 2011;29:3322–3327.
 56. Borthakur G, Ahdab SE, Ravandi F, et al. Activity of decitabine in patients with myelodysplastic syndrome previously treated with azacitidine. *Leuk Lymphoma*. 2008;49:690–695.
 57. List A, Dewald G, Bennett J, et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. *N Engl J Med*. 2006;355:1456–1465.
 58. Fenaux P, Giagounidis A, Selleslag D, et al. A randomized phase 3 study of

- lenalidomide versus placebo in RBC transfusion-dependent patients with low-/intermediate-1-risk myelodysplastic syndromes with del5q. *Blood*. 2011;118:3765–3776.
59. Sekeres MA, Maciejewski JP, Giagounidis AA, et al. Relationship of treatment-related cytopenias and response to lenalidomide in patients with lower-risk myelodysplastic syndromes. *J Clin Oncol*. 2008;26:5943–5949.
 60. Tehranchi R, Woll PS, Anderson K, et al. Persistent malignant stem cells in del(5q) myelodysplasia in remission. *N Engl J Med*. 2010;363:1025–1037.
 61. Raza A, Reeves JA, Feldman EJ, et al. Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. *Blood*. 2008;111:86–93.
 62. Molldrem JJ, Leifer E, Bahcec E, et al. Antithymocyte globulin for treatment of the bone marrow failure associated with myelodysplastic syndromes. *Ann Intern Med*. 2002;137:156–163.
 63. Saunthararajah Y, Nakamura R, Nam JM, et al. HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. *Blood*. 2002;100:1570–1574.
 64. Sloand EM, Wu CO, Greenberg P, et al. Factors affecting response and survival in patients with myelodysplasia treated with immunosuppressive therapy. *J Clin Oncol*. 2008;26:2505–2511.
 65. Sloand EM, Olnes MJ, Shenoy A, et al. Alemtuzumab treatment of intermediate-1 myelodysplasia patients is associated with sustained improvement in blood counts and cytogenetic remissions. *J Clin Oncol*. 2010;28:5166–5173.
 66. Canaani J, Nagler A. Established and emerging targeted therapies in the myelodysplastic syndromes. *Expert Rev Hematol*. 2016;9:997–1005.
 67. Giagounidis A, Platzbecker U, Germing U, et al. Luspatercept treatment leads to long term increases in hemoglobin and reductions in transfusion burden in patients with low or intermediate-1 risk myelodysplastic syndromes (MDS): preliminary results from the Phase 2 PACE-MDS Extension Study. *Blood*. 2015;126. 23 (2015): 92. Web. 04 Dec. 2017.
 68. Valcarcel D, Verma A, Platzbecker U, et al. Phase 2 Study of Monotherapy Galunisertib (LY2157299 Monohydrate) in Very Low-, Low-, and Intermediate-Risk Patients with Myelodysplastic Syndromes. *Blood* 2015;126.

The Myeloproliferative Neoplasms: Polycythemia Vera, Essential Thrombocythemia, Primary Myelofibrosis, and Chronic Myelomonocytic Leukemia

Matthew J. Olnes

The chronic myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell diseases characterized by overproduction of one or more blood cell lines, which were first recognized by William Dameshek in 1951.¹ Unlike myelodysplasia, the MPNs are associated with normal maturation and effective hematopoiesis (Fig. 8.1). Organomegaly is common and often symptomatic. Varying degrees of extramedullary hematopoiesis and leukemic transformation are also seen.

A diagnostic marker important to Philadelphia chromosome-negative MPNs was identified in 2005 in Janus Kinase 2 (JAK2) V617F, a tyrosine kinase in the Janus Kinase-signal transducers of activators of transcription (JAK–STAT) pathway responsible for erythropoietin (EPO) receptor signaling. Somatic mutation JAK2V617F is a valine to phenylalanine substitution at codon 617 on chromosome 9. This mutation is present in most of the polycythemia vera (PV) and to varying degrees in essential thrombocythosis (ET) and primary myelofibrosis (PMF), as well as other myeloid malignancies (Table 8.1).^{2,3} Mutations within the calreticulin gene (CALR) were recently identified in 15% to 35% of patients with ET and PMF,^{4,5} and mutations in the thrombopoietin receptor MPL are present in 5% of patients with ET and PMF.⁶ Mutations in JAK2, CALR, and MPL are mutually exclusive, and one of these mutations occurs in approximately 85% of MPNs.⁶ Other mutations including LNK, CBL, TET2, ASXL1, IDH, IKZF1, EZH2, and DNMT3A have also been identified in the subsets of MPN patients, but their pathogenic role is less clear at present.

Although algorithms have been devised for PV, ET, and PMF, diagnosis may remain problematic in some instances because of a significant overlap of hematological manifestations. This chapter focuses on PV, ET, and PMF. Chronic myelogenous leukemia (CML) is also included in the category of MPNs but is discussed in another chapter. Chronic myelomonocytic leukemia (CMML) is included as well, although it is placed in a separate disease category (myeloproliferative/ myelodysplastic neoplasms) by the World Health Organization (WHO).⁷

POLYCYTHEMIA VERA

PV was first described by Vaquez in 1892. In the early 1900s, Osler recommended phlebotomy as treatment for PV, and Dameshek classified PV as a myeloproliferative disorder in 1951.¹ In 1967, Wasserman organized the Polycythemia Vera Study Group (PSVG) designed to define the natural history of PV and determine optimal therapeutic management.

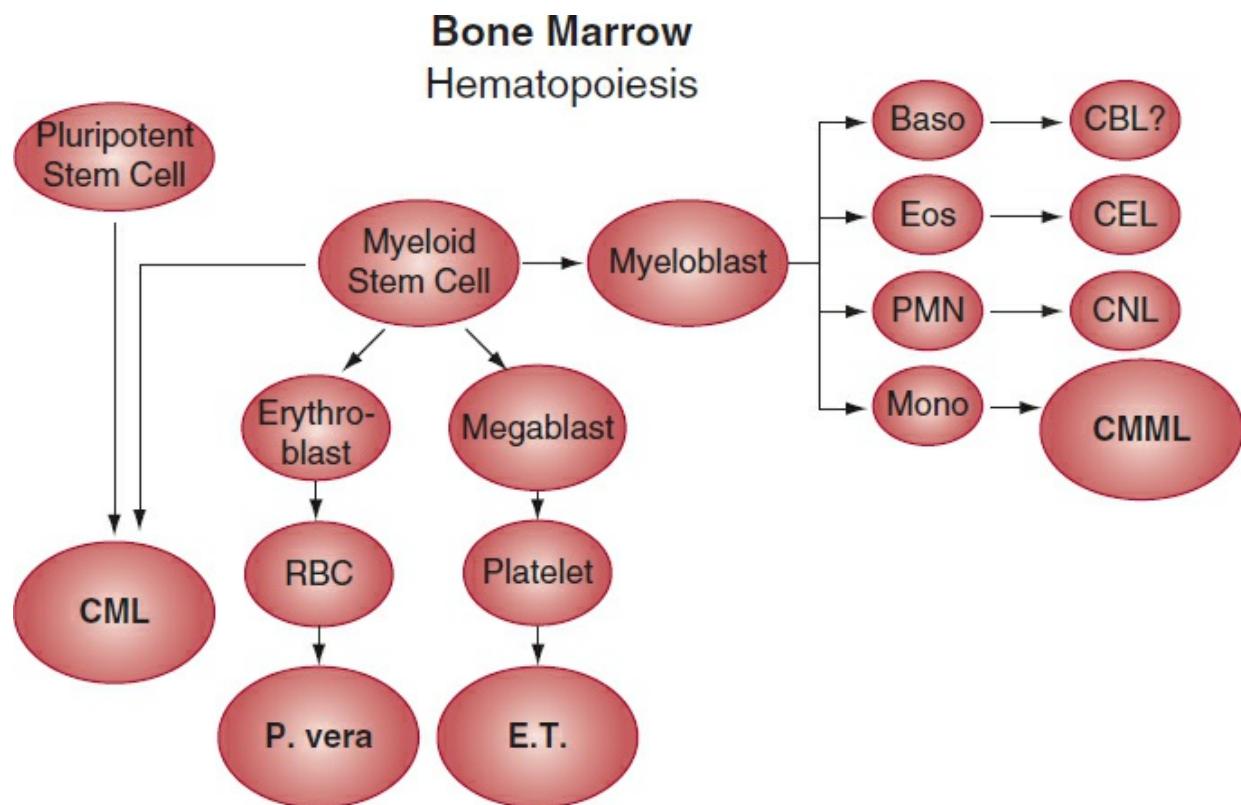


FIGURE 8.1 Bone marrow hematopoiesis.

Baso, basophil; CBL, chronic basophilic leukemia; CEL, chronic eosinophilic leukemia; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; CNL, chronic neutrophil leukemia; Eos, eosinophil; ET, essential thrombocythosis; Mono, monocytes; PMN, polymorphonuclear leukocytes; P. vera, polycythemia vera; RBC, red blood cells.

Table 8.1 Occurrence of the JAK2V617F Mutation^a

Disease	Frequency (%)
Polycythemia vera	96
Essential thrombocythosis	55
Primary myelofibrosis	65
Chronic myelomonocytic leukemia	3–9
Myelodysplastic syndromes	3–5
Acute myeloid leukemia	<5

^aThe JAK2V617F mutation is present in many myeloid neoplasms. It is present in most of the patients with polycythemia vera.

Epidemiology

The incidence of PV is 2 per 100,000. Rare familial cases have also been described.⁸ The median age at presentation is 60 years, and there is a slight male predominance of the disease. The median survival of untreated symptomatic PV is 6 to 18 months from diagnosis, 3.5 years for PV treated with phlebotomy, and 7 to 12 years for PV treated with myelosuppression. The incidence of leukemic transformation is 5% to 10% in the first 15 years after diagnosis.

Pathophysiology

PV is a clonal stem cell disorder with trilineage myeloid involvement. Some studies suggest that PV involves the B lymphocytes as well. PV is characterized by growth factor-independent erythroid proliferation producing an elevated red cell mass; *in vitro*, endogenous erythroid colony growth means that progenitors form colony forming units-erythrocyte-derived (CFU-E-derived) and burst forming units-erythrocyte derived (BFU-E-derived) colonies in the absence of EPO. PV may evolve from a proliferative phase of increased marrow activity and splenomegaly to a spent phase characterized by a leukoerythroblastic blood smear and extramedullary hematopoiesis producing massive hepatosplenomegaly, known as fibrotic transformation.

The JAK2V617F mutation was identified in 96% of patients with PV. In ET and PMF the JAK2V617F mutation is heterozygous, while in PV this mutation is homozygous.⁸ A higher JAK2V617F mutant allele burden is associated with fibrotic transformation and pruritus in patients with PV.⁹ In a minority of patients with PV that lack JAK2V617F, there are frameshift or point mutations in exon 12 of JAK2. These patients have erythrocytosis without thrombocytosis or leukocytosis, a low serum EPO level, and marrow erythroid hyperplasia without megakaryocyte or granulocyte abnormalities.^{10,11} Recent gene expression and mutational profiling studies indicate that JAK–STAT signaling plays a key role in the pathogenesis of PV.¹²

Although a few cases of congenital polycythemia caused by abnormal expression of a truncated form of the EPO receptor have been described,¹³ there is no evidence that EPO receptor mutations are involved in the pathogenesis of PV. Splicing defects in the EPO receptor RNA in some patients with PV are of unclear significance.¹⁴

At diagnosis, 10% to 20% of patients with PV have abnormal cytogenetics, including trisomy 8, trisomy 9, and deletion 20q. Loss of heterozygosity at chromosome 9p24, undetectable on routine cytogenetics, is found in 33% of patients. The frequency of chromosomal abnormalities increases with disease progression.⁷

Clinical Features

The elevated red cell mass in PV may result in a myriad of clinical signs and symptoms including:

Hypertension

Thrombosis, venous or arterial

Pruritis

Erythromelalgia (a sudden, severe burning pain in the hands or feet, usually accompanied by a reddish or bluish coloration of the skin)

Ulceration of fingers and toes

Joint pain

Epigastric pain

Weight loss

Headache

Weakness

Paresthesias

Visual disturbances

Vertigo

Tinnitus

Ruddy cyanosis

Conjunctival plethora

Pruritis aggravated by bathing is a distinctive feature of PV and is present in almost 50% of the patients. PV is the most common cause of erythromelalgia, which often responds to aspirin therapy. Increased cellular turnover in PV may result in gout or kidney stones. Palpable splenomegaly is found in 70% of the patients. Both bleeding and thrombosis can occur in PV. Less than 10% of patients experience major bleeding episodes, and hemorrhage is the cause of death in only 2% to 10% of PV. Various platelet defects are detectable and acquired von Willebrand disease exists in 33% of

the patients. An increased risk of osteoporotic fractures was recently observed in a cohort of patients with PV and ET.¹⁵

Thrombotic events (coronary events, cerebral vascular accidents, deep venous thrombosis [DVT], pulmonary embolism [PE], mesenteric thrombosis, and many others) are a major complication of PV. They are likely to arise from abnormalities in blood viscosity, platelets, and leukocytes.¹⁶ Multiple series have documented the incidence of major thrombosis to be 34% to 39% at diagnosis; 66% of these are arterial events and one-third are venous.^{16–18} Increased risk of thrombosis is associated with age >65 years, hematocrit >45%,¹⁹ leukocytosis $\geq 15 \times 10^9 / L$,²⁰ and a history of thrombosis. Patients at high risk of thrombosis and thrombocytosis (i.e., older individuals, patients with a history of thrombosis or atherosclerotic disease), should be treated with hydroxyurea to lower platelet counts to <400,000 cells/ μL .²¹

Although erythrocytosis distinguishes PV from the other MPN, only 20% of PV patients present with erythrocytosis alone, while 40% have trilineage hyperplasia at the onset of disease. PV can also present with isolated leukocytosis or thrombocytosis. Laboratory abnormalities include elevated leukocyte alkaline phosphatase, lactate dehydrogenase (LDH), uric acid, and elevated serum B12 (in 40% of patients). Secondary causes of an elevated red cell mass should also be excluded. Typical bone marrow findings in PV include hypercellularity, atypical megakaryocyte hyperplasia and clustering, and a decreased stainable iron.

The risk of transformation to acute leukemia is 1.5% in patients treated with phlebotomy alone. Patients with PV have a 10% to 25% risk of fibrotic transformation at 10 and 25 years of follow-up, respectively. Fibrotic transformation is characterized by normalization of the red cell mass associated with cytopenias, increasing splenomegaly due to extramedullary hematopoiesis, progressive reticulin deposition, and collagen fibrosis of the bone marrow.

Diagnostic Criteria

The WHO criteria for the diagnosis of PV are based on clinical and laboratory characteristics (Table 8.2).⁷ A bone marrow biopsy is not mandatory to make a diagnosis of PV in a patient who otherwise fulfills the WHO criteria. In the 2016 WHO guidelines, an elevated red cell mass is not an absolute requirement for diagnosis, while a JAK2V617F or similar exon 12 mutation can be used to diagnose PV. Although erythrocytosis distinguishes PV from the other MPNs, not all patients with PV have elevated hematocrits and not all patients with elevated hematocrits have PV. Although dehydration can cause spurious elevation of the hematocrit, resulting in apparent erythrocytosis, a hematocrit greater than 60% in men or 55% in women is usually caused by an elevated red cell mass. Conversely, erythrocytosis may be masked by

expanded plasma volume secondary to splenomegaly or by occult blood loss. Iron deficiency can also cause a decrease in the hematocrit in patients with PV. Secondary erythrocytosis caused by an elevation of serum EPO must also be excluded.

Conditions associated with physiologically appropriate production of EPO caused by hypoxemia, as well as diseases associated with inappropriate EPO production that result in erythrocytosis are listed in [Table 8.3](#).

Laboratory studies that may be useful in the evaluation of erythrocytosis are:

Arterial blood gas measurement

Iron studies

Serum EPO level

Liver and kidney function studies

Abdominal ultrasound or computed tomography (CT) scan

Bone marrow aspirate and biopsy

Red cell mass

[Table 8.4](#) shows clinical findings and assay results other than JAK2 mutational status that can be useful for distinguishing secondary polycythemia from PV.^{22,23} Gene expression profiling and mutational analysis are being investigated to help discriminate PV from secondary polycythemia.^{21,22,23} For example, a polymerase chain reaction (PCR)-based assay for overexpression of PRV1 (CD177) mRNA in peripheral granulocytes is positive in most patients with PV but not in secondary erythrocytosis.²⁴ Reduced thrombopoietin (TPO) receptor (c-MPL) levels have been described in PV megakaryocytes and platelets and in some patients with ET and PMF. Production of endogenous erythroid colonies in vitro is seen in PV but not in secondary erythrocytosis.²⁵ When it is impossible to make a definitive diagnosis, laboratory evaluation should be repeated in 3 months.

Table 8.2 The 2016 World Health Organization Criteria for Diagnosis of Polycythemia Vera

WHO Criteria	Polycythemia Vera ^a	Essential Thrombocythemia ^a	Primary Myelofibrosis ^a
Major criteria	<ol style="list-style-type: none"> 1. Hgb >16.5 g/ dL (men), >16.0 g/ dL (women), or Hgb or Hct >49% in men, Hct >48% in women, or red cell mass >25% above mean normal predicted value 2. BM biopsy showing hypercellularity for age with trilineage growth including prominent erythroid, 	<ol style="list-style-type: none"> 1. Platelet count $\geq 450 \times 10^9 \text{ L}^{-1}$ 2. Megakaryocyte proliferation with large and mature morphology. No or little granulocyte or erythroid proliferation, and rarely a minor (grade 1) increase in reticulin fibers. 	<ol style="list-style-type: none"> 1. Megakaryocyte proliferation and atypia^b accompanied by either reticulin and/or collagen fibrosis. 2. Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm

	granulocytic, and megakaryocytic proliferation with pleomorphic mature megakaryocytes	3. Not meeting WHO criteria for CML, PV, PMF, MDS, or other myeloid neoplasm	3. Demonstration of JAK2V617F, CALR, or MPL mutation or other clonal marker, or no evidence of reactive marrow fibrosis
	3. JAK2V617F or similar mutation	4. Demonstration of JAK2V617F, CALR, or MPL mutation	
Minor criteria	1. Subnormal serum Epo level	1. Presence of a clonal marker or absence of reactive thrombocytosis	1. Leukoerythroblastosis 2. Increased serum LDH 3. Anemia 4. Palpable splenomegaly 5. Leukocytosis $> 11 \times 10^9 / L$

^aDiagnosis of PV requires meeting either all three major criteria or the first two major criteria and the minor criterion. Diagnosis of essential thrombocythemia requires meeting all four major criteria, or the first three major criteria and the minor criterion. Diagnosis of PMF requires meeting all three major criteria and at least one minor criterion.

^bSmall to large megakaryocytes with an aberrant nuclear/ cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.

CML, chronic myelogenous leukemia; EEC, endogenous erythroid colony; Epo, erythropoietin; Hct, hematocrit; Hgb, hemoglobin; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; PMF, primary myelofibrosis; PV, polycythemia vera; WHO, World Health Organization.

Staging and Prognostic Features

In untreated PV, median survival is only 6 to 18 months; death most frequently results from thrombosis. Age greater than 65 years and a previous history of thrombosis are the major risk factors for thrombosis.²¹ The other causes of mortality include transformation to acute leukemia or fibrotic transformation.

Treatment

Treatment goals are to (1) relieve the clinical symptoms that result from an elevated red cell mass, (2) decrease thrombotic risk, and (3) slow or prevent leukemic transformation. The efficacy of therapies must be balanced against their toxicities.

Table 8.3 Conditions Related to Erythropoietin Production

EPO Overproduction Secondary to Hypoxia

Lung disease

High altitude

Smoking (carboxyhemoglobin)

Cyanotic heart disease

Methemoglobinemia

High oxygen affinity hemoglobin

Cobalt

EPO Overproduction

Tumors— renal, brain, hepatoma, uterine fibroids, pheochromocytoma

Renal artery stenosis

Neonatal

Inappropriate EPO secretion

Bartter syndrome

Renal cysts, hydronephrosis

Other Causes

EPO receptor hypersensitivity

Congenital erythrocytosis

Androgen therapy

Adrenal tumors

Autotransfusion (blood doping), self injection of EPO

Polycythemia vera^a

^aEPO levels in PV may be either low or normal; high EPO levels are not consistent with PV.

EPO, erythropoietin; PV, polycytemia vera.

Table 8.4 Features Distinguishing Polycytemia Vera from Secondary Polycytemia and Apparent Polycytemia^a

Findings	Polycytemia Vera	Secondary Polycytemia	Apparent Polycytemia
Splenomegaly	+	-	-
Leukocytosis	+	-	-
Thrombocytosis	+	-	-
Red blood cell volume	↑	↑	Normal
Arterial oxygen saturation	Normal	↓	Normal
Serum vitamin B ₁₂ level	↑	Normal	Normal
Leukocyte alkaline phosphatase	↑	Normal	Normal
Marrow	Panhypertrophy	Erythroid hyperplasia	Normal
EPO level	↓	↑	Normal
Endogenous CFU-E growth	+	-	-

^aThe differences listed are not present in all patients.

CFU-E, colony forming units-erythrocytes; EPO, erythropoietin.

Reprinted with permission from Beutler E. Williams Hematology. New York, NY: McGraw Hill; 2001.

The international PVSG began to organize large randomized trials in 1967. Patients were randomly assigned to phlebotomy, chlorambucil, or P32. The thrombosis rate for patients treated with phlebotomy alone was 37.3%, significantly higher than for those treated with chlorambucil or P32. However, there were several deaths secondary to leukemia in the chlorambucil and P32 arms of the study. The PVSG08 study showed that hydroxyurea significantly lowered the risk of thrombosis compared with phlebotomy alone, but patients who received hydroxyurea exhibited a trend toward increase in the risk of leukemic transformation. The European Collaboration on Low-Dose Aspirin in PV (ECLAP) trial followed a cohort of 518 patients with PV without a contraindication to aspirin therapy who received low-dose aspirin and were undergoing phlebotomy; major thrombosis was decreased by 60% in this cohort compared to controls, without a significant increase in bleeding.²⁰

Therapy in PV is based on risk of thrombohemorrhagic complications. The current risk stratification is outlined as follows:

Low Risk

Age <60 years

No history of thrombosis

Low Risk With Extreme Thrombocytosis

Low risk with platelet count >1 million/ μL

High Risk

Age ≥ 60 years

Previous history of thrombosis

Phlebotomy is the treatment of choice for most patients. The hematocrit should be maintained at <45% in men, 42% in women, and <37% in late pregnancy. In addition, for patients aged 60 years or older, myelosuppression is recommended to decrease the thrombotic risk. Paradoxically, the initiation of phlebotomy is transiently associated with an increase in thrombotic risk and is greatest in the elderly. The Janus kinase inhibitor ruxolitinib was recently approved by the Food and Drug Administration (FDA) to treat patients with PV who are refractory to hydroxyurea.²⁶ Interferon- α has also been used for cytoreduction in younger patients, and during pregnancy. Busulfan or P32 may be used in the elderly who may be unable to tolerate hydroxyurea. or ruxolitinib. A suggested treatment algorithm is seen in [Figure 8.2](#).

Additional therapies may be required for other complications related to PV. Low-dose aspirin appears effective for the alleviation of microvascular sequelae including headache, vertigo, visual disturbances, distal paresthesias, and erythromelalgia. The

safety and benefits of low-dose aspirin in PV have been investigated in a multicenter project (ECLAP).^{14,27} Aspirin lowered the risk of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, and total mortality; but the treatment nonsignificantly increased major bleeding. Aspirin should not be used in patients with a history of hemorrhage. All PV patients with extreme thrombocytosis (more than 1 million platelets/ μL) should be assessed for acquired von Willebrand syndrome with a ristocetin cofactor activity level, and aspirin should be avoided in those with ristocetin cofactor levels of <30%.

Pruritis occurs in 40% to 50% of patients with PV. Variably effective measures include reduction of water temperature and the use of antihistamines. Other agents of uncertain efficacy for these symptoms include cholestyramine, psoralen + UVA (PUVA), and interferon- α . The selective serotonin reuptake inhibitors paroxetine (20 mg every day) or fluoxetine (10 mg every day) have been shown to provide relief in many patients suffering from pruritis.²⁸

Patients with PV undergoing surgery are at high risk of postoperative complications. Elective procedures should be postponed until the hematocrit has normalized for more than 2 months.

Fibrotic transformation occurs on an average 10 years after the initial diagnosis and is heralded by the development of cytopenias and splenomegaly. Hydroxyurea and interferon- α may alleviate cytopenias due to splenomegaly. Although splenectomy may provide some relief from these symptoms, hepatomegaly secondary to extramedullary hematopoiesis may be a consequence. Low-dose splenic irradiation usually provides only short-term relief.

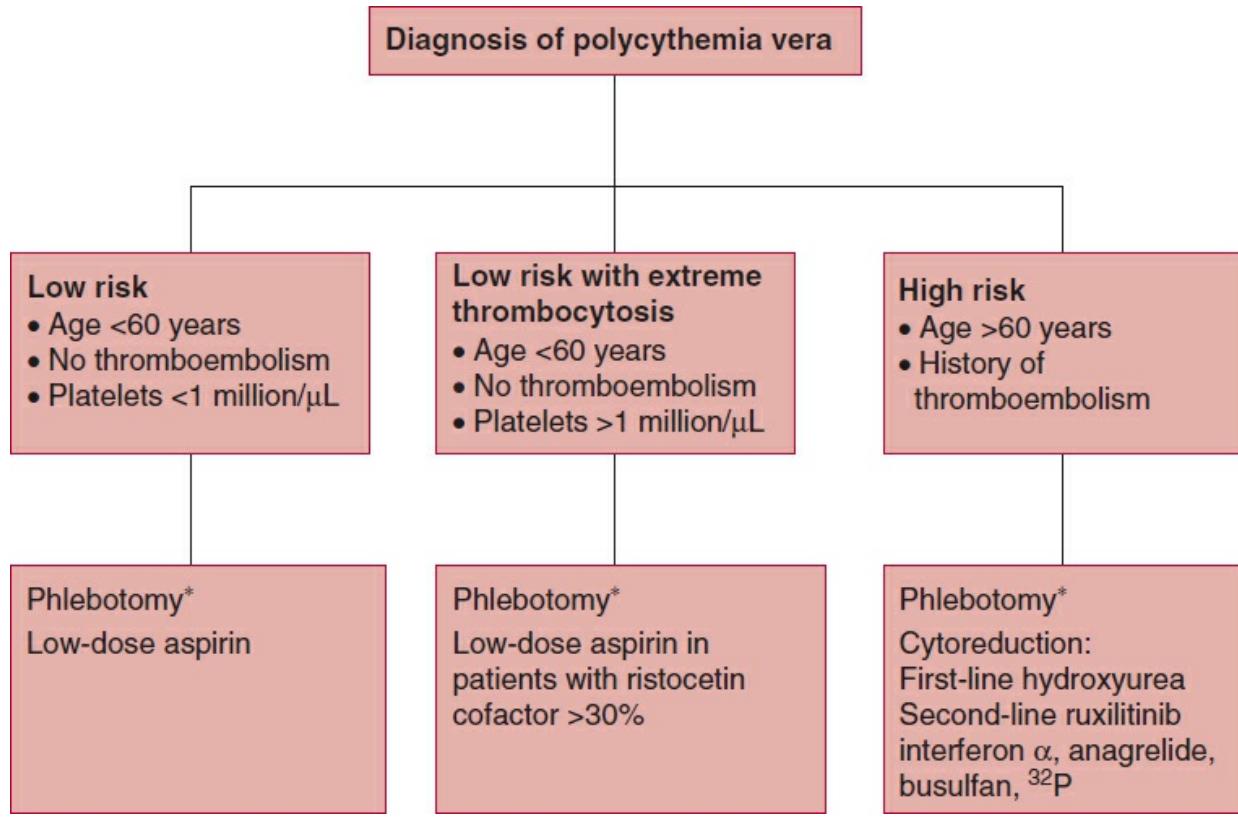


FIGURE 8.2 Treatment algorithm for polycythemia vera.

^aPhlebotomy HCT goals are <45% in men, <42% in women, and <37% in third-trimester pregnancy.

Allogeneic stem cell transplantation remains an option for advanced PV and can be curative. Outcomes are more favorable in those transplanted in fibrotic transformation than in those after evolution to acute leukemia.²⁹

ESSENTIAL THROMBOCYTOSIS

ET was first described by Epstein and Goedel in 1934^{29a} and called hemorrhagic thrombocythemia. Dameshek classified it as one of the myeloproliferative disorders in 1951.

Epidemiology

The annual incidence of ET is estimated at 1 to 2.5 per 100,000. Most patients are between ages 50 and 60 years at presentation, and there is no gender predilection. A second peak occurs around the age of 30 years when females are more often affected. Prevalence is higher in women than men 1.5 to 2 : 1. The median survival of ET is more than 10 years.⁷ Most patients with ET have a normal life expectancy without disease-related complications. The etiology of the disease is unknown.

Pathophysiology

Although ET has been traditionally described as a clonal disorder, X-chromosome inactivation studies suggest polyclonal hematopoiesis in some patients.³⁰ JAK2V617F is found in 55% of patients with ET; the mutation is associated with elevated hemoglobin and neutrophil counts, lower EPO levels, and increased progression to polycythemia.³¹ Somatic mutations in the CALR gene are present in 20% to 25% of patients with ET.⁶ Five percent of patients with ET have a mutation in the gene encoding the TPO receptor (c-MPL 515). Patients with ET tend to have normal to high TPO levels and many have low TPO receptor (c-MPL) levels.³² The rate of clonal cytogenetic abnormalities in ET is approximately 5%.

Clinical Features

As many as half of the patients are asymptomatic at presentation. Vasomotor symptoms occur in approximately 40% of patients and include visual disturbances, light-headedness, headaches, palpitations, atypical chest pain, erythromelalgia, livedo reticularis, and acral paresthesias. Thrombosis occurs in 15% of cases at presentation and in 10% to 20% during the course of the disease. Associated thrombotic events include DVT and PE, digital ischemia, portal vein thrombosis, and cerebrovascular and coronary ischemia. Major hemorrhage occurs in 5% to 10% of patients during the disease course. Other disease associations include recurrent first trimester abortions greater than the unaffected population and palpable splenomegaly, which is present in less than 50% of patients. The risk of leukemic transformation is low in the first decade after diagnosis but increases with each subsequent decade, but overall it is less than for other MPNs.

Diagnostic Testing

ET is characterized by persistent nonreactive thrombocytosis. The 2016 WHO diagnostic criteria are shown in [Table 8.2](#), and a recent update to these criteria include the inclusion of CALR and MPL mutations as diagnostic criteria, and the distinction between ET and pre-fibrotic PMF.⁷ The differential diagnosis for ET includes reactive thrombocytosis and other MPNs, as well as chronic myeloid disorders. The causes of thrombocytosis other than ET are listed as follows:

- Asplenia
- Acute hemorrhage
- Infections
- Hemolysis
- Postthrombocytopenic-rebound

Cancer

Inflammatory states (infection, collagen vascular disorders)

Iron deficiency

Pregnancy

MPN (Note that most of the MPNs may present with isolated thrombocytosis.)

Often a careful patient history excludes reactive thrombocytosis. In addition to JAK2, CALR, and MPL mutational analysis, other laboratory testing to assist in diagnosis include:

Iron studies to exclude iron deficiency.

C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and fibrinogen to rule out an occult inflammatory or malignant process.

Blood smear: Howell–Jolly bodies indicate anatomic or functional asplenia.

Bone marrow morphology.

Cytogenetics including fluorescence in situ hybridization (FISH) or PCR for BCR/ABL to exclude chronic myeloid leukemia (CML).

Decreased megakaryocyte c-MPL expression and increased granulocyte PRV1 and endogenous erythroid colony formation can be seen in both PV and ET, and do not distinguish between them.³³ When a definitive diagnosis is not initially possible, later periodic evaluation may be revealing.

Treatment

The decision to treat must be based on risk-based management because life expectancy in this disease is nearly normal. High-risk patients are those older than 60 years or those who have a history of thrombosis. Low-risk patients are younger than 60 years old with no history of thrombosis and may have extreme thrombocytosis (platelet count > 1 million/ μL).

The choice of therapy is based on its efficacy and toxicity.²¹ Therapeutic options include mechanical reduction of counts using plateletpheresis (in acute situations), myelosuppressive agents (alkylating agents, hydroxyurea, or radiophosphorus), maturation modulators (interferon- α or anagrelide), or antiplatelet agents ([Table 8.5](#)).^{34,35} Treatment should be focused on a platelet count goal of less than 400,000/ μL .

The treatment algorithm for ET is shown in [Table 8.6](#). All patients older than 60 years are at high risk. All ET patients with extreme thrombocytosis (more than 1 million platelets/ μL) should be tested for acquired von Willebrand syndrome with a ristocetin cofactor activity level. Low-dose aspirin can be used if ristocetin cofactor level is greater than 30%. Women of childbearing age not using birth control should be treated

with interferon- α based on anecdotal evidence of safety in pregnancy.

Low-risk patients (with or without extreme thrombocytosis) should be observed and not treated with cytoreductive therapy unless they develop high-risk features. Cytoreductive therapy should be administered to high-risk patients. Hydroxyurea is usually the first choice in high-risk patients, with interferon- α or busulfan used as the second line.

As in PV, low-dose aspirin is safe, and may even lower thrombotic complications in patients who do not have a significant bleeding risk.^{35,36} Aspirin is efficacious for treating vasomotor symptoms. It is contraindicated in patients who have experienced bleeding episodes, and in those with acquired von Willebrand syndrome.

Alkylating agents are generally avoided because of the risk of leukemia but are useful in the very elderly whose comorbidities make them intolerant to other therapies.

Hydroxyurea decreases thrombotic complications in patients with ET,²¹ but can cause bone marrow suppression. Questions remain about its leukemogenic potential in the absence of controlled randomized clinical trials. Hydroxyurea is contraindicated in women of childbearing age.

Anagrelide acts by interfering with platelet maturation but is associated with toxicities including fluid retention, headache, and palpitations, and is extremely expensive compared to hydroxyurea. Most side effects abate within 2 to 4 weeks after initiation of therapy, so it is prudent to slowly titrate the dose. Anagrelide should be avoided in patients with cardiovascular comorbidities because of its side effect profile. In a trial where randomized patients received aspirin plus hydroxyurea or anagrelide, there were lower rates of venous thromboembolism in the anagrelide arm, but arterial thrombosis, hemorrhage, and marrow fibrosis were increased.³⁷

Interferon- α is effective in reducing platelet count but is associated with significant side effects including flu-like symptoms and depression. Plateletpheresis is used in emergent thrombosis where abrupt decrease in platelet count is mandated. All patients with ET should be instructed to avoid smoking and avoid nonsteroidal anti-inflammatory drugs.

PRIMARY MYELOFIBROSIS

Myelofibrosis was first described in 1879 by Hueck^{37a} and was first included as one of the myeloproliferative diseases by Dameshek in 1951.¹

Epidemiology

The annual incidence of PMF is 0.5 to 1.5 per 100,000. The median age at presentation is 67 years. The male to female ratio is 1 : 1. PMF has the worst prognosis among the MPNs, with a median survival of 3 to 5 years. The etiology of the disease is unknown,

but a familial occurrence has been reported in rare kindreds. A high incidence of PMF was observed in individuals exposed to radiation at Hiroshima.

PMF that develops in late-stage PV or ET is referred to as postpolycythemic metaplasia (PPMM) or postthrombocythemic myeloid metaplasia (PTMM), respectively. De novo PMF is referred to as idiopathic myelofibrosis.

Pathophysiology

PMF is characterized by marrow fibrosis and extramedullary hematopoiesis. The marrow fibroblasts in PMF are not derived from the abnormal clone. Increased levels of platelet derived growth factor (PDGF), transforming growth factor (TGF) β , and other cytokines produced by megakaryocytes may contribute to the marrow fibrosis. Cytogenetic abnormalities are seen in approximately 50% of patients and include 13q, 20q, 12p, trisomy 8, and trisomy 9. High levels of CD34 $^{+}$ cells and of hematopoietic colony forming cells are typical in the circulation of patients with PMF and appear to correlate with the extent of myeloproliferation.³⁸

Table 8.5 Properties of Agents Used to Treat Polycythemia Vera and/ or Essential Thrombocytosis

Property	Hydroxyurea	Anagrelide	Interferon Alfa	Phosphorus-32
Drug class	Antimetabolite	Imidazoquinazolin	Biologic response modifier	
Mechanism of action	Not genotoxic, impairs DNA repair by inhibiting ribonucleotide reductase	Interferes with terminal differentiation of megakaryocytes	Myelosuppressive	β -Particle emitter, myelosuppressive
Specificity	Affects all cell lines	Affects platelet production, primarily	Affects all cell lines	Affects all cell lines
Pharmacology	Half-life approx. 4 h, renal excretion	Half-life approx. 1.5 h, renal excretion	Kidney is main site of metabolism	Half-life approx. 14 d
Starting dose	500 mg orally 2 or 3 times daily	0.5 mg orally 3 or 4 times daily	3–5 million units sc 3–5 d/ week	2.3 mCi/ m ² , may be repeated in 3–6 mo
Onset of action	Approx. 3–5 d	Approx. 6–10 d	3–26 weeks to obtain remission	4–8 weeks
Side effects observed in >10% of patients	Neutropenia anemia, oral ulcers, hyperpigmentation rash, nail changes	Headaches, forceful heartbeats, palpitations, diarrhea, fluid	Flu-like syndrome, fatigue, anorexia, weight loss, lack of ambition, alopecia	Transient mild cytopenia(s)

		retention		
Side effects observed in <10% of patients	Leg ulcers, lichen planus-like lesions of the mouth and skin, nausea, diarrhea	Congestive heart failure, arrhythmias, anemia, light-headedness, nausea	Confusion, depression, autoimmune thyroiditis or arthritis, pruritus, myalgia	Prolonged pancytopenia in elderly patients
Rare side effects	Fever, liver function test abnormalities	Pulmonary hypertension, pulmonary fibrosis	Pruritus, hyperlipidemia, transaminasemia	Delayed development of acute leukemia
Contraindications	Pregnancy, childbearing potential, breastfeeding	Congestive heart failure, pregnancy, childbearing potential	Anecdotal reports suggest safety during pregnancy	Pregnancy, childbearing potential

Reprinted with permission from Tefferi A. Recent progress in the pathogenesis and management of essential thrombocythemia. Leuk Res. 2001;25:373.

Table 8.6 Essential Thrombocytosis Treatment Algorithm

Low risk: Low-dose aspirin therapy

Low risk with extreme thrombocytosis: Low-dose aspirin for patients in whom von Willebrand syndrome has been excluded

High risk: Low-dose aspirin and cytoreductive therapy

The JAK2V617F mutation is found in 65% of patients, and the CALR mutation occurs in 20% to 25% of PMF patients. The MPL 515 mutation, also identified in ET, is present in approximately 5% of the patients. The other somatic mutations currently being investigated are LNK, TET2, ASXL1, IDH1/ IDH2, EZH2, DNMT3A, CBL, IKZF1, TP53, and SF3B1.²¹

Clinical Features

Approximately one-third of the patients are asymptomatic at diagnosis. Presenting complaints include profound fatigue, symptoms of anemia, abdominal discomfort, early satiety, or diarrhea caused by splenomegaly, bleeding, weight loss, and peripheral edema. The constitutional symptoms of fever and night sweats occur in most patients during the course of the disease. Splenomegaly is common in PMF and may be marked. Episodic left upper quadrant pain can occur secondary to splenic infarction. Palpable hepatomegaly is found in the majority of cases. Extramedullary hematopoiesis may occur in almost any organ.

Laboratory abnormalities in patients with PMF may include leukocytosis or leukopenia, and thrombocytosis or thrombocytopenia. The classic blood smear shows

leukoerythroblastosis but bone marrow morphologic findings vary from mild to marked fibrosis.³⁹ Osteosclerosis and periostitis can cause severe bone pain. Elevations of LDH, serum B12, and alkaline phosphatase are commonly seen. Transformation to acute leukemia occurs in approximately 20% of patients during the first decade after diagnosis.

Diagnostic Testing

The 2016 WHO diagnostic criteria for PMF are listed in [Table 8.2](#). The bone marrow is often inaspirable, a “dry tap.” The classic peripheral smear shows teardrop-shaped red cells, nucleated red cells, and granulocyte precursors (leukoerythroblastosis). However, other marrow infiltrative processes can cause a similar picture and must be excluded ([Table 8.7](#)). Absence of splenomegaly should make the diagnosis of PMF suspect. Many benign and malignant conditions mimic PMF, including metastatic cancer, granulomatous disease, connective tissue disease, lymphoma, systemic mast cell disease, hypereosinophilic syndrome, and other myeloid disorders. Both ET and PV can transform to PMF. Cytogenetics and FISH or PCR for BCR/ABL should be performed to exclude fibrotic CML.

Staging and Prognostic Features

PMF often progresses to marrow failure. The features associated with decreased survival include the following:

Advanced age

Hypercatabolic symptoms*

Anemia (hemoglobin < 10 g/ dL)**

Leukopenia (white cell count < 4,000/ mm³)**

Leukocytosis (white cell count > 30,000/ mm³)

Abnormal cytogenetics or the circulating granulocyte precursors or blasts*

(*May be an indication for splenectomy or **transplantation.)

Splenic irradiation may provide short-term improvement in patients with symptoms referable to organomegaly who are not surgical candidates.

Median survival in high-risk patients is less than 2 years, while patients with low-risk features have median survivals of over 10 years. Up to 30% of patients may progress to acute myeloid leukemia (AML), and this is thought to be more common after splenectomy.

Table 8.7 Causes of Marrow Fibrosis

Nonhematologic

Hematologic

Infections	Myeloproliferative disorders ET, PV, MMM
TB	
Leishmaniasis	Hypereosinophilic syndrome
Histoplasmosis	Systemic mastocytosis
HIV	CML
Connective tissue disease	AML-M7
Renal osteodystrophy	MDS
Metastatic cancer	Multiple myeloma
Vitamin D deficiency	Hairy cell leukemia
Hypothyroidism	Lymphoma
Hyperthyroidism	ALL
Paget disease	Gray platelet syndrome
Gaucher's disease	

ALL, acute lymphocytic leukemia; AML-M7, acute megakaryoblastic leukemia; CML, chronic myelomonocytic anemia; ET, essential thrombocythosis; MDS, myelodysplastic syndrome; MMM, myelofibrosis with myeloid metaplasia; PV, polycythemia vera; TB, tuberculosis.

A number of prognostic scoring systems have been developed for PMF. The International Prognostic Scoring Scale (IPSS) developed by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) is based on five adverse prognostic features noted on multivariate analysis: presence of constitutional symptoms, age greater than 65 years, hemoglobin less than 10 g/ dL, leukocyte count greater than 25,000/ μ L, and circulating blast greater than or equal to 1%.⁴⁰ Each category received one point and subjects with zero (low risk), one (intermediate risk-1), two (intermediate risk-2), or greater than or equal to 3 (high risk) at presentation had median survivals of 135, 95, 48, and 27 months, respectively. A dynamic IPSS (DIPSS) score has been developed, which can be used at any time during the disease course, and a more recent scoring system called DIPSS-plus incorporates the previous factors plus platelet count, red cell transfusion need, and an unfavorable karyotype.⁴¹

Treatment

Treatment for PMF is largely palliative. Approximately 30% of patients with anemia show improvement with a combination of androgen (oxymethalone 50 mg 4 times/ day or fluoxymesterone 10 mg 3 times/ day) and prednisone (30 mg/ day) therapy. Responses are usually brief in duration. EPO is most often ineffective. In patients with a more favorable prognosis who require transfusions for symptomatic anemia, timely initiation of chelation therapy is warranted.

Hydroxyurea, busulfan, interferon, or melphalan may be used to control thrombocytosis, leukocytosis, or organomegaly. Lower doses of hydroxyurea are used in

PMF than in ET or PV (start at 20 to 30 mg/ kg two or three times/ week). None of these agents is effective in preventing disease progression or improving survival. Anagrelide and imatinib are not effective. Thalidomide and prednisone may treat anemia and lenalidomide can be used if there is a deletion 5q abnormality.⁴² JAK2 inhibitors have shown significant reduction in splenic size and relief of symptoms in some patients. The JAK2 inhibitor ruxolitinib has been approved for the treatment of intermediate and high-risk myelofibrosis, and recent data indicate that it favorably impacts the natural history of PMF, although it is not a curative therapy.⁴³ There are ongoing studies using mTOR kinase inhibitors and proteasome inhibitors.

Allogeneic stem cell transplantation remains the only treatment with curative potential for patients with PMF.⁴⁴ Debate continues about the value of splenectomy before transplantation. Concerns about graft failure because of marrow fibrosis have proven unwarranted and, in fact, successful transplantation is associated with the resolution of marrow fibrosis. In both the European multicenter cooperative studies and Seattle single institution trials, overall survival after myeloablative transplantation was 60%. Reduced intensity conditioning is exploration of potential option for older patients and for those who are not candidates for myeloablative protocols.

CHRONIC MYELOMONOCYTIC LEUKEMIA

Epidemiology

The annual incidence of CMML is estimated at 4 cases per 100,000. There is a male predominance of 1.5 to 3:1. The median age at presentation is 70 years. Median survival is estimated at 12 to 18 months. The etiology of the disease is unknown.

Pathophysiology

The WHO classification places CMML in the category labeled myelodysplastic/myeloproliferative,⁷ which is appropriate because the marrow cells in this disease show dysplastic features, and there are many characteristics of myeloproliferation as well. The spleen, liver, and lymph nodes are the most common sites of extramedullary involvement. Clonal cytogenetic abnormalities are present in 20% to 40% of CMML and include trisomy 8, deletion 7q, and translocations involving 5q31 to 35; the latter activate the platelet derived growth factor receptor β (PDGFRβ) and are associated with eosinophilia.^{7,45,46} Mutational spectrum analysis has demonstrated the heterogeneity of CMML with various mutations in TET2, ASXL1, CBL, IDH1/ 2, KRAS, NRAS, JAK2V617F, UTX, DNMT3A, and EZH2.⁴⁷ One or more of these mutations are present in 86% of CMML patients,⁴⁷ and mutations in ASXL1, SRSF2, CBL, and IDH2 predict for an inferior survival.⁴⁸

Clinical Features

CMMI frequently presents with fatigue, fever, weight loss, or night sweats. There is a risk of infection because of neutropenia and bleeding secondary to thrombocytopenia. In approximately 50% of patients, the white count at presentation may be normal or decreased, while in the remainder it is elevated. In all cases there is persistent peripheral blood monocytosis, the defining feature of the disease. Progression to acute leukemia occurs in 15% to 30% of cases.⁴⁹

Diagnostic Testing

WHO diagnostic criteria include the following⁷:

Persistent peripheral blood monocytosis (greater than 1×10^9 per liter) with monocytes accounting for at least 10% of the white blood cells (WBC) for more than three months

Not meeting WHO criteria for BCR-ABL1+ CML, PMF, PV, or ET.

No evidence of PDGFRA, PDGFRB, or FGFR1 rearrangement or PCM1-JAK2.

Less than 20% blasts in the blood or bone marrow

Dysplasia of one or more myeloid lineages

Clonal cytogenetic abnormality

If dysplasia is absent, the diagnosis can be made if there is a clonal abnormality and no other causes of monocytosis.

Staging and Prognostic Features

Based on peripheral blood leukocyte counts, the French American British (FAB) group proposed dividing CMML into a dysplastic and a proliferative form with a white count greater than 13,000/ mm³. Attempts to evaluate the prognostic value of these distinctions have yielded disparate results. The recent analysis of CMML diagnosed based on FAB classification identified the following factors as independently associated with shorter survival: Hemoglobin < 12 g/ dL; lymphocyte count > 2,500/ mm³; medullary blast count 10% or more, and presence of circulating immature myeloid cells. Median survival was 12 months.⁵⁰ A recent investigation of 414 patients revealed an abnormal karyotype was associated with poorer overall survival and a higher risk of leukemic transformation. Low-risk category included a normal karyotype or loss of the Y chromosome as single anomaly; high-risk patients had trisomy 8, abnormalities of chromosome 7, or complex karyotype. All other abnormalities were intermediate risk. Five-year overall survival for low, intermediate, and high-risk cytogenetics were 35%, 26%, and 4%, respectively.⁵¹ Similarly, the presence of mutations in ASXL1 confers a poor prognosis, and a new prognostic score including age, hemoglobin, WBC, platelet

count, and ASXL1 status appears to be more predictive of clinical outcomes than previous scores.⁴⁸

Treatment

Treatment approaches are all experimental, and none has proven effective in modifying the natural course of the disease. Evaluation of treatment responses of patients with CMMI specifically is difficult, because they have historically been grouped under the myelodysplastic syndromes. Growth factors have been used in an attempt to treat cytopenias and low-dose chemotherapy during the preleukemic phase of the disease. Hydroxyurea is effective in controlling cell counts in the proliferative phase. Although many patients respond initially to chemotherapy, complete responses are rare and remissions generally short-lived. Various low-dose chemotherapeutic agents including cytarabine, topotecan, fludarabine, oral idarubicin, and oral etoposide have showed little success in altering long-term survival rates. Imatinib mesylate is effective in the rare CMMI patients who have PDGFR β translocations.^{52,53} Hypomethylating agents can induce complete or partial remissions in subsets of patients. Allogeneic stem cell transplantation remains the only option for cure.

References

1. Dameshek W. Some speculations on the myeloproliferative disorders. *Blood*. 1951;6:372–375.
2. Levine RL, Pardanani A, Tefferi A, et al. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat Rev Cancer*. 2007;7:673–683.
3. Vainchenker W, Delhommeau F, Constantinescu SN, et al. New mutations and pathogenesis of myeloproliferative neoplasms. *Blood*. 2011;118:1723–1735.
4. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Eng J Med*. 2013;369:2379–2390.
5. Nangalia J, Massie CE, Baxter EJ et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Eng J Med*. 2013;369:2391–2405.
6. Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia*. 2014;28:1472–1477.
7. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016; 127:2391–2405.
8. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 2005;365:1054–1061.
9. Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with

- polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. *Leukemia*. 2010;(24):1574–1579.
10. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356:459–468.
 11. Passamonti F, Elena C, Schnittger S, et al. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. *Blood*. 2011;117:2813–2816.
 12. Rampal R, Al-Shahrour F, Abdel-Wahab O, et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood*. 2014;123:e123–133.
 13. Kralovics R, Indrak K, Stopka T, et al. Two new EPO receptor mutations: truncated EPO receptors are most frequently associated with primary familial and congenital polycythemias. *Blood*. 1997;90(5):2057–2061.
 14. Spivak JL, Barosi G, Tognoni G, et al. Chronic myeloproliferative disorders. *Hematology Am Soc Hematol Educ Program*. 2003:200–224.
 15. Farmer S, Horváth-Puhó E, Vestergaard H et al. Chronic myeloproliferative neoplasms and risk of osteoporotic fractures: a nationwide population-based cohort study. *Br J Haematol*. 2013;163:603–610.
 16. Tefferi A, Elliot M. Thrombosis in myeloproliferative disorders: prevalence, prognostic factors, and the role of leukocytes and JAK2V617F. *Semin Hematol*. 2007;33:313–320.
 17. Gruppo Italiano Studio Policitemia. Polycytemia vera: the natural history of 1213 patients followed for 20 years. *Ann Intern Med*. 1995;123:514–515.
 18. Marchioli R, Finazzi G, Landolfi R, et al. Vascular and neoplastic risk in a large cohort of patients with polycytemia vera. *J Clin Oncol*. 2005;23:2224–2232.
 19. Marchioli R, Finazzi G, Specchia G et al. Cardiovascular events and intensity of treatment in polycytemia vera. *N Eng J Med*. 2013;368:22–33.
 20. Landolfi R, Di GL, Barbui T, et al. Leukocytosis as a major thrombotic risk factor in patients with polycytemia vera. *Blood*. 2007;109:2446–2452.
 21. Tefferi A, Vainchenker W. Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies. *J Clin Oncol*. 2011;29:573–582.
 22. Beutler E. *Williams Hematology*. New York, NY: McGraw Hill; 2001.
 23. Goerttler PS, Kreutz C, Donauer J, et al. Gene expression profiling in polycythaemia vera: overexpression of transcription factor NF-E2. *Br J Haematol*. 2005;129:138–150.
 24. Klippel S, Strunck E, Temerinac S, et al. Quantification of PRV-1 mRNA distinguishes polycytemia vera from secondary erythrocytosis. *Blood*. 2003;102:3569–3574.

25. Streiff MB, Smith B, Spivak JL. The diagnosis and management of polycythemia vera in the era since the Polycythemia Vera Study Group: a survey of American Society of Hematology members' practice patterns. *Blood*. 2002;99:1144–1149.
26. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *N Eng J Med*. 2015;372:426–435.
27. Landolfi R, Marchioli R, Kutt J, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. *N Engl J Med*. 2004;350:114–124.
28. Diehn F, Tefferi A. Pruritus in polycythaemia vera: prevalence, laboratory correlates and management. *Br J Haematol*. 2001;115:619–621.
- 29a. Fruchtman SM. Transplant decision-making strategies in the myeloproliferative disorders. *Semin Hematol*. 2003;40:30–33.
- 29b. Epstein E, Goedel A. Hemorrhagic thrombocythemia with a vascular, sclerotic spleen. *Virchows Archiv A Pathol Anat Histopathol*. 1934;293:233–248.
30. Harrison CN, Gale RE, Machin SJ, et al. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. *Blood*. 1999;93:417–424.
31. Campbell PJ, Scott LM, Buck G, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*. 2005;366:1945–1953.
32. Cerutti A, Custodi P, Duranti M, et al. Thrombopoietin levels in patients with primary and reactive thrombocytosis. *Br J Haematol*. 1997;99:281–284.
33. Kralovics R, Buser AS, Teo SS, et al. Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. *Blood*. 2003;102:1869–1871.
34. Tefferi A. Recent progress in the pathogenesis and management of essential thrombocythemia. *Leuk Res*. 2001;25:373.
35. Landolfi R, Marchioli R. European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP): a randomized trial. *Semin Thromb Hemost*. 1997;23:473–478.
36. van Genderen PJ, Mulder PG, Waleboer M, et al. Prevention and treatment of thrombotic complications in essential thrombocythaemia: efficacy and safety of aspirin. *Br J Haematol*. 1997; 97:179–184.
- 37a. Harrison CN. Essential thrombocythaemia: challenges and evidence-based management. *Br J Haematol*. 2005;130:153–165.
- 37b. Heuck G. Two cases of leukemia with peculiar blood and bone marrow findings, respectively. *Arch Pathol Anat*. 1879;78:475–496.
38. Barosi G. Myelofibrosis with myeloid metaplasia. *Hematol Oncol Clin North Am*. 2003;17:1211–1226.
39. Tefferi A. Myelofibrosis with myeloid metaplasia. *N Engl J Med*. 2000;342:1255–1265.

40. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*. 2009;113:2895–2901.
41. Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol*. 2011;29:392–397.
42. Tefferi A. Myeloproliferative neoplasms: a decade of discoveries and treatment advances. *Am J Hematol*. 2016; 91:50–58.
43. Cervantes F, Vannucchi AM, Kiladjian JJ et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. *Blood*. 2013;122:4047–4053.
44. Guardiola P, Anderson JE, Bandini G, et al. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Societe Francaise de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study. *Blood*. 1999;93:2831–2838.
45. Gunby RH, Cazzaniga G, Tassi E, et al. Sensitivity to imatinib but low frequency of the TEL/ PDGFRbeta fusion protein in chronic myelomonocytic leukemia. *Haematologica*. 2003;88:408–415.
46. Magnusson MK, Meade KE, Brown KE, et al. Rabaptin-5 is a novel fusion partner to platelet-derived growth factor beta receptor in chronic myelomonocytic leukemia. *Blood*. 2001; 98:2518–2525.
47. Jankowska A, Makishima H, Tiu R, et al. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. *Blood*. 2011;118(14):3932–3941.
48. Itzykson R, Kosmider O, Renneville A, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. *J Clin Oncol*. 2013;31: 2428–2436.
49. Cortes J. CMML: a biologically distinct myeloproliferative disease. *Curr Hematol Rep*. 2003;2:202–208.
50. Onida F, Kantarjian HM, Smith TL, et al. Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. *Blood*. 2002;99:840–849.
51. Such E, Cervera J, Costa D, et al. Cytogenetic risk stratification in chronic myelomonocytic leukemia. *Haematologica*. 2011;96:375–383.
52. Apperley JF, Gardembas M, Melo JV, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med*. 2002;347:481–487.
53. Magnusson MK, Meade KE, Nakamura R, et al. Activity of STI571 in chronic

myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene. *Blood*. 2002;100:1088–1091.

Neutrophil Disorders and Neutropenias

Matthew M. Hsieh and Harry L. Malech

Neutrophils or polymorphonuclear cells (PMNs) are 5 μm in diameter with distinctive multilobed nuclei and many small granules. Neutrophil maturation begins with myeloblasts in the bone marrow. These differentiate into promyelocytes characterized by the appearance of primary (azurophil) granules containing myeloperoxidase (MPO), followed by myelocytes characterized by the formation of secondary granules containing lactoferrin and gelatinase, and progress through metamyelocytes, band form, and finally mature neutrophils. This process is typically 10 to 14 days but may be accelerated in the setting of infection, in some cases leading to forms retaining excessive numbers of large azurophil granules (toxic granulation). Once mature neutrophils exit the bone marrow, they remain in circulation for about 6 to 12 hours. At sites of infection or inflammation, neutrophils adhere to and migrate between post-venule endothelial cells to exit blood vessels into the tissues where they last about 1 to 3 days. In the absence of overt infection, most neutrophils in the circulation progress to apoptosis and are taken up by macrophages in the spleen. Even without infection, there is a baseline rate of neutrophil migration into the mouth and gastrointestinal tract, where, together with the barrier function of the mucosa, they prevent entry of bacteria into tissues at those sites. This is why in the setting of severe neutropenia, the gastrointestinal tract is often the first site of invasive bacterial infection.

Neutrophils circulate in a metabolically quiescent state. When stimulated by inflammation or infection-related cytokines or chemotactic factors, they exit the circulation by adherence to endothelial cells and migrate to sites of inflammation. Neutrophils are one of the first cells to migrate to sites of inflammation, and thus represent the first line of defense against microbes. They internalize microbial particles by phagocytosis via Fc receptors and complement C₃, and granule contents and reactive oxidants are released into phagosomes to kill microbes. Increased numbers of life-threatening bacterial infections occur in association with inherited or acquired

disorders characterized by abnormal granule formation, poor neutrophil adherence, failure to produce microbicidal oxidants, or where there is very low production or increased destruction of neutrophils. The summary of neutrophil disorders and neutropenia is provided in [Table 9.1.](#)¹

NEUTROPHIL DISORDERS

Leukocyte Adhesion Deficiency

The β_2 integrins in neutrophils are particularly important for normal neutrophil egress from blood postcapillary venules, for migration through tissues and for complement-mediated phagocytosis. There are three leukocyte β_2 integrin adhesion molecules that share the CD18 protein antigen as a common subunit; CD11a/ CD18 (lymphocyte function-associated antigen-1), CD11b/ CD18 (macrophage 1 antigen), and CD11c/ CD18 (also known as p150/ 95). Mutations in the gene encoding CD18, leading to near-absent CD18 expression, are responsible for a disease known as leukocyte adhesion deficiency 1 (LAD-1). LAD-2 and -3 have been described in a handful of individuals, and relate to an abnormality of fucose glycosylation (ligand required for selectin binding) and FERMT3 protein-mediated integrin activation, respectively.² In general, “LAD” used without the numerical identification refers to LAD-1 because this is the defect responsible for the great majority of cases. LAD has an autosomal recessive inheritance pattern and affects only a few individuals per million. LAD is associated with recurrent life-threatening infections and other characteristic clinical manifestations. Diagnosis is usually made by flow cytometry measurement of the amount of CD11b or CD18 on the surface of neutrophils using specific antibodies. The severity of disease manifestations, including risk of early death from infection, appears to be correlated with the amount of β_2 integrins present. A moderate phenotype has 1% to 10% of normal levels of β_2 integrins, while the severe phenotype is associated with the presence of less than 1% detectable β_2 integrins. On the cellular level, there is poor neutrophil adhesion to endothelial and other immune cells, and neutrophils do not egress from the vasculature and migrate to sites of inflammation. Baseline peripheral blood neutrophil count, even in the absence of infection, is characteristically about two to three times normal; and when infections are present, neutrophil counts can exceed 60 k/ uL. Sometimes this is mistaken for a leukemic condition. Despite the very high circulating levels of neutrophils, there may be only mild erythema or pain at sites of infection and the patients fail to form pus, a condition that has been called “tissue neutropenia.” One of the hallmarks of severe LAD is delayed separation of umbilical cord, indicating a role for neutrophils in providing the proteases and hyaluronidases required for that event. A prominent manifestation of severe LAD is recurrent infections with large

nonhealing ulcers of the skin, particularly on the lower abdomen, perineum, and legs. Patients also have recurrent infections of the oral cavity (gingivitis, periodontitis with early loss of primary and secondary teeth), respiratory tract (sinusitis, otitis media, and pneumonia), gastrointestinal tract, and genital mucosa. Infection of the wall of the small bowel or colon complicated by perforation is a particular risk that often leads to a fatal outcome. Infections are commonly caused by *Staphylococcus aureus*, enteric organisms, and candida and aspergillus species. In patients with milder forms of LAD, who are not transplanted and survive past the first decade, the chronic large nonhealing ulcers of the lower extremities and groin become a particularly characteristic chronic problem and are very difficult to control or treat. Treatments include bacterial prophylaxis with trimethoprim/ sulfamethoxazole (TMP/ SMX), supportive antibiotics during acute infections, surgical debridement of skin when necessary, and skin grafting. There is a high mortality rate (~75%) of severe LAD in the first year of life. Successful bone marrow transplant is curative and should be considered for all patients with severe LAD.³

Table 9.1 Summary of Neutrophil Disorder and Neutropenia

Disease	Molecular or Genetic Defect	Pathogenic Organisms and Sites Affected	Clinical Presentation
LAD	CD18	Gram-negative enteric bacteria, <i>Staphylococcus aureus</i> , candida species, aspergillus species	Leukocytosis; recurrent infections of skin, soft tissue, and respiratory and GI tracts; periodontal disease; delayed separation of umbilical cord
MPO deficiency	Reduced MPO from multiple defects	Candida species in those with diabetes	Typically with no or mild clinical disease
CGD	Defective NADPH oxidases	Catalase-positive organisms: <i>S. aureus</i> , <i>Burkholderia cepacia</i> , aspergillus species, nocardia species, <i>Serratia marcescens</i>	Cellulitis, lymphadenitis, pneumonia; abscess formation in lungs, liver, brain, and bone; granuloma in GI or genitourinary tract
CHS	LYST mutation → giant granules	<i>S. aureus</i> , oropharyngeal organisms	Albinism, peripheral neuropathy, recurrent bacterial infections, periodontitis, easy bruising
SGD	C/ EBP ϵ	<i>S. aureus</i> , <i>Staphylococcus epidermidis</i> , enteric bacteria	Recurrent skin and lung infections
Drug-induced neutropenia	Peripheral clearance or marrow suppression	Not applicable	Infection severity dependent on degree of neutropenia

Infection-related neutropenia	Immune clearance or marrow suppression	Nonspecific	Nonspecific; anemia and thrombocytopenia may also occur
Severe congenital neutropenia	Some with ELA2 mutation	<i>S. aureus</i> , <i>Pseudomonas aeruginosa</i> , cellulitis, stomatitis, meningitis, perirectal abscess	Recurrent infections starting at 3–6 mo of age; responsive to G-CSF injections; increased risk of MDS/ AML
Cyclic neutropenia	Some with ELA2 19p13.3 mutation	Aphthous ulcers, gingivitis, stomatitis, cellulitis	21-day pattern of neutropenia; some may require G-CSF; no risk of MDS/ AML
Autoimmune neutropenia	Antineutrophil antibodies	Nonspecific	Coexisting autoimmune disorders
Idiopathic neutropenia	Unknown	Skin and oropharynx	Usually mild infections; rare severe infections; negative antineutrophil antibodies
Benign ethnic neutropenia	Unknown	Asymptomatic	Seen mostly in those of African descent, neutrophil count may range from 1,000 to 1,500/ uL

AML, acute myelogenous leukemia; CGD, chronic granulomatous disease; CHS, Chédiak–Higashi syndrome; G-CSF, granulocyte-colony stimulating factor; GI, gastrointestinal; LAD, leukocyte adhesion deficiency; MDS, myelodysplastic syndrome; MPO, myeloperoxidase; NADPH, nicotinamide adenine dinucleotide phosphate; SGD, specific granule deficiency.

Data from Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in phagocytes. *N Engl J Med.* 2000;343:1704.

Myeloperoxidase Deficiency

MPO is the most abundant protein in neutrophil granules. It resides in the primary (azurophilic) granules, and has antimicrobial functions (catalyzes the production of hypochlorous acid from chloride and the hydrogen peroxide product of the phagocyte nicotinamide adenine dinucleotide phosphate [NADPH] oxidase). MPO deficiency is the most common neutrophil abnormality, at an incidence of about 1 per 2,000 for partial deficiency and 1 per 4,000 for complete deficiency.⁴ Most individuals with MPO deficiency do not manifest any clinical problems, though ex vivo assays of bacterial and fungal killing demonstrate a defect associated with MPO deficiency. MPO deficiency is inherited through an autosomal recessive pattern, although it can also be manifested as an acquired abnormality associated with leukemia or myelodysplasia. Specific mutations in the MPO gene have been identified, which may affect transcription, translation, and/ or insertion of the heme group. Neutrophils with MPO deficiency mature, migrate, and phagocytose normally, but as noted there are defects in microbial killing. Some individuals do appear to have mildly increased frequency in bacterial infections, and in the setting of cofactors such as diabetes, may have difficulty

in clearance of infection by candida species (*albicans*, *tropicalis*, *stelatoidea*, and *krusei*). Diagnosis of MPO deficiency may be made by the measurement of peroxidase activity using flow cytometry or using certain types of automated blood count devices that use peroxidase activity to perform differential counts of blood leukocytes. Because most of these patients have clinically mild disease, antimicrobial and supportive therapy is sufficient. Prophylactic antibiotics should be limited to those with recurrent infections or with another disorder predisposing to infections, such as diabetes.

Chronic Granulomatous Disease

Chronic granulomatous diseases (CGDs) are a group of closely related inherited disorders characterized by defective phagocyte NADPH oxidase manifested by a failure of stimulated neutrophils, monocytes, eosinophils, and macrophages to produce superoxide and hydrogen peroxide.⁵ CGD affects approximately five individuals per million, appearing to equally affect all nationalities and ethnic groups. CGDs are caused by mutations in any of the four subunit components of the phagocyte NADPH oxidase. The clinically most severe form is the X-linked, gp91phox subunit-deficient form of CGD, usually associated with the total absence of any oxidant production and affecting almost 70% of patients with CGD. The other three types of CGD are inherited in an autosomal recessive pattern and consist mostly of p47phox-deficient CGD patients (25% of CGD patients), with the remainder comprised of the much less common p67phox-or p22phox-deficient forms of CGD. Clinical manifestations of CGDs involve both recurrent infections and formation of inflammatory granulomas, where the severity and individual manifestations can vary widely. The average age of diagnosis of X-linked CGD is 3 years, but the average age of diagnosis of females with the p47phox form of CGD is 9 years. Thus, some patients with no family history may reach young adulthood before the disease is recognized. Unlike patients with severe neutropenia or LAD who get infected primarily with commensal organisms (such as enteric bacterial normally found in the gastrointestinal tract), CGD patients are generally not susceptible to commensal organisms, such as *Escherichia coli*. They are particularly susceptible to a defined group of environmental organisms that generally have the characteristic of being catalase positive. The usual bacterial pathogens are *S. aureus*, *nocardia*, *Burkholderia cepacia* (and other *Burkholderia* species), and *Serratia marcescens*. Fungal pneumonia and other fungal infections are primarily caused by *aspergillus* species, with *Aspergillus nidulans* being a problem particular to CGD patients. However, infections with *paecilomyces*, and other fungi, including dematiaceous molds, are an increasing problem and must be considered because they may be resistant to voriconazole, but sensitive to posaconazole. Interestingly, CGD patients do not seem to be particularly susceptible to *Candida albicans* infections, though other candida species

such as *Candida glabrata* are a problem. Although the infections are usually recurrent and prolonged, they are episodic, meaning that CGD patients who are on appropriate effective prophylaxis may go many months or even years between severe infection. In infancy, *Serratia marcescens* osteomyelitis or soft tissue infection is a very common first presenting infection leading to diagnosis. In older children and adults with CGD, the most common life-threatening infections are bacterial or fungal pneumonias, though local soft tissue infections and lymph node infections are more common. All other tissues can be infected, including sites as diverse as osteomyelitis or brain abscess. After pneumonia, the most common severe infections are liver abscesses. It is noteworthy that in CGD patients taking TMP-SMX daily prophylaxis, severe staphylococcal deep tissue infections are relatively uncommon, yet almost 90% of liver abscesses appear to be caused by *S. aureus*. Methicillin-resistant *S. aureus* liver abscess is an increasing problem. Also of note is that liver abscess is generally not an easily drained pustular lesion, but most often consists of a solid granulomatous mass with micro-abscesses that require surgical extirpation together with prolonged antibiotic therapy for most effective cure. In some CGD individuals, granuloma formation may be the predominant problem rather than the infection, and in some cases the granulomatous inflammation can cause gastroesophageal junction or gastric outlet obstruction, bladder outlet obstruction, or chronic abdominal pain with diarrhea. In some cases, the gastrointestinal granulomatous process may be indistinguishable from Crohn's disease and appears to respond to similar treatments as those used for Crohn's disease. CGD granulomas are distinguished from granulomas of autoimmune diseases in that the CGD granulomas in some cases are particularly amenable to treatment with a tapering dose of steroids and controllable long term on very-low-dose alternate-day prednisone. Autoimmune disorders of the Th2 cytokine pattern type (Crohn's disease, rheumatoid arthritis, lupus, sarcoidosis) occur with increased frequency in CGD. Whether this is triggered by the recurrent infections, by the hyperinflammation associated with CGD, or by something intrinsic to CGD lymphocytes is yet to be determined.

CGDs should be suspected in those with a family history of unexplained deaths in infant or young boys, in those infected with suspected organisms (e.g., *serratia* osteomyelitis in an infant is almost diagnostic of CGD), and in children with pneumonia that does not rapidly resolve with conventional therapy. Diagnosis is made by a dihydrorhodamine flow cytometry measurement demonstrating defective oxidase activity in neutrophils, and confirmed by quantitative assays of superoxide production. Acute infections are managed with antibiotics and supportive therapy. Because of the propensity of CGD patients to get infections with unusual organisms such as nocardia or aspergillus, aggressively seeking a pathogenic organism is essential to achieve correct

antimicrobial therapy. When infections resolve, a prophylactic regimen is implemented by good oral hygiene with chlorhexidine-and/ or peroxide-based mouthwash, daily oral TMP/ SMX (5 to 6 mg/ kg/ day TMP equivalent), daily oral itraconazole (4 to 5 mg/ kg/ day), and three-times-a-week subcutaneous injections of recombinant interferon gamma (0.05 mg/ m²). Surgical intervention may be necessary to identify pathogens, debride devitalized tissues, or accelerate recovery and response to therapy. The granulomatous process can occur with or without infections; thus, appropriate microbial cultures are an important part of the evaluation. Gastrointestinal or genitourinary granulomas not associated with any pathogen can be treated with 0.5 to 1 mg/ kg of prednisone for 2 weeks, followed by gradual taper, though some patients require 0.1 to 0.25 mg/ kg prednisone on a long-term basis for control of granuloma gastrointestinal (GI) and/ or genitourinary (GU) problems. Some CGD patients may have trouble with dehiscence of surgical wounds, particularly on the abdomen or neck, and paradoxically this may require a course of low-dose corticosteroid to suppress granulomas in the wound and allow healing. Bone marrow or other hematopoietic stem cell transplantation may be considered for some patients with severe disease and/ or many recurrent infections and who have a human leukocyte antigen (HLA)-matched sibling donor.⁶ Gene therapy appears to be a promising alternative for those who are not eligible for stem cell transplantation.⁷

Chédiak–Higashi Syndrome

Chédiak–Higashi syndrome (CHS) is a rare autosomal recessive disorder caused by lysosomal trafficking regulator (LYST) gene mutations, leading to abnormal intracytoplasmic protein transport and vacuole formation.⁸ This results in the fusion of intracellular granules, and uneven distribution of giant granules in the cytoplasm of neutrophils and many other cells, such as platelets, melanocytes, renal tubular cells, Schwann cells, thyroid follicular cells, and mast cells. Cells containing giant granules have impaired function, and can manifest as recurrent bacterial infections; bleeding or easy bruising; hypopigmentation of skin, eyes, and hair; recurrent infections; peripheral nerve defect (neuropathy, nystagmus); or abnormal natural killer cell functions. Diagnosis is made by detecting large granules in neutrophils from a peripheral smear. The treatment includes supportive therapy and bacterial prophylaxis with TMP/ SMX. Not all patients appear to have recurrent infections; and the main problems arise from the progressive peripheral neuropathy that become manifest during the third decade of life and the risk of developing a lymphoma-like condition, which can be fatal. Vitamin C was shown to partially reverse some of the cellular defects observed in vitro, and this led to its use in patients, though it is unclear if it reduces infection or alters any other aspect of the course of the disease. Bone marrow transplantation, immunosuppression,

or rituximab can be considered for those who develop an “accelerated phase” with lymphoproliferative lymphoma-like syndrome.

Specific Granule Deficiency

Neutrophil secondary (or specific) granules contain a variety of proteases and other antimicrobial molecules. These proteins perform important normal functions in infection control and possibly also wound healing. Specific granule deficiency (SGD) occurs as a very rare inherited disorder, or more commonly appears associated with leukemia or myelodysplasia.⁹ Acute burn injury has also been noted to result in neutrophils deficient in specific granules, though this could be secondary to degranulation. It has recently been shown that inherited SGD can result from a mutation in the gene encoding a key regulatory factor required for late events during myeloid differentiation (CCAAT/enhancer-binding protein ϵ). The failure of function of this DNA-binding differentiation factor protein results in its inability to produce the specific granule itself, the contents of the specific granule, as well as failure to produce some other proteins normally made during the late phase of myeloid differentiation. Clinically, individuals with SGD have recurrent bacterial infections starting in early childhood. The common sites of infections are skin (cellulitis) and respiratory tract (sinusitis, pneumonia, otitis media). Similar to LAD, there is no erythema or pus at the site of infections, and recurrent large nonhealing ulcers are a chronic problem. The presence of nonhealing ulcers in both SGD and in LAD probably points to an important role of neutrophils not only for infection control but possibly also in wound healing. Treatment includes antibiotics for acute infections and prophylaxis with daily TMP/ SMX and itraconazole.

NEUTROPENIAS

Neutropenia is typically defined by an absolute neutrophil count (ANC) less than 1.5×10^9 per liter (or $<1,500/\text{uL}$). It is present in certain hereditary syndromes, and can result from infections, drugs or toxins, or autoimmune disorders. The risk of infection from neutropenia depends on three factors: the ANC, the neutrophil reserve in the bone marrow, and the duration of neutropenia. This risk is increased with a neutrophil count of 0.5 to $1.0 \times 10^9/\text{L}$ (500 to $1,000/\text{uL}$) and is the greatest with less than $0.5 \times 10^9/\text{L}$ ($<500/\text{uL}$). A falling neutrophil count or a significant decrease over steady-state levels, with a failure to increase neutrophil counts in the setting of infection or other bone marrow stress carries a higher risk of complication than a stable chronically low neutrophil count over many months or years that rises significantly in response to infection.

Acquired Neutropenias

Drug-Induced Neutropenia

Drugs can cause neutropenia in one or more of the following mechanisms: direct cytotoxic effect to rapidly dividing bone marrow cells, and immune mediated or other non-immune-mediated neutrophil destruction. A recent reviewed indicated that the duration of drug exposure to the onset of neutropenia can vary from less than 1 week to 60 days.¹⁰ The degree of neutropenia can be severe (e.g., ANC less than $0.1 \times 10^9/\text{L}$ or $100/\text{uL}$) but usually requires only that the sensitizing drug be discontinued. Neutrophil counts usually begin to recover within 5 to 10 days after the offending drug is stopped. Readministration of the sensitizing drug may decrease neutrophil counts abruptly. It is important to note that although some drugs ([Table 9.2](#)) have more often been cited as a cause of drug-related neutropenia, severe immune-mediated drug-induced neutropenia can be associated with any drug, including such unlikely agents as aspirin or acetaminophen.

Infection-Related Neutropenia

Neutropenia following infections is common, and can result from one or more of the following: destruction, margination, sequestration, or marrow suppression. Neutropenia from viral infections can be seen as early as a few days, and can persist for the duration of viremia. The degree and duration of virus-induced neutropenia is usually mild and short, but neutropenia from Epstein–Barr virus, hepatitis, and HIV can be severe and protracted. Gram-negative bacterial infections can cause neutropenia in those with impaired marrow neutrophil reserve, such as neonates, the elderly, and the chronically immunosuppressed. Protozoal (*Leishmania*) and rickettsial (Rocky Mountain spotted fever [RMSF] and *Ehrlichia*) infections can also cause neutropenia, often with accompanying anemia and/ or thrombocytopenia.

Immune-Related Neutropenia

This form of neutropenia is typically associated with specific antibodies directed to neutrophil antigens (these are not to be confused with antinuclear antibodies). These antibodies may occur with or without autoimmune disorders. Many syndromes appear clinically similar and are briefly discussed in the following.

In alloimmune (or isoimmune) neonatal neutropenia,¹¹ maternal immunoglobulin G (IgG) antibodies are directed toward paternal antigens on fetal neutrophils, causing moderate neutropenia that is self-limiting, lasting only a few weeks to a few months. These neonates have an increased risk of infections and can develop pulmonary, skin, or urinary tract infections from gram-positive or negative organisms. The treatment is supportive with antibiotics, intravenous immunoglobulin (IVIg), and sometimes granulocyte colony stimulating factor (G-CSF).

Table 9.2 Abbreviated List of Common Drugs Causing Neutropenia

Antiplatelet agents: ticlopidine
Sulfa-containing drugs: sulfasalazine, dapsone
Anti-thyroid agents: methimazole, propylthiouracil
Calcium dobesilate
Antimicrobials: particularly penicillins, cephalosporins, carbepenems
NSAIDs: dipyrone, indomethacin
Tricyclic antidepressants: clomipramine
Cardiac medications: anti-arrhythmic agents, digoxin, diuretics, ACE inhibitors
Reflux/ ulcer agents: cimetidine, ranitidine
Antipsychotic agents: clozapine, chlorpromazine
Antiviral agents (against HIV, HSV, and CMV)
Rheumatoid arthritis agents: penicillamine, gold compounds
Chemotherapy

ACE, angiotensin-converting enzyme; CMV, cytomegalovirus; HSV, herpes simplex virus; NSAIDs, non-steroidal anti-inflammatory drugs.

Data from Palmblad JE, von dem Borne AE. Idiopathic, immune, infectious, and idiosyncratic neutropenias. *Semin Hematol*. 2002;39:113–120.

Autoimmune neutropenia of infancy/ childhood¹² is typically seen in those younger than 2 years. The degree of neutropenia is variable, and infections in the oropharynx, ear, sinus, and upper respiratory tract can occur. The neutropenia may resolve spontaneously over many months or years, and typically does not require treatment. Antibiotics and G-CSF are given during acute infections, and TMP/ SMX is often given for prophylaxis.

Large granular lymphocytosis or leukemia¹³ is caused by abnormally expanded T or NK cells infiltrating the bone marrow, spleen, and liver, resulting in variable degrees of pancytopenia and splenomegaly. This may be an oligoclonal or monoclonal disease, and in its more aggressive form is considered a form of leukemia. It is typically described in individuals older near 60 years. Laboratory evaluation will reveal multiple abnormalities: 80% of affected individuals will have lymphocytosis $> 2 \times 10^9/ L$, 80% with ANC $< 1.5 \times 10^9/ L$, 50% with hemoglobin $< 11 \text{ g/ dL}$, 20% with platelet $< 150 \times 10^9/ L$. Large granular lymphocyte (LGL) can also occur with other autoimmune disorders (rheumatoid arthritis most commonly), myeloid and B cell malignancies, or solid tumors. Bone marrow examinations are also variable, but majority will have hypercellular marrow. No treatment is necessary until there are recurrent infections, severe neutropenia, or symptomatic anemia.¹⁴ Corticosteroids, methotrexate, cyclophosphamide, and other immunosuppressive therapy have been used with generally good response rates. However, aggressive monoclonal LGL disease should be considered a form of leukemia requiring specific chemotherapies appropriate to control the disease.

Congenital Neutropenias

Severe Congenital Neutropenia (Kostmann Syndrome and Autosomal Dominant Forms)

Dr. Kostmann in 1956 described severe neutropenia associated with recurrent bacterial infections in several families in northern Sweden. This syndrome was later observed in other geographic locations. Kostmann syndrome is an autosomal recessive form of severe congenital neutropenia that is a rare clinical entity with an incidence rate of about 1 to 2 per million.¹⁵ Neutrophil elastase (ELA2 or ELANE) mutations appear to be responsible for almost half of the individuals with the autosomal-dominant or sporadic forms of severe congenital neutropenia. Mutations in ELA2 have been hypothesized to cause defective signal transduction and programmed cell death (apoptosis) at the myelocyte level.¹⁶ These effects may be a result of cellular mechanisms that detect protein misfolding. There are additional abnormalities that can be acquired, which may lead to myelodysplasia and/ or acute myeloid leukemia: G-CSF receptor mutation, RAS oncogene mutation, or chromosome 7 monosomy. An autosomal dominant form of neutropenia has been reported to result from heterozygous mutations in the GFI1 gene that may affect ELA2.¹⁷ Recent studies have shown that Kostmann syndrome could be caused by mutations in the HAX1,¹⁸ G6PC3,¹⁹ or other genes.

Clinically, these individuals are infected at as early as 2 to 3 months of age by gram-positive or negative bacteria in one or more of the following sites: skin, ears, oral or gastrointestinal mucosa, upper or lower respiratory tract, urinary tract, or blood. Blood counts usually reveal a neutrophil count less than 500/ μL ($<0.5 \times 10^9/\text{L}$) with compensatory monocytosis and eosinophilia. Bone marrow biopsies show maturation arrest at the promyelocyte–myelocyte level, and absent band forms or mature neutrophils. Treatment includes supportive therapy and antibiotics for acute infections. G-CSF between 3 and 10 $\mu\text{g}/\text{kg}$ has been used successfully to increase the neutrophil count and reduce the frequency of infections. A minority of individuals will require an excess of 30 $\mu\text{g}/\text{kg}/\text{day}$. G-CSF is not currently thought to be associated with the acquisition of G-CSF mutations and is not itself thought to be a cause of the leukemia associated with this disorder. However, individuals requiring longer durations or high cumulative doses of G-CSF may have more severe forms of the disorder, and therefore are at higher risk of malignant transformation to leukemia. Side effects of chronic G-CSF administration include bone pain from marrow expansion, osteopenia or osteoporosis, and splenomegaly. Bone marrow transplantation is a curative option for those with HLA-matched sibling donors.

Cyclic Neutropenia

The incidence of inherited cyclic neutropenia is not known.^{20,21} Its cause is also not

completely understood, although neutrophil elastase mutations are associated with this disorder and have been hypothesized to cause neutrophil apoptosis and thus initiating the cycling. Clinically, neutrophil count oscillates predictably between very low or agranulocytic and low normal range; the average cycle length is 21 days with neutropenic duration of 3 to 6 days. The nadir neutrophil count can go to zero or be as low as 200/ uL ($0.2 \times 10^9/ L$). Platelet, reticulocyte, lymphocyte, and monocyte counts may also “counter-cycle” between normal and high range, and coincide or not with the neutrophil cycles. Serial bone marrow examinations will appear normal with normal neutrophil count and show decreased myeloid precursors in the neutropenic phase. Individuals with cyclic neutropenia may be asymptomatic during periods of normal neutrophil counts, and may have fever, lymphadenopathy, mild skin infections, and/ or oral mucosal ulcers during periods of neutropenia. Mild skin infections and/ or mouth ulcers are treated symptomatically. G-CSF, at 2 to 3 ug/ kg per 1 or 2 days, appears to improve the neutrophil nadir, shorten the cycles, and thus reduce infections. GM-CSF does not effectively treat inherited cyclic neutropenia.

Other Inherited Disorders Associated With Clinically Significant Neutropenia

There are a number of inherited disorders where clinically significant neutropenia is observed, but where neutropenia is not considered the most prominent feature of the inherited syndrome. Three examples are provided. There is a specific mutation responsible for Wiskott–Aldrich syndrome that can be associated with neutropenia.²² Patients with warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (WHIM) syndrome, which is caused by inherited C-terminal truncations in CXCR4, suffer from clinically significant neutropenia that is responsive to treatment with plerixafor.²³ A third example is that of a subset of patients with CD40 ligand deficiency (X-linked Hyper-IgM syndrome) having clinically significant neutropenia.²⁴

Other Neutropenias

Idiopathic Neutropenia

Idiopathic neutropenia, or chronic idiopathic neutropenia, affects about two to four individuals per million, and can be seen in both children and adults.²⁵ Clinically, it behaves very similar to autoimmune neutropenia, except that antineutrophil antibodies were not detected and other studies are nondiagnostic. Majority of these individuals have moderate neutropenia with mild symptoms. Pro-inflammatory cytokines that promote neutrophil apoptosis and myelosuppression from activated lymphocytes have been proposed as possible triggers. There is a small subset of individuals who have severe neutropenia, recurrent fevers, oropharyngeal infections (mucosal ulcers,

gingivitis), or severe systemic infections. Treatments are largely tailored for symptomatic relief and antibiotics dictated by sites of infection. G-CSF, 1 to 3 µg/ kg per dose weekly or on alternate days, is used in those with severe clinical syndromes. Development of myelodysplastic syndrome or leukemias is very rare. Generally, patients with increase in their neutrophil counts because of infection or other stress setting have a benign clinical course.

Benign Ethnic Neutropenia

Benign ethnic neutropenia (BEN) is a condition seen mostly in individuals of African descent, including African Americans, Yemenite Jews, and certain populations in the Caribbean and Middle East. Prior studies showed that up to 25% of non-U.S. individuals of African descent and about 4% of African Americans have neutrophil counts between 1.0×10^9 and 1.5×10^9 cells/ L.²⁶ The cause for this observation is unknown, but several investigators earlier have excluded stem cell disorder, excessive margination, and differentiation defect, suggesting that this may be a normal population-based variant. The physiologic mechanisms controlling the normal set point for circulating levels of neutrophils is unknown, but there is accumulating evidence that the Duffy antigen and receptor for chemokine (DARC) is associated with those of African descent with lower leukocyte/ neutrophil counts.^{27,28} CXCR4 chemokine receptor for the SDF-1 chemokine may also play a role in egress of neutrophils from the marrow and, at least theoretically, differences in expression or function of this cytokine/ cytokine receptor could affect this set point. Perhaps, normal variants in this or other receptors may be responsible for these population differences observed in average circulating neutrophil counts. Clinically, individuals with this ethnically based neutropenia variant are asymptomatic, without recurrent oral, skin, or systemic infections. When these individuals acquire typical viral or bacterial infections, these infections are not more severe and do not need longer period of treatment. Laboratory evaluations will show many blood counts that are abnormal over many years and the bone marrow examinations should be normal. Other than usual symptomatic treatment and antibiotics as needed (as for a normal healthy adult), no additional treatment is required, but it is important to note this variation to avoid unnecessary medical evaluation.

References

1. Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in phagocytes. *N Engl J Med.* 2000;343:1703–1714.
2. van de Vijver E, Maddalena A, Sanal O, et al. Hematologically important mutations: leukocyte adhesion deficiency (first update). *Blood Cells Mol Dis.* 2012;48:53–61.

3. Qasim W, Cavazzana-Calvo M, Davies EG, et al. Allogeneic hematopoietic stem-cell transplantation for leukocyte adhesion deficiency. *Pediatrics*. 2009;123:836–840.
4. Dinauer MC. Disorders of neutrophil function: an overview. *Methods Mol Biol*. 2014;1124:501–515.
5. Malech HL, Hickstein DD. Genetics, biology and clinical management of myeloid cell primary immune deficiencies: chronic granulomatous disease and leukocyte adhesion deficiency. *Curr Opin Hematol*. 2007;14:29–36.
6. Kang EM, Marciano BE, DeRavin S, et al. Chronic granulomatous disease: overview and hematopoietic stem cell transplantation. *J Allergy Clin Immunol*. 2011;127:1319–1326; quiz 1327–1318.
7. Ott MG, Schmidt M, Schwarzwälder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat Med*. 2006;12:401–409.
8. Kaplan J, De Domenico I, Ward DM. Chediak-Higashi syndrome. *Curr Opin Hematol*. 2008;15:22–29.
9. Gombart AF, Koeffler HP. Neutrophil specific granule deficiency and mutations in the gene encoding transcription factor C/ EBP(epsilon). *Curr Opin Hematol*. 2002;9:36–42.
10. Andersohn F, Konzen C, Garbe E. Systematic review: agranulocytosis induced by nonchemotherapy drugs. *Ann Intern Med*. 2007;146:657–665.
11. Palmblad JE, von dem Borne AE. Idiopathic, immune, infectious, and idiosyncratic neutropenias. *Semin Hematol*. 2002;39:113–120.
12. Audrain M, Martin J, Fromont P, et al. Autoimmune neutropenia in children: analysis of 116 cases. *Pediatr Allergy Immunol*. 2011;22:494–496.
13. Bareau B, Rey J, Hamidou M, et al. Analysis of a French cohort of patients with large granular lymphocyte leukemia: a report on 229 cases. *Haematologica*. 2010;95:1534–1541.
14. Lamy T, Loughran TP, Jr. How I treat LGL leukemia. *Blood*. 2011;117:2764–2774.
15. Welte K, Zeidler C, Dale DC. Severe congenital neutropenia. *Semin Hematol*. 2006;43:189–195.
16. Makaryan V, Zeidler C, Bolyard AA, et al. The diversity of mutations and clinical outcomes for ELANE-associated neutropenia. *Curr Opin Hematol*. 2015;22:3–11.
17. Person RE, Li FQ, Duan Z, et al. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. *Nat Genet*. 2003;34:308–312.
18. Klein C, Grudzien M, Appaswamy G, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). *Nat Genet*. 2007;39:86–92.
19. Boztug K, Rosenberg PS, Dorda M, et al. Extended spectrum of human glucose-6-

- phosphatase catalytic subunit 3 deficiency: novel genotypes and phenotypic variability in severe congenital neutropenia. *J Pediatr.* 2012;160(4):679–683.
- 20. Horwitz MS, Duan Z, Korkmaz B, et al. Neutrophil elastase in cyclic and severe congenital neutropenia. *Blood.* 2007;109:1817–1824.
 - 21. Dale DC, Welte K. Cyclic and chronic neutropenia. *Cancer Treat Res.* 2011;157:97–108.
 - 22. Devriendt K, Kim AS, Mathijs G, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. *Nat Genet.* 2001;27:313–317.
 - 23. McDermott DH, Liu Q, Velez D, et al. A phase 1 clinical trial of long-term, low-dose treatment of WHIM syndrome with the CXCR4 antagonist plerixafor. *Blood.* 2014;123:2308–2316.
 - 24. Leven EA, Maffucci P, Ochs HD, et al. Hyper IgM syndrome: a report from the USIDNET registry. *J Clin Immunol.* 2016;36:490–501.
 - 25. Papadaki HA, Pontikoglou C. Pathophysiologic mechanisms, clinical features and treatment of idiopathic neutropenia. *Expert Rev Hematol.* 2008;1:217–229.
 - 26. Hsieh MM, Everhart JE, Byrd-Holt DD, et al. Prevalence of neutropenia in the U.S. population: age, sex, smoking status, and ethnic differences. *Ann Intern Med.* 2007;146:486–492.
 - 27. Nalls MA, Couper DJ, Tanaka T, et al. Multiple loci are associated with white blood cell phenotypes. *PLoS Genet.* 2011;7:e1002113.
 - 28. Reiner AP, Lettre G, Nalls MA, et al. Genome-wide association study of white blood cell count in 16,388 African Americans: the continental origins and genetic epidemiology network (COGENT). *PLoS Genet.* 2011;7:e1002108.

Childhood Hematologic Diseases

Julie Jaffray and Alan S. Wayne

Despite some overlap with disorders encountered in adults, many congenital and acquired hematologic diseases manifest primarily during childhood. In addition, pediatric hematology is distinguished by developmental differences in normal physiology and blood parameters.¹ The purpose of this chapter is to highlight unique features in the evaluation, diagnosis, and treatment of common pediatric hematologic conditions. The reader is referred to other chapters in this edition for additional details of the management of specific disorders.

ANEMIA

Anemia is defined as an overall reduction in red cell mass or hemoglobin (Hb) concentration, 2 standard deviations below the mean normal value for the specific population. Normal red blood cell (RBC) indices vary with age and are affected by factors such as race, sex, and altitude (Table 10.1).² At birth, the Hb concentration is elevated due to low (i.e., venous) oxygen tension in utero, but as tissue oxygenation increases on delivery, the Hb gradually decreases until reaching a physiologic nadir at 2 to 4 months (earlier for premature infants), at which point erythropoiesis is stimulated.³

In children, anemia is commonly classified according to RBC size (Table 10.2). Microcytic anemias account for most of the cases of anemia in early childhood (Table 10.3). The initial diagnostic evaluation of a child with anemia should consist of a detailed history and physical examination and the following minimal laboratory testing: complete blood count (CBC), reticulocyte count, and examination of the peripheral blood smear. Consideration of the physiologic basis for anemia can be helpful in guiding further investigation (Table 10.4).

Microcytic Anemias

Iron deficiency is the most common cause of anemia during childhood and may result from a combination of low stores at birth, high requirements because of growth and blood volume expansion, inadequate nutrition, and poor bioavailability of dietary iron. Iron deficiency due to blood loss is usually a result of gastrointestinal tract irritation and occult hemorrhage associated with the introduction of cow's milk before the first year of life or heavy menstrual bleeding during adolescence. On history, additional risk factors for iron deficiency may include prematurity, limited or prolonged breastfeeding, non-iron-fortified formula or excessive intake of whole milk (generally more than 1 quart/ day). Early iron deficiency may result only in a low ferritin level. Falling iron stores leads to decreased serum iron and transferrin saturation and an increase in total iron-binding capacity (TIBC) and free erythrocyte protoporphyrin (FEP). Iron deficiency also leads to RBC morphology abnormalities, such as hypochromia, microcytosis, and anisocytosis, as well as thrombocytosis. Iron supplementation (3 mg/kg/ day for mild anemia and 6 mg/kg/ day for moderate to severe anemia) should be provided for 3 to 6 months.^{4,5} A response to a trial of elemental iron is often helpful in differentiating iron deficiency from other common causes of microcytic anemia such as thalassemia trait. A Hb increase of more than 1 g/ dL at one month and reticulocyte peak at 10 to 14 days is diagnostic. If anemia persists, other etiologies should be considered (Table 10.2).

Table 10.1 Normal Hematologic Parameters in Children

Hemoglobin (g/ dL)			Hematocrit (%)		MCV (fL)		Neutrophils (10^3 / μ L)	
Age	Mean	-2 SD	Mean	-2 SD	Mean	-2 SD	Mean	Range
Birth	16.5	13.5	51	42	108	98	11	6–26
1 mo	14	10	43	31	104	85	3.8	1–9
3–6 mo	11.5	9.5	35	29	91	74	3.8	1–8.5
0.5–2 y	12	10.5	36	33	78	70	3.5	1.5–8.5
2–6 y	12.5	11.5	37	34	81	75	4.3	1.5–8
6–12 y	13.5	11.5	40	35	86	77	4.4	1.8–8
12–18 y								
Female	14	12	41	36	90	78	4.4	1.8–8
Male	14.5	13	43	37	88	78	4.4	1.8–8

MCV, mean corpuscular volume; SD, standard deviation.

Adapted from Dallman PR, Shannon K. Developmental changes in red blood cell production and function. In: Rudolph A, ed. Pediatrics. 20th ed. New York, Appleton-Lange; 1995; chap 17:1170.

Table 10.2 Classification of Childhood Anemia

Microcytic	Normocytic	Macrocytic
------------	------------	------------

<ul style="list-style-type: none"> ■ Iron deficiency ■ Lead poisoning ■ Thalassemia syndromes ■ Sideroblastic anemias ■ Chronic inflammation ■ Hypo-proteinemia 	<ul style="list-style-type: none"> ■ Chronic inflammation, infection, bone marrow suppression, or infiltration ■ Congenital hemolytic anemias ■ Acquired hemolytic anemia (auto or alloimmune, microangiopathic) ■ Acute or subacute blood loss ■ Splenic sequestration/ hypersplenism ■ Transient erythroblastopenia of childhood (TEC) 	<ul style="list-style-type: none"> ■ Reticulocytosis ■ Vitamin B₁₂, folate deficiency ■ Congenital pure red cell aplasia (Diamond–Blackfan) ■ Bone marrow failure (aplastic anemia, Fanconi) ■ Liver disease ■ Hypothyroidism ■ Drug related ■ Normal newborn
-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Table 10.3 Evaluation of Microcytic Anemia

Laboratory Assay	Iron Deficiency	Thalassemia Trait	Thalassemia Major	Lead Toxicity	Chronic Disease
RDW	↑	NL	↑↑	NL	NL/ ↑
MCV	↓	↓	↓	↓	NL/ ↓
RBC #	↓	↑/ NL	↓	↓	↓
FEP	↑	NL	NL	↑↑	↑
Iron	↓	NL	↑	NL	↓
Transferrin (TIBC)	NL/ ↑	NL	NL/ ↑	NL	NL/ ↓
% TIBC Saturation	↓	NL	↑	NL	NL/ ↓
Ferritin	↓	NL	↑	NL	NL/ ↑
Hb A ₂	↓	β > 3.5% α < 3.5%	β > 3.5% α < 3.5%	NL	NL

FEP, free erythrocyte protoporphyrin; Hb, hemoglobin; MCV, mean corpuscular volume; NL, normal; RBC, red blood cells; RDW, red cell distribution width; TIBC, total iron binding capacity.

Table 10.4 Characteristics of Anemia Based on Pathophysiology

Decreased Production	Increased Destruction	Blood Loss	Mixed Pathophysiology
<ul style="list-style-type: none"> ■ ↓ Reticulocytes ■ +/ - ↓ WBC, platelets ■ Erythroid hypoplasia on marrow 	<ul style="list-style-type: none"> ■ ↑ Reticulocytes ■ ↑ Indirect bilirubin, LDH ■ ↓ Haptoglobin ■ Hemoglobinuria ■ Abnormal morphology on smear ■ May have splenomegaly 	<ul style="list-style-type: none"> ■ Acute or subacute blood loss (may be occult) ■ ↑ Reticulocytes ■ Evolving iron deficiency 	<ul style="list-style-type: none"> ■ ↓ Reticulocytes in setting of increased destruction (as in parvovirus B19 associated aplastic crisis in sickle cell disease or other congenital hemolytic anemia)

LDH, lactate dehydrogenase; WBC, white blood cell.

Recommendations by the American Academy of Pediatrics include screening for anemia by testing Hb and serum transferrin receptor between the ages of 9 and 12 months with additional screening between the ages of 1 and 5 years for patients at risk and adolescent females postpuberty ([Table 10.5](#)).^{4,5} The most common reason for a lack of response to oral iron therapy is a lack of adherence, but malabsorption syndromes should also be considered for which parenteral iron infusions may be required.

Lead toxicity often coexists with iron deficiency in at-risk populations and may further inhibit gastrointestinal absorption of iron. Lead poisoning should be suspected if there is a history of pica or exposure to lead-based paint. An elevated FEP and basophilic stippling on peripheral smear may be seen. Therapy should include oral treatment with succimer or, in severe cases, parenteral treatment with dimercaprol (BAL) or calcium–sodium ethylenediaminetetraacetic acid (EDTA).⁶ The Centers for Disease Control and Prevention (CDC) provides recommendations for the management of children with elevated lead levels.⁷

Thalassemia syndromes are common causes of microcytic anemia in childhood. They are classified into α -and β -thalassemias based on the affected globin chain. The α -thalassemias can present in utero or at birth, while the β -thalassemias are not evident until approximately 6 months of age when β -globin synthesis becomes predominant. Thalassemia trait is often mistaken for iron deficiency, but the patient remains microcytic even with iron therapy.⁸ In contrast to iron deficiency, the β -thalassemia trait is associated with a normal red cell distribution width (RDW), basophilic stippling and targeting on the blood smear, and an elevated HbA₂ on electrophoresis. The α -thalassemia trait is associated with a normal Hb electrophoresis outside of the newborn period, although Hb Barts (γ_4) is present on newborn screening samples. When suspecting α or β thalassemia, a Hb electrophoresis can help differentiate, but caution should be taken to correct any iron deficiency, for this can cause a falsely low production of HbA₂, thus obscuring the diagnosis of β -thalassemia trait. To confirm the diagnosis, testing should be sent for α -or β -globin gene sequencing. Prenatal diagnosis of thalassemia can be made as early as the 10th week of gestation using chorionic villus sampling.⁹ In the evaluation of thalassemias, ethnic heritage is often suggestive and microcytosis should be present in at least one parent. The thalassemia trait (heterozygous β -1 and 2-gene deletion α -thalassemias) requires no therapy. In contrast, patients with thalassemia major require aggressive packed RBC transfusion, which should be initiated early in life to eliminate the increased erythropoietic drive and allow normal linear growth and bone development. In utero transfusion has been used to prevent hydrops fetalis in 4-gene deletion α -thalassemia (Hb Bart's disease), which is otherwise fatal. Care should be given to address iron overload with chelation therapy in transfusion-dependent children to prevent end-organ damage later in life. As an

alternative to lifelong transfusion and chelation therapy, allogeneic hematopoietic stem cell transplantation (SCT) is a curative approach for children with thalassemia major who have human leukocyte antigen (HLA)-matched sibling donors.

Table 10.5 Recommendations for Anemia Screening

American Academy of Pediatrics (AAP) Recommendations⁵

Option 1: Universal screening (communities with high-risk populations)
9–12 mo, 15–18 mo

Adolescents: Males at peak growth, Females at routine examinations

Option 2: Selective screening (communities with low-risk populations)

Screen high-risk patients at 9–12 mo, 15–18 mo, yearly until 5 y

Adolescents: Males at peak growth, Females at routine examinations

Centers for Disease Control and Prevention (CDC)⁶

High-risk populations: 9–12 mo, 15–18 mo, yearly until 5 years

Adolescent females every 5–10 y, yearly if risk factors

Adolescent males at peak growth

Normocytic Anemias

Anemia is a common manifestation of numerous systemic conditions in pediatrics. The anemia of acute inflammation and chronic disease is frequently mild and usually normocytic, although the mean cell volume (MCV) is occasionally low and transferrin is frequently diminished (Table 10.3). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) may be elevated in patients with inflammatory conditions leading to anemia. Treatment is aimed at the primary underlying condition. Viral infection is the most common cause of transient bone marrow suppression in children and may result in anemia, leukopenia, and/ or thrombocytopenia. The hallmark of viral suppression is failure of the reticulocyte count to increase in the face of anemia. Usually, only close observation is required because the bone marrow suppression is self-limited.

Transient erythroblastopenia of childhood (TEC) is an acquired pure red cell aplasia that can also follow viral illness in previously healthy children.¹⁰ The median age for presentation is 2 years, in contrast to congenital pure red cell aplasia (Diamond–Blackfan syndrome), which commonly presents in infancy. Reticulocytopenia and, occasionally, leukopenia and thrombocytopenia are seen. Most children with TEC recover in 1 to 2 months. Observation alone is usually sufficient, although short-term transfusion therapy may be required for cardiovascular compromise associated with severe anemia.

Normocytic anemia can also be due to hemolysis. There are several congenital and acquired hemolytic conditions of childhood, including RBC membrane disorders,

hemoglobinopathies, metabolic defects, enzymopathies, and immune-mediated hemolysis. Immune-mediated hemolysis, either isoimmune or alloimmune due to blood group incompatibility, presents in neonates; autoimmune hemolytic anemia (warm-or cold-agglutinin disease) is seen in older children. Hemoglobinopathies such as sickle cell disease (SCD), enzyme deficiencies such as glucose-6-phosphate dehydrogenase deficiency, and membrane disorders such as hereditary spherocytosis (HS) should be considered in the differential diagnosis of hemolysis. Microangiopathic hemolysis may be seen in hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and disseminated intravascular coagulation (DIC). Laboratory features consistent with hemolysis include reticulocytosis, elevated lactate dehydrogenase (LDH), indirect hyperbilirubinemia, decreased serum haptoglobin, and, in severe cases, hemoglobinuria. A positive direct Coomb's test indicates immune-mediated hemolysis. Examination of the peripheral smear may reveal characteristic RBC morphology, such as the schistocytes, spherocytes, or bite cells. Thrombocytopenia and renal impairment are additional features of HUS. RBC enzyme evaluation or osmotic fragility should be sent once patients recover from the hemolytic crisis when there is a concern for an enzymopathy or RBC membrane protein deficiency, respectively. The treatment of hemolysis should be directed toward the underlying cause, with transfusions reserved for severe anemia and cardiovascular compromise. Immune hemolysis often requires corticosteroids and/ or other immunosuppressive medications.

The diagnosis of SCD, which is secondary to a single amino acid substitution in the β -globin gene, is usually made during routine newborn screening by Hb electrophoresis. Children diagnosed with SCD should be cared for by practitioners with specific expertise in the management and prevention of its complications, and thus consultation with a pediatric hematologist is strongly recommended. Vaso-occlusive crises, which are manifested by recurrent episodes of pain, organ damage, and hemolysis, are the hallmark of SCD.¹¹ Dactylitis, vaso-occlusion of the small bones of the hands and feet, is an early presentation of SCD in infants and young children.¹² Specialized preventative care is imperative due to their high risk of infection (from functional asplenia) and multiple sites of end-organ damage such as stroke and pulmonary hypertension. Children should receive influenza vaccine annually starting at 6 months of age, the 13-valent conjugated pneumococcal vaccine series in the first year of life, and 23-valent polysaccharide pneumococcal vaccine and meningococcal vaccine at 2, 4, and 6 months, respectively, and 12 to 15 months of age.¹¹ Penicillin prophylaxis should begin at diagnosis (ideally in infancy) and continue until the age of 5 years and completion of the pneumococcal vaccination series. It is of importance that the risk of pneumococcal sepsis is lifelong and individuals with SCD require immediate medical attention for fever or signs of infection. Because of the risk of cerebrovascular accident

and the difficulty of clinical diagnosis in the pediatric population, children should undergo annual transcranial Doppler ultrasound (TCD) starting at the age of 2 years until at least 16 years.¹¹ Chronic blood transfusions can prevent stroke in children with abnormal TCD (velocities ≥ 200 cm/ s).^{13,14} Hydroxyurea is one of the few medications shown to decrease both morbidity and mortality in individuals with SCD by increasing fetal Hb and reducing vaso-occlusive pain crises, acute chest syndrome, and the need for transfusion.^{15,16} Other routine preventative care measures include annual echocardiograms to detect pulmonary hypertension starting at 15 years and annual eye examination at 10 years to evaluate for retinopathy.

Macrocytic Anemias

Vitamin B₁₂ deficiency causes megaloblastic changes in the bone marrow.¹⁷ In infants, B₁₂ deficiency may be the result of maternal depletion and decreased stores at birth. In older children and adolescents, etiologies include pernicious anemia, malabsorption, dietary deficiency, and inborn errors of metabolism. Unrecognized, severe deficiency early in life may cause failure to thrive and even permanent neurologic damage. Symptoms in older children may include anorexia, weight loss, diarrhea, constipation, weakness, glossitis, peripheral neuropathy, ataxia, and dementia. Anemia due to vitamin B₁₂ deficiency is commonly accompanied by neutropenia, hypersegmented neutrophils, and thrombocytopenia. Low serum B₁₂, elevated methylmalonic acid and homocysteine levels, as well as a response to replacement therapy, are confirmatory.

Folate deficiency also results in a megaloblastic bone marrow.¹⁷ The newborn infant has increased demands for folate. Risk factors for early deficiency include prematurity, low levels in maternal breast milk, and a predominance of goat's milk intake. In older children, folate deficiency is usually the result of malnutrition, although it may also be caused by certain medications (e.g., methotrexate, malarial medications, anti-epileptics), chronic hemolysis, malabsorption, and inborn errors of metabolism. Serum and erythrocyte folate levels will be low with elevated homocysteine levels and normal methylmalonic acid levels. The anemia should respond to small replacement doses of folic acid.

Bone marrow failure syndromes should also be considered when pediatric patients present with macrocytic anemia. Diamond-Blackfan anemia (DBA) or congenital pure red cell aplasia is usually noted soon after birth or during the first year of life. Twenty-five percent of patients with DBA have associated anomalies, such as short stature and/or abnormalities of the head, face, and upper limbs. Laboratory features include reticulocytopenia, high MCV (often only mildly elevated), increased Hb F, elevated adenosine deaminase activity, normal or decreased white blood cell (WBC) count, normal or increased platelet count, and erythroid hyperplasia in the bone marrow.¹⁸

Mutations in genes that encode ribosomal proteins have been found in about 50% of patients,¹⁹ and large deletions or changes in regulatory regions likely account for the remainder. The main differential diagnosis is TEC, which more commonly presents after the first year of life and in children with a previously normal CBC. Most of the children with DBA respond to corticosteroids. Chronic RBC transfusion and chelation therapy is used in patients refractory to corticosteroids or those with steroid induced toxicity. SCT can be curative.²⁰

Fanconi anemia (FA) typically presents with macrocytic anemia, elevated fetal Hb and pancytopenia in mid-childhood, although the first hematologic signs of FA may manifest in infancy. Severe pancytopenia usually develops later in life. FA can often be differentiated from acquired aplastic anemia by characteristics such as impaired growth and/ or anomalies of the thumbs, radii, kidneys, head, eyes, ears, skin, and/ or genitourinary system. Inheritance is autosomal recessive and the family history may be positive for marrow failure and leukemia. Diagnosis is made by the demonstration of increased chromosomal breakage of T lymphocytes on exposure to diepoxybutane (DEB) in peripheral blood (preferred) or bone marrow. Molecular testing is also available. There is a 10% to 35% risk of developing leukemia or myelodysplastic syndrome.²¹ The differential includes other familial or acquired bone marrow failure syndromes. Abnormal chromosomal breakage analysis or FA genotyping confirms the diagnosis. Anemia is frequently responsive to androgen therapy. SCT is curative for the hematologic manifestations of FA, but a modified pretransplant conditioning is critical to avoid severe toxicity caused by chemotherapy and radiation sensitivity.

BLEEDING

Many congenital and acquired disorders of hemostasis, including platelet abnormalities, present in infancy and childhood (Fig. 10.1). Hemorrhagic disorders in infancy may manifest as bleeding from the umbilicus, circumcision site, unusually large cephalohematomas, intracranial hemorrhages, or mucocutaneous bleeding, such as bruising or oral bleeding. The normal ranges for coagulation assays are age dependent and differ greatly from the neonatal period to infancy and later childhood (Table 10.6).^{22–24} Most coagulation proteins increase in parallel with gestational age due to developmental hemostasis. Because physiologic levels of many clotting factors are low at birth (except factors V, VIII, and fibrinogen), it is often difficult to diagnose disorders of hemostasis in newborns.²⁵

Acquired Coagulation Disorders

Vitamin K deficiency bleeding (VKDB), otherwise known as hemorrhagic disease of the newborn (HDN), is a complication of physiologically low levels of vitamin K–

dependent factors in the newborn.²⁶ The three types of VKDB are early, classical, and late. Early onset is typically due to maternal ingestion of medications, such as anticonvulsants, which cross the placenta, and infants present with bleeding in the first 24 hours of life. Classic VKDB presents on days 2 to 7 of life in otherwise healthy, full-term infants and occurs in 1/ 10,000 live births due to the physiologic deficiency of vitamin K, combined with a lack of vitamin K administration. Risk factors include poor placental transfer of vitamin K, marginal levels in breast milk, inadequate milk intake, and the sterile newborn gut. Late onset VKDB typically occurs between 2 weeks and 6 months of age in exclusively breastfed infants. Vitamin K deficiency can also be seen in children with liver disease, chronic antibiotic use, inadequate intake, or disorders that interfere with vitamin K absorption, such as chronic diarrhea, cystic fibrosis, or other fat malabsorption syndromes. Therapy should include vitamin K administration as well as measures directed at any underlying cause.

Diagnosis can be confirmed by prolonged screening coagulation tests (prothrombin time [PT] and partial thromboplastin time [aPTT]) and decreased vitamin K–dependent factor activity levels (factors II, VII, IX, and X). Determination of decarboxylated forms of vitamin K–dependent factors induced by vitamin K antagonists also may be helpful. VKDB should be prevented in all newborns by prophylactic administration of vitamin K at birth with a single dose of 0.5 to 1 mg intramuscularly (preferred route) or an oral dose of 2 to 4 mg, followed by continued supplementation in breastfed infants.

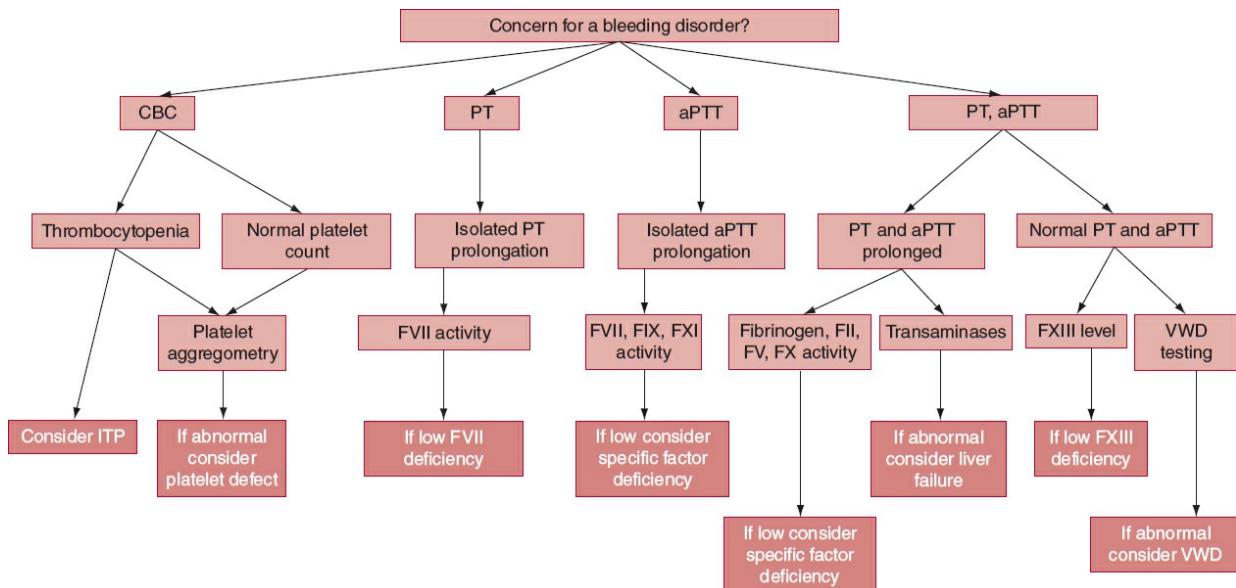


FIGURE 10.1 Algorithm and diagnostic considerations for children with a concern for an underlying bleeding disorder.

aPTT, activated thromboplastin time; CBC, complete blood count; FII, Factor II; FV, Factor V; FVII, Factor VII; FVIII, Factor VIII; FIX, Factor IX; FX, Factor X; FXI, Factor XI; FXIII, Factor XIII; ITP, immune thrombocytopenia purpura; PT, prothrombin time; VWD, von Willebrand disease.

Table 10.6 Normal Age-Specific Coagulation Values^a

Coagulation Test	30–36 Week Gestation at Birth	Term Infant at Birth	1–5 Y	6–10 Y	11–16 Y	Adult
PT (s)	13 (10.6–16.2)	13 (10.14–15.9)	11 (10.6–11.4)	11.1 (10.1–12.1)	11.2 (10.2–12)	12 (11–14)
aPTT (s)	53.6 (27.5–79.4)	42.9 (31.3–54.5)	30 (24–36)	31 (26–36)	32 (26–37)	33 (27–40)
Fibrinogen (g/L)	2.43 (1.5–3.73)	2.83 (1.67–3.99)	2.76 (1.7–4.05)	2.79 (1.57–4)	3 (1.54–4.48)	2.78 (1.56–4)
II (U/mL)	0.45 (0.2–0.77)	0.48 (0.26–0.7)	0.94 (0.71–1.16)	0.88 (0.67–1.07)	0.83 (0.61–1.04)	1.08 (0.7–1.46)
V (U/mL)	0.88 (0.41–1.44)	0.72 (0.36–1.08)	1.03 (0.79–1.27)	0.9 (0.63–1.16)	0.77 (0.55–0.99)	1.06 (0.62–1.5)
VII (U/mL)	0.67 (0.21–1.13)	0.66 (0.28–1.04)	0.82 (0.55–1.16)	0.85 (0.52–1.2)	0.83 (0.58–1.15)	1.05 (0.67–1.43)
VIII (U/mL)	1.11 (0.5–2.13)	1.0 (0.22–1.78)	0.9 (0.59–1.42)	0.95 (0.58–1.32)	0.92 (0.53–1.31)	0.99 (0.5–1.49)
IX (U/mL)	0.35 (0.19–0.65)	0.53 (0.15–0.91)	0.73 (0.47–1.04)	0.75 (0.63–0.89)	0.82 (0.59–1.22)	1.09 (0.55–1.63)
X (U/mL)	0.41 (0.11–0.71)	0.4 (0.12–0.68)	0.88 (0.58–1.16)	0.75 (0.55–1.01)	0.79 (0.5–1.17)	1.06 (0.7–1.52)
XI (U/mL)	0.3 (0.08–0.52)	0.38 (0.1–0.66)	0.97 (0.56–1.5)	0.86 (0.52–1.2)	0.74 (0.5–0.97)	0.97 (0.67–1.27)
XII (U/mL)	0.38 (0.1–0.66)	0.53 (0.13–0.93)	0.93 (0.64–1.29)	0.92 (0.6–1.4)	0.81 (0.34–1.37)	1.08 (0.52–1.64)
XIIIa (U/mL)	0.7 (0.32–1.08)	0.79 (0.27–1.31)	1.08 (0.72–1.43)	1.09 (0.65–1.51)	0.99 (0.57–1.4)	1.05 (0.55–1.55)
vWF (U/mL)	1.36 (0.78–2.1)	1.53 (0.19–2.87)	0.82 (0.6–1.2)	0.95 (0.44–1.44)	1 (0.46–1.53)	0.92 (0.5–1.58)
ATIII (U/mL)	0.38 (0.14–0.62)	0.63 (0.39–0.87)	1.11 (0.82–1.39)	1.11 (0.9–1.31)	1.05 (0.77–1.32)	1.0 (0.74–1.26)
Protein C (U/mL)	0.28 (0.12–0.44)	0.35 (0.17–0.53)	0.66 (0.4–0.92)	0.69 (0.45–1.44)	0.83 (0.55–1.53)	0.96 (0.64–1.58)

Protein S, total (U/ mL)	0.26 (0.14–0.38) 0.36 (0.12– 0.6)	0.86 (0.54– 1.18)	0.78 ^{0.93)} (0.41– 1.14)	0.72 ^{1.11)} (0.52– 0.92)	0.81 ^{1.28)} (0.6– 1.13)
Protein S, free (U/ mL)	N/A	N/A	0.45 (0.21– 0.69)	0.42 (0.22– 0.62)	0.38 (0.26– 0.55)

Values expressed in units per milliliter are compared with pooled plasma, which contains 1.0 U/ mL. However, pooled plasma contains 0.4 U/ mL of free protein S.

^aValues at individual laboratories may vary based on reagents and analyzers available.

Data from Refs. 22–24.

DIC is the consumption of both pro-and anticoagulant proteins, as well as platelets. It is always caused by an underlying disorder. Diagnosis is based on clinical features as well as prolonged PT and aPTT, thrombocytopenia, hypofibrinogenemia, and increased fibrin degradation products. Therapy should be directed at the underlying cause, although supportive measures may include treatment with fresh-frozen plasma (FFP).

Inherited Factor Deficiencies

Deficiencies of factor (F) VIII and IX, known as hemophilia A and B, respectively, are often present in early childhood. Persons with hemophilia are classified as mild, moderate, or severe, based on their factor activity levels. Newborns with hemophilia can bleed with circumcision and, rarely, may suffer an intracranial hemorrhage after delivery.²⁷ Infants can present with bruising in the absence of trauma. Most children with hemophilia do not present until later in infancy and toddlerhood when they are more mobile. Common presenting signs and symptoms include large hematomas, hemarthrosis in weight-bearing joints, and deep intramuscular hemorrhages. Laboratory evidence for hemophilia includes a prolonged aPTT, which corrects on mixing studies. An abnormally low FVIII or FIX activity level confirms the diagnosis. The treatment of hemophilia in children is similar to that in adults and includes factor replacement dosed according to the site, type, and severity of hemorrhage.²⁸ In patients with severe hemophilia, prophylactic dosing of factor after the first joint bleed is recommended to prevent the later development of chronic arthropathy.²⁹ Many factor products are currently available for patients with both hemophilia A and B. These include the older, virally inactivated plasma derived products, the standard recombinant factor products, and the new, extended half-life recombinant products. For dose determination of factor concentrates, factor VIII levels typically increase by approximately 2% for every 1 unit/kg given and factor IX levels by about 0.8% for every 1 unit/kg administered. In patients with mild hemophilia A, desmopressin is often effective for short-term management of mild bleeding. Inhibitor development remains the most significant cause of morbidity in patients with hemophilia, and is typically present in severe hemophilia

patients prior to 50 exposure days. Treatment for bleeding episodes in patients with inhibitors includes bypassing agents such as recombinant factor VIIa and activated prothrombin complex concentrates.³⁰

Von Willebrand disease (VWD), the most common inherited bleeding disorder, is caused by a decrease in either the quantity or function of von Willebrand factor (VWF). Children are typically present with mucocutaneous bleeding, such as epistaxis, bruising, oral bleeding, and gastrointestinal bleeding, but they can also bleed after a surgery or trauma. Because recurrent bruising and epistaxis are relatively common in children, history should be directed toward prolonged, unusual, or severe bleeding. A careful family history may reveal similar symptoms in parents or siblings. The diagnosis is confirmed by abnormal assays for factor VIII, VWF antigen and activity, and multimer analysis.³¹ Factor VIII and VWF are acute phase reactants. Consequently, interval illnesses or stress may lead to falsely elevated levels, which may obscure the diagnosis. Thus, repeat testing should be performed once the acute illness has resolved. The therapeutic approach for children with VWD is similar to that employed in adults.³²

Rare factor deficiencies, such as fibrinogen, factors II, V, VII, X, XI, or XIII, can also present in childhood. These deficiencies represent 3% to 5% of all coagulation disorders, with an incidence of 1:500,000 to 1:2,000,000.³³ Children can present with mucocutaneous bleeding, bleeding from the umbilical cord stump, or bleeding after a surgery or trauma. Depending on the factor, patients can present with a prolonged PT, aPTT, or both. Testing assays of the specific factor in question is used to make a definitive diagnosis.³⁴ Specific factor concentrates are available for the replacement of fibrinogen, FVII, and FX; otherwise FFP can be used for bleeding with any factor deficiency.

Platelet Disorders

Neonatal alloimmune thrombocytopenia (NAIT) results from the placental transfer of maternal alloantibodies against paternally inherited antigens (most commonly HPA-1a) on fetal platelets. Newborns present with transient, isolated, but severe thrombocytopenia that must be distinguished from other causes, including maternal immune thrombocytopenic purpura (ITP), severe infection, DIC, hypersplenism, and Kasabach–Merritt syndrome. Approximately 10% of affected neonates experience intracranial hemorrhage, either in utero or in the immediate postnatal period.³⁵ Unlike Rh disease of the newborn, prior sensitization is not required, and thus NAIT may occur with the first pregnancy. A normal platelet count in the mother helps to differentiate NAIT from maternal ITP. Immunophenotyping of maternal and paternal platelets is useful to confirm the diagnosis. The treatment of choice in severe NAIT is transfusion of maternal platelets. When they are not readily available, platelets obtained from a known

HPA-1a-negative donor or from random donors may be used for active bleeding. Intravenous immunoglobulin (IVIG) or corticosteroids may also be used as a temporary measure, with dosing as in ITP.³⁶ Subsequent pregnancies result in more severe cases of thrombocytopenia, and prenatal treatment with IVIG and/ or steroids in the mother is indicated.

ITP affects approximately 1 in 10,000 children annually in the United States. In contrast to adults, ITP in childhood is usually a self-limited, benign condition, and 80% have spontaneous resolution within 12 months.³⁷ Children typically present under the age of 10 years. Infants and adolescents are more likely to have prolonged thrombocytopenia. The typical presentation in acute ITP is the abrupt onset of mucosal bleeding, petechiae, and bruising in healthy children, often preceded by a viral illness. Most children have severe thrombocytopenia (platelet counts < 20,000/ μL) with large platelets but an otherwise normal CBC. Acute ITP is a diagnosis of exclusion. However, extensive laboratory testing is rarely required when ITP is suspected in otherwise healthy children with no significant medical history or findings on physical examination. Testing for HIV infection can be considered. The diagnostic utility of bone marrow examination in suspected acute ITP is low. Evaluation of chronic ITP should include bone marrow studies and testing for immunodeficiency and autoimmune diseases. The need for treatment in acute ITP is often debated; however, current guidelines recommend therapy for significant bleeding.³⁸ Although the risk of intracranial hemorrhage is small, precautions should be taken to prevent head trauma, especially when the patient is first diagnosed.

Inherited platelet disorders may be qualitative or quantitative; they are a rare cause of thrombocytopenia in infancy and childhood. The various qualitative disorders includes Glanzmann thrombasthenia (GT), Bernard–Soulier syndrome (BSS), platelet-type pseudo-VWD, and platelet storage granule defects. Quantitative defects are seen in congenital amegakaryocytic thrombocytopenia, thrombocytopenia-absent radii (TAR), X-linked thrombocytopenia, Wiskott–Aldrich syndrome (WAS), and May–Hegglin anomaly. Children with these disorders present with mucocutaneous bleeding, such as petechiae, easy bruising, or bleeding from surgery or trauma. Rarely, gastrointestinal or intracranial bleeding may occur. Screening for qualitative disorders requires platelet aggregation studies. The characteristic features of specific disorders should be sought, such as forearm deformities in TAR, immunodeficiency in WAS, and macrothrombocytes in May–Hegglin anomaly and BSS. Treatment for bleeding is usually supportive, and can consist of platelet transfusions, antifibrinolytic agents (aminocaproic acid or tranexamic acid) or activated, recombinant factor VII.³⁹ Platelet transfusions should be avoided if possible in patients with BSS and GT because of the risk of developing alloantibodies to the missing platelet antigens GPIb–IX and αIIb-β3,

respectively.

THROMBOSIS

As with coagulation factor levels, normal ranges for endogenous antithrombotic proteins are age and gestation dependent (Table 10.6). Venous thromboembolic events (VTE) are less common in children than in adults, but overall the incidence of hospital-acquired VTE is increasing.⁴⁰ VTE affects children in a bimodal age distribution, with a peak in neonates and then adolescents. The central venous catheter (CVC) is one of the most frequently identified risk factors.⁴¹ Anticoagulant and thrombolytic therapy should be dosed according to age and weight (Tables 10.7 and 10.8). Currently, most recommendations for therapy choice and duration are based on adult studies.

Congenital Prothrombotic Disorders

Protein C and S deficiencies, in the homozygous state, classically present as purpura fulminans within hours or days of birth. Purpura fulminans is more common with protein C deficiency and is characterized by acute DIC with rapidly progressive hemorrhagic necrosis of the skin and other thrombotic/ hemorrhagic complications, including death. Homozygous infants usually have undetectable levels of protein C or S, and their parents have heterozygous deficiency. Both functional and immunologic assays for protein C and S should be used. Acquired causes of protein C and S deficiency, such as liver disease and sepsis, should be excluded. Purpura fulminans should be treated with FFP and, if available, purified protein C concentrate. Warfarin-induced skin necrosis has been described in children with heterozygous protein C and S deficiency, and extreme caution is required when converting such individuals from heparin to warfarin anticoagulation.

Table 10.7 Non–Vitamin K Antagonist Anticoagulation Therapy in Children

Age	Heparin Bolus (U/kg)	Heparin Infusion (U/kg/hr) ^a	Enoxaparin (Treatment) ^b	Fondaparinux
Infants	75	28	<2 mo 1.5 mg/kg q12 h	NA
Children	75	20	≥2 mo 1 mg/kg q12 h	≥1 y 0.01 mg/kg q24 h
Adults	80	18	1 mg/kg q12 h	7.5 mg q24 h

^aGoal is aPTT of 1.5–2.5× control (60–85 s).

^bGoal is antifactor Xa level of 0.5–1.0 U/mL 2–6 h after injection.

Data from Monagle P, Chan AK, Goldenberg NA, et al.; American College of Chest Physicians.

Antithrombotic therapy in neonates and children: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 suppl):e737S–801S.

Table 10.8 Vitamin K Antagonist Therapy in Children^a

Day 1	Days 2–4		Maintenance	
	INR	Action	INR	Action
Load 0.2 mg/ kg po if baseline INR 1.0–1.3	1.1–1.3	Repeat initial loading dose	1.1–1.4	Increase by 20% of dose
	1.4–1.9	50% of loading dose	1.5–1.9	Increase by 10% of dose
	2.0–3.0	50% of loading dose	2.0–3.0	No change
	3.1–3.5	25% of loading dose	3.1–3.5	Decrease by 10% of dose
	>3.5	Hold until INR < 3.5, restart at 50% less than previous dose	>3.5	Hold until INR < 3.5, restart at 20% less than previous dose

^aDo not initiate warfarin until therapeutic heparinization. Heparin should not be discontinued until INR is therapeutic.

INR, international normalized ratio.

Adapted with permission from Monagle P, Chan AK, Goldenberg NA, et al.; American College of Chest Physicians. Antithrombotic therapy in neonates and children: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 suppl):e737S–801S.

Other inherited thrombophilic states, including antithrombin deficiency, factor V Leiden, prothrombin G20210A mutations, homocysteinemia, and elevated lipoprotein(a), have also been associated with recurrent thromboembolism in children and adolescents. The significance of an inherited thrombophilia and the risk it places on pediatric patients is yet to be determined.^{42,43}

Acquired Prothrombotic Disorders

As in adult patients, thromboembolism in children is usually secondary. Besides the uses of CVCs, other risk factors include malignancy, surgery, trauma, pregnancy, congenital heart disease, nephrotic syndrome, immobility, and inflammatory states. Complete evaluation for possible underlying conditions should be undertaken. The laboratory examination should be guided by clinical findings and risk factors, and in some cases should include lupus anticoagulant or antiphospholipid antibody assay (especially in adolescents where another cause is not identified).

NEUTROPENIA

Normal neutrophil counts vary with age and are affected by race and other factors (Table 10.1). For example, the lower limit of normal in blacks may be 200 to 600/ μ L less than that in whites. Neutropenia is commonly encountered in pediatrics, most often caused by viral suppression, which typically resolves without intervention. Autoimmune neutropenia of childhood is the most common cause of chronic neutropenia in pediatrics, primarily affecting children younger than 3 years of age. The ANC at presentation can be below 250/ μ L with associated monocytosis, and neutrophil antibodies are usually negative.⁴⁴ Usually unwarranted, bone marrow aspiration can show myeloid maturation arrest at the band stage due to antibody-mediated destruction. The other causes of neutropenia should be considered and ruled out, such as immunodeficiency, drug-related, transient postinfectious, and congenital neutrophil disorders. Although the ANC is often extremely low or absent, most children experience only minor infections and thus this condition is sometimes referred to as chronic benign neutropenia of childhood. Nonetheless, empiric, broad-spectrum parenteral antibiotics are recommended for the first few episodes of fever. If a child appears to have a benign course, subsequent febrile episodes might be managed more routinely unless there is documented infection or signs of sepsis. G-CSF is usually effective in low doses (1 to 2 μ g/ kg/ day) and should be considered for children with recurrent, severe neutropenic complications. Spontaneous remission within the first few years of diagnosis is common, especially in young children.

Cyclic neutropenia is characterized by periodic oscillations in the ANC. Cycles commonly occur every 21 days and the nadir is usually below 200/ μ L. Symptoms typically begin in the first year of life and commonly include recurrent fever, gingivitis, stomatitis with oral aphthous ulcers, and pharyngitis. Diagnosis is confirmed by monitoring serial CBCs twice per week for 6 to 8 weeks to establish the periodicity of the neutropenia, which may also be accompanied by asymptomatic oscillations in other blood counts. Cyclic neutropenia is an autosomal dominant disorder caused by mutation in the gene encoding neutrophil elastase (ELANE). Parental history and/ or CBCs may be helpful. Although in most cases cyclic neutropenia is a benign condition, serious infectious complications may occur and treatment with G-CSF is sometimes indicated. Low doses of G-CSF often suffice, and doses of 2 to 3 μ g/ kg daily or every other day can be titrated to maintain the neutrophil count greater than 500/ μ L.

Severe congenital neutropenia (Kostmann disease) is a disorder associated with severe, chronic neutropenia from birth. The ANC is usually less than 200/ μ L and recurrent bacterial infections are common, as well as life-threatening sepsis, meningitis, and gastrointestinal tract infections. Neonates may present with omphalitis. Bone marrow examination reveals neutrophil developmental arrest. Inheritance is most often due to autosomal recessive mutations in ELANE, although other genetic defects and

inheritance patterns are well described.⁴⁵ Standard therapy consists of daily G-CSF, but high doses may be required. Patients undergoing long-term therapy are at risk for myelodysplastic syndrome and acute myelogenous leukemia, and SCT should be considered as a curative approach.

Shwachman–Diamond syndrome and Chédiak–Higashi syndrome are both autosomal recessive constitutional neutropenias. Shwachman–Diamond, caused by compound heterozygous or homozygous mutations in the SBDS gene, is characterized by progressive marrow failure, pancreatic exocrine insufficiency, short stature, skeletal deformities, and predisposition to myelodysplastic syndrome and acute myeloid leukemia. Two-thirds of patients have a moderate neutropenia that can be intermittent and responsive to G-CSF. Chédiak–Higashi syndrome is also a multiorgan disease that includes oculocutaneous albinism, recurrent bacterial infection, a mild bleeding disorder due to platelet dysfunction, and neuropathy. The accumulation of giant granules in neutrophils leads to premature destruction. This disorder is caused by homozygous or compound heterozygous mutations in the lysosomal trafficking regulator gene (LYST or CHS1). Affected individuals commonly progress to an accelerated phase characterized by hemophagocytic lymphohistiocytosis, which is often fatal. Therapy is mostly supportive and SCT is the only known cure for the hematologic manifestations.

Other causes of neutropenia include drug-induced, inborn errors of metabolism, nutritional deficiency, or bone marrow infiltration. Treatment should be directed at the underlying etiology.

LEUKOCYTOSIS

Leukocytosis refers to an increase in total WBC count for age. Neutrophilia is an increase in the ANC above 7,500/ μL , but the upper limit of normal may be higher in newborns and infants (Table 10.1). Neutrophilia may result from increased production, mobilization from the bone marrow, or peripheral demargination. In children, acute neutrophilia is most often due to bacterial or viral infection. Absolute lymphocytosis usually indicates an acute or chronic viral process. Evaluation of leukocytosis should include a detailed history and physical examination directed to symptoms and signs of infection and for lymphadenopathy and hepatosplenomegaly. Examination of the peripheral blood smear is essential to distinguish normal from atypical and malignant WBCs.

Leukocyte adhesion deficiency I (LADI) is a disorder of impaired leukocyte adhesion, chemotaxis, and ingestion, caused by partial or total deficiency of CD18-related surface glycoproteins due to deficiency of the gene encoding the β -2 integrin chain (ITGB2).⁴⁶ The hallmark of this disorder is the occurrence of repeated, severe bacterial infections of the skin and mucosal surfaces in the absence of pus despite persistent

neutrophilia. Infants typically present in the newborn period with omphalitis or delayed umbilical cord separation. The diagnosis of LAD can be confirmed by flow cytometry, which reveals deficiency of surface expression of the CD18/ CD11 molecules in patients with marked leukocytosis. Treatment is supportive, consisting primarily of prophylaxis against and treatment of infection. SCT may be curative, and more recently investigational gene therapy has been studied.

Infectious mononucleosis (IM) is classically associated with atypical lymphocytosis and is caused by infection with Epstein–Barr virus (EBV). In adolescents and young adults, a prodrome of fatigue and anorexia usually precedes development of fever, lymphadenopathy, exudative pharyngitis, and hepatosplenomegaly. Young children commonly present with a mild respiratory illness only. Hematologic complications, including immune-mediated hemolytic anemia, thrombocytopenia, aplastic anemia, and hemophagocytosis can be seen. Other rare complications include central nervous system (CNS) involvement, myocarditis, orchitis, and splenic rupture. Children with acquired or congenital immunodeficiency states can develop EBV-associated lymphoproliferative syndrome, which can evolve into non-Hodgkin lymphoma. EBV in boys with X-linked lymphoproliferative syndrome, results in fulminant IM, hemophagocytic lymphohistiocytosis, lymphoma, and/ or other severe EBV-associated complications that are often fatal.⁴⁷ Therapy of IM is supportive. Rarely, short course corticosteroids are used to manage life-threatening manifestations, such as upper airway obstruction from tonsillar/ adenoidal hypertrophy. To reduce the risk of splenic rupture, contact sports should be avoided until splenomegaly resolves.

HEMATOLOGIC MANIFESTATIONS OF SYSTEMIC CONDITIONS

Many systemic conditions can result in secondary hematologic abnormalities. Evaluation of the CBC and peripheral smear may also provide important clues during the evaluation of a diagnostic dilemma. Systemic disorders that have prominent hematologic findings and present predominantly in childhood are detailed in the following.

Lysosomal storage diseases are caused by a deficiency in specific enzymes of the lysosomal metabolic pathway. This results in pathologic accumulation of normal substrates, leading to dramatic changes in the central nervous and hematologic systems, as well as enlargement of organs that comprise the reticuloendothelial system, including the liver and spleen. Vacuolated lymphocytes and hypergranulated neutrophils in the peripheral blood and lipid-laden macrophages (“foam” or “storage” cells) in the bone marrow are commonly observed. Other characteristic cell types include the sea-blue histiocyte in Niemann–Pick disease and the Gaucher cell. Specific enzyme replacement therapy is available for some of these disorders, while therapy is only supportive for

others and SCT is curative in certain conditions.⁴⁸

Autoimmune lymphoproliferative syndrome (ALPS) is a rare disorder of early childhood caused by defective lymphocyte apoptosis.⁴⁹ Symptoms include lymphadenopathy, splenomegaly, autoimmunity, and an increased risk of lymphoid malignancy. Autoimmune cytopenias are common. The increased numbers of double negative (CD4-/ CD8-) T-cells on flow cytometry supports the diagnosis. Several molecular defects have been identified, most commonly mutations in Fas (TNFRSF6, CD95). Therapy is mainly supportive, although immunosuppressive drugs may be needed to manage complications of autoimmunity and lymphoproliferation. SCT can be curative.

Collagen vascular diseases commonly have hematologic manifestations, most often anemia of chronic illness and/ or autoimmune-mediated cytopenias. Patients with SLE may present with mild thrombocytopenia and are also at an increased risk for developing antiphospholipid antibodies. Although such lupus anticoagulants result in prolongation of PT and PTT, they predispose to thromboembolism rather than bleeding.

TRANSFUSION SUPPORT

The indications for transfusion in infants and children are similar to those in adults. Patient size, blood volume, and underlying condition mandate special precautions in regard to dosing and risks. In all cases, careful consideration should be given to the indication for, appropriate dose of, and potential toxicities of the specific blood product. Formulas to calculate pediatric transfusion requirements are detailed in [Table 10.9](#).

Packed red blood cells (PRBCs) should be transfused in children according to age-specific blood volumes and target Hb levels. Unless rapid replacement is required for shock or rapid loss, the recommended infusion rate is 2 to 4 mL/ kg/ hr or 10 to 15 mL/ kg aliquots over 3 to 4 hours. With volume intolerance, gradual correction can be achieved by infusing small aliquots (5 to 10 mL/ kg) over 4 to 6 hours. Diuretics may be helpful when there is a concern for fluid overload. When rapid correction is required but is limited by fluid intolerance, partial exchange transfusion can be used with whole blood removed in small aliquots and replaced with equal volumes of PRBCs.⁵⁰

Table 10.9 Transfusion Dosing in Pediatrics

Total blood volume (TBV) Estimate

Newborn	100 mL/ kg
Child	80 mL/ kg
Adult	65 mL/ kg

Packed red blood cells (PRBC)

Manual partial red blood cell (RBC) exchange^a

Platelets

0.1 unit/ kg should increase the platelet count by approximately 50,000/ μ L

Fresh frozen plasma (FFP)

10 mL/ kg should increase the factor activity level by approximately 20%

Cryoprecipitate

0.3 unit/ kg should increase the fibrinogen level by approximately 200 mg/ dL

HCT should be in fractions (e.g., 40% = 0.4); HCT_i, initial; HCT_d, desired; HCT_{prbc} usually 0.65–0.8.

^aAdapted from Neiburg PI, Stockman JA. Rapid correction of anemia with partial exchange transfusion. Am J Dis Child. 1977;131:60–61.

Platelets transfused at a dose of 0.1 U/ kg are expected to increase the platelet count by approximately 50,000/ μ L in most infants and children.⁵¹ The target posttransfusion platelet count varies with the clinical scenario. In general, the aim should be to raise the count to a level at which bleeding stops. Values of around 50,000/ μ L usually suffice, although counts about 100,000/ μ L should be maintained for life-threatening circumstances, such as CNS, vascular, or surgical hemorrhage. In the setting of myelosuppression without additional risk factors for severe bleeding, prophylactic platelet transfusion is recommended at a level of 10,000/ μ L. To minimize the risk of bleeding in newborns, standard recommendations are to maintain the platelet count above 30,000/ μ L for full-term and 50,000/ μ L for premature infants. Prophylactic transfusions are not indicated in the setting of ITP and other antibody-mediated forms of platelet destruction where no transfusion-related increment is expected; rather, transfusions should be reserved for life-threatening bleeding. To decrease the risk of alloimmunization in children who require multiple transfusions, single-donor (apheresis) and leukocyte-depleted platelets should be used whenever possible.

FFP is recommended for children with coagulopathy, as evidenced by prolonged PT and/ or PTT, who have active bleeding or to prevent hemorrhage in those at high risk (e.g., preoperatively). FFP should be used to replace clotting factors for which specific concentrates are not available and a dose of 10 to 15 mL/ kg usually raises the clotting activity by approximately 20%. Multiple doses may be required if there is an ongoing consumption. The rate of transfusion is limited by citrate toxicity, and vital signs and

ionized calcium levels should be monitored closely when large or rapid infusions are used. Cryoprecipitate is used primarily to combat bleeding with hypofibrinogenemia. A dose of 0.3 U/ kg will increase the fibrinogen level by approximately 200 mg/ dL.

Specialized Blood Products to Prevent Toxicities

WBC removal by leukofiltration should be performed for recipients of blood products who require multiple transfusions to decrease the risk of sensitization to leukocyte antigens. Leukodepletion also decreases the risks of febrile reactions and CMV transmission. Irradiation of cellular blood products with 2,500 cGy should be used to prevent transfusion-associated graft-versus-host disease in the following situations: (1) potential immunocompromised host, including very low birth weight infants, immunodeficiency, malignancy, marrow or organ transplantation; (2) blood from first-degree family members or HLA-matched donors; and (3) all granulocyte transfusions.

References

1. Orkin SH FD, Nathan DG, Ginsburg D, et al. Nathan and Oski's Hematology of Infancy and Childhood. Philadelphia, PA: Elsevier Health Sciences; 2009.
2. Dallman PR, Shannon K. Developmental changes in red blood cell production and function. In: Rudolph A, ed. Pediatrics. 20th ed. New York: Appleton-Lange; 1995; chap 17:1170.
3. O'Brien RT, Pearson HA. Physiologic anemia of the newborn infant. *J Pediatr*. 1971;79(1):132–138.
4. Baker RD, Greer FR, Committee on Nutrition American Academy of P. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). *Pediatrics*. 2010;126(5):1040–1050.
5. Kohli-Kumar M. Screening for anemia in children: AAP recommendations—a critique. *Pediatrics*. 2001;108(3):E56.
6. Woolf AD, Goldman R, Bellinger DC. Update on the clinical management of childhood lead poisoning. *Pediatr Clin North Am*. 2007;54(2):271–294, viii.
7. Prevention CfDCa. Managing elevated blood lead levels among young children: recommendations from the Advisory Committee on Childhood Lead Poisoning Prevention. 2002;
http://www.cdc.gov/nceh/lead/casemanagement/casemanage_chap3.htm-Table%203.1. Accessed October 2016.
8. Earley A, Valman HB, Altman DG, et al. Microcytosis, iron deficiency, and thalassaemia in preschool children. *Arch Dis Child*. 1990;65(6):610–614.
9. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. *Blood*. 2011;118(13):3479–3488.
10. van den Akker M, Dror Y, Odame I. Transient erythroblastopenia of childhood is

- an underdiagnosed and self-limiting disease. *Acta Paediatr.* 2014;103(7):e288–294.
11. Amankwah EK, Atchison CM, Arlikar S, et al. Risk factors for hospital-associated venous thromboembolism in the neonatal intensive care unit. *Thromb Res.* 2014;134(2):305–309.
 12. Gill FM, Sleeper LA, Weiner SJ, et al. Clinical events in the first decade in a cohort of infants with sickle cell disease. Cooperative Study of Sickle Cell Disease. *Blood.* 1995;86(2):776–783.
 13. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med.* 1998;339(1):5–11.
 14. Scothorn DJ, Price C, Schwartz D, et al. Risk of recurrent stroke in children with sickle cell disease receiving blood transfusion therapy for at least five years after initial stroke. *J Pediatr.* 2002;140(3):348–354.
 15. McGann PT, Ware RE. Hydroxyurea for sickle cell anemia: what have we learned and what questions still remain? *Curr Opin Hematol.* 2011;18(3):158–165.
 16. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). *Lancet.* 2011;377(9778):1663–1672.
 17. Rosenblatt DS, Whitehead VM. Cobalamin and folate deficiency: acquired and hereditary disorders in children. *Semin Hematol.* 1999;36(1):19–34.
 18. Lipton JM, Ellis SR. Diamond-Blackfan anemia: diagnosis, treatment, and molecular pathogenesis. *Hematol Oncol Clin North Am.* 2009;23(2):261–282.
 19. Boria I, Garelli E, Gazda HT, et al. The ribosomal basis of Diamond-Blackfan anemia: mutation and database update. *Hum Mutat.* 2010;31(12):1269–1279.
 20. Vlachos A, Federman N, Reyes-Haley C, et al. Hematopoietic stem cell transplantation for Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Bone Marrow Transplant.* 2001;27(4):381–386.
 21. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood.* 2003;101(3):822–826.
 22. Jaffray J, Young G. C: clinical implications from the fetus to the adolescent. *Pediatr Clin North Am.* 2013;60(6):1407–1417.
 23. Andrew M, Vegh P, Johnston M, et al. Maturation of the hemostatic system in childhood. *Blood.* 1992;80:1998–2005.
 24. Andrew M, Paes B, Milner R, et al. Development of the coagulation system in the healthy premature infant. *Blood.* 1988;72:1651–1657.
 25. Andrew M, Paes B, Milner R, et al. The development of the human coagulation system in the full term infant. *Blood.* 1987;70:165–170.
 26. Sutor AH, von Kries R, Cornelissen EA, et al. Vitamin K deficiency bleeding (VKDB) in infancy. ISTH Pediatric/ Perinatal Subcommittee. International Society

- on Thrombosis and Haemostasis. *Thromb Haemost*. 1999;81(3):456–461.
- 27. Davies J, Kadir RA. Mode of delivery and cranial bleeding in newborns with haemophilia: a systematic review and meta-analysis of the literature. *Haemophilia*. 2016;22(1):32–38.
 - 28. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2013;19(1):e1–e47.
 - 29. Manco-Johnson M. Comparing prophylaxis with episodic treatment in haemophilia A: implications for clinical practice. *Haemophilia*. 2007;13(suppl 2):4–9.
 - 30. Kempton CL, Meeks SL. Toward optimal therapy for inhibitors in hemophilia. *Blood*. 2014;124(23):3365–3372.
 - 31. Ng C, Motto DG, Di Paola J. Diagnostic approach to von Willebrand disease. *Blood*. 2015;125(13):2029–2037.
 - 32. The diagnosis, evaluation, and management of von Willebrand Disease. U.S. Department of Health And Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, NIH Publication No. 08-5832, December 2007; www.nhlbi.nih.gov/files/docs/guidelines/vwd.pdf.
 - 33. Acharya SS, Coughlin A, Dimichele DM, North American Rare Bleeding Disorder Study G. Rare bleeding disorder registry: deficiencies of factors II, V, VII, X, XIII, fibrinogen and dysfibrinogenemias. *J Thromb Haemost*. 2004;2(2):248–256.
 - 34. Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. *Blood*. 2015;125(13):2052–2061.
 - 35. Bussel JB, Zacharoulis S, Kramer K, et al. Clinical and diagnostic comparison of neonatal alloimmune thrombocytopenia to non-immune cases of thrombocytopenia. *Pediatr Blood Cancer*. 2005;45(2):176–183.
 - 36. Bussel JB, Berkowitz RL, Hung C, et al. Intracranial hemorrhage in alloimmune thrombocytopenia: stratified management to prevent recurrence in the subsequent affected fetus. *Am J Obstet Gynecol*. 2010;203(2):135, e131–e114.
 - 37. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386–2393.
 - 38. Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood*. 2011;117(16):4190–4207.
 - 39. Franchini M. The use of recombinant activated factor VII in platelet disorders: a critical review of the literature. *Blood Transfus*. 2009;7(1):24–28.
 - 40. Raffini L, Huang YS, Witmer C, et al. Dramatic increase in venous thromboembolism in children's hospitals in the United States from 2001 to 2007. *Pediatrics*. 2009;124(4):1001–1008.
 - 41. Mahajerin A, Branchford BR, Amankwah EK, et al. Hospital-associated venous

- thromboembolism in pediatrics: a systematic review and meta-analysis of risk factors and risk-assessment models. *Haematologica*. 2015;100(8):1045–1050.
42. Manco-Johnson MJ, Grabowski EF, Hellgreen M, et al. Laboratory testing for thrombophilia in pediatric patients. On behalf of the Subcommittee for Perinatal and Pediatric Thrombosis of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis (ISTH). *Thromb Haemost*. 2002;88(1):155–156.
 43. Monagle P, Chan AK, Goldenberg NA, et al. Antithrombotic therapy in neonates and children: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 suppl):e737S–801S.
 44. Bux J, Behrens G, Jaeger G, et al. Diagnosis and clinical course of autoimmune neutropenia in infancy: analysis of 240 cases. *Blood*. 1998;91(1):181–186.
 45. Ward AC, Dale DC. Genetic and molecular diagnosis of severe congenital neutropenia. *Curr Opin Hematol*. 2009;16(1):9–13.
 46. Hanna S, Etzioni A. Leukocyte adhesion deficiencies. *Ann N Y Acad Sci*. 2012;1250:50–55.
 47. Rezaei N, Mahmoudi E, Aghamohammadi A, et al. X-linked lymphoproliferative syndrome: a genetic condition typified by the triad of infection, immunodeficiency and lymphoma. *Br J Haematol*. 2011;152(1):13–30.
 48. Boelens JJ, Prasad VK, Tolar J, et al. Current international perspectives on hematopoietic stem cell transplantation for inherited metabolic disorders. *Pediatr Clin North Am*. 2010;57(1):123–145.
 49. Oliveira JB, Bleesing JJ, Dianzani U, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood*. 2010;116(14):e35–e40.
 50. Neiburg PI, Stockman JA. Rapid correction of anemia with partial exchange transfusion. *Am J Dis Child*. 1977;131:60–61.
 51. Luban NL, McBride E, Ford JC, et al. Transfusion medicine problems and solutions for the pediatric hematologist/ oncologist. *Pediatr Blood Cancer*. 2012;58(7):1106–1111.

Acute Myeloid Leukemia

Christopher S. Hourigan and Vera
Malkovska

Acute myeloid leukemia (AML) is a heterogeneous group of diseases characterized by uncontrolled proliferation of myeloid progenitor cells that gradually replace normal hematopoiesis in the bone marrow. The genetic changes arising in the neoplastic clone lead to cascades of molecular events that cause abnormal proliferation and aberrant differentiation of the malignant cells and ultimately result in the inhibition of normal hematopoiesis.

Characterization of genetic aberrations is becoming increasingly important in establishing diagnosis, defining prognosis, and planning therapy in AML. Aggressive chemotherapy with optimal supportive treatment has improved outcomes in younger patients with AML. Most of these patients achieve a complete remission (CR) but many relapse and their 5-year survival remains below 50% in large studies. Patients older than 65 years have a median survival of less than a year and long-term survival rates around 10%. The current major challenges in AML care are to diagnose, prevent, and treat relapsed disease in younger patients and to design, based on improved understanding of the molecular mechanisms of AML, leukemia-specific treatments that are effective in chemotherapy-resistant disease and applicable to older patients.

EPIDEMIOLOGY

The age-adjusted incidence rate of AML in the United States is 4.1 per 100,000, accounting for more than 10,000 deaths per year. AML accounts for about 15% to 20% of acute leukemias in children and adolescents and 85% in adults. The incidence of AML rises rapidly after the age of 60 and the median age at diagnosis is 67 years (Fig. 11.1).^{1–3}

ETIOLOGY

AML is a diagnostic term encompassing a diverse collection of myeloid malignancy, with no single unifying etiological mechanism. Inherited genetic predisposition, environmental mutagens such as radiation, drugs, and other toxins, and acquired somatic mutations associated with aging may all play a role in the development of AML.⁴ Genetic causes are suggested by the increased incidence of AML in identical twins, as well as the known association of AML with various congenital disorders.

Known risks factors for the development of AML are shown in [Table 11.1](#).

A large European population-based study, however, found no significant familial aggregation for AML or myelodysplastic syndromes (MDS)⁴ suggesting that in most adults with AML/ MDS environmental factors and acquired somatic mutations are a more common etiology than inherited germline mutations. Genetic predisposition is important in the development of some AML in children and young adults and is increasingly recognized as playing a role in an important subset of cases in older adults.^{5,6} The latest World Health Organization (WHO) classification explicitly includes reference to myeloid neoplasms with germline predisposition ([Table 11.2](#)).

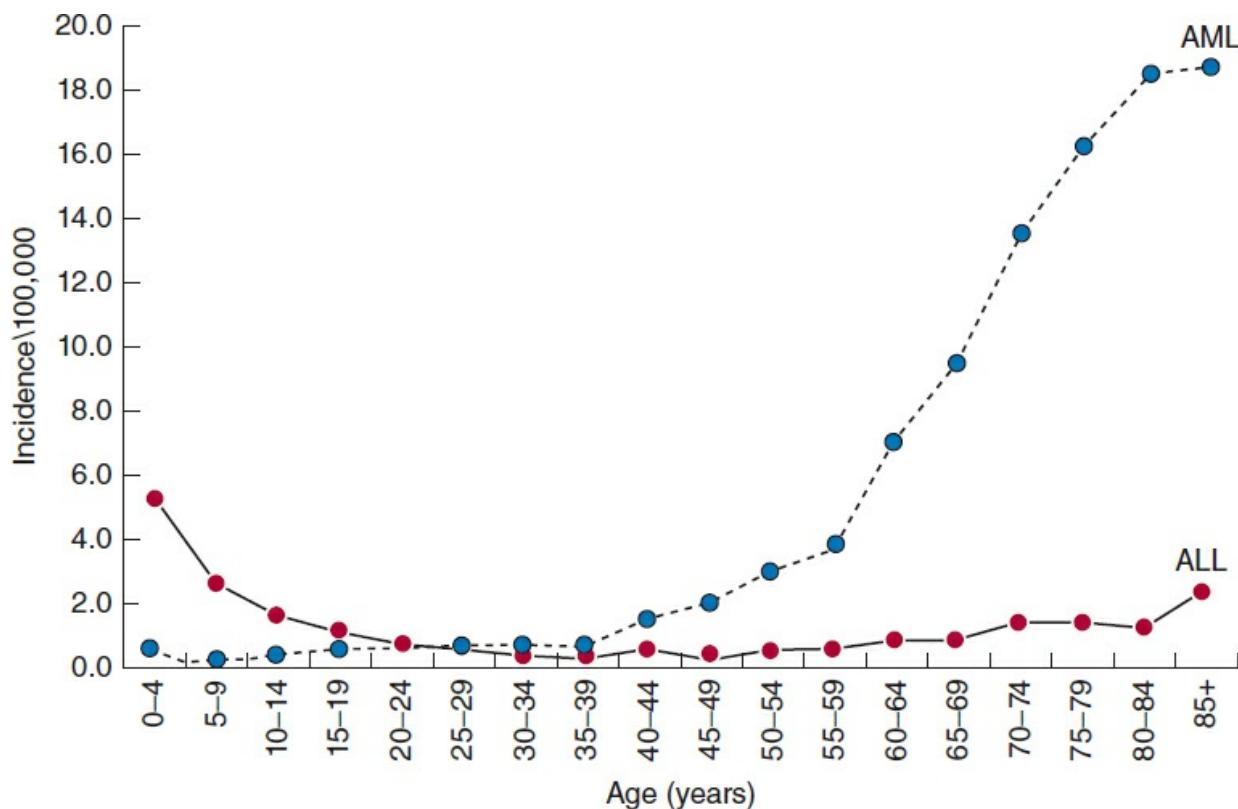


FIGURE 11.1 The age-related incidence of AML in the United States.

AML, acute myeloid leukemia.

Table 11.1 Risk Factors for Development of Acute Myeloid Leukemia**Environmental Exposures**

Benzene
Ionizing radiation
Smoking

Genetic Disorders

Down syndrome
Bloom syndrome
Fanconi anemia
Dyskeratosis congenita
Ataxia telangiectasia
Li-Fraumeni syndrome
Kostmann syndrome
Klinefelter syndrome

Preexisting Hematologic Disorders

MDS
Myeloproliferative disorders
Aplastic anemia

Treatment Associated

Alkylating agents: AML usually arises from MDS, after a 3- to 10-year latency period and is associated with deletions involving chromosomes 5 or 7.

Topoisomerase II inhibitors: AML lacks preceding myelodysplasia, has a shorter latency (1–3 y), exhibits monocytic morphology, and is associated with changes involving the long arm of chromosome 11 (11q23).

Radiotherapy alone or in combination with chemotherapy.

AML, acute myeloid leukemia; MDS, myelodysplastic syndromes.

Table 11.2 WHO Classification of Myeloid Neoplasms With Germline Predisposition**Myeloid Neoplasms With Germline Predisposition Without a Preexisting Disorder or Organ Dysfunction**

AML with germline CEBPA mutation
Myeloid neoplasms with germline DDX41 mutation^a

Myeloid Neoplasms With Germline Predisposition and Preexisting Platelet Disorders

Myeloid neoplasms with germline RUNX1 mutation^a
Myeloid neoplasms with germline ANKRD26 mutation^a
Myeloid neoplasms with germline ETV6 mutation^a

Myeloid Neoplasms With Germline Predisposition and Other Organ Dysfunction

Myeloid neoplasms with germline GATA2 mutation
Myeloid neoplasms associated with BM failure syndromes
Myeloid neoplasms associated with telomere biology disorders
JMML associated with neurofibromatosis, Noonan syndrome or
Noonan syndrome-like disorders

Myeloid neoplasms associated with Down syndrome^a

AML, acute myeloid leukemia; CEBPA, CCAAT/ enhancer binding protein alpha; WHO, World Health Organization.

^aLymphoid neoplasms also reported.

Reprinted with permission from Arber et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*, 2016;127(20):2391–2405.

AML arising from preexisting hematologic disorders, most commonly MDS or myeloproliferative disorders, have inferior prognosis. AML following exposure to chemotherapy and radiation is characterized by resistance to treatment and short survival.

PATHOGENESIS

It had long been known that AML has heterogeneous pathogenesis; unlike other clonal hematological disorders where a single molecular event may be pathognomonic and disease defining. Studies of cytogenetics in AML revealed a wide diversity of underlying genetics with associated differences in response to treatment and overall prognosis.⁷ Advances in sequencing technology over the past 8 years have revolutionized our understanding of the underlying biology of the diverse group of myeloid malignancies covered by the diagnostic umbrella of “AML.”^{8–12} It is now clear that the disease phenotype of AML is generated by many different combinations of chromosomal or DNA abnormalities.^{7,9,11} It is also now clear that these genetic abnormalities happen in a defined sequence with a temporal hierarchy that often branches at later events resulting in a polyclonal disease at clinical presentation, with differential sensitivity to treatment such that the predominant clone present at initial diagnosis prior to treatment is not necessarily the same malignant clone ultimately responsible for relapse and death.^{13–20} Recurrent “hotspots” of somatic mutations commonly seen across patients have been identified (see Tables 11.6 and 11.7). These can provide mechanistic insight informing targeted treatment approaches and also predictive and prognostic information for current therapy.

CLINICAL FEATURES

Patients with AML usually present with bone marrow failure that causes anemia, bleeding from thrombocytopenia, and neutropenic infections. Tissue infiltration with leukemic blasts involving gums, skin, meninges, and other organs is most commonly

associated with monocytic morphology. Striking bruising and life-threatening hemorrhage should raise suspicion of disseminated intravascular coagulation (DIC) frequently seen in acute promyelocytic leukemia (APL). However, DIC can occur in any type of AML. Leukostasis and hyperviscosity causing organ dysfunction usually occur with blast cell counts greater than 100,000/ μ L. Manifested by confusion, visual impairment, and shortness of breath, leukostasis can also lead to hemorrhage in the retina, brain, lungs, and other organs. Rare but striking manifestations of AML include the Sweet syndrome—a skin rash with neutrophilic infiltrates in the dermis, and chloromas—tumors of myeloid blasts. Extramedullary leukemia portends a worse prognosis.

Symptoms and signs on presentation include the following:

Marrow failure

- | Fatigue
- | Shortness of breath
- | Fever
- | Focal bacterial infections
- | Petechiae
- | Bruising
- | Bleeding (if severe, suspect promyelocytic leukemia)

Tissue involvement

- | Bone pain, tenderness
- | Moderate splenomegaly
- | Gingival hyperplasia
- | Central nervous system (CNS) and cranial nerve dysfunction
- | Visual changes (retinal involvement, hemorrhage, papilledema)

Rare manifestations

- | Sweet syndrome
- | Chloromas

LABORATORY FINDINGS

The most common laboratory findings in AML are anemia, thrombocytopenia, neutropenia, and myeloid blasts on the blood smear. The blasts have distinct immunophenotypes detected by flow cytometry. In aleukemic leukemia, blasts are seen only in the bone marrow. Coagulopathy resulting from DIC is common in promyelocytic leukemia. Hyperuricemia from high cell turnover is often seen on presentation and worsens during chemotherapy. Rapidly rising serum levels of uric acid, potassium, and

phosphate with decreasing calcium herald a tumor lysis syndrome that can cause acute renal failure. Renal tubular dysfunction caused by muramidase released from leukemic blasts can worsen the electrolyte abnormalities commonly seen in AML. Lactic acidosis tends to occur with leukostasis while high lactate dehydrogenase (LDH) is associated with CNS involvement. High numbers of leukemic blasts in blood samples may cause spurious hypoglycemia, hypoxemia, hypokalemia, and other abnormalities resulting from cellular metabolic activity in vitro. Rapid processing of anticoagulated blood samples avoids this artifact.

In addition to routine chest X-rays, imaging studies, including computerized tomography (CT) and magnetic resonance imaging (MRI) scans, directed according to symptoms can reveal leukemic infiltrates, hemorrhage, or infection.

Laboratory findings in AML include the following:

Hematologic

- | Increased white blood cell count with blasts in peripheral blood
- | Anemia
- | Granulocytopenia
- | Thrombocytopenia
- | DIC

Chemistry

- | Hyperuricemia
- | Elevated blood urea nitrogen and creatinine (urate nephropathy)
- | High LDH
- | Hypokalemia (tubular dysfunction)
- | Lactic acidosis (leukostasis)
- | Hypercalcemia, rarely hypocalcemia
- | Spurious hypoxemia, hypoglycemia, hyperkalemia or hypokalemia

Imaging studies

- | Intracranial hemorrhage (often with hyperviscosity) (CT)
- | Thickened nerve sheaths (MRI)
- | Lung infiltrates (CT)

CLASSIFICATION

The classification of AML has evolved from the mainly morphology-based French–American–British (FAB) to the more comprehensive WHO classification. The older FAB classification based on morphology, cytochemical staining, and immunophenotype of the predominant cells divided AML into eight subtypes (M0–M7) ([Table 11.3](#)).

The 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia integrates new clinical, prognostic, morphologic, immunophenotypic, and genetic data that have emerged since the last edition in 2008.⁶

In particular, molecular information from cytogenetics and genetic mutations is being used for disease definition supplanting classification purely by morphological characteristics, in recognition to the predictive and prognostic importance of these underlying etiological lesions (Table 11.4).

Table 11.3 French–American–British Classification of Acute Myeloid Leukemia

Subtype	Description
M0	Minimally differentiated AML: negative peroxidase reaction, two or more myeloid markers by flow cytometry, frequently has complex cytogenetic abnormalities associated with poor prognosis.
M1	AML without maturation: less than 10% promyelocytes or more mature myeloid forms.
M2	AML with maturation: subset of patients have the t(8;21) translocation associated with favorable prognosis.
M3	APL: in most cases heavy granulation and bilobed nuclear contour; rarely microgranular variant with inconspicuous granules. Most cases have t(15;17) translocation and favorable prognosis.
M4	Acute myelomonocytic leukemia: monocytes and promonocytes in the marrow exceed 20%. M4Eo variant contains more than 5% abnormal eosinophils; associated with the inv(16) cytogenetic abnormality and favorable prognosis.
M5	Acute monocytic leukemia: 80% or more of nonerythroid cells are monoblasts, monocytes, or promonocytes. Nonspecific esterase stain is positive. Associated with extramedullary disease and abnormalities of the long arm of chromosome 11 (11q).
M6	Acute erythroleukemia: more than 50% nucleated marrow cells are erythroid, often severely dyserythropoietic. Erythroblasts are strongly periodic acid-Schiff (PAS)-positive and glycophorin A positive.
M7	Acute megakaryocytic leukemia: may have micromegakaryoblasts. Diagnosis is confirmed by immunophenotyping (CD41) or electron microscopy (platelet peroxidase)

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; FAB, French–American–British.

Table 11.4 World Health Organization Classification of Acute Myeloid Leukemia

AML and Related Neoplasms

AML with recurrent genetic abnormalities

AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11

APL with PML-RARA

AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A

AML with t(6;9)(p23;q34.1);DEK-NUP214

AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM

AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1

Provisional entity: AML with BCR-ABL1

AML with mutated NPM1

AML with biallelic mutations of CEBPA

Provisional entity: AML with mutated RUNX1

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, NOS

AML with minimal differentiation

AML without maturation

AML with maturation

AML

Acute monoblastic/ monocytic leukemia

Pure erythroid leukemia

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

TAM

Myeloid leukemia associated with Down syndrome

Acute leukemias of ambiguous lineage

Myeloid neoplasms with germline predisposition (see [Table 11.2](#))

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CEBPA, CCAAT/ enhancer binding protein alpha; NOS, not otherwise specified; TAM, transient abnormal myelopoiesis.

Adapted with permission from Arber et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*, 2016;127(20):2391–2405.

Notably, this latest WHO classification also includes a new section on myeloid neoplasms with germline predisposition, including AMLs that occur in patients with a predisposing germline mutation.⁶ The guidelines state that the presence of the specific underlying genetic defect or predisposition syndrome should be documented as part of the diagnosis. A thorough and directed family history is necessary to make such a diagnosis.⁵

DIAGNOSTIC EVALUATION

Leukemic myeloblasts are usually seen on the blood smear and are almost always found on examination of the bone marrow biopsy.

A comprehensive history of any antecedent hematologic or cancer diagnosis in the

patient or the family together with any occupational, medication (chemotherapy, radiation, immunosuppression) or environment exposures in the patient should be taken. Specifically any family or personal history of low blood counts, bleeding disorders, skin pigmentation or nail abnormalities, premature hair loss or greying, liver disease, pulmonary fibrosis, oral leukoplakia, limb anomalies, lymphedema, cutaneous warts, sensorineural deafness, immune deficiencies, or atypical infections should be sought. The history should be communicated to the pathologist, together with results of relevant examination, laboratory, radiographic, and other findings. A historical trend of complete blood count results can be particularly informative, and attempts should be made to secure these records.

The evaluation of a patient with AML at diagnosis includes the following:

- Blood count and inspection of blood smear
- Bone marrow aspirate and biopsy
 - Morphology with Wright–Giemsa stain
 - Immunophenotyping by multiparameter flow cytometry
 - Karyotype (metaphase cytogenetics +/ – FISH)
 - Molecular analysis (including NPM1, FLT3, KIT, CEBPA, TP53, RUNX1)
- Lumbar puncture (after blasts cleared from blood): if CNS symptoms, monocytic morphology, or high blast count

The morphologic diagnosis of AML can be supported by the presence of Auer rods in the cytoplasm, positive cytochemistry with Sudan black, and staining for myeloperoxidase and esterases. Immunophenotyping with a panel of monoclonal antibodies is particularly useful for distinguishing AML from acute lymphoblastic leukemia (ALL) and for identification of the subtypes, including AML with minimal differentiation, erythroleukemia, and megakaryoblastic leukemia (Table 11.5). Cytogenetic and molecular abnormalities associated with the morphologic subtypes can further support the diagnosis and help predict treatment outcomes.

Table 11.5 Diagnostic Markers in Acute Myeloid Leukemia

FAB Class	Cytochemistry	Immunophenotype	Cytogenetics
MO: Minimally differentiated	MPO < 3%	CD34+, HLA DR+ CD33+/-, CD13+/-	11q13
M1: Without maturation	MPO < 3%	CD34+, HLA DR+ CD33+, CD13+	-5, -7, -17 Del 3p, +21, +8
M2: With maturation	MPO > 10%	CD34-, HLA DR+ CD33+, CD13+	t(8;21), del3p or inv 3 -5, -7, +8, t(6;9)
M3: Promyelocytic	MPO++	CD 34-, HLA DR-,	t(15;17)

M4: Myelomonocytic	MPO+, Esterase+	CD 13+, CD 33+ CD 34-, HLA DR+, CD 13+, CD 33+ CD 11+, CD14+,	Inv 16, t(16;16) -5, -7, t(6;9)
M5: Monocytic	Esterase++, PAS+	CD 34-, HLA DR+, CD 13+/ -, CD 33+ CD11+, CD14+,	t(9;11) (p21;23), +8
M6: Erythroid	PAS++ Esterase-	Glycophorin A+	-5q, -5, -7, -3. +8
M7: Megakaryocyte	PAS+/ -	CD41+	+8, +21, inv or del 3p

Myeloid markers: CD 13, CD33; monocytic markers: CD11, CD14.

CD, cluster designation; FAB, French–American–British; HLA DR, human leukocyte antigens D-related; MPO, myeloperoxidase; PAS, periodic acid-Schiff.

PROGNOSTIC FACTORS

Established over decades, the powerful prognostic factors that include age greater than 60 years, cytogenetics, prior MDS, and treatment-related AML, have been recently supplemented by molecular genetic abnormalities. Prognostic variables associated with treatment outcomes are listed as follows:

Clinical prognostic factors

- | Age
- | AML arising from preexisting MDS
- | Treatment-related AML
- | Performance status (PS)
- | Extramedullary disease
- | Comorbid conditions

The best pretreatment predictors of long-term outcome, together with age, are chromosomal and molecular genetic findings in leukemic cells. Cytogenetic analysis is the basis of stratification of patients into three (or in Europe, four) risk groups with different responses to chemotherapy and long-term outcomes ([Table 11.6](#)).

Approximately 40% to 50% of all patients have a normal karyotype at the time of diagnosis, which in the absence of NPM1 or FLT3-ITD or isolated biallelic CEBPA mutations, is associated with an intermediate-risk category ([Table 11.6](#)). The molecular genetic changes in cytogenetically normal AML that correlate with prognosis have been used to subdivide this group and create more refined treatment algorithms.[21,22](#)

The frequency and reported prognostic impact of gene mutations in AML with normal karyotype are listed in [Table 11.7](#). Some of these mutations, including those in NPM1, FLT3-ITD, isolated bilallelic CEBPA, and KIT are already represented in clinical

guidelines, while the others are under investigation. These mutations provide substantial insights into the pathogenesis of AML and help identify molecular targets for treatment.

Table 11.6 Risk Status Based on Cytogenetic and Molecular Abnormalities

Risk Status	Cytogenetic and Molecular Abnormalities
Favorable risk	Inv(16) or t(16;16) t(8;21) t(15;17) Normal karyotype with NPM1 mutation in absence of FLT3-ITD Normal karyotype with isolated biallelic CEBPA mutation
Intermediate risk	Normal cytogenetics +8 t(9;11) t(8;21), inv(16), t(16;16) with c-KIT mutation Not defined as better-or poor-risk
Poor risk	Complex karyotype (≥ 3 clonal chromosomal abnormalities) Monosomal karyotype -5, 5q-, -7, 7q- 11q23— non t(9;11) Inv(3), t(3;3) t(6;9) t(9;22) Normal cytogenetics with FLT3-ITD mutation TP53 mutation

CEBPA, CCAAT/ enhancer binding protein alpha; c-KIT, CD117 receptor tyrosine kinase; FLT3-ITD, Fms-like tyrosine kinase3-internal tandem duplications; NPM1, nucleophosmin.

Table 11.7 Frequency and Prognostic Impact of Gene Mutations in AML

Gene Mutation	Frequency (%)	Notes
NPM1	25–30	Seen in ~50% of normal karyotype AML. Favorable in NK-AML if no FLT3-ITD mutation. Commonly co-mutated with DNMT3A and/ or FLT3-ITD. Co-mutations in NRAS, TET2 and PTPN11 also seen.
FLT3-ITD	20–25	Associated with poor risk in NK-AML. Common, but no prognostic significance, in APL.
CEBPA^{biallelic}	~5	Favorable for NK-AML only if isolated biallelic CEBPA mutation.
KIT	~5	Associated with intermediate risk when associated with a t(8;21), inv(16) or t(16;16) karyotype.
TP53	~6	Associated with poor risk in NK-AML. Often associated with complex karyotype or chromosomal aneuploidy (poor risk).
DNMT3A	~18–25	Prognostic significance unclear— likely depending on co-mutations.

		Commonly found with mutations in NPM1, IDH2R172 and chromatin-spliceosome genes. Age associated. Early event.
IDH1 and IDH2	17–20	Impact varied based on combinations with other genotypes eg: DNMT3A mutations.
TET2	~10	Co-mutated with NPM1, chromatin-spliceosome genes.
WT1	~6	Higher rates of resistant disease and lower overall survival reported.
RUNX1	9–10	Higher rates of resistant disease and lower overall survival reported. Age associated. MDS association.
ASXL1	~5	Higher rates of resistant disease and lower overall survival reported. Age associated. Early event.

Overview only from various sources.^{6,9,11} Mutations of genes in bold are already found within clinical guidelines.^{21,22}

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CEBPA, CCAAT/ enhancer binding protein alpha; DNMT3A, DNA-methyltransferase 3A; FLT3-ITD, Fms-like tyrosine kinase 3-internal tandem duplications; IDH1/ 2, isocitrate dehydrogenase 1 and 2; NPM1, nucleophosmin; RUNX1, Runt-related transcription factor; TET2, ten-eleven-translocation 2; WT1, Wilms tumor 1.

It is clear that mutations only have relevance in the context of disease, and any prognostic significance is typically based on retrospective analysis of sample banks from historical cohorts. Although targeted panels allowing sequencing of dozens of genes known to be mutated in AML are now widely available for clinical use, it is clear that the limits of current technology underestimate how common early “founder” mutations (e.g., DNMT3A) may be present in older healthy adults,²³ or the true clonal complexity of those with an AML diagnosis before or after treatment.^{24,25} The physician should therefore exert considerable caution and humility in interpretation of genomic testing, especially in a patient treated in a different manner from a historical cohort or in an individual without a disease diagnosis.

Overall, the outcome of younger patients with AML has markedly improved in the last three decades, mainly due to advances in treatment of APL and in supportive care. More than 50% of patients younger than 60 years in the favorable prognostic category can be cured with current chemotherapy. The cure rates of younger patients without better-risk chromosomal and molecular markers are about 30% to 40%. Unfortunately, little progress has been made in the long-term survival of older adults with AML. These patients have more poor-risk disease characteristics, higher frequency of comorbid conditions, and poor tolerance of toxic therapy. Novel treatment strategies are needed for most of the older patients.

TREATMENT

AML is heterogeneous and needs individualized treatments. Initial AML treatment plans are determined by age, PS, and patient wishes, then subsequently by prognostic factors (e.g., cytogenetic and molecular/ genomic characterization with increasing emphasis on minimal residual disease testing) for those achieving a remission after initial therapy.

With the exception of APL (discussed later), the first decision for treating the physician is to determine if the patient is a candidate for intensive cytotoxic therapy. For such individuals, therapy is divided into two phases: remission induction and postremission therapy. The goal of the former is to achieve a CR, currently defined as less than 5% blasts in a bone marrow that is 20% or more cellular, absent extramedullary leukemia, a neutrophil count greater than 1,000/ μ L, and a platelet count greater than 100,000/ μ L. The achievement of CR translates into improved survival. CR defined by these simple criteria does not imply freedom from persisting residual leukemia. It is therefore to be expected that most patients relapse if they only receive induction chemotherapy. For long-term survival, postremission therapy (consolidation) is required.

Initial Management

The early management of AML patients should be organized and implemented by an experienced team.

The initial evaluation includes:

- | History and physical examination
- | Complete blood count with differential
- | Examination of the peripheral blood smear
- | Coagulation studies: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer
- | Serum chemistries with uric acid, calcium, and phosphorus
- | Evaluation of renal and liver functions
- | Hepatitis B and C, herpes simplex virus (HSV), cytomegalovirus (CMV), varicella, and HIV serologies
- | Pregnancy test in females of reproductive age
- | Bone marrow aspirate for morphology, cytochemistry, cytogenetics, molecular genetic studies, and flow cytometry
- | Bone marrow biopsy
- | Human leukocyte antigen (HLA) typing of patients who are candidates for hematopoietic stem cell transplant
- | Lumbar puncture in patients at high risk of CNS involvement (CNS symptoms, elevated leukocyte count, extramedullary disease, or monocytic morphology FAB M4)

- or M5)—this should ordinarily be delayed until blasts are cleared from blood
- Chest radiograph and electrocardiogram
- Evaluation of cardiac function by echocardiogram or multigated acquisition scan (MUGA) in selected patients
- Central venous-access catheter placement in those who will receive intensive induction

The workup must be accompanied by an unhurried discussion with the patient about the diagnosis, prognosis, side effects of therapy, probable impact on lifestyle, and anticipated requirements for support by family and friends. In frail elderly patients, a decision to give less intensive therapy or only supportive treatment may be reached jointly by the patient and the attending physician. Given the suboptimal outcomes for this disease with current approaches, all patients diagnosed with non-APL AML should be offered, in the first instance, enrollment in an appropriate clinical trial.

The final outcome depends not only on the choice of chemotherapy but also on close monitoring, prevention, and meticulous management of complications. Many events are associated with AML therapy and their timing is predictable. For example, hyperleukocytosis, tumor lysis, and DIC tend to occur early, while marrow aplasia with resulting complications can be expected from the second week of chemotherapy. Patients should be monitored for side effects such as cardiotoxicity due to anthracyclines or neurotoxicity from high doses of cytosine arabinoside. Infection prophylaxis includes meticulous care for indwelling central venous catheters and prevention of mucositis. Empirical broad-spectrum intravenous antibiotics should be administered immediately if the patient becomes febrile during neutropenia. Cultures should be obtained before administration of antibiotics. Prompt treatment or prophylaxis of oral and perianal herpetic ulcerations prevents discomfort and bacterial superinfection. Prophylaxis against herpes viruses can prevent these complications and decrease the severity of mucositis. Prophylactic antifungal therapy may lead to a decrease in fungal infections. Transfusion support with irradiated and leukodepleted blood products should be provided to prevent symptoms of severe anemia and to maintain the platelet count above 10,000/ μ L. Transfusions of blood products in AML are guided by the same principles as described for ALL ([Chapter 12](#)).

Induction Therapy

The most commonly used chemotherapy regimen in patients younger than 60 years is a combination of cytarabine 100 to 200 mg/ m^2 by continuous intravenous (IV) infusion over 7 days with 3 days of an anthracycline (e.g., daunorubicin 60 to 90 mg/ m^2 or idarubicin 12 mg/ m^2). This “3+7” regimen results in CR rates of approximately 60% to 85% in patients younger than 60 years.^{3,21,26} There is, however, a wide variation in CR

achievement depending on the AML subtype.⁷

Numerous attempts to intensify the “7+3” combination have been made: The addition of cladribine (5 mg/ m²), but not fludarabine, to “3+7” therapy improved remission rates and overall survival in a multicenter randomized phase III study and is a reasonable approach.²⁷ Fludarabine in combination with cytarabine, granulocyte colony-stimulating factor (CSF), and idarubicin (FLAG-Ida) is an effective remission induction treatment in younger patients, with reduced relapse compared with “7+3” but with perhaps greater toxicity.²⁸ The addition of etoposide to “3+7” therapy has not however been shown to result in improved overall survival compared with “3+7.”²⁸ For those patients with FLT3-ITD mutations, addition of a targeted agent, in the context of a clinical trial, may be considered.^{29,30} Further strategies to intensify induction chemotherapy including in particular using anti-CD33 monoclonal antibody therapy are promising and ongoing.^{31,32}

Selected older patients with no comorbidities may also fare better with intensive chemotherapy induction than with supportive care. In older patients unlikely to benefit from intensive cytotoxic chemotherapy, agents such as azacitidine and decitabine may prolong survival even in the absence of CR. The goals and end points of therapy for elderly patients are therefore shifting (discussed further).

Postremission Treatment

Nearly all patients in CR after induction therapy have residual disease that, without further treatment, leads to relapse. The main strategies to prevent relapse involve postremission treatment with high-dose cytarabine and/ or allogeneic hematopoietic stem cell transplantation (HSCT). Maintenance therapy is not part of current standard treatment for AML. However, maintenance therapies including immunotherapy, demethylating agents, and targeted therapies are being tested with the intention of preventing post-consolidation relapse.

Intensive consolidation improves survival in younger patients with AML. A dose-dependent response to cytarabine has been found in randomized controlled trials.²⁶ Consolidation with high-dose cytarabine (HDAC)-based therapy using daily doses of 1 to 6 g/ m² (e.g., 2 to 3 g/ m² twice daily on days 1, 3, and 5 or twice daily for 6 days) is the standard for patients younger than 60 years. HDAC appears to be most beneficial for younger patients with better-risk cytogenetics, especially those with core-binding factor mutations t(8;21) and inv (16).³³ Patients older than 60 years do not benefit from HDAC consolidation²⁶ The optimal number of courses of HDAC-based consolidation has not been determined, but evidence suggests that three or four courses are appropriate.³³ For older patients, there are also studies suggesting no particular value in intensifying postremission therapy beyond a single course of consolidation. The optimal cumulative

dose of cytarabine in the initial therapy of younger patients with AML is still an open issue. The reduction in cytarabine doses (cumulative dose not exceeding 12 g/ m² within first consolidation) during conventional chemotherapy did not appear to worsen treatment outcome.³⁴ For younger AML patients, the choice between consolidation chemotherapy and allogeneic HSCT (allo-HSCT) should be based on the risk of relapse. In practice, consolidation chemotherapy is often given while arrangements for transplantation are being made.

Hematopoietic Stem Cell Transplantation

High doses of marrow-ablative chemotherapy and total body radiation followed by autologous or allogeneic stem cell rescue are widely used in AML. Autologous HSCT (auto-HSCT) requires stem cell collection from the patient while in CR and is now infrequently used in modern AML care in the United States with the sole exception of relapsed APL achieving polymerase chain reaction (PCR) negative remission after salvage therapy. Allogeneic stem cells are obtained from HLA-matched siblings, or haploidentical family donors, unrelated donors, or cord blood. Allogeneic HSCT (allo-HSCT) confers immune-mediated antileukemic activity, the so-called graft-versus-leukemia (GVL) effect. Multiple prospective randomized trials comparing standard consolidation chemotherapy with HSCT have demonstrated that allo-HSCT provides the lowest risk of recurrence ([Table 11.8](#)).

The benefit of the GVL of allo-HSCT is compromised by treatment-related mortality (TRM), which reduces overall survival ([Table 11.9](#)). The availability of a donor has been used as a surrogate for randomization in studies comparing HSCT with nontransplant approaches. These donor–no donor comparisons may be confounded by limited application of the assigned therapy, and the results of comparative studies have not always been consistent. Recent analyses demonstrate superior OS after allo-HSCT for AML patients excluding those with better-risk cytogenetics, and this benefit is most pronounced in younger patients. A large meta-analysis of 24 prospective studies including more than 6,000 patients with AML in first CR (CR1) compared the role of HSCT and non-HSCT treatments. Overall, 3,638 patients were analyzed by cytogenetic risk category, including 547 better-risk, 2,499 intermediate-risk, and 592 poor-risk patients. Compared with non-allogeneic therapy, the hazard ratio of relapse or death with an allo-HSCT for patients in CR1 was 0.80 (95% confidence interval: 0.74 to 0.86). When the analysis was broken down by risk category and outcome, there was a significant relapse-free survival and OS benefit for allo-HSCT during CR1 in intermediate-risk and poor-risk AML patients, but not for better-risk AML patients.³⁵ Allo-HSCT appears to be particularly beneficial for those patients with FLT3-ITD mutations.^{36,37}

Table 11.8 Relapse Rates Following Allo-HSCT, Auto-HSCT, and Chemotherapy in Prospective Randomized Studies

Study	Allo-HSCT	Auto-HSCT	Chemotherapy
GIMEMA 8(24)	24	40	57
MRC 10(25)	19	35	53
ECOG/ SWOG(26)	29	48	61

Allo-HSCT, allogeneic hematopoietic stem cell transplantation; Auto-HSCT, autologous hematopoietic stem cell transplantation.

Table 11.9 Trials Evaluating Allo-HSCT on an Intent-to-Treat Basis

Trial	DFS (%)		OS (%)	
	Donor	No Donor	Donor	No Donor
GIMEMA 8(24)	46	33	48	40
GOELAM(28)	44	38	53	53
MRC AML 10(25)	50	42	55	50
ECOG/ SWOG(26)	43	35	46	52
GIMEMA 10(29)	51	41	58	49
HOVON-SAKK(30)	48	37	54	46

Allo-HSCT, allogeneic hematopoietic stem cell transplantation; DFS, disease-free survival; Donor, patient with HLA-matched donor; No donor, patient without donor; OS, overall survival.

It is increasingly clear that the burden of disease on entering allo-HSCT has a significant impact on clinical outcomes, with those entering transplantation without evidence of measurable residual disease (MRD) having superior outcomes.³⁸ The preference is that AML patients receive allo-HSCT with myeloablative conditioning, based in part on the results of a recent prospective randomized trial (BMT-CTN 0901), closed early due to increased relapse and worse relapse-free survival in the reduced-intensity conditioning (RIC) arm.³⁹ A role for RIC regimens may remain however in allografts for elderly patients and younger patients with comorbidities intolerant of myeloablative conditioning.⁴⁰

Less than one-third of patients have an HLA-matched sibling donor available. However, advances in high-sensitivity HLA typing for better matching of unrelated donors, cord blood banks and improved haploidentical grafts have made allo-HSCT available for almost all patients who need a transplant.⁴¹ The advantages of cord blood and mismatched-related donor sources include shorter time to transplant. For haploidentical HSCT, the posttransplant cyclophosphamide regimen has reduced the incidence of graft versus host disease (GVHD) and posttransplant lymphoproliferative disorder.^{41,42}

Not captured by statistical representations of relapse-free or overall survival is the morbidity associated with allo-HSCT, notably GVHD. This has promoted interest in the integration of “freedom from GVHD” into end points⁴³ together with increased study of posttransplantation quality of life and treatment late effects.^{44,45} Ultimately, the decision to refer an AML patient for consideration for allo-HSCT is complex and should weigh not only attempts at prognostication based on surrogates of disease biology, but also considerations regarding patient age, comorbidity and preferences.⁴⁶

Risk-Based Approach to Acute Myeloid Leukemia Treatment in Younger Patients

Better-Risk Acute Myeloid Leukemia

Patients with a better-risk karyotypes (core-binding factor AML with t[8;21] or inv[16]) or normal karyotype with an NPM1 mutation but no FLT3-ITD, or with normal karyotype and isolated biallelic CEBPA mutations represent approximately one-third of AML patients younger than 65 years.¹¹ They are generally consolidated with high-dose cytarabine alone ([Table 11.6](#)) achieving long-term DFS of 50% to 80%. Allo-HSCT does not offer any additional benefit and is therefore not appropriate for this subset of patients. APL, also a better-risk AML, is considered separately in the following.

Poor-Risk Acute Myeloid Leukemia

Patients with poor-risk cytogenetics or patients with normal karyotype and a FLT3-ITD or TP53 mutation, as well as patients with secondary AML, have very poor prognosis ([Table 11.6](#)). Outcome is dismal with conventional consolidation or autologous HSCT. Lack of an appropriate sibling or 10/ 10 unrelated HLA-matched donor should not preclude allo-HSCT as multiple alternative donor sources (haploidentical, cord blood, less than 10/ 10 unrelated) are now available. Full-intensity myeloablative HSCT is appropriate for all patients achieving CR except those with significant comorbidity or advanced age who may be offered RIC allo-HSCT. Patients with FLT3-ITD should be considered for clinical trials with FLT3 tyrosine kinase inhibitors such as midostaurin, lestaurtinib, sorafenib, and quizartinib.⁴⁷ Given poor outcomes, even with allo-HSCT, clinical trials are appropriate for all patients in this risk group.

Intermediate-Risk Acute Myeloid Leukemia

Treatment decisions are particularly complex in the largest and most heterogeneous prognostic group of patients with normal karyotype (NK-AML) as determined by metaphase cytogenetics. Detection of somatic mutations can help refine prognostic risk in these NK-AML patients, with a NPM1 mutation in the absence of FLT3-ITD or an isolated bi-allelic CEBPA mutation associated with a better risk classification and a

FLT3-ITD or TP53 mutation associated with poor risk.^{3,22,48} It is likely additional mutations, and in particular combinations of mutations, will continue to be added to refine increasingly sophisticated clinical guidelines for risk stratification on the basis of studies showing prognostic relevance (Tables 11.6 and 11.7).^{10–12} In absence of randomized clinical trials, the role of allo-HSCT in the NK-AML patient without such risk-modifying molecular abnormalities will continue to be an individualized decision based on patient factors and the performance characteristics of the transplant option available.⁴⁶

Treatment of Acute Promyelocytic Leukemia

APL is a well-defined disease entity with a distinct epidemiology, characteristic morphology, and potentially life-threatening coagulopathy at presentation but overall better prognosis than any other AML subgroup. It is the first example of leukemia in which targeted therapy directed against the leukemogenic event, the t(15;17) PML-RAR α fusion transcript, lead to improved outcome. With better management of the associated coagulopathy and the introduction of the non-cytotoxic chemotherapy agents, all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), APL now represents the most curable subtype of AML with long-term event-free survival rates in excess of 90% quoted in large clinical trials.^{49–51}

Despite these excellent long-term outcomes, there remains a high risk of mortality during the initial phase of management. Because of the associated coagulopathy, newly diagnosed APL should be treated as a medical emergency. ATRA and aggressive supportive measures should be started as soon as the diagnosis is suspected, even before the confirmatory genetic tests are available. Bleeding is the commonest cause of early death in APL. Coagulopathy should be treated by maintaining a fibrinogen level greater than 150 mg/ dl with cryoprecipitate and fresh-frozen plasma together with aggressive transfusion to keep platelets above 50,000/ μ L until resolution of coagulopathy. The APL differentiation syndrome is characterized by fever, often associated with rising WBC count >10,000 with dyspnea, hypoxemia, pulmonary infiltrates, pleural and pericardial effusions. It requires prompt treatment with intravenous administration of dexamethasone 10 mg twice daily. Patients receiving ATO should be also closely monitored for prolongation of QT interval and their electrolytes maintained within normal range. Conventional cytotoxic chemotherapy (in the form of 3 or 4 days of an anthracycline) is now only indicated in high-risk disease (white count above 10,000/ mcL at presentation), but is not mandatory.⁵⁰ The mainstay of both induction and consolidation in APL is ATRA and ATO combination therapy.

The goal of induction and consolidation therapy should be the attainment of PCR negativity for the PML-RAR α rearrangement.⁵² Stem cell transplantation has a limited

role in the care of APL patients, but is considered for the patient in second CR after a morphologic or molecular relapse (autologous transplant if PCR negative CR2, allogeneic transplant if PCR-positive CR2).²²

Acute Myeloid Leukemia in Older or Unfit Patients

Older adults with AML have a dismal prognosis that has not changed significantly in decades.⁵³ Care of these patients is challenging due to the triple contribution of patient status (e.g., comorbidities, delayed mucosal recovery after cytotoxic therapy), disease (e.g., poor-risk genetics, typically evolving from antecedent hematological disorder and/or mutated clone), and physician (e.g., preferences regarding offering treatment to elderly AML patients) factors.^{54–57}

Older age per se, however, should not be a reason to withhold intensive therapy. Multiple studies suggest that treatment provides better quality of life and longer survival than supportive care only.^{54–56} Patients aged 60 years and older without significant comorbidities can receive standard “3+7” induction therapy. Their outcomes worsen with advancing age and decreasing PS (Table 11.10). Patients who benefit most from standard chemotherapy can be reasonably identified by the following criteria: age 60 to 69 years, no secondary AML or preexisting MDS, good PS, no pretreatment infection, and normal organ function.^{56,58} Calculators are available to estimate likelihood of CR and early death in older patients receiving intensive chemotherapy.⁵⁹ It has been suggested that genomic profiling may be able to identify those who may benefit most from intensive induction¹⁰; however this awaits prospective validation.

Optimal post remission therapy for older patients remains undefined. While high-dose cytarabine is difficult to tolerate and associated with significant toxicity in this age group,²⁶ intermediate-dose cytarabine is reasonable for those with good PS, normal renal function, and without any genetic markers of poor-risk disease biology.^{3,22} One or two cycles of standard-dose cytarabine is a reasonable consolidation strategy in older patients.

Table 11.10 Mortality Within 30 Days of Initiation of 7+3 Induction Therapy According to Age and Performance Status in Southwest Oncology Group Studies

Performance Status	Age < 56 Y	Age 56–65 Y	Age 66–75 Y	Age > 75 Y
PS 0	3/ 129 (2%)	8/ 72 (11%)	9/ 73 (12%)	2/ 14 (14%)
PS 1	6/ 180 (3%)	6/ 11 (5%)	20/ 126 (16%)	7/ 40 (18%)
PS 2	1/ 46 (2%)	6/ 34 (18%)	16/ 52 (31%)	7/ 14 (50%)
PS 3	0/ 9	7/ 24 (29%)	9/ 19 (47%)	9/ 11 (82%)

Adapted from Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. Blood. 2006;107:3481–3485.

Clofarabine has activity against AML as a single agent. Although not superior to daunorubicin in induction in older patients,⁶⁰ it may be beneficial in combination with low dose cytarabine as a less toxic alternative to an anthracycline.⁶¹

Increasingly, however, elderly AML patients are being offered less intensive hypomethylating therapy.²² The regimen of azacitidine 75 mg/ m²/ day for 7 days every 4 weeks, for example, may be used as outpatient therapy in older adult patients and may result in outcomes comparable to that of intensive chemotherapy and superior to that of other palliative treatment approaches such as low-dose cytarabine or hydroxyurea.^{62,63} Similarly a 10-day regimen of 20 mg/ m² of decitabine in previously untreated AML patients aged 60 or older was associated with an impressive overall response rate in a phase 2 study.⁶⁴ A phase 3 study in older patients with intermediate-and poor-risk cytogenetics using a 5-day regimen of decitabine showed a median overall survival of 7.7 months,⁶⁵ sufficient for drug approval for this indication in Europe.⁶⁶

For most of the elderly AML patients, however, clinical trials with novel agents should be considered at the time of diagnosis due to poor results with conventional therapy.^{3,22,53,67}

Treatment of Relapsed and Refractory Acute Myeloid Leukemia

Initial cytotoxic induction chemotherapy is often able to reduce tumor burden to a level sufficient to meet the current criteria for “complete” remission. Nevertheless, most AML patients ultimately die from their disease, most commonly as clinically evident relapsed AML. Moreover, approximately 25% of younger patients are refractory to standard induction chemotherapy. Patients with recurrent AML experience lower CR rates with reinduction chemotherapy compared to initial treatment. If second CR is achieved, it tends to be shorter, although it has been suggested that patients transplanted in MRD negative CR2 tend to do better than those with MRD positive CR1.³⁸

Clinical relapse may be caused by: (1) chemo-sensitive disease that was only partially treated and returns, sometimes with additional genetic mutations; (2) a subclone, derived from the same founder clone as the predominant clone, initially present at low frequency but given a clonal advantage during treatment due to decreased chemotherapy sensitivity; or (3) de novo generation of AML due to toxicity from treatment. Experimental evidence suggests that the first two mechanisms are more common, although the last one may explain late relapses 3 or more years after initial CR.⁶⁸

For patients with CR1 greater than 12 months, reinduction with the original regimen or an HDAC-containing regimen is reasonable. For patients with shorter initial CR durations, the priority is treatment on a clinical trial. Novel agents are currently being investigated (see Table 11.11).

When treating relapsed or refractory AML with curative intent, the ultimate goal of therapy is typically an allo-HSCT. Data suggest, however, that allo-HSCT may not be beneficial in relapsed core-binding factor leukemia as superior survival has been observed for those not undergoing transplantation.⁶⁹ Although this impression has not yet been tested in randomized trials, avoiding allo-HSCT in this subset of patients is reasonable particularly if a fully HLA-matched donor is not available. Hypomethylating agents (in combination with a tyrosine kinase inhibitor for those with FLT3-ITD mutation) can be used successfully as a bridge to allo-HSCT or as palliative care in those who do not wish to pursue intensive treatment.

The outcome for those relapsing after allo-HSCT is particularly dire.^{70,71} Clinical trials should be considered in all relapsed and refractory patients because response rates with all currently available agents remain suboptimal.

Table 11.11 Clinical Trials With Novel Agents in Acute Myeloid Leukemia

Therapeutic Class	Target/ Type	Examples
FLT3 inhibitor	FLT3-ITD	Sorafenib Midostaurin Crenolanib
IDH inhibitor	IDH1 inhibitor	AG-120 FT-2102 IDH305
IDH2 inhibitor	AG-221	
IDH1+IDH2 inhibitor	AG-881	
Hypomethylating agents	Oral decitabine	ASTX727
Oral azacytidine	CC-486	
“Next generation” HMA	Guadecitabine	
Bcl2 inhibitor	ABT-199 (venetoclax)	NCT01994837
Targeted therapy	Genomic or ex vivo drug sensitivity testing	Beat AML NCT02779283
Cytotoxic chemotherapy	Liposomal cytarabine–daunorubicin	CPX-351
Antibody-conjugate	Anti-CD33	Mylotarg SGN-CD33A
Anti-CD123	SGN-CD123A JNJ-56022473	
Immunotherapy	CAR-T cells	Anti-CD33, Anti-CD123
Bi-specific antibody	MGD006 (CD3/ 123) XmAb14045 (CD3/ 123)	
Leukemia CTls	NCT02895412	
Vaccine	NCT01096602	
Immune Checkpoint	Anti-PD1, anti-CTLA4	

AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CD, cluster designation; CTL, cytotoxic lymphocyte; FLT3-ITD, Fms-like tyrosine kinase3-internal tandem duplications; HMA, hypomethylating agents; IDH1/ 2, isocitrate dehydrogenase 1 and 2; PD, progressive disease.

Hematopoietic Growth Factors

CSFs shorten the duration of neutropenia during AML treatment. Both granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) accelerate neutrophil recovery after induction chemotherapy. Large studies demonstrate that CSFs reduce neutropenic and febrile (FTI) days. However, despite numerous clinical trials, a survival benefit of G-CSF and GM-CSF given to sensitize blasts to chemotherapy by recruiting cells into the cell cycle has not been shown and the addition of CSFs to chemotherapy provides no benefit in all-cause mortality, CR, or relapse rates in patients with AML.⁷² Growth factors may be considered in the elderly after chemotherapy is complete. However, G-CSF and GM-CSF should be stopped for a minimum of 7 days before obtaining bone marrow to document remission because CSFs may confound interpretation of the bone marrow biopsy. Myeloid growth factors should not be used in APL or during initial induction therapy but may be considered during consolidation therapy in selected life-threatening cases.²²

Acute Myeloid Leukemia in Pregnancy

The prevalence of leukemia during pregnancy is low, roughly 1 in 75,000 to 100,000 pregnancies. AML accounts for two-thirds of cases.⁷³ Acute leukemia is usually reported during the second and third trimesters of pregnancy. This could be a result of a selection bias of unreported cases that occurred early and resulted in termination of pregnancy. The management of pregnant patients with AML poses major challenges. Patients are at high risk for pregnancy-associated complications due to the bleeding and infection. Acute leukemia needs immediate treatment irrespective of gestational stage because delay or modification of therapy results in inferior maternal outcome. A recent systematic review identified 87 AML patients (88 pregnancies) treated with systemic therapy during the course of pregnancy. With few exceptions, patients were electively treated following the end of the first trimester.⁷⁴ Nearly 50% of those who were exposed to chemotherapy during the first trimester had poor fetal outcomes. Chemotherapeutic agents and target therapies, such as ATRA, should be avoided during the first trimester. ATRA, highly effective in APL, confers substantial toxicity to the fetus in the first trimester, including CNS and cardiovascular malformations. Hence, women diagnosed early in gestation should be offered a termination of pregnancy. Administration of chemotherapy and ATRA in the second and third trimesters is less

likely to result in teratogenesis, although it increases the risk of intrauterine growth retardation.⁷³ In summary, management of AML in pregnancy should focus on survival of the mother, while minimizing treatment-related fetal toxic effects.

Evaluation of Minimal Residual Disease

Posttreatment response criteria in AML rely on the detection, by morphology, of greater than 5% residual AML blasts in blood and bone marrow. These criteria were established 60 years ago and do not reflect the many improvements in technology or biological understanding in the interim.⁷⁵ Patients achieving a “complete response” as currently defined therefore represent a heterogeneous group, with a wide range of disease burden (from patients who are cured to those with many millions of residual leukemic cells remaining) with consequently diverse clinical outcomes.

It is now possible to use higher sensitivity tools to measure leukemia remaining after treatment. Both PCR and multiparametric flow cytometry have been shown to have efficacy in risk stratifying patients in CR before allogeneic transplant into high and low risks of posttransplant relapse.^{76,77} This minimal or measurable residual disease (MRD) can also be used at other timepoints, for example post-consolidation surveillance monitoring.⁷⁸ The role of AML MRD in regulatory approval as a surrogate endpoint is under discussion. MRD has been shown, in multivariate analysis, to supersede pretreatment surrogates of disease biology (cytogenetics, molecular markers) currently used to determine prognosis. MRD monitoring is considered standard of care for APL, and is likely to increasingly be part of monitoring for other AML types.⁵²

Novel Therapeutic Targets in Acute Myeloid Leukemia

Standard treatment for AML is based on aggressive cytotoxic chemotherapy given in repetitive cycles in order to eradicate disease. Only a minority of patients is cured by this approach. The success of ATRA and ASO in APL demonstrates that more selective and less toxic drugs can substantially increase cure rates of AML. The concept of targeting leukemic cells and sparing normal ones from the broad attack of chemotherapy is now under investigation in other types of AML. With improved understanding of the mechanisms underlying leukemogenesis, novel classes of drugs have entered clinical trials (Table 11.11). Most of these drugs have limited activity as single agents and their full potential may be only realized in combination therapy. To advance progress with new treatments in this high-risk disease, all patients with AML including the elderly should be offered therapy on well-designed clinical trials whenever possible.

SUMMARY

AML is a genetically, morphologically, and clinically heterogeneous group of

hematopoietic malignancies characterized by a rapid growth of myeloid blasts and suppression of normal hematopoiesis. The initiating genetic events and the pathways involved in the pathogenesis of AML are the subject of intense investigations. These events determine the type of AML, response to therapy, and to some extent, the final outcome. The well-established prognostic factors including age and cytogenetic changes are being continuously expanded by molecular characterization. Mutation testing is now incorporated into prognostic models and used to identify treatment targets. Unfortunately, less than half of younger patients and less than 10% of patients older than 60 years are cured of their disease with current therapies. In younger patients, the longest disease-free survival is achieved with repeated cycles of intensive chemotherapy containing anthracyclines and cytarabine. Postremission consolidation therapy is crucial, while a role of maintenance therapy remains unproven. HDAC is the consolidation of choice for younger patients with better-risk cytogenetics. Patients with intermediate-or poor-risk disease who have an HLA-matched donor and good PS benefit from allo-HSCT. Although questions remain about the optimal drugs for induction and about the numbers of consolidation cycles, it is unlikely that further modifications of standard chemotherapy will result in dramatic improvement in survival. Aggressive chemotherapy is not suitable for older patients with comorbidities and decreased PS. These patients can derive modest benefit from hypomethylating agents or low-dose cytarabine. New targeted treatments including antibody-based therapies and tyrosine kinase inhibitors have shown some activity and acceptable toxicity in clinical studies. Combinations of these agents based on studies of underlying pathophysiology should lead to better outcomes than can be achieved with unselective cytotoxic therapy.

References

1. Siegel, RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
2. Howlader N, Noon AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2011. 2014.
3. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia . N Engl J Med. 2015;373(12):1136–1152.
4. Goldin LR, Kristinsson SY, Liang XS, et al. Familial aggregation of acute myeloid leukemia and myelodysplastic syndromes. J Clin Oncol. 2012;30(2):179–183.
5. Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;128(14):1800–1813.
6. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.

7. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116(3):354–365.
8. Ley TJ, Mardis ER, Ding L, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature*. 2008;456(7218):66–72.
9. Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059–2074.
10. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–1376.
11. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23):2209–2221.
12. Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079–1089.
13. Ding L, Ley TJ, Larson DE, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature*. 2012;481(7382):506–510.
14. Jan M, Snyder TM, Corces-Zimmerman MR, et al. Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med*. 2012;4(149):149ra118.
15. Klco JM, Spencer DH, Miller CA, et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell*. 2014;25(3):379–392.
16. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506(7488):328–333.
17. Klco JM, Miller CA, Griffith M, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. *JAMA*. 2015;314(8):811–822.
18. Shih AH, Jiang Y, Meydan C, et al. Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. *Cancer Cell*. 2015;27(4):502–515.
19. Welch JS. Mutation position within evolutionary subclonal architecture in AML. *Semin Hematol*. 2014;51(4):273–281.
20. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012;150(2):264–278.
21. Döhner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453–474.
22. O'Donnell MR, Tallman MS, Abboud CN, et al. Acute myeloid leukemia, version 2.2013. *J Natl Compr Canc Netw*. 2013;11(9):1047–1055.

23. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Commun.* 2016;7:12484.
24. Griffith M, Miller CA, Griffith OL, et al. Optimizing cancer genome sequencing and analysis. *Cell Syst.* 2015;1(3):210–223.
25. Wong TN, Miller CA, Klco JM, et al. Rapid expansion of preexisting nonleukemic hematopoietic clones frequently follows induction therapy for de novo AML. *Blood.* 2016;127(7):893–897.
26. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *N Engl J Med.* 1994;331(14):896–903.
27. Holowiecki J, Grosicki S, Giebel S, et al. Cladribine, but not fludarabine, added to daunorubicin and cytarabine during induction prolongs survival of patients with acute myeloid leukemia: a multicenter, randomized phase III study. *J Clin Oncol.* 2012;30(20):2441–2448.
28. Burnett AK, Russell NH, Hills RK, et al. Optimization of chemotherapy for younger patients with acute myeloid leukemia: results of the medical research council AML15 trial. *J Clin Oncol.* 2013;31(27):3360–3368.
29. Rollig C, Serve H, Huttmann A, et al. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukaemia (SORAML): a multicentre, phase 2, randomised controlled trial. *Lancet Oncol.* 2015;16(16):1691–1699.
30. Midostaurin +chemo ups AML survival. *Cancer Discov.* 2016;6(2):Of2.
31. Hills RK, Castaigne S, Appelbaum FR, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol.* 2014;15(9):986–996.
32. Kung Sutherland MS, Walter RB, Jeffrey SC, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. *Blood.* 2013;122(8):1455–1463.
33. Byrd JC, Ruppert AS, Mrózek K, et al. Repetitive cycles of high-dose cytarabine benefit patients with acute myeloid leukemia and inv(16)(p13q22) or t(16;16) (p13;q22): results from CALGB 8461. *J Clin Oncol.* 2004;22(6):1087–1094.
34. Schaich M, Röllig C, Soucek S, et al. Cytarabine dose of 36 g/ m(2) compared with 12 g/ m(2) within first consolidation in acute myeloid leukemia: results of patients enrolled onto the prospective randomized AML96 study. *J Clin Oncol.* 2011;29(19):2696–2702.
35. Koreth J, Schlenk R, Kopecky KJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. *JAMA.* 2009;301(22):2349–2361.

36. Deol A, Sengsayadeth S, Ahn KW, et al. Does FLT3 mutation impact survival after hematopoietic stem cell transplantation for acute myeloid leukemia? A Center for International Blood and Marrow Transplant Research (CIBMTR) analysis. *Cancer*. 2016;122(19):3005–3014.
37. DeZern AE, Sung A, Kim S, et al. Role of allogeneic transplantation for FLT3/ITD acute myeloid leukemia: outcomes from 133 consecutive newly diagnosed patients from a single institution. *Biol Blood Marrow Transplant*. 2011;17(9):1404–1409.
38. Walter RB, Buckley SA, Pagel JM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood*. 2013;122(10):1813–1821.
39. Scott BL, Pasquini MC, Logan BR, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. *J Clin Oncol*. 2017;35(11):1154–1161.
40. Reshef R, Porter DL. Reduced-intensity conditioned allogeneic SCT in adults with AML. *Bone Marrow Transplant*. 2015;50(6):759–769.
41. Kanakry CG, de Lima MJ, Luznik L. Alternative donor allogeneic hematopoietic cell transplantation for acute myeloid leukemia. *Semin Hematol*. 2015;52(3):232–242.
42. Kanakry JA, Kasamon YL, Bolaños-Meade J, et al. Absence of post-transplantation lymphoproliferative disorder after allogeneic blood or marrow transplantation using post-transplantation cyclophosphamide as graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant*. 2013;19(10):1514–1517.
43. Holtan SG, DeFor TE, Lazaryan A, et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood*. 2015;125(8):1333–1338.
44. Visentin S, Auquier P, Bertrand Y, et al. The impact of donor type on long-term health status and quality of life after allogeneic hematopoietic stem cell transplantation for childhood acute leukemia: a Leucemie de l'Enfant et de L'Adolescent Study. *Biol Blood Marrow Transplant*. 2016;22(11):2003–2010.
45. Armenian SH, Chemaitilly W, Chen M, et al. National institutes of health hematopoietic cell transplantation late effects initiative: the cardiovascular disease and associated risk factors working group report. *Biol Blood Marrow Transplant*. 2016;23(2):201–210.
46. Cornelissen JJ, Gratwohl A, Schlenk RF, et al. The European LeukemiaNet AML Working Party consensus statement on allogeneic HSCT for patients with AML in remission: an integrated-risk adapted approach. *Nat Rev Clin Oncol*. 2012;9(10):579–590.
47. Grunwald MR, Levis MJ. FLT3 tyrosine kinase inhibition as a paradigm for targeted drug development in acute myeloid leukemia. *Semin Hematol*.

- 2015;52(3):193–199.
48. Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453–474.
 49. Lo-Coco F, Di Donato L, Schlenk RF. Targeted therapy alone for acute promyelocytic leukemia. *N Engl J Med*. 2016;374(12):1197–1198.
 50. Burnett AK, Russell NH, Hills RK, et al. Arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2015;16(13):1295–1305.
 51. Lo-Coco F, Avvisati G, Vignetti M, et al. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med*. 2013;369(2):111–121.
 52. Grimwade D, Jovanovic JV, Hills RK, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. *J Clin Oncol*. 2009;27(22):3650–3658.
 53. Burnett AK. Treatment of acute myeloid leukemia: are we making progress? *Hematology Am Soc Hematol Educ Program*. 2012;2012:1–6.
 54. Medeiros BC, Satram-Hoang S, Hurst D, et al. Big data analysis of treatment patterns and outcomes among elderly acute myeloid leukemia patients in the United States. *Ann Hematol*. 2015;94(7):1127–1138.
 55. Juliusson G, Antunovic P, Derolf A, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*. 2009;113(18):4179–4187.
 56. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. 2006;107(9):3481–3485.
 57. Wheatley K, Brookes CL, Howman AJ, et al. Prognostic factor analysis of the survival of elderly patients with AML in the MRC AML11 and LRF AML14 trials. *Br J Haematol*. 2009;145(5):598–605.
 58. Kantarjian H, Ravandi F, O'Brien S, et al. Intensive chemotherapy does not benefit most older patients (age 70 years or older) with acute myeloid leukemia. *Blood*. 2010;116(22):4422–4429.
 59. Krug U, Röllig C, Koschmieder A, et al. Complete remission and early death after intensive chemotherapy in patients aged 60 years or older with acute myeloid leukaemia: a web-based application for prediction of outcomes. *Lancet*. 2010;376(9757):2000–2008.
 60. Burnett AK, Russell NH, Hills RK, et al. A comparison of clofarabine with ara-C, each in combination with daunorubicin as induction treatment in older patients with acute myeloid leukaemia. *Leukemia*. 2016;31(2):310–317.
 61. Takahashi K, Kantarjian H, Garcia-Manero G, et al. Clofarabine plus low-dose cytarabine is as effective as and less toxic than intensive chemotherapy in elderly

- AML patients. *Clin Lymphoma Myeloma Leuk.* 2016;16(3):163–168, e1–e2.
- 62. Fenaux P, Mufti GJ, Hellström-Lindberg E, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol.* 2010;28(4):562–569.
 - 63. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood.* 2015;126(3):291–299.
 - 64. Blum W, Garzon R, Klisovic RB, et al. Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. *Proc Natl Acad Sci USA.* 2010;107(16):7473–7478.
 - 65. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol.* 2012;30(21):2670–2677.
 - 66. Nieto M, Demolis P, Béhanzin E, et al. The European medicines agency review of decitabine (dacogen) for the treatment of adult patients with acute myeloid leukemia: summary of the scientific assessment of the committee for medicinal products for human use. *Oncologist.* 2016;21(6): 692–700.
 - 67. Walter RB, Estey EH. Management of older or unfit patients with acute myeloid leukemia. *Leukemia.* 2015;29(4):770–775.
 - 68. Ramos NR, Mo CC, Karp JE, et al. Current approaches in the treatment of relapsed and refractory acute myeloid leukemia. *J Clin Med.* 2015;4(4):665–695.
 - 69. Burnett AK, Goldstone A, Hills RK, et al. Curability of patients with acute myeloid leukemia who did not undergo transplantation in first remission. *J Clin Oncol.* 2013;31(10):1293–1301.
 - 70. Breems DA, Van Putten WL, Huijgens PC, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *J Clin Oncol.* 2005;23(9):1969–1978.
 - 71. Schroeder T, Czibere A, Platzbecker U, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapse of AML or MDS after allogeneic stem cell transplantation. *Leukemia.* 2013;27(6):1229–1235.
 - 72. Gurion R, Belnik-Plitman Y, Gafter-Gvili A, et al. Colony-stimulating factors for prevention and treatment of infectious complications in patients with acute myelogenous leukemia. *Cochrane Database Syst Rev.* 2012;(6):Cd008238.
 - 73. Brenner B, Avivi, I Lishner M. Haematological cancers in pregnancy. *Lancet.* 2012;379(9815):580–587.
 - 74. Azim HA, Jr, Pavlidis N, Peccatori FA. Treatment of the pregnant mother with cancer: a systematic review on the use of cytotoxic, endocrine, targeted agents and

- immunotherapy during pregnancy. Part II: Hematological tumors. *Cancer Treat Rev.* 2010;36(2):110–121.
- 75. Hourigan CS, Karp JE. Minimal residual disease in acute myeloid leukaemia. *Nat Rev Clin Oncol.* 2013;10(8):460–471.
 - 76. Araki D, Wood BL, Othus M, et al. Allogeneic hematopoietic cell transplantation for acute myeloid leukemia: time to move toward a minimal residual disease-based definition of complete remission? *J Clin Oncol.* 2015;34(4):329–336.
 - 77. Hourigan CS, Goswami M, Battiwalla M, et al. When the minimal becomes measurable. *J Clin Oncol.* 2016;34(21):2557–2558.
 - 78. Ivey A, Hills RK, Simpson MA, et al. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med.* 2016;374(5):422–433.
 - 79. Lee SC, Dvinge H, Kim E, et al. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. *Nat Med.* 2016;22(6):672–678.

Acute Lymphoblastic Leukemia

Deepa Bhojwani and Alan S. Wayne

Approximately 6,500 new cases of acute lymphoblastic leukemia (ALL) are diagnosed each year in the United States, more than half of them in children.¹ There has been significant progress in the development of curative therapy, such that currently children and adults with ALL have expected disease-free survival (DFS) rates of about 90% and 50%, respectively.^{2–4}

EPIDEMIOLOGY

ALL represents the most common pediatric malignancy, accounting for approximately 25% of childhood cancer. The peaks in prevalence of ALL occur between the ages of 2 and 4 years and after the age of 50 years. There is a slight male predominance, and Caucasians have a twofold increased risk compared with African Americans, while the highest incidence is seen in Hispanic children.⁵

ETIOLOGY AND RISK FACTORS

Certain conditions predispose to ALL, most notably trisomy 21 (Down syndrome), in which the relative risk is increased 15-fold. Other predisposing conditions include immunodeficiency and chromosomal breakage syndromes, but most often no such underlying disorder is found. Environmental exposure risks have been suggested, but with the exception of ionizing radiation, few have been shown to be causal. Acquired chromosomal abnormalities confined to lymphoblasts are found in more than 90% of cases, including aneuploidy (most commonly hyperdiploidy) and/ or translocations that in some cases are prenatal in origin.^{6,7} The genes involved in leukemogenesis are frequently transcription factors expressed in hematopoietic tissues.⁸

CLINICAL FEATURES

Presenting signs and symptoms are almost always caused by lymphoblast infiltration of the bone marrow with resultant blood count abnormalities ([Table 12.1](#)). Other organs may also be involved, most commonly the central nervous system (CNS) and the testes. T-cell ALL frequently presents with bulky adenopathy, mediastinal mass, pleural effusion, and/ or hyperleukocytosis. There are several life-or organ-threatening presentations that require emergent intervention ([Table 12.2](#)).

Table 12.1 Presenting Features

Common Presenting Signs and Symptoms (%)	Sites of Involvement (%)
Hepatosplenomegaly (70)	Bone marrow (100)
Fever (60)	Anterior mediastinal mass (10)
Fatigue (50)	CNS (5)
Lymphadenopathy (50)	Testicular (2)
Bleeding (40)	Others (e.g., eye, skin, pericardium, pleura, kidney, breast, ovary, priapism, intussusception) (<5)
Bone or joint pain (40)	
Anorexia (20)	
Abdominal pain (10)	

CNS, central nervous system.

Table 12.2 Emergency Presentations

Emergent Presentation	Intervention
Hyperleukocytosis	Cytoreductive low-dose chemotherapy, leukapheresis
Neutropenia with fever or infection	Broad-spectrum IV antibiotics
Thrombocytopenia	Platelet transfusion
Disseminated intravascular coagulation	Fresh frozen plasma, cryoprecipitate
Tumor lysis syndrome	IV hydration, allopurinol or rasburicase, and supportive measures as clinically indicated (e.g., dialysis)
Airway obstruction	Oxygen, corticosteroids, and/ or radiation
Superior vena cava syndrome	Corticosteroids and/ or radiation
Pericardial tamponade	Pericardiocentesis, corticosteroids
CNS manifestations	Corticosteroids and/ or radiation
Ocular involvement	Radiation

Spinal cord compression Corticosteroids and/ or radiation
CNS, central nervous system; IV, intravenous.

LABORATORY FEATURES

The diagnosis is readily confirmed by the demonstration of lymphoblasts in the blood and/ or bone marrow. Routine hematopathologic analysis, immunohistochemistry, flow cytometry, cytogenetics, and molecular studies are used to define the subtype and further identify prognostic factors. Most ALL is of precursor B-cell (pre-B) phenotype (CD10, CD19, CD22, HLA-DR, TDT+), 10% to 20% is T-cell (CD2, CD7+). Certain cytogenetic abnormalities are not apparent on routine karyotyping, and thus molecular testing may be required, most notably for t(12;21) seen in about 25% of cases in children. Lumbar puncture is required to evaluate the possibility of meningeal leukemia (Table 12.3).

CLASSIFICATION

Historically, ALL was classified according to blast morphology into three categories (L1, L2, and L3) by the French–American–British (FAB) system. Since 2008, the World Health Organization (WHO) classification system is widely used.^{9,10} It is based on immunophenotypic, cytogenetic, and molecular features that impact prognosis. ALL is included in the group of precursor lymphoid neoplasms, of either B-cell or T-cell origin. Of note, Burkitt leukemia (CD20, surface-IgM κ or λ+) is classified as a mature B-cell neoplasm along with Burkitt lymphoma and warrants a different treatment approach. The 2016 revision of the WHO classification of ALL is detailed in Table 12.4.

Table 12.3 Definitions

Bone Marrow Status

M1: <5% blasts

M2: 5%–25% blasts

M3: >25% blasts

Cerebrospinal Fluid Cytology

CNS-1: no blasts

CNS-2: WBC < 5/ μL with blasts

CNS-3: WBC ≥ 5/ μL with blasts or symptomatic CNS involvement (e.g., cranial nerve palsy)

CNS, central nervous system; WBC, white blood cell.

Table 12.4 World Health Organization Classification of Acute Lymphoblastic Leukemia

B-Lymphoblastic Leukemia/ Lymphoma

B-lymphoblastic leukemia/ lymphoma, NOS

B-lymphoblastic leukemia/ lymphoma with recurrent genetic abnormalities

B-lymphoblastic leukemia/ lymphoma with t(9;22)(q34.1;q11.2);BCR-ABL1

B-lymphoblastic leukemia/ lymphoma with t(v;11q23.3);KMT2A rearranged

B-lymphoblastic leukemia/ lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1

B-lymphoblastic leukemia/ lymphoma with hyperdiploidy

B-lymphoblastic leukemia/ lymphoma with hypodiploidy

B-lymphoblastic leukemia/ lymphoma with t(5;14)(q31.1;q32.3) IL3-IGH

B-lymphoblastic leukemia/ lymphoma with t(1;19)(q23;p13.3);TCF3-PBX1

Provisional entity: B-lymphoblastic leukemia/ lymphoma, BCR-ABL1-like

Provisional entity: B-lymphoblastic leukemia/ lymphoma with iAMP21

T-Lymphoblastic Leukemia/ Lymphoma

Provisional entity: Early T-cell precursor lymphoblastic leukemia

Provisional entity: NK cell lymphoblastic leukemia/ lymphoma

ALL, acute lymphoblastic leukemia; NK, natural killer; NOS, not otherwise specified; WHO, World Health Organization.

Adapted from Duffield AS, Racke F, Borowitz MJ. Precursor B-and T-cell neoplasms. In: Jaffe ES, Arber DA, Campo E, Harris NL, Quintanilla-Martinez L, eds. Hematopathology. 2nd ed. Philadelphia, PA: Elsevier Inc.; 2011:761–773; Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.

PROGNOSTIC FACTORS

Clinical and biologic features, as well as initial response to therapy, are used to determine risk-directed treatment for individuals with pre-B ALL (Table 12.5). Age is a strong prognostic determinant, and outcome is inferior in infants and adults in comparison to children. T-cell ALL has historically had lower DFS rates than pre-B ALL; however, stratified treatment has minimized this difference in children.¹¹ A higher initial leukocyte count and presence of CNS involvement by leukemia also determine higher-risk disease. Early response to therapy, best measured by minimal residual disease (MRD) after 4 to 6 weeks of therapy is highly predictive for outcome in adults and children.^{12,13} Recently, genomic analysis has revealed molecular alterations and profiles associated with poor outcome, thus allowing further discrimination of diagnostic subtype, risk classification, and treatment-response prediction.¹⁴

Table 12.5 Risk-Group Assignment in B-Precursor Acute Lymphoblastic Leukemia

Clinical Feature	Standard Risk	High Risk	Very High Risk
Age (y)	1–9	10–35	<1 >35

			>55
WBC (μ L)	<30,000 <50,000	\geq 30,000 \geq 50,000	
CNS	Negative	Positive	
Chromosomes	t(12;21), <Double or triple Trisomy 4/ 10/ 17	11q23, t(1;19), t(9;22)	
Ploidy	Hyperdiploidy	Hypodiploidy	
Treatment response	RER	SER	Induction failure
Post-induction MRD (%)	<0.01	0.01–0.1 \geq 1.0	>0.1

ALL, acute lymphoblastic leukemia; CNS, central nervous system; MRD, minimal residual disease; RER, rapid early responder; SER, slow early responder; WBC, white blood cell.

TREATMENT

Many chemotherapeutic regimens are effective for children and adults with ALL. Therapy is stratified based on clinicopathologic features, and treatment should be directed by physicians familiar with subtype-specific regimens. The following core recommendations are based on results of large cooperative group clinical trials.^{24,15,16}

- Therapy should be instituted as soon as possible after diagnosis.
- Treatment is based on phenotype and prognostic factors and includes the following phases: induction, consolidation, CNS sterilization, intensification, and maintenance for a total of 2 to 3 years (Table 12.6). Initial induction therapy for children and most adults consists of 3 to 5 drugs given in a 28-day cycle. An alternate approach for adults is the hyper-CVAD regimen, which repeats two alternating intensive chemotherapy cycles for a total of eight cycles.¹⁷ A 7-day steroid prophase is included in some studies, which helps in gradual tumor reduction and permits determination of early response. Various consolidation and intensification regimens are commonly employed, some of which are detailed in Table 12.6. Multiple consolidation/intensification blocks are often advised for high-risk patients. A late reinduction phase, also known as delayed intensification, improves DFS for children who are slow early responders (SERs).¹⁸ The application of pediatric regimens to adult ALL has improved the DFS rates especially for adolescents and young adults, but at the expense of increased toxicity in older individuals.^{3,15} Prolonged maintenance with a total treatment duration of 24 to 36 months improves DFS for both adults and children.
- Allogeneic stem cell transplantation (SCT): Although relapse rates are lower after allogeneic SCT compared with chemotherapy, treatment-related mortality rates are higher after transplantation. Thus, SCT is rarely used for children in first remission (CR1) except within the context of clinical trials for individuals with extremely poor prognostic features such as induction failure.¹⁹ The indications for SCT in CR1 in

adults with ALL continue to evolve toward recommendations based on early response to chemotherapy.²⁰ When SCT is employed in the treatment of ALL, the conditioning regimen typically utilizes total body irradiation (TBI), which has been shown to decrease the relapse risk.²¹

- | Risk-group assignment for B-precursor ALL: Although there is protocol-specific variability in the approach to risk-adapted therapy, in general, age, white blood cell (WBC) count, CNS involvement, and immunophenotype are used for the initial risk-group assignment (Table 12.5).^{2,4,20} Subsequently, the risk group may be elevated based on cytogenetics and response to therapy, the latter of which is defined by morphologic blast reduction (in peripheral blood on day 7 or bone marrow on day 7 or 14) and further quantified by MRD determination by flow cytometry or polymerase chain reaction amplification.^{12,13}
- | Infant ALL: Most children younger than 1 year at diagnosis harbor rearrangements in the KMT2A (formerly MLL) gene and have a poor prognosis. These infants should be treated on age-specific protocols with certain agents dosed by weight to decrease the risk of severe toxicity.
- | T-ALL: Patients with T-cell phenotype are treated similarly to higher-risk group pre-B ALL. Improved outcome has been associated with the use of intensified therapy that commonly includes high-dose methotrexate, dexamethasone, and asparaginase.²²
- | Philadelphia-chromosome positive ALL: Early incorporation of a tyrosine kinase inhibitor has significantly improved cure rates in this very high-risk subtype of ALL.^{23,24}
- | CNS-directed therapy: All patients require CNS sterilization. Intensive intrathecal chemotherapy in combination with systemic agents that have good CNS penetration, most notably dexamethasone and high-dose methotrexate, are highly effective (Table 12.7). To minimize neurotoxicity, radiation is usually reserved for those with active meningeal leukemia or at very high risk of CNS relapse (Table 12.8).²⁵
- | Testicular leukemia: Males with testicular involvement have historically received radiation to both testes, although recent studies suggest that radiation can be spared when testicular involvement resolves completely during the initial induction phase of therapy.²⁶

Table 12.6 Common Treatment Regimens for Pre-B-Cell Acute Lymphoblastic Leukemia and T-Cell Acute Lymphoblastic Leukemia

Induction Regimen (Week 1–4)

3-Drug

- Prednisone 40–60 mg/ m²/ day or dexamethasone 6 mg/ m²/day in divided doses PO × 21–28 days (days 0–28)
- Vincristine 1.5 mg/ m² (maximum dose 2 mg) IV weekly × 4 doses (days 0, 7, 14, 21)
- Pegylated L-asparaginase 2,500 IU/ m² IV × 1 dose (day 4)
- IT methotrexate: (or triple IT therapy with methotrexate, hydrocortisone, and cytarabine)

- CNS-1: Every 1–2 weeks × 3 doses (days 0, 8/ 14, 29)
- CNS-2 or CNS-3: Weekly (or biweekly) × at least 4 doses and until 2 successive CNS-1 (days 0, 7, 14, 21)

4-Drug: Add the following to above

- Doxorubicin 25–30 mg/ m² or daunorubicin 25–45 mg/ m² IV weekly × 4 doses (days 0, 7, 14, 21) or IV daily × 2 to 3 doses (days 0, 1, +/ -2)

5-Drug: Add the following to above

- Cyclophosphamide 800–1,200 mg/ m² IV × 1 dose (day 0)

Response evaluation

Day 14 bone marrow

- M1: Rapid early responder
- M2 or M3: SER

Day 29 bone marrow

- M1: Remission, continue as below. If MRD is present, may be allocated to a higher-risk group and receive intensified therapy.
- M3: Induction failure, salvage reinduction required

Post-Induction Regimens

Pretreatment criteria

- ANC ≥ 750/ μL, platelets ≥75,000/ μL
- ALT <2 times the upper limit of normal, direct bilirubin normal for age
- Serum creatinine normal for age
- No active infection or life-threatening organ dysfunction

Consolidation (Week 5)

Standard Berlin-Frankfurt-Munster Study Group (BFM)

- Cyclophosphamide 1,000 mg/ m² IV × 2 doses (days 0, 14)
- Mercaptopurine (6-MP) 60 mg/ m² PO once daily × 28 days (days 0–27)
- Vincristine 1.5 mg/ m² (maximum dose 2 mg) IV × 4 doses (days 14, 21, 42, 49)
- Cytarabine 75 mg/ m² IV or SQ (days 1–4, 8–11, 15–18, 22–25)
- IT methotrexate weekly × 4 doses (days 1, 8, 15, 22)

Augmented BFM

- Cyclophosphamide 1,000 mg/ m² IV × 2 doses (days 0, 28)
- Mercaptopurine (6-MP) 60 mg/ m² PO once daily × 28 days (days 0–13, 28–41)
- Vincristine 1.5 mg/ m² (maximum dose 2 mg) IV × 4 doses (days 14, 21, 42, 49)
- Cytarabine 75 mg/ m² IV or SQ (days 1–4, 8–11, 29–32, 36–39)
- Pegylated L-Asparaginase 2,500 IU/ m² IV × 2 doses (days 15, 43)
- IT methotrexate weekly × 4 doses (days 1, 8, 15, 22)

High-dose methotrexate with leucovorin rescue

- Refer to protocol-specific dosing, administration, and leucovorin rescue guidelines

Capizzi

- Cytarabine (Ara-C) 3,000 mg/ m² IV over 3 h every 12 h × 4 doses, weekly × 2 (days 0, 1 and days 7, 8)
- Erwinia Asparaginase 25,000 IU/ m² IM at hour 42 following Ara-C (3 h after the completion of the fourth Ara-C infusion on days 1 and 8)

Ifosfamide/ etoposide

- Etoposide (VP-16): 100 mg/ m² IV × 5 doses (days 1–5)
- Ifosfamide: 1.8 g/ m² IV × 5 doses (days 1–5). Begin immediately upon completion of VP-16 infusion
- Mesna: 360 mg/ m² IV prior to ifosfamide and every 3 h × 8 doses/ day (days 1–5)

Interim maintenance

- Commonly employed between consolidation and delayed intensification/ reinduction courses

Delayed intensification/ reinduction

- Dexamethasone 10 mg/ m² in divided doses PO × 14 days (days 0–7, 14–21)
- Vincristine 1.5 mg/ m² (maximum dose 2 mg) IV weekly × 5 doses (days 0, 14, 21, 42, 49)
- Doxorubicin 25–30 mg/ m² IV weekly × 3 doses (days 0, 7, 14)
- Cyclophosphamide 1,000 mg/ m² IV (day 28)
- 6-Thioguanine (6-TG) 60 mg/ m² PO once daily × 14 days (days 28–41)
- Cytarabine 75 mg/ m² IV or SQ (days 29–32, 36–39)
- IT methotrexate × 2 doses (days 29, 36)

With or without:

- Pegylated l-asparaginase 2,500 IU/ m² IM × 2 doses (days 4, 43)

Maintenance/ Continuation Regimen

Repeat cycles to complete 24–36 mo of total treatment.

- Prednisone 40–60 mg/ m²/ day or dexamethasone 6 mg/ m²/ day in divided doses PO × 5 days every 4 weeks
- Vincristine 1.5 mg/ m² (maximum dose 2 mg) IV every 4 weeks
- Mercaptopurine (6-MP) 75 mg/ m²/ dose^b PO once daily
- Methotrexate 20 mg/ m²/ dose^b PO once weekly
- IT methotrexate every 4–12 weeks for 1–3 y of treatment

^aA BCR/ ABL kinase inhibitor should be incorporated into the treatment regimen for individuals with Philadelphia chromosome-positive ALL.

^b6-MP and methotrexate doses should be adjusted to maintain the ANC between 750–1,500/ μL and the platelet count >75,000/ μL.

ALL, acute lymphoblastic leukemia; ALT, alanine transferase; ANC, absolute neutrophil count; BCR, breakpoint cluster region; CNS, central nervous system; IM, intramuscular; IT, intrathecal; IV, intravenous; MP, mercaptopurine; MRD, minimal residual disease; PO, oral; SER, slow early responder; SQ, subcutaneous.

Table 12.7 Intrathecal Chemotherapy

- Intrathecal chemotherapy delivered by lumbar puncture is part of all phases of treatment (unless full dose CNS radiation is required).
- Single agent intrathecal methotrexate has been the standard treatment.
- Triple agent intrathecal chemotherapy is sometimes employed especially for those with high-risk disease, CNS leukemia, or meningeal relapse.
- To minimize the risk of meningeal contamination due to traumatic lumbar puncture, spinal taps should be performed by clinicians experienced in the procedure. In addition, intrathecal chemotherapy should always be administered at the time of the initial (i.e., diagnostic) lumbar puncture and platelet count should be maintained above 50–100,000/ μL.
- To facilitate CNS delivery, the volume of CSF removed should equal the volume administered and patients should remain prone for 30–60 minutes.
- Intrathecal chemotherapy is dosed by age as follows:

Age (y)	Methotrexate (mg)	Hydrocortisone (mg)	Cytarabine (mg)	Volume (mL)
<1	7.5	7.5	15	5
1	8	8	16	6
2	10	10	20	7
3–8	12	12	24	8
≥9	15	15	30	10

Induction schedule

- CNS-1: Every 2 weeks × 2 doses
- CNS-2 or CNS-3: Weekly × at least 4 doses and until 2 successive CNS-1

Consolidation schedule

- Every 1–4 weeks

Maintenance schedule

- Every 4–12 weeks for 1–3 y of treatment

CNS, central nervous system; CSF, cerebrospinal fluid.

Table 12.8 Radiation Guidelines

- CNS radiation should be avoided in children younger than 2 y.
- Radiation dose should be based on the specific indication and overall treatment regimen.

Site	Total Dose (cGy)	Fractional Dose (cGy)
Cranium	1,200–2,400	150–200
Spine	600–1,200	150–200

CNS, central nervous system.

DOSE MODIFICATION

Improved outcome is associated with greater drug exposure, and every attempt should be made to administer protocol-specified doses unless toxicity precludes their delivery. Importantly, 6-mercaptopurine (6-MP) and methotrexate dosing should be increased during maintenance to achieve a targeted degree of myelosuppression (Table 12.6). In the event of significant chemotherapy-related toxicity, individual agents should be dose-reduced or discontinued as clinically indicated. Specific agents may require dose adjustment for renal or hepatic dysfunction. Patients with thiopurine S-methyltransferase deficiency (approximately 1 : 300 incidence) require significant dose reductions of 6-MP to avoid severe toxicity. Individuals with Down syndrome tolerate methotrexate poorly and may require dose reduction of that agent. Asparaginase may have to be discontinued in cases of severe pancreatitis.

EXTRAMEDULLARY LEUKEMIA

Current chemotherapy regimens are associated with low rates of extramedullary relapse in both the CNS and testes. Importantly, patients with isolated extramedullary relapse also require systemic therapy. Radiation is currently reserved primarily to treat overt CNS leukemia (Table 12.8).

NEW TREATMENT APPROACHES

Molecularly targeted agents have been incorporated into the treatment of ALL resulting

in improved outcomes. For example, the BCR/ABL tyrosine kinase inhibitors, imatinib mesylate (Gleevec), dasatinib, and nilotinib have been successfully utilized in combination with chemotherapy to improve survival in Philadelphia chromosome-positive ALL.^{23,24,27} Monoclonal antibody-based therapies that target differentiation antigens expressed on the surface of lymphoblasts (e.g., CD19, CD20, CD22, CD52) have also been used in the setting of ALL.^{28,29} In particular, rituximab combined with conventional chemotherapy significantly improved survival in a randomized study of adults with CD20-positive, Philadelphia chromosome-negative ALL.²⁹

Various novel immunotherapeutic strategies have shown promise in early phase studies. Blinatumomab is a bispecific antibody that engages the patient's T-cells via CD3+ to target CD19+ B-ALL cells. This agent was recently approved by the Food and Drug Administration (FDA) for second-line treatment of B-ALL.³⁰ Inotuzumab ozogamycin, an immunotoxin targeting CD22, significantly improved complete remission rates in patients with relapsed ALL compared to standard therapy;³¹ however, hepatotoxicity was notable and further studies are ongoing to define its role in frontline therapy. Adoptive transfer of T-cells expressing chimeric antigen receptors (CARs) against leukemia-specific antigens (e.g., CD19) is an emerging therapeutic modality with very encouraging initial results.^{32,33} Although this therapy can be associated with life-threatening adverse events necessitating intensive supportive care, durable remissions have been reported in patients with multiple relapsed and chemotherapy-refractory ALL. The FDA recently approved once such CAR T cell product (tisagenlecleucel) for children and young adults up to age 25 with multiply relapsed or primary refractory CD19+ ALL.

MANAGEMENT OF RELAPSE

The chance of cure decreases substantially after relapse. Attaining a second remission is critical and often times can be achieved with standard four-or five-drug reinduction regimens (Table 12.6).³⁴ The likelihood of prolonged DFS with standard regimens varies based in large part on immunophenotype, duration of the initial complete remission, and site of relapse. Curative salvage using standard chemotherapy and radiation is more likely in the setting of isolated extramedullary relapse.³⁵ For children with bone marrow or combined relapse and CR1 durations of more than 36 months, approximately 50% achieve prolonged DFS with intensive retreatment. Outcome is guarded with shorter CR1 durations, multiple relapses, T-cell ALL, or induction failure.^{34–36} Outcomes are dismal in adults with relapsed ALL with standard approaches;³⁷ thus, these patients should be offered novel therapies on clinical trials.

Allogeneic Stem Cell Transplantation

For adult patients with relapsed ALL, allogeneic SCT in second CR is standard care.²⁰ In general, survival is better for HLA-matched sibling donor SCTs as the risks of transplant-related morbidity and mortality are increased with alternative donors (unrelated, HLA-mismatched related, and cord blood). With recent and ongoing improvements in SCT conditioning and supportive care regimens, alternate donor SCTs are being performed more frequently and with improved outcomes. In children, transplant in CR2 is beneficial for patients with high-risk relapse (i.e., shorter CR1 duration).²¹ Achieving MRD negativity prior to transplant increases the probability of a favorable outcome.³⁸

SUPPORTIVE CARE

Aggressive monitoring and supportive care are essential throughout all phases of treatment.

Antiemetics

Nausea and vomiting, common during induction, consolidation, intensification, and CNS-directed therapy, are managed with routine antiemetic prophylaxis and treatment.

Tumor Lysis Syndrome

Rapid blast lysis can result in life-threatening metabolic complications. Tumor lysis syndrome is usually seen within the first few hours to days of initiation of induction chemotherapy. Patients with WBC higher than 100,000/ μL , elevated serum lactate dehydrogenase (LDH), and/ or elevated uric acid are at increased risk. Tumor lysis precautions should be started as soon as possible after diagnosis and at least 6 to 12 hours prior to the start of induction therapy. Prophylaxis and monitoring should continue until disease burden is reduced, peripheral blasts are clear, and it is apparent that no tumor lysis has developed, usually for 3 to 7 days. The following measures are indicated for all patients around initial induction therapy:³⁹

- | Allopurinol: 100 mg/ m^2 per dose orally, three times daily. Urate oxidase (rasburicase) is an effective alternative for management of extreme hyperuricemia, especially in the setting of renal insufficiency. Rasburicase can cause severe hemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and thus should be avoided in such patients.
- | Hydration: Intravenous fluids at a rate of 1.5 to twice the maintenance requirements (120 mL/ m^2/ h) should be titrated to maintain a urine specific gravity of 1.010 or less and normal urine output. Because of the risk of hyperkalemia, potassium should be avoided. Although alkalinization with sodium bicarbonate added to hydration fluids increases the solubility of uric acid, it enhances calcium-phosphate precipitation in the

- kidney and should be avoided, especially in the presence of hyperphosphatemia.
- Frequent serial laboratory monitoring is required during initiation of induction chemotherapy. Complete blood count (CBC), potassium, phosphorous, calcium, creatinine, blood–urea nitrogen (BUN), and uric acid should be assayed every 4 to 6 hours for the first 24 to 48 hours, then less frequently once stable.

Transfusions

Blood transfusion should be used to prevent complications related to severe cytopenias. To decrease the risk of transfusion-associated complications, specialized products should be used.

- Platelets: To prevent bleeding, platelet counts should routinely be maintained above 10,000/ μL . Higher levels are recommended for management of bleeding, prior to invasive procedures such as lumbar puncture, and to reduce the risk of CNS hemorrhage related to leukostasis in the setting of hyperleukocytosis. Single-donor platelets are recommended whenever possible to decrease donor exposure and the risk of HLA alloimmunization.
- Red blood cells (RBCs): Concomitant anemia partially offsets the hyperviscosity associated with severe hyperleukocytosis. Thus, RBC transfusion should be avoided if possible when the WBC is higher than 100,000/ μL . If transfusion is necessary, the hemoglobin and hematocrit should be increased slowly using small aliquots of packed RBCs until the peripheral blast count is reduced.
- Irradiation: To reduce the risk of transfusion-associated graft-versus-host disease, all cellular blood products should be irradiated.
- Leukodepletion: Platelets and red cells should be leuko-reduced to decrease the risk of febrile reactions, HLA-alloimmunization with subsequent platelet-refractoriness, and transmission of cytomegalovirus (CMV) infection.

Infection Prophylaxis

Aggressive surveillance, prophylaxis, and treatment for bacterial, fungal, viral, and opportunistic infections are essential to prevent morbidity and mortality.

- Pneumocystis jiroveci pneumonia (PCP): Patients should receive PCP prophylaxis with trimethoprim/ sulfamethoxazole continuing until 6 months after chemotherapy is completed.
- Neutropenic fever: Patients with an absolute neutrophil count (ANC) less than 500/ μL and temperature 38.3°C or higher should be evaluated for possible infection and treated empirically with parenterally administered broad-spectrum antibiotics. Antifungal therapy should be initiated for neutropenic fever that persists for 5 days. Antibiotics should be continued until the ANC rises above 500/ μL , fever resolves, cultures are negative, and any suspected infection is fully treated.

- Intravenous immunoglobulin (IVIG): Hypogammaglobulinemia is common during treatment for ALL. Immunoglobulin G (IgG) levels should be assayed for those with recurrent infections, and if low, IVIG supplementation should be considered (approximately 500 mg/ kg every 4 weeks as needed to maintain an IgG level of 500 mg/ dL).
- Myeloid growth factors: Granulocyte colony-stimulating factor (G-CSF) during induction has been shown to improve outcome for adults, but no benefit was demonstrated in a pediatric study. Myeloid growth factor support may be employed in cases of severe fungal infection in an attempt to hasten neutrophil recovery.

Chemotherapy Prophylaxis

Agent-specific prophylaxis should be utilized as clinically indicated. For example, gastritis prophylaxis may be required during corticosteroid administration. Leucovorin rescue is indicated to prevent severe toxicity after high-dose methotrexate. To reduce the risk of conjunctivitis associated with high-dose Ara-C, corticosteroid or saline ophthalmic solution should be administered during and for 24 to 48 hours after treatment. Mesna should be used to prevent hemorrhagic cystitis associated with high-dose ifosfamide and cyclophosphamide.

Nutritional Support

Nutritional status should be monitored and supplementation provided as indicated. Patients should be counseled on healthy eating habits as increased appetite and obesity often develop during ALL therapy secondary to corticosteroid use. Routine folic acid use should be avoided with methotrexate administration because it may counteract the therapeutic efficacy of folate antagonism.

Psychosocial Support

Multidisciplinary support for the patient and family is an important part of successful treatment.

EVALUATIONS

Serial evaluations to monitor for response, relapse, complications, and therapy-associated toxicity should be conducted throughout all treatment phases.

Evaluations During Treatment

- History, physical examination, and routine laboratory assessments including CBC and chemistry panel should be performed regularly throughout treatment.

Bone marrow aspiration should be obtained at the following times:

- Induction day 7 or 14 to assess early response (some studies substitute peripheral blood MRD assessment on day 8).
- Induction day 29 to assess remission status. If indeterminate, repeat every 1 to 2 weeks until recovery to confirm remission or induction failure.
- Suspected relapse.
- Flow cytometry, cytogenetics, and/ or molecular genetic studies can be used to monitor MRD, which is prognostic.
- CSF cell count and cytology should be performed at the time of all intrathecal chemotherapy administrations. Lumbar puncture should also be performed if CNS relapse is suspected.

Evaluations After Treatment

- Follow-up evaluations to include history, physical examination, and routine laboratory studies (CBC, chemistry panel) should be conducted to monitor for toxicity and recurrent disease until at least 5 years after completion of treatment on the following schedule (or as clinically indicated):
 - Every 1 to 2 months during the first year
 - Every 2 to 3 months during the second year
 - Every 3 to 4 months during the third year
 - Every 6 months during the fourth year
 - Yearly thereafter

Late Effects

- Lifelong follow-up to monitor various possible late complications of treatment is recommended.⁴⁰ The following are among the most frequent late effects.
- Cardiomyopathy: To decrease the risk of cardiotoxicity, cumulative anthracycline doses are usually limited to less than 400 mg/ m². Echocardiograms for left ventricular function determination should be performed at baseline, at completion of treatment, every 1 to 2 years after treatment until serial studies remain normal, and as clinically indicated.
- Neurologic toxicity: Children are at especially high risk of neurotoxicity from chemotherapy and radiation. All patients should be monitored for neurologic toxicity including neurodevelopmental dysfunction.
- Endocrinologic dysfunction: Patients should be monitored for endocrinopathies, including growth retardation, hypothyroidism, and infertility.
- Osteonecrosis: Corticosteroids, especially dexamethasone, are associated with a high incidence of osteonecrosis.
- Secondary malignancy: Patients should be monitored for secondary malignancies because these continue to develop even in the second decade after treatment.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
2. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. N Engl J Med. 2015;373(16):1541–1552.
3. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol. 2011;29(5):532–543.
4. Pui CH. Acute lymphoblastic leukemia. Childhood Leukemias. 3rd ed. Cambridge, UK: Cambridge University Press; 2012:332–366.
5. Ward E, DeSantis C, Robbins A, et al. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin. 2014;64(2):83–103.
6. Bhojwani D, Yang JJ, Pui CH. Biology of childhood acute lymphoblastic leukemia. Pediatr Clin North Am. 2015;62(1):47–60.
7. Zuna J, Madzo J, Krejci O, et al. ETV6/ RUNX1 (TEL/ AML1) is a frequent prenatal first hit in childhood leukemia. Blood. 2011;117(1):368–369; author reply 370–361.
8. Roberts KG, Mullighan CG. Genomics in acute lymphoblastic leukaemia: insights and treatment implications. Nat Rev Clin Oncol. 2015;12(6):344–357.
9. Duffield AS, Racke F, Borowitz MJ. Precursor B-and T-cell neoplasms. In: Jaffe ES, Arber DA, Campo E, Harris NL, Quintanilla-Martinez L, eds. Hematopathology. 2nd ed. Philadelphia, PA: Elsevier Inc.; 2011:761–773.
10. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.
11. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol. 2012;30(14):1663–1669.
12. Bruggemann M, Gokbuget N, Kneba M. Acute lymphoblastic leukemia: monitoring minimal residual disease as a therapeutic principle. Semin Oncol. 2012;39(1):47–57.
13. Campana D. Should minimal residual disease monitoring in acute lymphoblastic leukemia be standard of care? Curr Hematol Malig Rep. 2012;7(2):170–177.
14. Hunger SP, Mullighan CG. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. Blood. 2015;125(26):3977–3987.
15. Curran E, Stock W. How I treat acute lymphoblastic leukemia in older adolescents and young adults. Blood. 2015;125(24):3702–3710.
16. Paul S, Kantarjian H, Jabbour EJ. Adult acute lymphoblastic leukemia. Mayo Clin Proc. 2016;91(11):1645–1666.
17. Kantarjian HM, O'Brien S, Smith TL, et al. Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. J Clin

- Oncol. 2000;18(3):547–561.
- 18. Nachman JB, Sather HN, Sensel MG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *N Engl J Med.* 1998;338(23):1663–1671.
 - 19. Oliansky DM, Camitta B, Gaynon P, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the treatment of pediatric acute lymphoblastic leukemia: update of the 2005 evidence-based review. ASBMT Position Statement. *Biol Blood Marrow Transplant.* 2012;18(7):979–981.
 - 20. Hoelzer D, Bassan R, Dombret H, et al. Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2016;27(suppl 5):v69–v82.
 - 21. Eapen M, Raetz E, Zhang MJ, et al. Outcomes after HLA-matched sibling transplantation or chemotherapy in children with B-precursor acute lymphoblastic leukemia in a second remission: a collaborative study of the Children’s Oncology Group and the Center for International Blood and Marrow Transplant Research. *Blood.* 2006;107(12):4961–4967.
 - 22. Moricke A, Zimmermann M, Valsecchi MG, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. *Blood.* 2016;127(17):2101–2112.
 - 23. Schultz KR, Carroll A, Heerema NA, et al. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children’s Oncology Group study AALL0031. *Leukemia.* 2014;28(7):1467–1471.
 - 24. Fielding AK. Treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: a broader range of options, improved outcomes, and more therapeutic dilemmas. *Am Soc Clin Oncol Educ Book.* 2015:e352–359.
 - 25. Vora A, Andreano A, Pui CH, et al. Influence of cranial radiotherapy on outcome in children with acute lymphoblastic leukemia treated with contemporary therapy. *J Clin Oncol.* 2016;34(9):919–926.
 - 26. Hijiya N, Liu W, Sandlund JT, et al. Overt testicular disease at diagnosis of childhood acute lymphoblastic leukemia: lack of therapeutic role of local irradiation. *Leukemia.* 2005;19(8):1399–1403.
 - 27. Bassan R, Rossi G, Pogliani EM, et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. *J Clin Oncol.* 2010;28(22):3644–3652.
 - 28. Kantarjian H, Thomas D, Wayne AS, et al. Monoclonal antibody-based therapies: a new dawn in the treatment of acute lymphoblastic leukemia. *J Clin Oncol.* 2012;30(31):3876–3883.
 - 29. Maury S, Chevret S, Thomas X, et al. Rituximab in B-lineage adult acute lymphoblastic leukemia. *N Engl J Med.* 2016;375(11):1044–1053.

30. Topp MS, Gokbuget N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. *Lancet Oncol.* 2015;16(1):57–66.
31. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med.* 2016;375(8):740–753.
32. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet.* 2015;385(9967):517–528.
33. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med.* 2014;371(16):1507–1517.
34. Ko RH, Ji L, Barnette P, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. *J Clin Oncol.* 2010;28(4):648–654.
35. Nguyen K, Devidas M, Cheng SC, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children’s Oncology Group study. *Leukemia.* 2008;22(12):2142–2150.
36. Bhojwani D, Pui CH. Relapsed childhood acute lymphoblastic leukaemia. *Lancet Oncol.* 2013;14(6):e205–217.
37. Fielding AK, Richards SM, Chopra R, et al. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ ECOG 2993 study. *Blood.* 2007;109(3):944–950.
38. Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J Clin Oncol.* 2009;27(3):377–384.
39. Howard SC, Jones DP, Pui CH. The tumor lysis syndrome. *N Engl J Med.* 2011;364(19):1844–1854.
40. Oeffinger KC, Mertens AC, Sklar CA, et al. Chronic health conditions in adult survivors of childhood cancer. *N Engl J Med.* 2006;355(15):1572–1582.

Chronic Myelogenous Leukemia

Agnes S. M. Yong and A. John Barrett

Although chronic myelogenous leukemia (CML) is rare, it has achieved great prominence in the medical literature because its biologic basis has been elucidated in unprecedented detail. As a result, CML became the model for the development of effective molecularly targeted and immune-based treatments for leukemia. Nowell and Hungerford reported the unique, unusually small G group chromosome in patients with CML in 1960 and named it the Philadelphia (Ph) chromosome.¹ This was the first association of a human malignant disease with a consistent chromosomal marker. In 1973, the Ph chromosome was identified as the truncated chromosome 22 consequent to a reciprocal translocation involving chromosome 9.² It was not until the 1980s that the fusion partners of the translocation were identified as the ABL1 oncogene on chromosome 9 and the breakpoint cluster region (BCR) on chromosome 22.^{3,4} The BCR-ABL1 oncoprotein was found to have tyrosine kinase activity, and when the gene was inserted into mouse stem cells it induced leukemia in recipient animals.⁵ Until the 1990s, allogeneic stem cell transplantation (SCT) was the preferred first-line treatment in eligible CML patients as the disease is highly susceptible to a graft-versus-leukemia effect from transplanted donor lymphocytes.⁶ The advent of imatinib mesylate (Gleevec), the first of a new class of small molecule drugs designed specifically to block the BCR-ABL1 tyrosine kinase, has supplanted SCT for most of the patients as these drugs confer durable disease control, particularly in the earlier stages of CML.⁷ Second-generation tyrosine kinase inhibitors (TKIs) dasatinib and nilotinib, which are more potent pharmacologically, have recently been shown to be more efficient in rapidly reducing the leukemic load compared with imatinib, and are now advocated as first-line treatment for CML.^{8,9} Despite progress in CML biology and treatment, fundamental questions about its origin remain unanswered. Evidence suggests that a predisposition to develop CML precedes the clonal expansion of BCR-ABL1 translocated stem cells,¹⁰ and the discovery of very low levels of BCR-ABL1 in the

blood of normal individuals who do not develop CML raises the possibility that the BCR-ABL1 translocation alone is not sufficient to cause leukemia.¹¹ The emergence of drug resistance to TKIs in the era of molecular targeting for CML has driven the development of third-generation TKIs such as ponatinib. However, only a proportion of TKI-resistant CMLs are attributable to mutations of the BCR-ABL1 kinase domain. Drugs targeting alternate non-kinase dependent and stem cell pathways are also being pursued. Unfortunately, advanced phases of CML still are largely refractory to available treatments, and SCT remains the only curative option.

EPIDEMIOLOGY

Rare incidence of 1.5 in 100,000

Represents 10% to 15% of all leukemias

Incidence increases with age (median age of diagnosis = 65); exceedingly rare in children (incidence 0.6 to 1.0/ 1,000,000¹²)

Male predominance (1.5:1)

Worldwide distribution: no sociogeographic preponderance

Ionizing radiation is the only known causative factor, leukemia occurs usually within 6 to 8 years of exposure

No known genetic factors determine susceptibility to CML

PATHOPHYSIOLOGY

Leukemic Hematopoiesis Originates in a Multipotent Stem Cell

BCR-ABL1 translocation is found in all cells of the myeloid lineage (erythroid and granulocyte precursors and megakaryocytes) as well as in B cells but not in T cells.¹³ There are two major hypotheses for this observation: first, the acquisition of BCR-ABL1 may occur in a multipotent stem cell with little or no T differentiation capacity; second, T cells bearing BCR-ABL1 may be systematically eliminated. Unregulated proliferation of BCR-ABL1-positive stem cells is responsible for massive expansion, primarily in granulocyte production, which leads to leukocytosis.

Clonal Dominance

The BCR-ABL1–positive clone outcompetes normal hematopoiesis. At diagnosis, it is common to find a mixed population of Ph-positive and Ph-negative cells in the bone marrow. With time, normal stem cells are progressively replaced by CML stem cells. CML CD34+ progenitor cells require less hematopoietic growth factors than do normal progenitors for survival and proliferation, a characteristic that may be partially due to the presence of an autocrine production of hematopoietic growth factors by CML

cells.¹⁴

Molecular Basis of Chronic Myelogenous Leukemia in the BCR-ABL1 Translocation

The BCR-ABL1 oncprotein is a constitutively activated tyrosine kinase that phosphorylates intermediate molecules in several important pathways, affecting proliferation, maturation, resistance to apoptosis, and cell adhesion, ultimately resulting in the typical leukemic phenotype.¹⁵

Genomic Instability

CML is characterized by progression to refractory acute leukemia. CML usually starts as a relatively benign disorder that evolves to an accelerated phase (AP), when the leukemia is much more difficult to control and additional chromosomal abnormalities appear, followed by a progressive increase in blast cells in blood and marrow, termed the blastic phase (BP) or blast crisis, when the disease transforms to an acute myeloid or B lymphocytic leukemia.¹⁶ Clonal evolution, which is matched by increasing malignant behavior of the leukemia, has a variable pace but is inevitable.

PRESENTATION

Classic Presentation

CML presents with an insidious history of increasing fatigue, lassitude, weight loss, night sweats, massive splenomegaly, and gout. Some patients have leukocyte counts greater than $300 \times 10^9/\text{L}$ and experience symptoms of leukostasis with headache, focal neurologic deficits, and priapism.

Typical Presentation in the Developed World

Overt symptoms and signs are rarely encountered because the diagnosis is made earlier. Commonly, patients present with fatigue, with or without moderate weight loss, abdominal discomfort, and early satiety from an enlarged spleen, or simply due to the chance observation of an elevated leukocyte count. CML should be considered in the differential diagnosis of a patient at any age presenting with splenomegaly and with an elevated white blood cell (WBC) count.

Rare Presentations

Rare presentations include chloroma, petechiae, and bruising. These features suggest progression of CML to an AP or BP. Unlike other leukemias, CML seldom if ever presents with bacterial or fungal infection because neutrophil function is preserved.

DIAGNOSIS

Although blood and bone marrow examinations share features with other myeloproliferative disorders, the typical presentation with high leukocyte count and a hypercellular marrow with basophilia is pathognomonic of CML. Chromosome and molecular analysis confirm the presence of a BCR-ABL1 translocation.

Blood Count

Leukocyte number varies between being slightly elevated to more than $200 \times 10^9/\text{L}$; counts as high as $700 \times 10^9/\text{L}$ are occasionally encountered. The platelet count is normal or elevated and there is often a mild normochromic normocytic anemia.

Blood Film

Blood film is of great diagnostic value, because many of the typical features of CML are unique—a left shift with circulating myeloblasts, myelocytes, metamyelocytes, and band forms. The hallmark of CML is basophilia with basophil counts often exceeding $1 \times 10^9/\text{L}$. Sustained basophilia is almost never encountered outside CML and some cases of mastocytosis. Eosinophilia and occasional nucleated red blood cells are also common findings. Platelet morphology is usually normal but giant forms can be seen.

Bone Marrow

The aspirate shows cellular spicules, and the biopsy is hypercellular with almost complete effacement of the fat spaces. There is granulocytic hyperplasia of the neutrophil, eosinophil, and basophil series. Megakaryocytes are normal or increased and may show reduced numbers of nuclei. Sea-blue histiocytes are commonly seen in CML marrows. Fibrosis of the marrow is a feature of AP CML, as is an increase in blasts over 10%. BP shows more than 20% blasts.

Chromosome Analysis

The typical karyotype of CML shows the reciprocal translocation t(9;22)(q34;q11) (Fig. 13.1). Variants include three-way translocations among chromosomes 9, 22 and 11, or 19. An additional chromosome abnormality or Ph chromosome duplication usually indicates a more advanced disease stage. Fluorescence in situ hybridization (FISH) is a rapid and sensitive technique to detect the Ph chromosome directly in blood or marrow as it does not rely on dividing cells.

Molecular Diagnosis

More than 95% of patients presenting with the clinical and morphologic features of CML have Ph chromosomes in the marrow. Of the 5% who are Ph-negative, most have a

cryptic BCR-ABL1 transcript detected by polymerase chain reaction (PCR). The remaining patients are described as atypical BCR-ABL1-negative CML. A few such cases are morphologically indistinguishable from Ph-positive CML but most have atypical features on careful examination and are classified under myelodysplastic/myeloproliferative neoplasms, and some have SETBP1 mutations.¹⁷ Molecular analysis provides further information of the precise transcript in CML. Depending on the BCR breakpoint, four common BCR-ABL1 transcript variants are possible: e13a2 and e14a2 (formerly b2a2 and b3a2), both encoding the 210 kD BCR-ABL1 oncoprotein (p210); e1a2, which is more common in Ph-positive acute lymphoblastic leukemia (ALL), encoding the 190 kD BCR-ABL1 oncoprotein (p190); and e19a2, which encodes the 230 kD BCR-ABL1 oncoprotein of chronic neutrophilic leukemia. Recently CML patients with the e13a2 transcript have been found to have poorer outcome compared to those with the e14a2 despite adequate TKI therapy.¹⁸ Rarely, unusual transcript variants, such as e1a3 or e6a2, have also been described.¹⁹

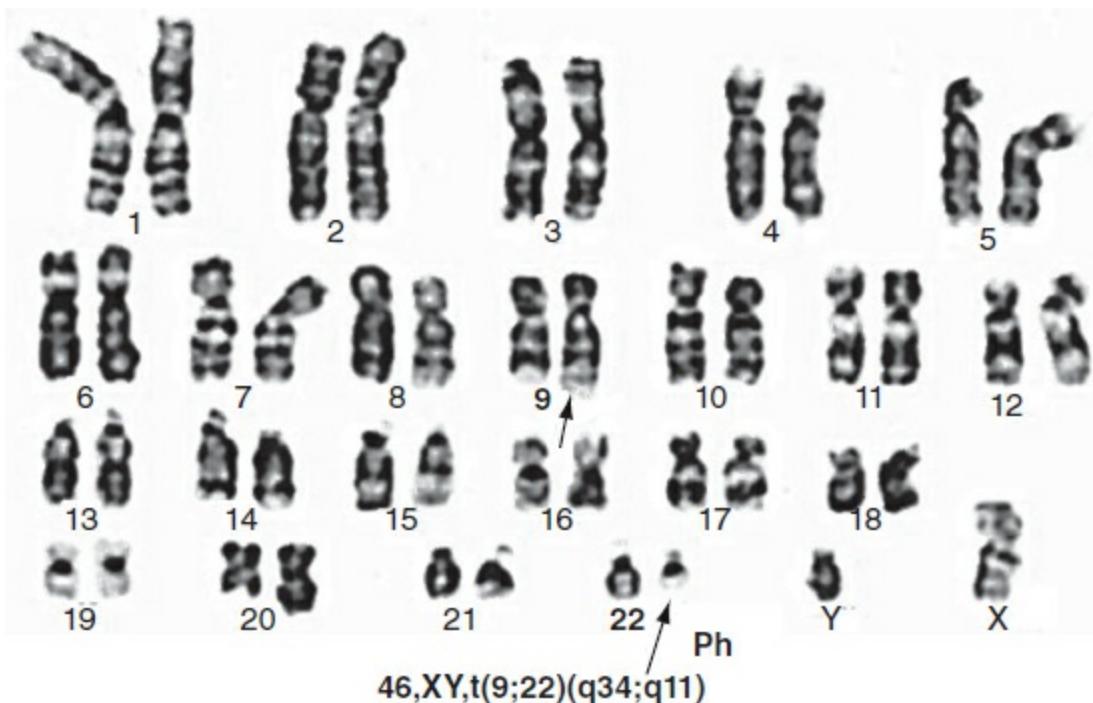


FIGURE 13.1 The Ph chromosome. G-banded metaphase preparation showing the diminutive Ph-positive chromosome and extrachromosomal material on the long arm of chromosome 9.

Ph, Philadelphia.

Differential Diagnosis

The diagnosis of CML is made in three stages (Fig. 13.2):

- Persisting leukocytosis without any obvious infective cause suggests a myeloproliferative neoplasm, prompting further examination of the blood and bone marrow.
- Morphology and blood count show either typical features of CML (basophilia being especially significant) or suggest other myeloproliferative neoplasms (high platelet counts, essential thrombocythemia; high red cell count, polycythemia vera, teardrop red cells, myelofibrosis). Dysplasia suggests a hyperproliferative myelodysplastic syndrome.
- Definitive diagnosis requires chromosome analysis of the bone marrow. Cytogenetics identifies the Ph-positive chromosome and the BCR-ABL1 translocation in all but a small percentage of patients with a morphologic diagnosis of CML. Confirmation of the presence of BCR-ABL1 transcripts by PCR is advised as it aids in disease monitoring following treatment²⁰ (Fig. 13.3).

COURSE OF CHRONIC MYELOGENOUS LEUKEMIA

CML is a multistage disease that progresses from chronic phase (CP) to AP and then to BP (Fig. 13.4).

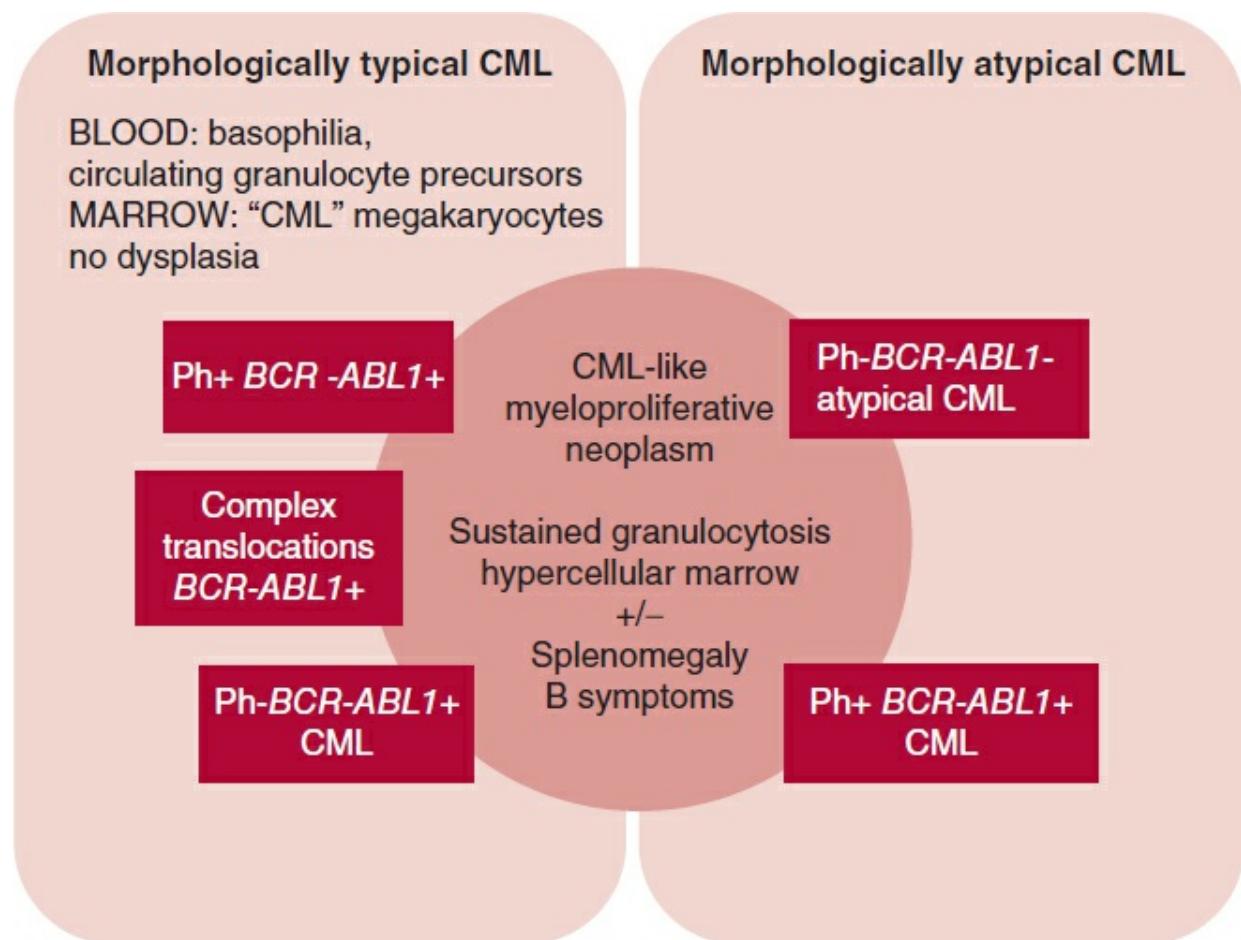


FIGURE 13.2 Differential diagnosis of CML and related disorders.

CML, chronic myelogenous leukemia.

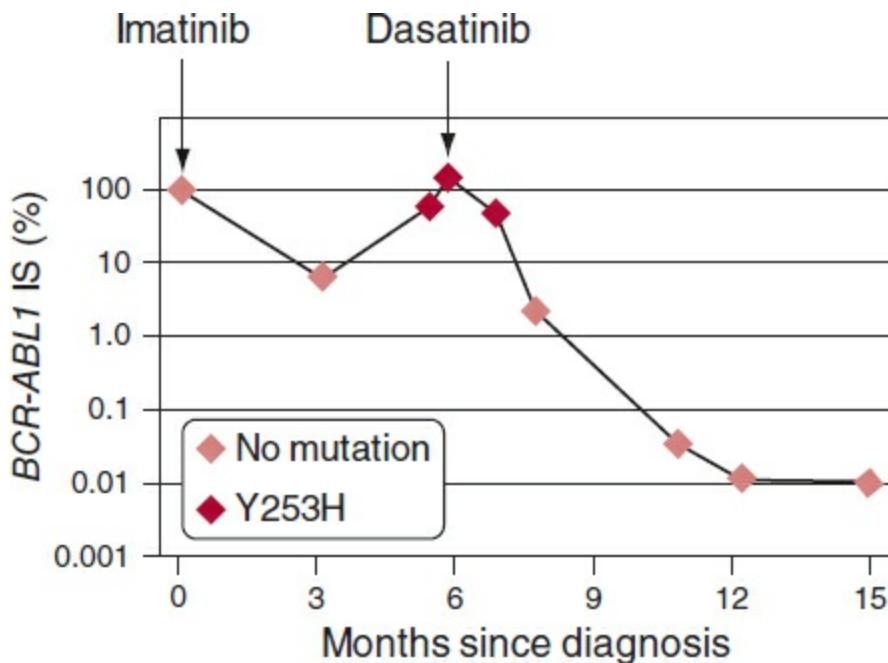


FIGURE 13.3 PCR for BCR-ABL1 transcripts to monitor treatment in CML.

CML, chronic myelogenous leukemia; PCR, polymerase chain reaction.

Courtesy S. Branford, IMVS/ SA Pathology, Adelaide, Australia.

Chronic Phase

Untreated patients in CP show a gradual rise in the leukocyte count with emergence of splenomegaly and ultimately the full picture of a myeloproliferative neoplasm with B symptoms, weight loss, and hyperleukocytosis.

Duration of CP was highly variable in the pre-TKI era: some patients progressed within months of diagnosis directly to AP and BP, whereas others remained for more than a decade in stable CP. Sometimes patients present in AP or BP without a clear preceding CP; in this circumstance, it is important to distinguish CML presenting as acute leukemia from de novo (Ph-negative) acute leukemia because the treatment approaches are distinct.

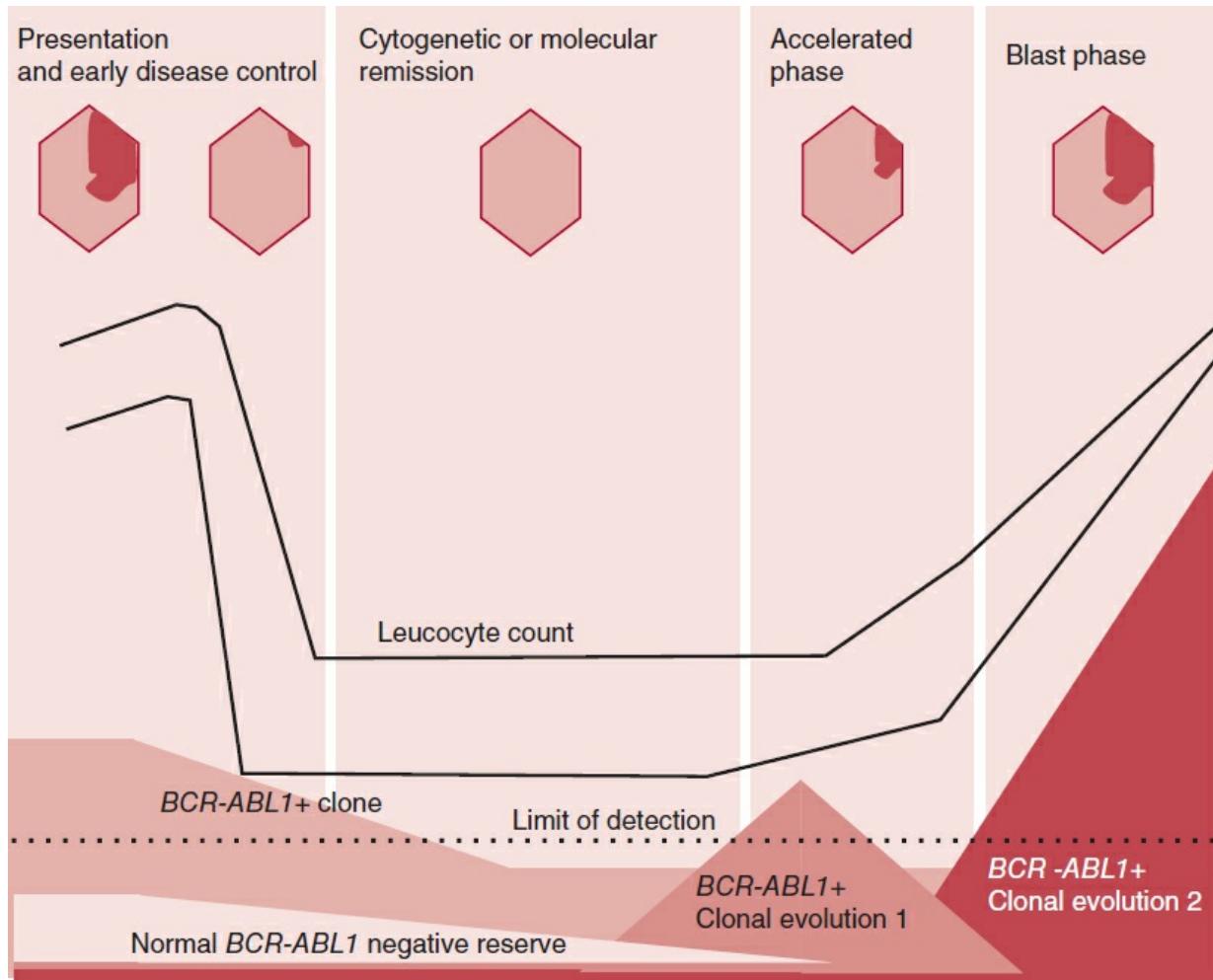


FIGURE 13.4 Course and clonal evolution of CML.

CML, chronic myelogenous leukemia.

Optimal response to TKI in most of the CML patients has greatly reduced the rate of transformation to AP or BC.

Accelerated Phase

The revised criteria for AP include “provisional” response-to-TKI, which may be more intuitive clinically, but still requires support by additional data¹⁷ (Table 13.1):

Blastic Phase

Signs and symptoms of acute leukemia: bone pains, weight loss, and B symptoms.

Marrow failure: decreasing red cell count and platelets. (Neutrophil counts are better conserved.)

Clonal evolution: further chromosomal abnormalities.

Blasts: 20% or more in blood or marrow, or infiltrative proliferation of blasts in an extramedullary site

Table 13.1 Criteria Distinguishing Chronic Myelogenous Leukemia at Different Stages of Evolution

Criterion	CP	AP	BP
Response to first TKI			
None or failed CHR		+	Failed ≥ 1 TKI
Failed CHR/ MR to 2 TKIs		+	
2 or more BCR/ ABL mutations		+	
Blasts in blood %	<10	10–19	>20
Blasts in marrow %	<10	10–19	>20
Basophils %	<20	>20	
Karyotype at diagnosis	Ph+	Additional to Ph+: tri 8, 17q, tri 19 complex karyotype 3q 26.2	
Karyotype evolution	—	Any new abnormality	
Marrow dysplasia	—	Abnormal megakaryocytes	
Marrow fibrosis	—	Reticulin/ collagen +	+
WBC escape from control	—	Persistently $>10 \times 10^9 / L$	+
Platelets:			
Thrombocytosis	—	$>1,000 \times 10^9 / L$	
OR Thrombocytopenia	—	$<100 \times 10^9 / L$	+
Splenomegaly	—	Persisting/ unresponsive to therapy	+
Extramedullary blasts	—	—	+
Large foci of blasts in marrow	—	—	+

BP is distinguished from AP mainly by blast cell counts, blast foci in marrow and extramedullary blasts and TKI failure (bold), with or without other criteria allocated to AP. CP patients may progress to BP without a perceptible AP.

AP, accelerated phase; BCR, breakpoint cluster region; BP, blastic phase; CHR, complete hematological remission; CP, chronic phase; MR, molecular remission; TKIs, tyrosine kinase inhibitors.

Adapted from Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–2405.

Characterization of Blastic Phase

Approximately 60% of patients develop myeloid BP resembling acute myeloid leukemia (AML); the remainder has lymphoid BP reminiscent of ALL. In both phenotypes, blasts are poorly differentiated. Auer rods are not seen and the lymphoid or myeloid origin of the leukemia is only reliably determined by cytochemical stains and surface phenotype, revealing either a pre-B ALL (TdT-positive, CD10+, CD19+, CD33±, CD34±) or an undifferentiated AML (peroxidase weak-positive, CD33+, CD34+, CD13±). A peculiar feature of CML is the variability of its subsequent

evolution. Patients achieving remission from AML can reenter CP only to relapse again with ALL or vice versa.

PROGNOSTIC FACTORS

Poor prognosis (tendency to rapid progression to BP)

Massive splenomegaly and constitutional symptoms

High basophil counts

High peripheral blood blast percentage

Of predictive scores using patient characteristics at diagnosis that were validated in the pre-imatinib era to determine outcome and survival,^{21,22} the Sokal score, which has some of the aforementioned criteria, still appears to be prognostic in patients who are treated with imatinib.^{7,23,24}

TREATMENT

Treatment of CML involves diverse, evolving approaches²⁰ outlined in the algorithm in Figure 13.5. The drugs commonly used to treat CML are detailed in Table 13.2.

CML treatment is guided by disease monitoring using regular blood counts and bone marrow examination to document hematologic changes, chromosome analysis of marrow or FISH analysis of blood or marrow to detect response or progression at the karyotypic level, and PCR for BCR-ABL1 mRNA transcripts in the blood to quantify response at the molecular level (Fig. 13.3). The degree of disease bulk reduction determines the appropriate monitoring approach, and the degree of response is defined as hematologic response (HR), cytogenetic response, and molecular response (MR) or complete molecular remission²⁰ (Fig. 13.6).

Treating Newly Diagnosed Chronic Myelogenous Leukemia in Chronic Phase

The great majority (>80%) of CML patients is diagnosed in CP. Initial treatment is aimed at reducing disease bulk and obtaining hematologic remission (normalization of blood counts). Subsequent therapy is tailored toward achieving either a “cure” or “minimal residual disease” (MRD). The choice of initial TKI depends on the aim of treatment and the patient’s existing comorbidities that may be exacerbated by the specific adverse effects of individual TKIs.²⁵ In a patient presenting with a high white cell count (e.g., > 80 to 100 × 10⁹/L), hydroxyurea 0.5 to 2.5g daily is given to reduce the white cell count. Allopurinol 300 mg daily is given to minimize tumor lysis syndrome until the white cell count normalizes. Once the diagnosis of CML is

confirmed, a TKI is commenced irrespective of the white cell count, and hydroxyurea ceased:

- Imatinib 400 to 600 mg daily
- Or nilotinib 300 mg twice daily
- Or dasatinib 100 mg daily

Monitoring Response to Imatinib

Full blood counts should be monitored every 2 weeks until complete HR, equivalent to normalization of blood counts, is achieved. Complete HR should be confirmed on two subsequent occasions.^{20,26}

Bone marrow aspiration every 6 months to assess cytogenetic response, analyzing at least 20 metaphases for the Ph chromosome. Patients who achieve complete cytogenetic response (0% Ph) have a prolonged period without disease progression (see definitions in Fig. 13.6). The cytogenetic response improves over time in responding patients, and once complete cytogenetic response is achieved and confirmed on two subsequent occasions, bone marrow examinations for cytogenetics can be performed every 12 months to detect possible onset of dysplasia or clonal changes in the Ph-negative cells.²⁷

Quantitative PCR for BCR-ABL1 transcripts in the blood should be performed at least every 3 months. Serial BCR-ABL1 measurements are clinically useful to document if patients are responding to treatment with declining transcripts, have stable (plateau) levels of transcripts, or are losing their response as signaled by rising transcripts. Reduction of BCR-ABL1 transcripts by 3 or more logs below a standardized baseline value for untreated patients (major molecular response, MMR) is associated with particularly good outcome.

Achieving Minimal Residual Disease

Administer imatinib at the maximum tolerated dose (up to 800 mg daily). Continue treatment indefinitely, unless loss of response occurs (see the following text).

More than 85% of CML-CP patients treated with imatinib from diagnosis achieve a complete cytogenetic response (0% Ph) and, of these, 80% have a 3 log reduction in BCR-ABL1 transcripts by the 4-year follow-up.⁷ This MRD status is associated with longer survival. Up-front treatment with imatinib may reduce several leukemic progenitors at risk of clonal evolution and disease progression. CP-CML patients who are treated with imatinib from diagnosis and achieve complete cytogenetic response appear to have decreasing annual rates of disease progression to AP or BP with longer follow-up.²⁶ Although the ultimate duration of imatinib treatment is still unclear, current recommendations are to continue treatment until relapse or progression of disease.²⁰ A complete molecular remission (undetectable BCR-ABL1 transcripts in the blood by

PCR) is achieved by less than 10% of patients in complete cytogenetic remission. Imatinib has been discontinued in patients with complete molecular remission for a minimum of 2 years in two clinical trials in France and Australia^{28,29} and found that up to 60% of patients relapse within a few months of cessation, suggesting that imatinib does not totally eradicate CML in most patients. Monitoring of patients who have not yet relapsed is ongoing, but some patients have ceased imatinib for up to 5 years without relapse, reflecting the heterogeneity of either disease biology or immune control.

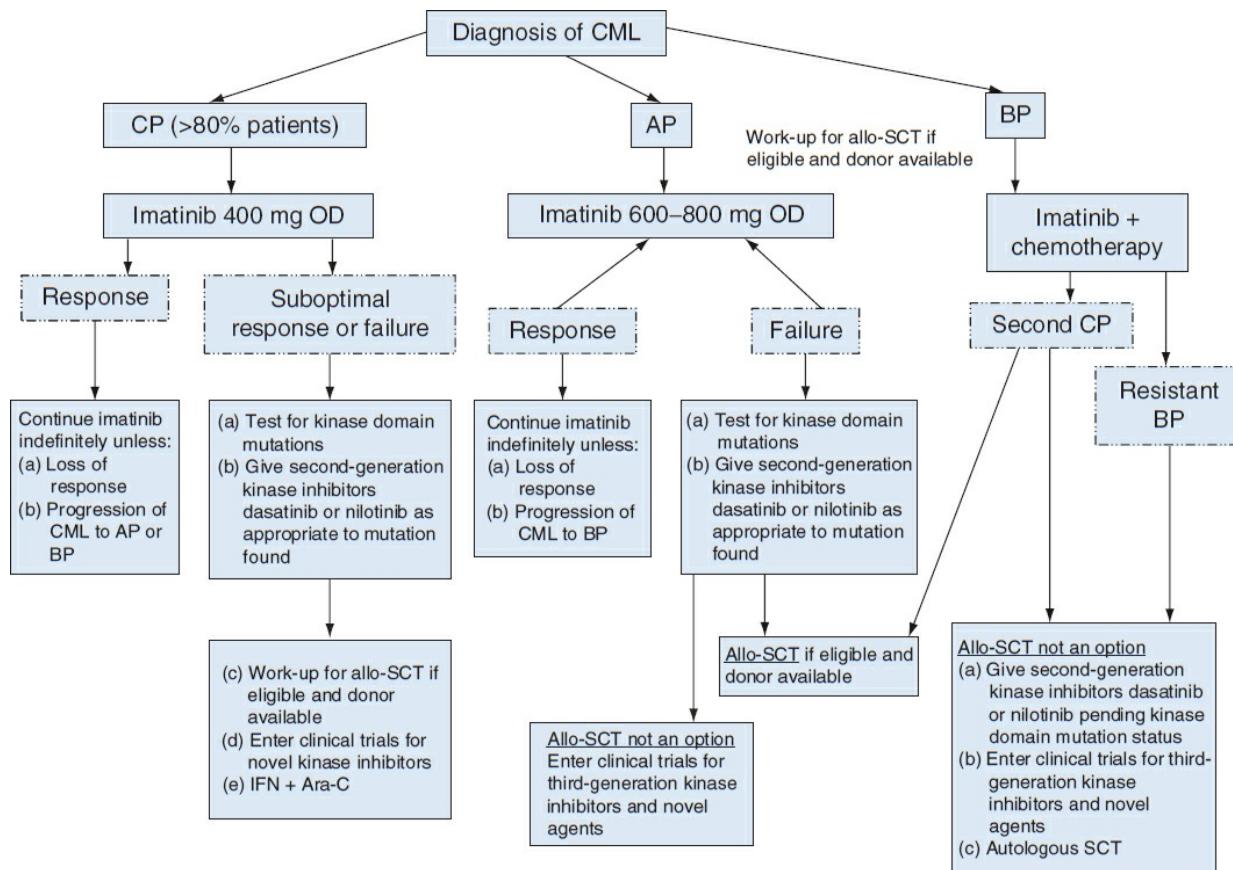


FIGURE 13.5 Treatment algorithm for CML.

AP, accelerated phase; BP, blastic phase; CML, chronic myelogenous leukemia; CP, chronic phase; IFN, interferon; SCT, stem cell transplantation.

Table 13.2 Commonly Used Drugs to Treat Chronic Myelogenous Leukemia

Indication	Drug	Dose
Cytoreduction at presentation	Hydroxyurea (Hydrea)	PO 0.5–2.5g daily
For WBC $100 \times 10^9 / L$	Allopurinol	PO 300 mg daily
Standard first-line treatment for CP	Imatinib	PO 400–800 mg daily
Standard first-line treatment for CP	Dasatinib	PO 100 mg daily
Standard first-line treatment for CP	Nilotinib	PO 300 mg twice daily

CP resistant to first-line or second-line TKI	Bosutinib	PO 500 mg daily
CP resistant to first-line or second-line TKI (T315I mutation)	Ponatinib	PO 30–45 mg daily
Alternative control of WBC	Busulfan	PO 50–100 mg/ 4–8 week
Used in clinical trials with TKI	Interferon α	sc 3–6 \times 10 ⁶ U 2–5 \times weekly

ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CP, chronic phase; PO, oral; sc, subcutaneous; TKI, tyrosine kinase inhibitor; WBC, white blood cells.

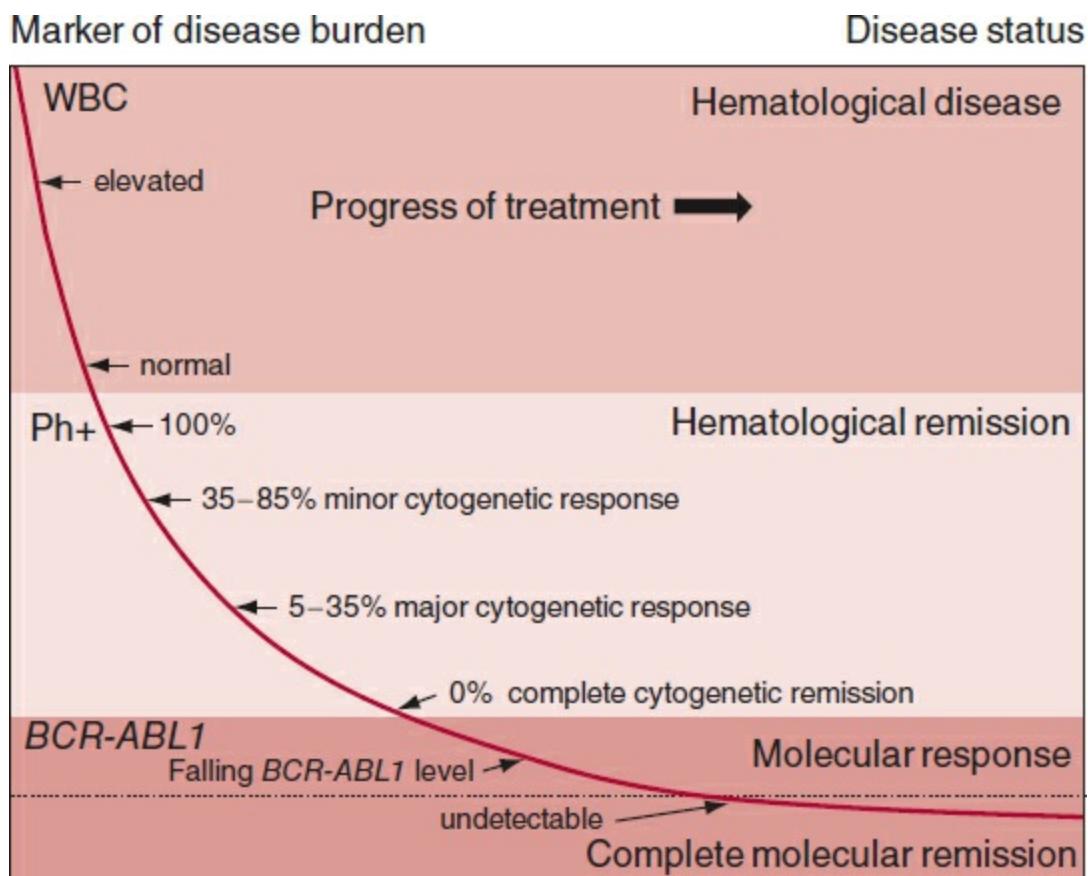


FIGURE 13.6 Disease monitoring in CML.

CML, chronic myelogenous leukemia; WBC, white blood cells..

Optimal or Failure of Response to Tyrosine Kinase Inhibitors

Failure to achieve a hematologic remission with TKI is uncommon. The patient's compliance to treatment should be ascertained.

Failure of TKI treatment²⁰

- ─ No HR in 3 months
- ─ No cytogenetic response (Ph > 95%) in 3 months
- ─ Less than partial cytogenetic response (Ph > 35%) in 6 months
- ─ BCR-ABL1 > 10% in 6 months

- No complete cytogenetic response (any Ph detected) in 12 months
- BCR-ABL1 > 1% in 12 months
- Loss of previously achieved responses—for example, loss of complete HR, complete cytogenetic response or MMR (BCR-ABL1 ≤ 0.1%).
- Development of TKI-resistant mutations

Suboptimal response to TKI treatment or “warning”²⁰

- Clonal cytogenetic abnormalities in Ph+ cells or “major route” cytogenetic abnormalities at baseline
- Less than partial cytogenetic response (Ph > 35%) in 3 months
- BCR-ABL1 > 10% in 3 months
- No complete cytogenetic response (any Ph detected) in 6 months
- BCR-ABL1 1% to 10% in 6 months
- No MMR in 12 months
- Additional chromosomal abnormalities in Ph-negative cells, especially monosomy 7 or 7q-in serial bone marrow examinations.

Patients who lose an initial response to TKI may have developed drug resistance due to point mutations of the BCR-ABL1 gene, which result in amino acid changes in the catalytic domain of the BCR-ABL1 protein (kinase domain mutation), resulting in impaired TKI binding.³⁰ Alternatively, CML may have progressed to AP or BP. A bone marrow aspirate is indicated to determine the status of the disease, and analysis for kinase domain mutations should be undertaken.

When failure of response to first-line TKI is encountered, treatment with an alternative TKI is indicated, guided by kinase domain mutation results.^{20,31}

Patients in CP who are resistant or intolerant to at least one of the second-generation TKIs should be offered an allogeneic SCT from a human leukocyte antigen (HLA)-identical sibling or a well-matched unrelated donor.²⁰

For patients unsuitable for SCT or without a matched donor, cytosine arabinoside (ARA-C) and interferon- α (IFN- α) improve the degree of response in a proportion of cases. Some patients may benefit from enrolling in clinical trials investigating the efficacy of new agents such as ABL001, the allosteric inhibitor of BCR-ABL1, or exploring the use of SCT from the alternative donor sources of cord blood or haploidentical SCT. More experimental approaches with novel TKIs, aurora kinase inhibitors, autologous SCT, and peptide vaccines are also being evaluated.

Allogeneic Stem Cell Transplantation: Treatment With Curative Intent

SCT from an HLA-matched sibling in CP within a year of diagnosis achieves long-term disease control and survival of 70% (approximately 60% for patients with CP who undergo transplantation more than a year from diagnosis). Age has a major impact on

outcome, results being especially favorable for the minority pediatric CML population, whereas patients older than 40 years have lower disease-free survival (DFS). Disease stage is the other major variable affecting transplantation success. Both transplant-related mortality (TRM) and relapse are higher in transplants performed for AP and BP (Fig. 13.7); however, patients who achieve a second CP have a better chance of DFS and results have improved in the imatinib era. Most reported results analyze survival in the first 5 years. Longer-term follow-up indicates that late relapses and deaths from chronic graft-versus-host disease (GVHD) continue to cause late mortality many years after transplant. In evaluating outcome after transplant for CML, measuring DFS underestimates the final cure rate because donor lymphocyte infusions (DLIs) can cure relapsed disease. In summary, the long-term, allogeneic SCT from a matched sibling results in cure in approximately 65% of individuals in CML CP. In the TKI era where SCT is usually a second-line option for TKI resistance, DFS of greater than 50% can be expected using reduced intensity SCT and applicable to patients up to the age of 75 years.

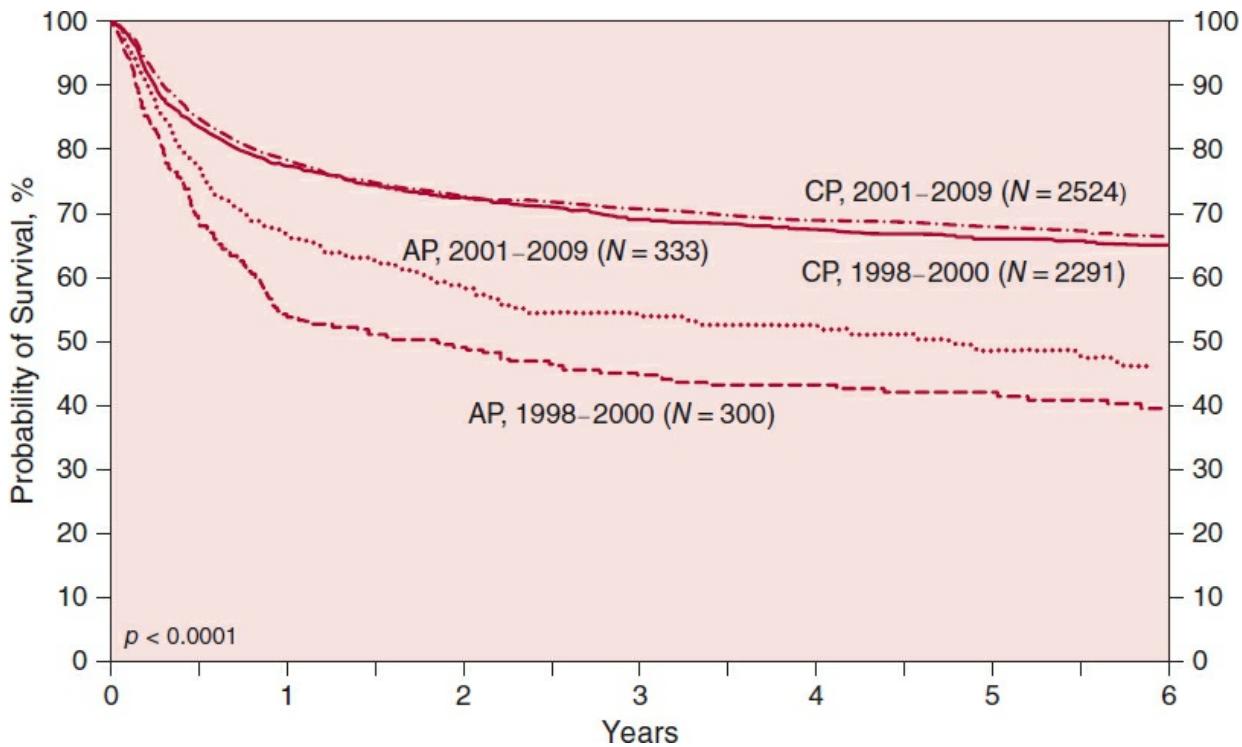


FIGURE 13.7 Probability of survival after HLA-identical sibling donor transplants for CML from 1998 to 2009 by disease status and year.

AP, accelerated phase; CML, chronic myelogenous leukemia; CP, chronic phase; HLA, human leukocyte antigen.

Unrelated Donor Transplants

There is now a large experience in transplants for CML using unrelated volunteer

donors. Age, timing of transplant (early or late CP, more advanced disease), and degree of matching each strongly affects the success of the transplant. For low-risk patients (defined as younger than 40 years, in first CP, less than 1 year from diagnosis and with an HLA-matched unrelated donor) DFS of approximately 60% can be achieved; poorer results can be anticipated from patients with less favorable presentations. However, reduced intensity transplants have improved the outlook for older patients. Thus it is appropriate to offer lower-intensity SCT to patients with CML up to the age of 70 years who have no significant comorbidities.

Selecting Patients for Allogeneic Transplantation

Gratwohl et al.³² described a simple scoring system to predict the chance of a successful transplant outcome (Table 13.3). SCT is no longer recommended as primary therapy for CP-CML for most of the patients.³³ However, as SCT has a definite curative potential with long-term survival documented over many decades, it is still appropriate to recommend it for young patients younger than 30 years who are expected to have a particularly low morbidity and mortality, and also in circumstances in which there is difficulty obtaining TKIs due to economic reasons. For other patients, TKI is the first-line treatment, followed by other TKIs as second-line or third-line, and SCT is reserved for patients who fail to respond, progress, or present with CML beyond CP.

Table 13.3 Gratwohl Score for Predicting Outcome After Bone Marrow Transplantation

Score	0	1	2	3	4	5	6
Survival at 5 y (%)	72	70	62	48	40	18	22
TRM (%)	20	23	31	46	51	71	73
Score	0			1		2	
Donor type		HLA-identical sibling		Matched unrelated		—	
Disease stage		First CP		AP		BP, CP2+	
Recipient age		<20 y		20–40 y		>40 y	
Donor/ recipient gender		M/ M, F/ F, M/ F		F/ M		—	
Diagnosis-to-transplantation		<12 mo		12 mo		—	

AP, acute phase; BP, blastic phase; CP, chronic phase; F, female; HLA, human leukocyte antigen; M, male; TRM, transplant-related mortality.

Treatment of Accelerated Phase

Allogeneic SCT is the most substantiated curative therapy for CML AP and should be offered to patients with a fully or partially matched HLA-identical donor. However, due to the heterogeneity of AP, many patients have good responses to imatinib and second-generation TKIs are effective in imatinib-resistant disease. Identification of high-risk or

low-risk AP may guide the decision for continuing TKI, particularly if there is a good response in low-risk disease, or opting for SCT.^{33,34} Alternatively interferon in combination with ARA-C can achieve disease control. Experimental treatment approaches include SCT from a mismatched-related donor and high-dose chemotherapy or radiation followed by autologous SCT.

Treatment of Blastic Phase

In the TKI era, progression to CML BP has fallen to 1% to 1.5% per year compared to more than 20% per year in the pre-TKI era.³⁵ The risk of progression appears to decrease the longer the duration of optimal response to TKI treatment. The first step in managing CML BP is to determine whether the leukemia has developed into lymphoid or myeloid BP. The average survival on progression to BP is between 6 and 10 months, slightly longer for lymphoid BP.³⁶ Imatinib-refractory patients should be given second-generation TKIs such as dasatinib or nilotinib based on their mutation profile. The hematological response rate to dasatinib in BP is 33% to 61% (lymphoid BP 36% to 80%);³⁵ however, up to one-third of patients develop pleural effusions and may require dose reduction, diuretics or corticosteroids. Dasatinib crosses the blood–brain barrier, and is effective for central nervous system (CNS) disease. Daily dose of dasatinib 140 mg daily is better tolerated than divided doses of 70 mg twice daily. Patients with rapidly progressing leukemia require combination with induction chemotherapy with standard regimens— ALL-based for lymphoid BP, and AML-based (anthracycline and ARA-C) induction therapy for patients with myeloid BP. Lymphoid BP patients also require prophylactic CNS treatment to prevent meningeal leukemia. Many patients in BP in the TKI era achieve an HR and second CP but most relapse rapidly, with TKI resistance, which may be driven by kinase domain mutations. BCR-ABL1 kinase domain mutations such as T315I are highly resistant to imatinib, dasatinib, and nilotinib, and when they develop in CP patients, they may herald the onset of advanced phase CML. Ponatinib, a third-generation TKI, is efficacious against T315I mutations, and results in 31% HR in such CML BP patients.³⁷

Allogeneic SCT, although considered salvage therapy and associated with significant TRM in patients who have progressed to BP, offers the only chance of cure and should be offered to eligible patients. A significant number of patients have prolonged cytopenia after successful eradication of blasts. Patients who have few clinical options may benefit from entering clinical trials of investigational agents.

SPECIAL ISSUES IN CHRONIC MYELOGENOUS LEUKEMIA MANAGEMENT

Treatment of Relapse After Transplantation

Durable molecular remissions after DLI are achieved between 3 and 12 months after DLI in as many as 80% of patients relapsing in CP and in more than 90% of molecular relapses. Predictably, the occurrence of GVHD results in a much higher probability of leukemic response, and the antileukemic effect of DLI is greatest in the absence of immunosuppression. Although DLI is often effective, it may cause bone marrow failure and lethal GVHD. Bone marrow failure is a greater risk in patients with no detectable residual donor marrow cells at relapse. Marrow aplasia in these patients can be prevented or treated by infusing more donor stem cells. Despite concerns that DLI in the setting of unrelated donor transplants would result in excessive toxicity, response and durable remission rates are similar to those seen after matched-sibling DLI. GVHD remains a hazard but does not appear to be more frequent or more severe than that encountered after matched-sibling DLI. Imatinib has been combined successfully with DLI in treatment of relapse.³⁸

Leukostasis

Leukostasis is an uncommon problem in CML and only occurs in a minority of patients with high leukocyte counts ($>300 \times 10^9/\text{L}$). In patients with priapism or neurologic deficit, emergency leukapheresis can be effective but may require several large-volume apheresis sessions to lower the leukocyte count significantly. At presentation, such patients should receive high-dose hydroxyurea (up to 4g daily) with allopurinol 300 mg daily, adequate hydration, and monitoring of blood chemistry. Imatinib may be started once control of the leukocyte count has been achieved.

Splenic Infarcts

Splenic infarcts usually occur when the disease is uncontrolled. Treatment is symptomatic, while attempts to lower the blood count are made. Splenectomy is not generally indicated.

Myelofibrosis

Myelofibrosis causing significant cytopenias can be treated by splenectomy but this maneuver is frequently followed by increasing symptomatic hepatomegaly. Myelofibrosis is not a contraindication for allogeneic SCT and diminishes after successful SCT.

Chloromas

Chloromas often respond poorly to chemotherapy and are best treated with local radiotherapy.

Chronic Myelogenous Leukemia in Pregnancy

Patients are living near-normal lives when they have optimal responses to TKI. Fertile male patients on TKIs have fathered healthy children; however, TKIs are contraindicated in pregnancy due to reports of fetal malformations. Female patients who intend to have children are advised to have optimal control of CML before conceiving. Ideally, the patient should be in MMR or deep molecular response (MR⁴ or BCR-ABL1 < 0.01%) and TKI ceased 3 months before conception and for the duration of the pregnancy. Leukapheresis is performed if there is significant leukocytosis off treatment. Hydroxyurea and IFN- α have also been used in pregnancy without complications.²⁵

Psychological Response of Patients With Chronic Myelogenous Leukemia

Patients presenting with CML are often asymptomatic and may have difficulty accepting that they have a potentially lethal disease. Perhaps for this reason some explore alternative treatments and attempt psychosomatic techniques to control their leukemia. The complexity of disease evolution and the dilemmas of treatment in CML make it essential to educate patients about their leukemia, in order to provide them an informed basis for making treatment decisions.

EVOLVING STANDARD OF CARE

Choice of Tyrosine Kinase Inhibitors as First-Line Treatment

CML patients diagnosed in CP may receive either imatinib, dasatinib, or nilotinib as first-line therapy. Up-front treatment with a second-generation TKI such as dasatinib or nilotinib results in more rapid achievement of therapeutic end points such as MMR compared with imatinib.^{8,39} Nilotinib has been shown to reduce the incidence of progression to CML BP;³⁹ however, a survival benefit has not been demonstrated. Second-generation TKIs are more costly than imatinib, particularly as generic imatinib is now available in many countries, including the United States, from 2016. The choice of up-front TKI in clinical practice depends on the patient's comorbidities as long-term side effects differ with specific TKIs.⁴⁰ For example, patients with significant atherosclerotic disease or diabetes should avoid nilotinib, as these are exacerbated with this particular TKI. Similarly patients with significant lung disease should avoid dasatinib because of its increased risk of troublesome pleural effusions in up to 20% of patients, which continue to occur several years after initiation of treatment.

Combination therapy of imatinib with IFN- α has been shown to achieve better MRs compared to imatinib alone in some recent clinical trials^{41–43} but not in others.^{44,45} Survival benefit has not been demonstrated in patients receiving combination therapy, who generally had more side effects than those on imatinib alone.

Trigger to Change Tyrosine Kinase Inhibitors

There still is controversy regarding earlier switching of TKI when BCR-ABL1 transcript levels are greater than 10% at 3 months. The rate of decline of BCR-ABL1 levels compared to baseline may be more prognostic.⁴⁶ For most of the patients, intolerance of specific adverse effects, loss of initial response, or progression are the reasons for changing TKIs.

Treatment-Free Remission

The success of imatinib cessation without recurrence of CML in about 40% of patients with complete molecular remission for a minimum of 2 years in two clinical trials in France and Australia^{28,29} has resulted in subsequent ongoing clinical trials testing cessation after a variable period in stable deep molecular response (MR⁴ or BCR-ABL1 <0.01%). There is emerging data that the deeper the response rate and the longer the patient has been on TKI, the greater the success rate of a treatment-free remission.^{47,48} Patients who have recurrence of CML after ceasing TKI have generally responded well to reintroduction of TKI with subsequent achievement of their optimal responses.⁴⁸ Although there are guidelines on which CML patient may be suitable for a trial of cessation of TKI, discontinuation is still currently recommended within clinical trials.

References

1. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science*. 1960;132:1497.
2. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973;243(5405):290–293.
3. Groffen J, Stephenson JR, Heisterkamp N, et al. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell*. 1984;36(1):93–99.
4. Heisterkamp N, Stephenson JR, Groffen J, et al. Localization of the c-abl oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature*. 1983;306(5940):239–242.
5. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/ abl gene of the Philadelphia chromosome. *Science*. 1990;247(4944):824–830.
6. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood*. 2008;112(12):4371–4383.
7. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355(23):2408–2417.

8. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2010;362(24):2260–2270.
9. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2010;362(24):2251–2259.
10. Raskind WH, Ferraris AM, Najfeld V, et al. Further evidence for the existence of a clonal Ph-negative stage in some cases of Ph-positive chronic myelocytic leukemia. *Leukemia.* 1993;7(8):1163–1167.
11. Biernaux C, Loos M, Sels A, et al. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. *Blood.* 1995;86(8):3118–3122.
12. de la Fuente J, Baruchel A, Biondi A, et al. Managing children with chronic myeloid leukaemia (CML): recommendations for the management of CML in children and young people up to the age of 18 years. *Br J Haematol.* 2014;167(1):33–47.
13. Takahashi N, Miura I, Saitoh K, et al. Lineage involvement of stem cells bearing the Philadelphia chromosome in chronic myeloid leukemia in the chronic phase as shown by a combination of fluorescence-activated cell sorting and fluorescence in situ hybridization. *Blood.* 1998;92(12):4758–4763.
14. Jiang X, Lopez A, Holyoake T, et al. Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. *Proc Natl Acad Sci USA.* 1999;96(22):12804–12809.
15. Quintas-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood.* 2009;113(8):1619–1630.
16. Perrotti D, Jamieson C, Goldman J, et al. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest.* 2010;120(7):2254–2264.
17. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391–2405.
18. Jain P, Kantarjian H, Patel KP, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. *Blood.* 2016;127(10):1269–1275.
19. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood.* 1996;88(7):2375–2384.
20. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood.* 2013;122(6):872–884.
21. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. *J Natl*

- Cancer Inst. 1998;90(11):85085–85088.
- 22. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in “good-risk” chronic granulocytic leukemia. *Blood*. 1984;63(4):789–799.
 - 23. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *New Engl J Med*. 2003;349(15):1423–1432.
 - 24. White D, Saunders V, Lyons AB, et al. In-vitro sensitivity to imatinib-induced inhibition of ABL kinase activity is predictive of molecular response in de-novo CML patients. *Blood*. 2005;106(7):2520–2526.
 - 25. Cortes J, Kantarjian H. How I treat newly diagnosed chronic phase CML. *Blood*. 2012;120(7):1390–1397.
 - 26. Hughes T, Deininger M, Hochhaus A, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood*. 2006;108(1):28–37.
 - 27. Hochhaus A, O’Brien SG, Guilhot F, et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia*. 2009;23(6):1054–1061.
 - 28. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol*. 2010;11(11):1029–1035.
 - 29. Ross DM, Branford S, Seymour JF, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. *Blood*. 2013;122(4):515–522.
 - 30. Weisberg E, Manley PW, Cowan-Jacob SW, et al. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat Rev Cancer*. 2007;7(5):345–356.
 - 31. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients after imatinib failure: does the BCR-ABL mutation status really matter? *Blood*. 2009;114(27):5426–5435.
 - 32. Gratwohl A, Hermans J, Goldman JM, et al. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Lancet*. 1998;352(9134):1087–1092.
 - 33. Innes AJ, Milojkovic D, Apperley JF. Allogeneic transplantation for CML in the TKI era: striking the right balance. *Nat Rev Clin Oncol*. 2016;13(2):79–91.
 - 34. Jiang Q, Xu LP, Liu DH, et al. Imatinib mesylate versus allogeneic hematopoietic stem cell transplantation for patients with chronic myelogenous leukemia in the accelerated phase. *Blood*. 2011;117(11):3032–3040.

35. Hehlmann R. How I treat CML blast crisis. *Blood*. 2012;120(4):737–747.
36. Wadhwa J, Szydlo RM, Apperley JF, et al. Factors affecting duration of survival after onset of blastic transformation of chronic myeloid leukemia. *Blood*. 2002;99(7):2304–2309.
37. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2013;369(19):1783–1796.
38. Savani BN, Montero A, Kurlander R, et al. Imatinib synergizes with donor lymphocyte infusions to achieve rapid molecular remission of CML relapsing after allogeneic stem cell transplantation. *Bone Marrow Transpl*. 2005;36(11):1009–1015.
39. Kantarjian HM, Hochhaus A, Saglio G, et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. *Lancet Oncol*. 2011;12(9):841–851.
40. Larson RA. Is there a best TKI for chronic phase CML? *Blood*. 2015;126(21):2370–2375.
41. Palandri F, Castagnetti F, Iacobucci I, et al. The response to imatinib and interferon-alpha is more rapid than the response to imatinib alone: a retrospective analysis of 495 Philadelphia-positive chronic myeloid leukemia patients in early chronic phase. *Haematologica*. 2010;95(8):1415–1419.
42. Preudhomme C, Guilhot J, Nicolini FE, et al. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med*. 2010;363(26):2511–2521.
43. Simonsson B, Gedde-Dahl T, Markevarn B, et al. Combination of pegylated IFN-alpha2b with imatinib increases molecular response rates in patients with low-or intermediate-risk chronic myeloid leukemia. *Blood*. 2011;118(12):3228–3235.
44. Hehlmann R, Lauseker M, Jung-Munkwitz S, et al. Tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus interferon-alpha in newly diagnosed chronic myeloid leukemia. *J Clin Oncol*. 2011;29(12):1634–1642.
45. Marin D, Ibrahim AR, Lucas C, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. *J Clin Oncol*. 2012;30(3):232–238.
46. Branford S, Yeung DT, Parker WT, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. *Blood*. 2014;124(4):511–518.
47. Hughes TP, Ross DM. Moving treatment-free remission into mainstream clinical practice in CML. *Blood*. 2016;128(1):17–23.
48. Saussele S, Richter J, Hochhaus A, et al. The concept of treatment-free remission in chronic myeloid leukemia. *Leukemia*. 2016;30(8):1638–1647.

Chronic Lymphocytic Leukemia

Inhye E. Ahn, Clare Sun, and Adrian
Wiestner

BACKGROUND

Chronic lymphocytic leukemia (CLL) is a disorder of morphologically mature but immunologically incompetent B lymphocytes and is manifested by progressive accumulation of these cells in the blood, bone marrow, and lymphatic tissues.¹ CLL accounts for about 25% of all leukemia and is the most common leukemia in Western countries. Incidence increases with age from <1/ 100,000 individuals younger than 40 years to >20/ 100,000 in persons older than 65 years and it is almost twice as frequent in men as in women. CLL is predominantly found in Caucasians and is less common in African Americans, Hispanics, and Asians. In 2016, it is estimated that there will be 18,960 new cases of CLL and 4,660 disease-related deaths in the United States.²

ETIOLOGY AND PATHOGENESIS

CLL has historically been viewed as a disease of clonal B cells that have a low proliferation rate and a defect in apoptosis. CLL cells express the B-cell receptor (BCR) and antigenic stimulation of the BCR appears to be a major pathogenic driver.³ Recent work highlights the importance of proliferation that occurs primarily in the tissue microenvironment of the bone marrow and lymph nodes. Key pathways promoting CLL cell proliferation and survival in these tissues are activation of the BCR and NF-KB pathways.⁴

A positive family history is associated with two-to eightfold risk for the development of CLL.⁵ The role of environmental factors and viral infections in the pathogenesis of CLL remains ill-defined.

CLINICAL MANIFESTATIONS

CLL is often diagnosed following the incidental detection of lymphocytosis on routine blood tests or asymptomatic lymphadenopathy. Abdominal fullness, fatigue, reduced exercise tolerance, or other constitutional symptoms can also be presenting complaints. Symptoms can precede the onset of anemia or clinically manifest organomegaly. In the advanced stage, patients may have recurrent infections, weight loss, or symptoms related to anemia and thrombocytopenia.

CLL can cause most of the signs or symptoms of non-Hodgkin's lymphoma, specifically those related to B symptoms (night sweats, fevers, and weight loss). The pace of disease is slower than in aggressive lymphomas, and the sudden onset of new symptoms, especially in previously untreated patients, should prompt efforts to exclude other diagnoses. Lymphadenopathy is typically non-tender but lymph node enlargement can cause abdominal discomfort, fullness, and malaise. Even bulky lymphadenopathy rarely leads to obstruction or organ impairment. Splenomegaly is frequent and hepatomegaly due to CLL infiltration of the liver can occur. Extranodal involvement is uncommon and can manifest as skin lesions or pulmonary nodules. Pleural infiltrations leading to effusions, renal or gastrointestinal tract involvement have been reported. Central nervous system involvement is unusual and neurologic symptoms should be investigated to determine other etiologies, especially infections. Although night sweats or low-grade fevers can be symptoms of CLL, it is important to also consider infectious etiologies. In addition, infections in CLL patients may lead to an exaggerated, albeit transient increase in lymphadenopathy or splenomegaly, which needs to be differentiated from transformation into high-grade lymphoma.

DIAGNOSIS, IMAGING, AND LABORATORY STUDIES

Diagnosis of CLL requires $>5 \times 10^9$ clonal B-lymphocytes/ L (5,000/ μ L) with a typical immunophenotype in the peripheral blood (see Immunophenotyping and Flow Cytometry).⁶ The World Health Organization classification of hematopoietic neoplasias describes CLL being distinguishable from small lymphocytic lymphoma (SLL) only by its leukemic appearance.¹ The diagnosis of SLL requires lymphadenopathy and/ or splenomegaly with $<5 \times 10^9$ / L circulating clonal B cells. Although histopathologic evaluation of a lymph node biopsy may be the standard diagnostic test for SLL, circulating clonal B cells of typical immunophenotype is sufficient in cases with an indolent presentation. Monoclonal B-cell lymphocytosis (MBL) is defined by $<5 \times 10^9$ / L clonal B cells in the absence of lymphadenopathy or organomegaly (as defined by physical examination or computerized tomography), cytopenias, or disease-related symptoms. CLL is often preceded by so-called high-count MBL (clonal B cells $>0.5 \times$

$10^9/\text{L}$) that progresses to CLL requiring treatment at a rate of around 1% to 2% per year.⁷ For the purposes of this chapter, the term “CLL” encompasses both CLL and SLL, and excludes MBL.

Complete Blood Count and Blood Smear

CLL cells are small-to medium-sized, mature appearing lymphocytes with round nuclei, clumped chromatin, and scant cytoplasm. Smudge cells, bare nuclei that appear squashed, are a classic feature. Prolymphocytes that are medium-to large-sized cells with prominent nucleoli making up less than 10% of the lymphocytes in typical cases but may increase in proportion in rapidly progressive disease. Cases with more than 55% prolymphocytes are recognized as a distinct diagnostic entity called prolymphocytic leukemia (PLL). In advanced disease, anemia or thrombocytopenia are common, most often due to replacement of the bone marrow by tumor cells, with a possible contribution of hypersplenism or autoimmune mechanisms.

Immunophenotyping and Flow Cytometry

Flow cytometry is the single most informative diagnostic study in CLL. CLL cells are B cells (CD19 positive) that coexpress CD5 and CD23. These cells typically have weak expression of CD20, CD22, surface immunoglobulin, and are negative for FMC7, CD10, and CD103. The low expression of CD20 is part of the characteristic signature of CLL.

Other Laboratory Evaluations

- Direct antiglobulin test (DAT) should be obtained before treatment and in patients with anemia. Conversion of the DAT from negative to positive may herald the onset of autoimmune hemolytic anemia (AIHA).
- Serum immunoglobulins typically decrease with disease duration. A small immunoglobulin M (IgM) M spike is consistent with a diagnosis of CLL. An occasional IgG M spike may suggest a concurrent diagnosis of monoclonal gammopathy of undetermined significance.
- Beta-2-microglobulin (B2M) may be elevated and often increases with disease bulk. High B2M levels ($>3 \text{ mg/L}$) have been associated with inferior treatment response and survival.⁸ Notably, renal insufficiency may also increase the level of B2M.
- In indolent CLL, lactate dehydrogenase (LDH) is typically normal. Elevated LDH is seen with AIHA and may be modestly increased in rapidly progressive disease. High or rapidly rising levels of LDH may be a sign of disease transformation.
- A mild elevation of alkaline phosphatase is common. Elevated transaminases should trigger evaluation for viral hepatitis, especially if treatment with rituximab or a kinase inhibitor is considered.

Bone Marrow Biopsy

The bone marrow is always involved in CLL and in most SLLs. Distinct patterns of infiltration, which have some prognostic value, are recognized: nodular, interstitial, diffuse, or mixed. Advanced disease is often associated with a diffuse pattern of infiltration. Bone marrow biopsy, outside of clinical studies, is not part of routine workup and can be reserved for cases presenting diagnostic difficulties or that have depressed peripheral blood counts.

Lymph Node Biopsy

A lymph node biopsy can distinguish between CLL and other lymphomas or may be necessary to exclude transformation in patients with rapidly enlarging nodes, especially if the growth preferentially affects a single nodal area. The lymph node architecture in CLL is effaced by an abundance of small lymphocytes with clumped chromatin. Mitotic activity is typically low.

Computed Tomography and Positron Emission Tomography

Computed tomography (CT), although not required for routine assessment of patients with CLL, can capture the extent of lymph node involvement in CLL and is the preferred imaging method to evaluate response to treatment. CLL lymph nodes typically have only weak metabolic activity on positron emission tomography (PET). Although PET is not helpful in the diagnosis of CLL, it can add valuable information in advanced stage or relapsed disease when transformation into high-grade lymphoma is a consideration.

DIFFERENTIAL DIAGNOSIS

Morphology, flow cytometry, immunohistochemistry and cytogenetics (see Cytogenetics) are the tests with the best diagnostic yield. Briefly summarized in [Table 14.1](#) are the main distinguishing features of closely related entities.

NATURAL HISTORY, STAGING, AND PROGNOSIS

The natural history of CLL is quite variable, ranging from an indolent disease with age-matched normal survival that never requires treatment to an incurable disease progressing from asymptomatic lymphocytosis to treatment-refractory leukemia within a few years. Two-staging systems have been used to risk-stratify CLL: The Rai classification utilized in North America (described in detail in [Table 14.2](#)),⁹ and the Binet classification commonly used in Europe. Both are based on clinical and laboratory parameters and confer prognostic information.

Biomarkers that are independent of clinical stage, especially immunoglobulin

variable region heavy chain gene (IGHV) mutation status and CD49d, are valuable in assessing early-stage patients as they reflect the pace of disease progression and the time it will take to develop active disease requiring treatment. Disease stage and several additional biomarkers refine the prognostic information and correlate with overall survival (OS).

Table 14.1 Immunophenotype of B-Cell Chronic Lymphocytic Leukemia and Lymphomas That Resemble It

Entity	slg	CD5	CD10	CD19	CD20	CD23	Karyotype/ FISH
B-CLL	Weak	++	-	++	+	++	13q-, 11q-, 17p-trisomy 12
FL	++	-	+	++	++	+/-	t(14;18), BCL-2 ^a
MCL	++	++	-	++	++	-	t(11;14), Cyclin D1 ^b
MZL	+	-	-	++	++	-	No consistent abnormality
PLL	++	+/-	-	++	++	-	Occasional t(11;18)

^aBCL-2 on chromosome 18; the t(14;18) translocation is also infrequently seen in CLL.

^bCyclin D1 on chromosome 11 is not expressed in normal B cells and can be detected by immunohistochemistry.

B-CLL, B-cell chronic lymphocytic leukemia; CD, cluster designation; FISH, fluorescence in situ hybridization; FL, follicular lymphoma, MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; PLL, prolymphocytic leukemia; slg, surface immunoglobulin.

Table 14.2 Rai Clinical Staging System

Stage	Modified Rai Staging		Median Survival (y) ^a
	Risk Group	Clinical Features	
0	Low	Blood and marrow lymphocytosis	11.5
I	Intermediate	Lymphocytosis and adenopathy	11
II	Intermediate	Lymphocytosis and splenomegaly or hepatomegaly, with or without adenopathy	7.8
III	High	Anemia (Hb < 11 g/ dL)	5.3
IV	High	thrombocytopenia (<100,000/ μ L)	7

^aBased on 2007 report of 1,674 patients from MD Anderson Cancer Center.

Adapted from Wierda WG, O'Brien S, Wang X, et al. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood. 2007;109(11):4679–4685.

Immunoglobulin Variable Region Heavy Chain Gene Mutation

The immunoglobulin expressed by B cells is composed of light and heavy chains encoded by distinct genes. The presence or absence of somatic mutations in the variable region of the heavy chain gene distinguishes two disease subsets: patients whose CLL cells express unmutated IGHV genes (U-IGHV) have a more rapid progression of

disease than patients whose CLL cells express mutated genes (M-IGHV). M-IGHV CLL can have a median survival of 20 or more years and may never require treatment. In contrast, U-IGHV CLL typically requires treatment within a few years from diagnosis and median survival of 8 to 10 years has been reported.¹⁰

Cytogenetics

In 1990, Juliusson et al. used metaphase karyotype to risk-stratify CLL patients into four distinct subgroups.¹¹ This study pioneered the concept that genetic features have prognostic value and complex karyotype is linked to shorter survival. However, metaphase chromosomal studies using G banding has limited sensitivity due to the low mitotic rate of CLL. To evaluate cytogenetic abnormalities in CLL fluorescence in situ hybridization (FISH) has become the standard as it does not require cell division and hundreds of cells can be scored. The drawback is that the results are limited to the specific FISH probes used in the assay. Using probes for chromosomal regions 13q, 11q, 17p, and 12 abnormalities are identified in approximately 80% of cases. In a pivotal study of mostly treatment-naïve patients, deletion 13q (del 13q) was present in 55%, deletion 11q (del 11q) in 18%, trisomy 12 in 16%, and deletion 17p (del 17p) in 7%. No abnormality was detected in 18%. Twenty-nine percent of patients had two or more aberrations.¹² These lesions are not diagnostic of CLL but confer prognostic information.

Del 17p (p53 locus) or 11q (ATM locus) are associated with inferior outcome. Median survival was estimated at 32 months for del 17p and 79 months for del 11q.¹² However, treatment-naïve patients in whom tumor cells with del 17p make up <25% of the clone and who often have M-IGHV CLL have been reported to have a rate of progression that is not significantly different from patients without this abnormality.¹³ Patients with isolated del 13q had the longest OS of 133 months.^{12,14} Because many patients have more than one abnormality, a hierarchical model of genetic subgroups was established, which assigns patients to the genetic subgroup of the prognostic dominant abnormality. For example, a combination of del 13q and del 17p is assigned to the prognostic group of del 17p. Del 17p is relatively infrequent in newly diagnosed patients but is more common in relapsed or refractory disease, and correlates with poor treatment response to chemotherapy¹⁴ and less durable response to targeted agents.¹⁵

Somatic Mutations

The genomic landscape of CLL is complex. More than 20 driver mutations were identified from whole-exome sequencing of 149 CLL patients,¹⁶ and recurrent mutations can also be found in noncoding genes.¹⁷ The TP53, SF3B1, ATM, or NOTCH1 mutations are associated with shorter time to first treatment irrespective of IGHV

mutation status.¹⁸

ZAP-70

The tyrosine kinase ZAP-70 is essential for T-cell receptor signaling in response to antigen. Relatively higher expression of ZAP-70 is typically seen in U-IGHV CLL and infrequent in M-IGHV CLL. ZAP-70 expression can be assessed by flow cytometry. Time from diagnosis to initial treatment is approximately 3 years in ZAP-70 positive CLL, and 9 years for ZAP-70-negative disease.¹⁹ Median OS of the two subtypes is approximately 9 and 25 years, respectively. ZAP-70 is a cytoplasmic protein that is expressed at lower levels in CLL B cells than in T cells, making the test technically challenging. Results close to the threshold should therefore be interpreted with caution.

CD38

Increased CD38 expression on the cell surface of CLL cells as measured by flow cytometry correlates with inferior outcome. CD38 does not add to the prognostic information derived from IGHV mutation status and/ or ZAP-70 or CD49d expression.²⁰

CD49d

CD49d is an integrin subunit that regulates trafficking of B and T cells. The expression of CD49d by flow cytometric analysis on CLL cells has been associated with advanced stage, earlier progression, and shorter OS.²⁰ It appears to be superior to other flow cytometry-based markers such as ZAP-70 and CD38 in prognosticating outcome.²⁰ Importantly, CD49d has been shown to be an independent prognostic marker for OS in addition to age, gender, absolute lymphocyte count, IGHV mutation status, and del 17p in a cohort of 1,117 patients.²⁰

Lymphocyte Doubling Time

A lymphocyte doubling time (LDT) of <12 months indicates progressive disease and is associated with decreased survival independent of stage. LDT <6 months is a feature of active disease and can be an indication to consider treatment.¹

TREATMENT

The Paradigm of Watchful Waiting

Most patients with early-stage CLL are asymptomatic and have a relatively good long-term prognosis. Several randomized trials investigating immediate versus deferred chlorambucil-based treatment in early-stage patients showed a slightly inferior survival with immediate chemotherapy.²¹ Deferral of treatment or “watchful waiting” has therefore become the standard of care for patients with early-stage CLL. Periodic

evaluation including basic laboratory testing at 3- to 6-month intervals is a reasonable strategy for asymptomatic patients with relatively stable disease. Treatment should be reserved for symptomatic or rapidly progressive disease.

Active disease is defined by one of the following criteria;¹

- | Constitutional symptoms due to CLL (fevers, night sweats, weight loss)
- | Symptomatic/ massive (>10 cm) lymphadenopathy
- | Symptomatic/ massive splenomegaly (>6 cm below costal margin)
- | Progressive marrow failure: worsening anemia and/ or thrombocytopenia
- | Rapidly progressive lymphocytosis (LDT < 6 months)
- | Autoimmune cytopenias (immune thrombocytopenia [ITP], AIHA, pure red cell aplasia [PRCA]) poorly responsive to corticosteroid treatment

Assessing Response to Therapy

The International Workshop on Chronic Lymphocytic Leukemia published standardized criteria for assessing response to therapy in 2008.¹ Response is determined by history and physical examination as well as evaluation of peripheral blood and bone marrow for disease. With the introduction of targeted agents, clinical endpoints have been revised to account for treatment-related lymphocytosis in the absence of progressive disease.²² In addition, the elimination of minimal residual disease, as detected by multicolor flow cytometry or polymerase chain reaction-based methods, improves survival and may represent an important therapeutic goal.^{23,24}

Chemoimmunotherapy

Chemoimmunotherapy (CIT) is an acceptable first-line treatment for physically fit patients without del 17p CLL. In the randomized CLL8 trial, the addition of rituximab to fludarabine and cyclophosphamide (FCR) resulted in better progression-free survival (PFS) and overall response rates (ORRs) than fludarabine and cyclophosphamide.²⁵ Notably, durable responses were observed in two-thirds of patients with M-IGHV at 5 years following FCR therapy.²⁶ Long-term follow-up of 300 patients treated with FCR at the MD Anderson Cancer Center revealed sustained minimal residual disease negativity at a median of 12.8 years in 15 patients with M-IGHV, suggesting that FCR may cure some patients within this subgroup.²⁷

Grade 3 to 4 hematologic toxicity occurs in more than 50% of patients treated with FCR. Cyclophosphamide-containing regimens are also associated with an increased risk of chemotherapy-related myeloid neoplasms.²⁸ Patients with del 17p CLL relapse quickly after FCR and should be treated with targeted therapies.^{25,29}

Bendamustine and rituximab (BR) may be the preferred CIT regimen for patients older than 65 years. The CLL10 trial randomized 564 treatment-naïve patients to

treatment with FCR or BR.³⁰ Although PFS was shorter in the BR group, there were also fewer cases of severe neutropenia, infections, and second cancers. Patients older than 65 years were more susceptible to myelosuppression and infections, particularly in the FCR arm, and PFS between the two treatment regimens was comparable.

The humanized anti-CD20 monoclonal antibody, ofatumumab, in combination with chlorambucil has been approved for the up-front treatment of patients considered inappropriate for fludarabine-based therapy.³¹ Compared to chlorambucil alone, the addition of ofatumumab improved median PFS from 13.1 to 22.4 months. At the time of reporting, no difference was observed in OS.

Obinutuzumab is a glycoengineered, humanized anti-CD20 antibody. Referred to as a type 2 antibody, it differs from rituximab and ofatumumab (both type 1 antibodies) in regards to the target epitope and effector mechanisms of cell killing. Obinutuzumab in combination with chlorambucil has been approved for the up-front treatment of patients with significant comorbidities. In a phase 3 study comparing chlorambucil alone, chlorambucil with rituximab or chlorambucil with obinutuzumab, PFS was prolonged in patients treated with chlorambucil–obinutuzumab relative to those who received chlorambucil–rituximab or chlorambucil alone (Table 14.3).³² OS was inferior in the chlorambucil only arm; however, there was no OS difference between the two antibody-based regimens due to a small number of deaths in each arm.³³

Table 14.3 Chemoimmunotherapy Options in CLL

Reference	Regimen	Phase	N	Median FU (mo)	ORR (%)	CR (%)	PFS	OS
CLL8 ^{25,26}	FCR	III	409	71	90	44	56.8 mo	NR
	FC		408		80	22	32.9 mo	86.0 mo
MDACC ^{8,34}	FCR	II	300	72	95	72	6 y 51%	6 y 77%
CLL10 ³⁰	BR	III	280	37	96	31	41.7 mo	3 y 92%
	FCR		284		95	40	55.2 mo	91%
CLL11 ^{32,33}	G-Clb	III	333	NA	77.3	22.3	26.7 mo	Death rate ^a 9%
	R-Clb		330		65.7	7.3	16.3 mo	15%
	Clb		118		31.4	0	11.1 mo	20%
COMPLEMENT ¹³¹	O-Clb	III	221	28.9	82	14	22.4 mo	2 y 89%
	Clb		226		69	1	13.1 mo	87%

^aThe rate of death in May 2013. Patients were enrolled between April 2010 and July 2012.

BR, bendamustine + rituximab; Clb, chlorambucil; CLL, chronic lymphocytic leukemia; CR, complete response rate; FC, fludarabine + cyclophosphamide + rituximab; FCR, fludarabine + cyclophosphamide + rituximab; FU, follow-up; G-Clb, obinutuzumab + chlorambucil; N, number of patients enrolled; NR, not

reached; O-Clb, ofatumumab + chlorambucil; ORR, overall response rate; OS, median overall survival or projected OS with year of projection; PFS, median progression-free survival or projected PFS with year of projection; R-Clb, rituximab + chlorambucil.

Although patients who experience a long remission (>3 years) after CIT may be considered for retreatment with a similar regimen,³⁴ the approval of targeted agents increases treatment options. For fludarabine refractory disease, defined by a lack of response or relapse within 24 months of a fludarabine containing regimen (or more generally a CIT regimen), targeted agents or investigational therapy on clinical trials are indicated.

Cellular Therapies

Owing to the introduction of highly effective, well-tolerated targeted therapies in recent years, the role of allogeneic stem cell transplantation (allo-HSCT) in CLL is diminishing. Allo-HSCT is potentially curative because of a potent graft-versus-leukemia effect with long-term disease-free survival of 35% to 40%.³⁵ However, even with reduced intensity conditioning, the rate of non-relapse mortality is 15% to 30% during the first 2 years after allo-HSCT.³⁵ The current European Research Initiative on CLL (ERIC) guidelines recommend treatment with targeted agents before consideration of allo-HSCT on an individual basis.

Chimeric antigen receptor-modified T cells targeting CD19 is an experimental treatment that can lead to prolonged remissions in multiple relapsed patients.³⁶ A severe cytokine release syndrome is quite common and significant neurologic toxicity has been reported.

Small Molecules

Orally bioavailable small molecules have changed the treatment paradigm of CLL. Since 2014, the U.S. Food and Drug Administration (FDA) approved three orally administered small molecule drugs— ibrutinib, idelalisib, and venetoclax— for the treatment of CLL (Table 14.4). All three agents are given as continuous therapy as opposed to the conventional time-limited approach of combination CIT.

Ibrutinib, given as 420 mg PO once daily, is the first Bruton tyrosine kinase (BTK) inhibitor to gain FDA approval for the treatment of CLL. In randomized phase III trials, ibrutinib improved survival of CLL patients compared to chlorambucil as a first-line therapy and compared to ofatumumab in relapsed/ refractory disease.^{40,38} Ibrutinib is remarkably effective in high-risk CLL. In relapsed/ refractory patients with del 17p, the ORR and 2-year PFS were 83% and 63%, respectively, while in a study population mixed with previously treated and untreated patients ORR and 2-year PFS were 94% and 82% respectively.^{29,45} Notable side effects of ibrutinib include bruising, rash,

diarrhea, arthralgia, myalgia and atrial fibrillation, which may, in part, be due to inhibition of kinases other than BTK, such as Tec, interleukin-2-inducible T-cell kinase (ITK), and epidermal growth factor receptor (EGFR).³ Several next-generation BTK inhibitors more specific for BTK are under clinical investigation. One such drug, acalabrutinib, demonstrated high efficacy and acceptable tolerability in a phase I/ II study.⁴¹ A phase III trial comparing ibrutinib and acalabrutinib in relapsed/ refractory CLL is ongoing (NCT02477696).

Idelalisib selectively inhibits PI3Ki, which is highly expressed in lymphoid cells. Combination of idelalisib with rituximab was superior to rituximab monotherapy in relapsed/ refractory CLL;⁴³ the combination is approved for CLL patients for whom rituximab alone would be considered appropriate due to comorbidities. Idelalisib carries a black box warning for fatal and serious toxicities: hepatic, severe diarrhea, colitis, pneumonitis, infections, and intestinal perforation. Several of these toxicities appear to be due to unmasking of autoimmunity.³

Venetoclax, a BCL-2 inhibitor, is approved for treatment of previously treated CLL with del 17p.^{44,46} Venetoclax can cause serious tumor lysis syndrome (TLS). The risk of TLS is minimized with ramp-up dosing, close monitoring, aggressive hydration, and administration of a hypouricemic agent.

Unique Aspects of Targeted Agents in Chronic Lymphocytic Leukemia

Small molecules are associated with characteristic patterns of treatment response. Both ibrutinib and idelalisib lead to a transient lymphocytosis due to the egress of CLL cells from tissues.⁴⁷ This lymphocytosis must not be mistaken as disease progression and does not predict inferior long-term disease control.¹⁵ Most patients on ibrutinib or idelalisib achieve a partial response; with time the rate of complete response increases.¹⁵

Table 14.4 Select Treatment Trials Using Small Molecules in Chronic Lymphocytic Leukemia

Reference	Regimen	Phase	N	Median FU (mo)	ORR(%)	CR(%)	PFS	OS
Previously untreated								
15,37	Ibrutinib	II/ III	29 ^a	35	84	23	96.3% at 30 mo	97% at 30 mo
RESONATE-2 ³⁸	Ibrutinib	III	269 ^a	18	86	4	90% at 18 mo	98% at 24 mo
	Chlorambucil				35	2	52% at 18 mo	85% at 24 mo
39	Idelalisib + R	II	64 ^a	22	97	19	83% at 36 mo	90% at 36 mo

Relapsed/ refractory CLL									
RESONATE ⁴⁰	Ibrutinib	III	391	9	63	0	88% at 6 mo	90% at 12 mo	
	Ofatumumab			4	0		65% at 6 mo	81% at 12 mo	
41	Acalabrutinib	I/ II	62	14	95	0	—	—	
HELIOS ⁴²	Ibrutinib + BR	III	578	17	83	10	79% at 18 mo	Median NR	
	Placebo + BR			68	3		24% at 18 mo	Median NR ^b	
43	Idelalisib + R	III	220 ^c	4 ^d	81	0	93% at 24 weeks	92% at 12 mo	
	Placebo + R			13	0		46% at 24 weeks	80% at 12 mo	
44	Venetoclax	I	116	17	79	20	66% at 15 mo	84% at 24 mo	
Deletion 17p									
29	Ibrutinib	II	48 ^e	24	94	10	82% at 24 Mo	80% at 24 mo	
RESONATE-17 ⁴⁵	Ibrutinib	II	144 ^f	28	83	8	63% at 24 Mo	75% at 24 mo	
46	Venetoclax	II	107	12	79	8	72% at 12 Mo	87% at 12 mo	
Ongoing phase III trials									
NCT02863718	Ibrutinib vs. placebo	III	540				Target population: asymptomatic and treatment-naïve CLL with intermediate to high-risk of disease progression.		
NCT02048813	Ibrutinib+R vs. FCR	III	519				Target population: treatment-naïve CLL meeting iwCLL treatment criteria.		
NCT02264574	Ibrutinib+Obi vs. chlorambucil+Obi	III	212				Target population: treatment-naïve CLL meeting iwCLL treatment criteria who are age 65 or older or have deletion 17p or have comorbidities.		
NCT01886872	Ibrutinib vs. Ibrutinib+R vs. BR	III	523				Target population: treatment-naïve CLL meeting iwCLL treatment criteria who are age 65 or older		
NCT02477696	Ibrutinib vs. acalabrutinib	III	500				Target population: relapsed/ refractory CLL with either deletion 17p or deletion 11q.		
NCT02756611	Venetoclax	IIIb	250				Target population: relapsed/ refractory CLL including those with deletion 17p or those who had received prior BCR inhibitor therapy		

^aEligible patients were 65 years of age or older and had previously untreated CLL.

^b31% of patients in the placebo arm crossed over to receive ibrutinib after disease progression.

^cEligible patients had relapsed/ refractory CLL within 24 months after their last treatment and were not able to receive cytotoxic agents due to coexisting medical conditions.

^dThe study was terminated early by the data and safety monitoring board owing to significant survival benefit in the idelalisib arm.

^eOf 48 evaluable patients, 33 were previously untreated and 15 had prior treatments.

^fAll 145 patients were previously treated. The median number of prior treatments was 2 (range 1–3).

BCR, B-cell receptor; BR, bendamustine, rituximab; CLL, chronic lymphocytic leukemia; CR, complete response rate; FCR, fludarabine and cyclophosphamide; FU, follow-up; mo, months; N, number of patients enrolled; NR, not reached; Obi, obinutuzumab; Ofa, ofatumumab; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R, rituximab; RR, relapsed/ refractory CLL; TN, treatment-naïve CLL; —, not reported.

Resistance to targeted agents has been reported. Resistance to ibrutinib is most often due to point mutations in BTK that remove the Cys481 residue required for covalent binding of ibrutinib, and/ or in PLCG2, which activate the BCR pathway downstream of BTK.^{48,49} Mechanisms of resistance to idelalisib are not well understood but signaling through PI3K other than the PI3K δ subunit may play a role.³ Resistance to venetoclax may involve upregulation of antiapoptotic proteins other than BCL-2.⁵⁰

Combination of Small Molecules and Conventional Agents

Combination regimens using targeted agents as a backbone are under active investigation. Two phase III trials showed that the addition of BR to ibrutinib or idelalisib were superior to BR alone in patients with relapsed/ refractory CLL.^{42,51} Other ongoing studies combine BCR-targeted agents with an anti-CD20 monoclonal antibody and/ or venetoclax (Table 14.4).

Individualized Therapy and Predictive Biomarkers

Factors that predict response to treatment are, at present, incompletely defined,^{14,25} but include del 17p, del 11q, high B2M, advanced clinical stage and selected mutations such as TP53 and SF3B1. More recently, complex karyotype was shown to predict poor outcome to ibrutinib in a single-center follow-up of 56 CLL patients.⁵² TP53 aberrations correlate with poor treatment response to CIT¹⁴ and less durable response to targeted agents.¹⁵

The expanding choice of effective therapies makes individualized treatment decisions possible that can be based on patient characteristics (i.e., age, comorbidities, patient's preference), disease biology (i.e., cytogenetic status, prior therapies), and treatment factors (i.e., side effects, duration of treatment, cost). For patients with del 17p and those with early (<2 years) relapse after CIT or refractory disease treatment with targeted agents is clearly preferred based on studies showing inferior outcome with CIT and high efficacy of ibrutinib and venetoclax in these patients.^{29,45,44,46} For

patients who cannot tolerate intensive therapy due to older age or comorbidities, orally dosed targeted agents may offer a low-intensity approach.^{38,43} For some patients, time-limited CIT may be preferred, especially when there is a good chance of long-lasting remissions. The pros and cons of the different approaches should be better defined when the results from randomized trials comparing novel agents to standard CIT become available.

COMPLICATIONS OF CHRONIC LYMPHOCYTIC LEUKEMIA AND THEIR TREATMENT

Autoimmune Manifestations

Autoimmune hematologic manifestations occur quite frequently in advanced disease or during treatment with purine analogs. AIHA and idiopathic thrombocytopenia purpura are more common than PRCA and autoimmune neutropenia is rare. These autoimmune complications often respond to prednisone or cyclosporine. The risk of serious autoimmune complications appears to be highest with single-agent fludarabine and mitigated by CIT containing an anti-CD20 antibody. Ibrutinib has been used in patients with subclinical autoimmune cytopenias and may be able to effectively control the autoimmune process in some patients.⁵³

Infections

Infections from bacterial, viral, and fungal agents are an important cause of morbidity and mortality in CLL and may be exacerbated by hypogammaglobulinemia typically found in advanced disease. Immunosuppression secondary to chemotherapy and biologic agents further increases the risk of infections. G-CSF maybe useful to reduce the duration of neutropenia associated with fludarabine combination regimens but should be used with caution as it may mask marrow toxicity of fludarabine that can result in persistent myelosuppression. Use of rituximab is associated with an increased risk of infections, including serious or fatal reactivation of hepatitis B virus. Patients should be screened before initiation of anti-B-cell therapy, which in addition to anti-CD20 antibodies probably should include small molecule-targeted agents, and antiviral therapy or regular monitoring needs to be considered for patients with evidence of prior infection including those who are seronegative for HBsAg but seropositive for antibodies against hepatitis B core antigen and/ or surface antigen. Despite inhibiting an essential kinase in the BCR signaling pathway, ibrutinib has been associated with a decreased rate of infections on continued therapy,^{40,54} and patients can respond to vaccines.⁵⁵ All CLL patients should be immunized according to the Centers for Disease Control and Prevention (CDC) recommendations for immunocompromised persons and

avoid live vaccines.⁵⁵ Prophylactic use of antibiotics in neutropenic or hypogammaglobulinemic patients is not recommended. Prophylaxis for pneumocystis and herpes infections are recommended during CIT but are not routinely used during treatment with BCR-targeting agents. However, a recent report suggests that BTK inhibition could increase the risk for pneumocystis infection.⁵⁶ Patients with recurrent sinopulmonary infections and with low serum IgG (<500 mg/ dL) can be considered for I.V. immunoglobulin (IVIG). Starting dose of IVIG is 0.3 to 0.5g/ kg given monthly. The interval and the dose can be adjusted to maintain the nadir IgG level above 500 mg/ dL.

Richter's Transformation

Transformation of CLL to large B-cell lymphoma or Hodgkin lymphoma is called Richter's syndrome. Characteristic findings include B symptoms, rapid lymph node enlargement, elevation of LDH, and highly active nodal disease on PET.⁵⁷ In the initial studies of high-risk CLL treated with ibrutinib and more recently venetoclax, few patients progressed, but many of these progression events were due to Richter's syndrome.^{29,40,46,48} We believe this observation reflects the nature of the disease and is not a drug-related effect; a notion supported by the even higher incidence of Richter's syndrome in high-risk CLL treated with FCR.²⁹ Improving treatment for Richter's syndrome is clearly a priority as response to chemotherapy is usually short-lived and survival short. A recent report identifies PD-1 blockade as a promising option for patients with Richter syndrome.⁵⁸

SELECT INTERNET RESOURCES

Ongoing clinical trials: <http://www.clinicaltrials.gov>

Ongoing NHLBI clinical trials: <http://www.nhlbi.nih.gov/studies/nhlbi-trials>

Education and patient information: <http://cllsociety.org>

Research funding, education and patient information: <http://www.lymphoma.org>

Patient run volunteer support groups: <http://www.acor.org>

ACKNOWLEDGMENTS

We thank Sarah E. M. Herman, Hannah Robinson, and Gerald E. Marti for critical reading and helpful comments.

References

1. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer

- Institute-Working Group 1996 guidelines. *Blood*. 2008;111(12):5446–5456.
2. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2014, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017. Accessed, November 2017. <http://seer.cancer.gov/statfacts/html/clyl.html>
 3. Wiestner A. The role of B-cell receptor inhibitors in the treatment of patients with chronic lymphocytic leukemia. *Haematologica*. 2015;100(12):1495–1507.
 4. Herishanu Y, Perez-Galan P, Liu D, et al. The lymph node microenvironment promotes B-cell receptor signaling, NF- κ B activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood*. 2011;117(2):563–574.
 5. Slager SL, Benavente Y, Blair A, et al. Medical history, lifestyle, family history, and occupational risk factors for chronic lymphocytic leukemia/ small lymphocytic lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. *J Natl Cancer Inst Monogr*. 2014;2014(48):41–51.
 6. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375–2390.
 7. Rawstron AC, Bennett FL, O'Connor SJ, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. *N Engl J Med*. 2008;359(6):575–583.
 8. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol*. 2005;23(18):4079–4088.
 9. Wierda WG, O'Brien S, Wang X, et al. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. *Blood*. 2007;109(11):4679–4685.
 10. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94(6):1848–1854.
 11. Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med*. 1990;323(11):720–724.
 12. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343(26):1910–1916.
 13. Tam CS, Shanafelt TD, Wierda WG, et al. De novo deletion 17p13.1 chronic lymphocytic leukemia shows significant clinical heterogeneity: the M. D. Anderson and Mayo Clinic experience. *Blood*. 2009;114(5):957–964.
 14. Grever MR, Lucas DM, Dewald GW, et al. Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US Intergroup Phase III Trial E2997. *J Clin Oncol*.

- 2007;25(7):799–804.
15. Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. *Blood*. 2015;125(16):2497–2506.
 16. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell*. 2013;152(4):714–726.
 17. Puente XS, Bea S, Valdes-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2015;526(7574):519–524.
 18. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*. 2016;127(17):2122–2130.
 19. Rassenti LZ, Huynh L, Toy TL, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med*. 2004;351(9):893–901.
 20. Bulian P, Shanafelt TD, Fegan C, et al. CD49d Is the Strongest Flow Cytometry-Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia. *J Clin Oncol*. 2014;32(9):897–904.
 21. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. *J Natl Cancer Inst*. 1999;91(10):861–868.
 22. Cheson BD, Byrd JC, Rai KR, et al. Novel targeted agents and the need to refine clinical end points in chronic lymphocytic leukemia. *J Clin Oncol*. 2012;30(23):2820–2822.
 23. Kovacs G, Robrecht S, Fink AM, et al. Minimal residual disease assessment improves prediction of outcome in patients with chronic lymphocytic leukemia (CLL) who achieve partial response: comprehensive analysis of two phase III studies of the German CLL Study Group. *J Clin Oncol*. 2016;34(31):3758–3765.
 24. Thompson PA, Wierda WG. Eliminating minimal residual disease as a therapeutic end point: working toward cure for patients with CLL. *Blood*. 2016;127(3):279–286.
 25. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet*. 2010;376(9747):1164–1174.
 26. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*. 2016;127(2):208–215.
 27. Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. *Blood*. 2016;127(3):303–309.

28. Smith MR, Neuberg D, Flinn IW, et al. Incidence of therapy-related myeloid neoplasia after initial therapy for chronic lymphocytic leukemia with fludarabine-cyclophosphamide versus fludarabine: long-term follow-up of US Intergroup Study E2997. *Blood*. 2011;118(13):3525–3527.
29. Farooqui MZ, Valdez J, Martyr S, et al. Ibrutinib for previously untreated and relapsed or refractory chronic lymphocytic leukaemia with TP53 aberrations: a phase 2, single-arm trial. *Lancet Oncol*. 2015;16(2):169–176.
30. Eichhorst B, Fink AM, Bahlo J, et al. First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol*. 2016;17(7):928–942.
31. Hillmen P, Robak T, Janssens A, et al. Chlorambucil plus ofatumumab versus chlorambucil alone in previously untreated patients with chronic lymphocytic leukaemia (COMPLEMENT 1): a randomised, multicentre, open-label phase 3 trial. *Lancet*. 2015;385(9980):1873–1883.
32. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N Engl J Med*. 2014;370(12):1101–1110.
33. Goede V, Fischer K, Engelke A, et al. Obinutuzumab as frontline treatment of chronic lymphocytic leukemia: updated results of the CLL11 study. *Leukemia*. 2015;29(7):1602–1604.
34. Tam CS, O'Brien S, Plunkett W, et al. Long-term results of first salvage treatment in CLL patients treated initially with FCR (fludarabine, cyclophosphamide, rituximab). *Blood*. 2014;124(20):3059–3064.
35. Dreger P, Schetelig J, Andersen N, et al. Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents? *Blood*. 2014;124(26):3841–3849.
36. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015;7(303):303ra139.
37. O'Brien S, Furman RR, Coutre SE, et al. Ibrutinib as initial therapy for elderly patients with chronic lymphocytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1b/ 2 trial. *Lancet Oncol*. 2014;15(1):48–58.
38. Burger JA, Tedeschi A, Barr PM, et al. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. *N Engl J Med*. 2015;373(25):2425–2437.
39. O'Brien SM, Lamanna N, Kipps TJ, et al. A phase 2 study of idelalisib plus rituximab in treatment-naive older patients with chronic lymphocytic leukemia. *Blood*. 2015;126(25):2686–2694.
40. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med*. 2014;371(3):213–223.

41. Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med.* 2016;374(4):323–332.
42. Chanan-Khan A, Cramer P, Demirkiran F, et al. Ibrutinib combined with bendamustine and rituximab compared with placebo, bendamustine, and rituximab for previously treated chronic lymphocytic leukaemia or small lymphocytic lymphoma (HELIOS): a randomised, double-blind, phase 3 study. *Lancet Oncol.* 2016;17(2):200–211.
43. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2014;370(11):997–1007.
44. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med.* 2016;374(4):311–322.
45. O'Brien S, Jones JA, Coutre SE, et al. Ibrutinib for patients with relapsed or refractory chronic lymphocytic leukaemia with 17p deletion (RESONATE-17): a phase 2, open-label, multicentre study. *Lancet Oncol.* 2016.
46. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol.* 2016;17(6):768–778.
47. Herman SE, Niemann CU, Farooqui M, et al. Ibrutinib-induced lymphocytosis in patients with chronic lymphocytic leukemia: correlative analyses from a phase II study. *Leukemia.* 2014;28(11):2188–2196.
48. Maddocks KJ, Ruppert AS, Lozanski G, et al. Etiology of ibrutinib therapy discontinuation and outcomes in patients with chronic lymphocytic leukemia. *JAMA Oncol.* 2015;1(1):80–87.
49. Ahn IE, Underbayev C, Albitar A, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. *Blood.* 2017;129(11):1469–1479.
50. Oppermann S, Ylanko J, Shi Y, et al. High-content screening identifies kinase inhibitors that overcome venetoclax resistance in activated CLL cells. *Blood.* 2016;128(7):934–947.
51. Zelenetz AD, Barrientos JC, Brown JR, et al. Idelalisib or placebo in combination with bendamustine and rituximab in patients with relapsed or refractory chronic lymphocytic leukaemia: interim results from a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* 2017;18(3):297–311.
52. Thompson PA, O'Brien SM, Wierda WG, et al. Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. *Cancer.* 2015;121(20):3612–3621.
53. Vitale C, Ahn IE, Sivina M, et al. Autoimmune cytopenias in patients with chronic lymphocytic leukemia treated with ibrutinib. *Haematologica.* 2016;101(6):e254–258.
54. Sun C, Tian X, Lee YS, et al. Partial reconstitution of humoral immunity and fewer

- infections in patients with chronic lymphocytic leukemia treated with ibrutinib. *Blood*. 2015;126(19):2213–2219.
55. Sun C, Gao J, Couzens L, et al. Seasonal influenza vaccination in patients with chronic lymphocytic leukemia treated with ibrutinib. *JAMA Oncol*. 2016;2(12):1656–1657.
 56. Ahn IE, Jerussi T, Farooqui M, et al. Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib. *Blood*. 2016;128(15):1940–1943.
 57. Falchi L, Keating MJ, Marom EM, et al. Correlation between FDG/ PET, histology, characteristics, and survival in 332 patients with chronic lymphoid leukemia. *Blood*. 2014;123(18):2783–2790.
 58. Ding W, Dong H, Call TG, et al. PD-1 blockade with pembrolizumab (MK-3475) in relapsed/ refractory CLL including richter transformation: An early efficacy report from a phase 2 trial (MC1485). *Blood*. 2015;126(23):834.

Hodgkin Lymphoma

Joseph Roswarski and Mark Roschewski

Hodgkin lymphoma (HL), an aggressive lymphoma of B-cell origin, was first described by the British pathologist Thomas Hodgkin in 1832. Dr. Hodgkin provided clinical histories and postmortem findings of seven patients with enlarged, pathologic lymph nodes and spleens that affected contiguous nodal groups in an orderly manner and was uniformly fatal. The 2008 World Health Organization (WHO) classification of hematologic neoplasms divides HL into two distinct types, classical Hodgkin lymphoma (CHL), which represents 95% of cases and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), which accounts for the remainder. CHL has a characteristic morphologic appearance: multinucleated giant cells of B-cell origin, known as Reed-Sternberg (RS) cells, in a background of reactive lymphocytes and inflammatory cells. The treatment of HL has resulted in successful cure of both localized and advanced disease since the 1950s, when it was first discovered that patients could be cured with extended fields of radiation therapy. Subsequently, HL became the first advanced malignancy of a major organ system to be effectively treated by combination chemotherapy alone.¹ In the modern treatment era, most patients with newly diagnosed HL are cured of disease, so the short-term and long-term toxicities of the treatment rendered are critical considerations from the very beginning. Current research efforts in HL involve development of functional imaging to guide treatment and novel therapies that offer the potential to improve the cure rate in HL.

EPIDEMIOLOGY

HL occurred at an estimated rate of 8,260 new cases in 2017 with a slight male predominance, and an estimated 1,070 patients died from the disease.² HL accounts for only 0.5% of all new cancer diagnoses per year. Caucasians and Asians/ Pacific Islanders exhibit a bimodal age distribution with primary peak incidence occurring

between 20 and 29 years old and secondary peak incidence after the age of 60 years old; other racial groups do not have this bimodal incidence.³ HL is more prevalent among individuals of higher socioeconomic status. Certain infections, genetic factors, and deficits in the immune system all have been shown to increase the risk of developing HL. Infectious mononucleosis from Epstein–Barr virus (EBV) is a predisposing risk factor for subsequent EBV⁺ CHL. EBV is found in the RS cells 40% to 60% of the time, and cases of EBV⁺ HL have a distinct molecular profile, but the exact oncogenic role of EBV in HL remains unclear.⁴ The risk of CHL is increased 5- to 15-fold in patients with HIV, particularly with certain histologic subtypes of HL.⁵ There may be familial clustering of HL, and first-degree relatives of individuals with CHL have a three-to ninefold increased risk of developing the disease. Specific HLA types and genetic susceptibility loci have also been associated with an increased risk of developing HL, and a small number of cases of NLPHL are associated with a germline mutation in NPAT.^{6,7}

HISTOPATHOLOGY AND BIOLOGY OF CLASSICAL HODGKIN LYMPHOMA

The distinction between CHL, which comprises 95% of all HL cases, and NLPHL is based on distinct immunophenotypes, morphologic appearance of the RS cell, and clinical features. CHL is defined by the presence of neoplastic multinucleated RS cells within a dense inflammatory infiltrate (see Fig. 15.1). These neoplastic cells are germinal center B cells. The inflammatory milieu includes reactive T cells, eosinophils, histiocytes, plasma cells, neutrophils, and fibroblasts. The typical immunophenotype of RS cells is CD30 positive (virtually 100%) and are frequently CD15 positive (85% of cases), with membrane and Golgi-staining patterns. PAX-5 is generally weakly expressed. There is a striking loss of pan-B and pan-T cell antigens (CD19, CD20, CD79a; CD3, CD7 are all frequently negative). Further subclassifications of CHL are made on histologic appearance.

Histopathologic Classifications of Classic Hodgkin Lymphoma

- . Nodular sclerosis (NSCHL): This most common subtype (80%) of CHL is characterized by fibrotic bands that separate the neoplastic and reactive cells into compartments. The neoplastic cells can have a lacunar appearance and are generally EBV negative.⁸

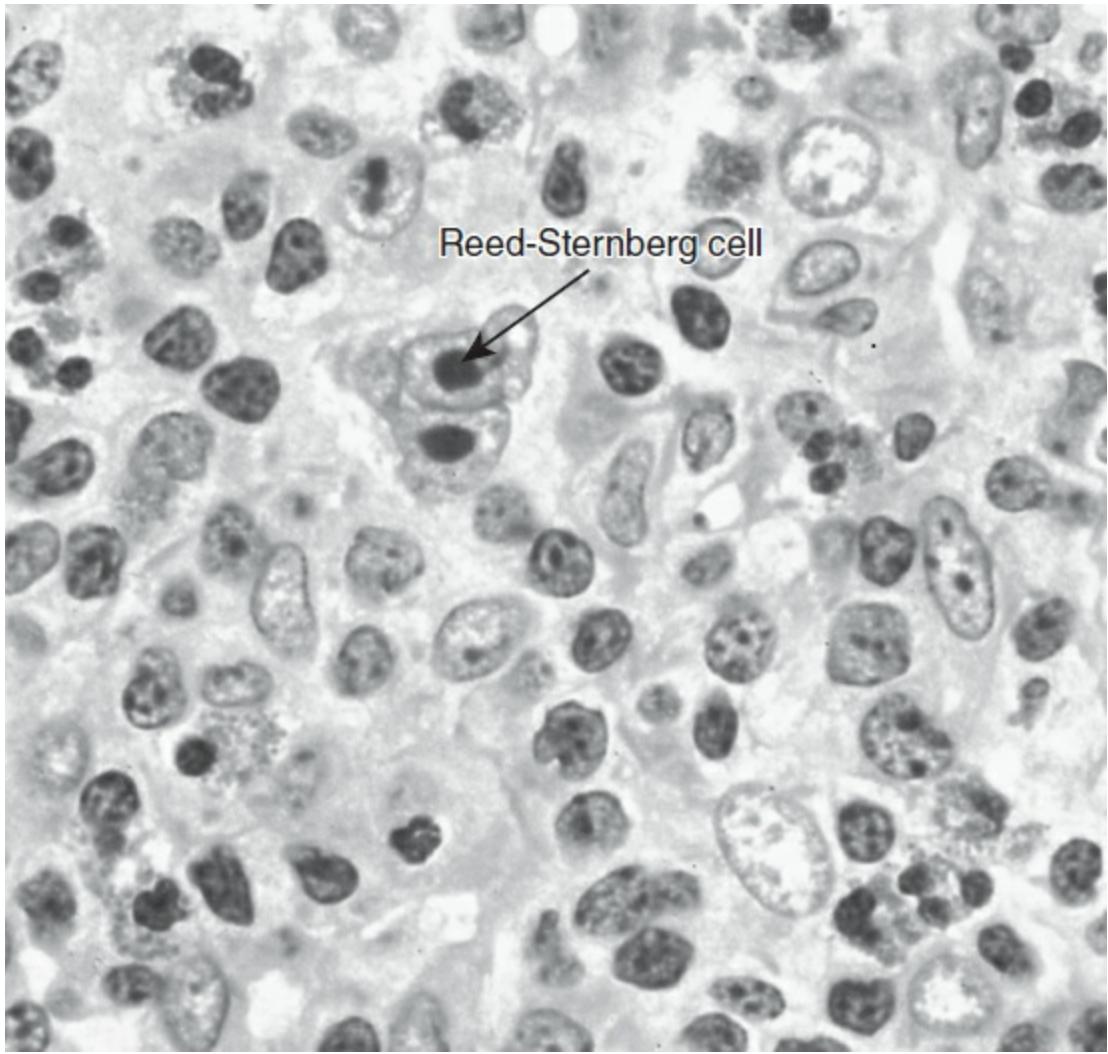


FIGURE 15.1 Diagnostic RS cells seen in classic types of HIs (mixed cellularity, nodular sclerosis, lymphocyte depletion). Neoplastic cells in NLPHLs are termed popcorn cells or L and H cells (lymphocytic or histiocytic predominance).

HL, Hodgkin lymphoma; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; RS, Reed-Sternberg.

- . Mixed cellularity (MCCHL): This subtype is the second most common overall and the most frequently associated with EBV positivity (up to 75% of cases). It occurs commonly in children or the elderly, and has a higher association with an immunocompromised state, including HIV infection. The inflammatory milieu generally contains many eosinophils and histiocytes. This subtype often disseminates hematogeneously, is more likely to present with advanced disease, and carries a poorer prognosis compared to NSCHL.
- . Lymphocyte-rich (LRCHL): Accounting for about 5% of CHL cases, this uncommon subtype is characterized histologically by neoplastic RS cells in a monomorphic

background of lymphocytes that are contained in small, confluent nodules. A pattern of palisading T-lymphocytes around RS cells can be seen on staining for PD-1. The RS cells show EBV positivity in 30% to 50% of cases.

- Lymphocyte-depleted (LDCHL): This rare subtype (<1% of CHL) tends to occur in immunocompromised patients, especially those with HIV infection. PAX5-negative cases of LDCHL must be differentiated from CD30-positive, ALK-negative anaplastic large cell lymphoma, as they share similar morphologic appearance and immunophenotype.

Signaling Pathways, Genetics, and Tumor Microenvironment of Hodgkin Lymphoma

The cell of origin in CHL is a germinal center B lymphocyte, which was established by the discovery of rearranged and somatically mutated immunoglobulin variable genes within RS cells. Despite being of mature B-cell origin, there is aberrancy of the B-cell phenotype within RS cells. This is in part mediated by downregulation of the necessary B-cell gene transcription factors E2A and EBF by various antagonists, notably ABF-1/ ID2/ Notch1.^{9,10} With this loss of differentiation, RS cells use signaling pathways other than the B-cell receptor signaling pathway to promote growth and survival. Constitutive activation of NF-κB signaling, through both canonical and noncanonical pathways, and the JAK/ STAT pathway promote RS cell survival in many cases. Recurrent somatic mutations in RS cells include single nucleotide variants and deletions of the genes involved in the NF-κB and JAK/ STAT pathways, which lead to constitutive activity of these pathways.¹¹ The JAK/ STAT pathway is also upregulated by an autocrine mechanism, by which IL-13 from RS cells drives activation of STAT6 and promotes survival of RS cells.¹²

CHL is characterized by an infiltration of immunologic, inflammatory cells into the lymphoma tissue, including lymphocytes, plasma cells, neutrophils, eosinophils and mast cells, such that the RS cells themselves usually represent about 1% of cells in the tumor. The RS cells have a direct effect on recruiting cells into this tumor microenvironment. For example, chemokines secreted by RS cells attract T helper-2 and regulatory T-cells. These cells suppress antitumor immune response and promote RS cell survival. RS cells further escape the immune system via increased secretion of galectin-1 and expression of programmed death-ligands, PD-L1/ PD-L2; the former helps to skew the inflammatory infiltrate toward an immunosuppressive phenotype and the latter leads to decreased effector capability and exhaustion of cytotoxic T-cell lymphocytes.^{13,14} Increased expression of PD-L1/ PD-L2 is due to various genetic alterations at the 9p24 locus, which contains the genes for both PD-L1 and PD-L2.¹⁵ The complexity of genetic alterations within individual CHL tumors demonstrate intra-patient heterogeneity, but virtually all cases of CHL have some level of alteration at

9p24, suggesting it is a defining genetic event for CHL.

CLINICAL FEATURES

The hallmark clinical finding in HL is painless nodal enlargements. Most cases display a central pattern of lymph node involvement that progresses contiguously from a head-to-toe orientation, usually beginning in the cervical lymph node chain. Over 80% of patients have cervical lymph node enlargement (particularly the left cervical and supraclavicular region), and more than 50% have mediastinal adenopathy. Mediastinal mass involvement can be prominent and is considered large if the mass is greater than one-third the maximum diameter of the thorax. The presence of “B” symptoms (fever, which may be cyclical as in the Pel-Ebstein pattern; drenching night sweats; weight loss > 10% of body weight preceding diagnosis) occurs in about a third of patients. Occurring less frequently are nonspecific symptoms of fatigue, anorexia, pain at nodal sites shortly after drinking alcohol, and pruritis.

PRETREATMENT EVALUATION AND STAGING

- | Excisional biopsy (and not needle biopsy) of a lymph node or lymphoid tissue is required for accurate diagnosis
- | Detailed history with attention to constitutional symptoms, comorbid conditions, fertility issues, and performance status
- | Complete physical examination, including the lymph node examination and evaluation for hepatosplenomegaly

Laboratory tests

- | Complete blood count
- | Erythrocyte sedimentation rate
- | Biochemical tests of liver function, renal function
- | Serum lactate dehydrogenase
- | Pregnancy test should be included for all women of childbearing age
- | HIV testing (if risk factors are present or with MCCHL or LDCHL)

Radiologic studies

- | Computed tomography (CT) scan of the chest, abdomen, and pelvis
- | Positron emission tomography (PET) or PET/ CT is recommended for initial staging

Other testing

- | Bone marrow biopsy of the posterior iliac crest should be performed in patients staged II to IV

- Pulmonary function tests (PFTs) with diffusing capacity of the lungs for carbon monoxide (DLCO)
- Determination of ejection fraction
- Adequate counseling on fertility preservation and risk should be considered before initiation of treatment. Gonadal toxicity with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) is low, but can be significant if pelvic radiation or other chemotherapy regimens are to be employed.

Prognostic Features

Unfavorable prognostic features in stage I/ II disease include:

- Erythrocyte sedimentation rate (ESR) > 50 mm/ hr
- Age > 50 years
- Presence of B symptoms
- More than three sites of nodal involvement
- More than one area of extranodal involvement
- Bulky adenopathy, including mediastinal disease more than one-third of the chest diameter on chest x-ray or tumor size >10 cm on CT scan

Staging (Ann Arbor/ American Joint Committee on Cancer [AJCC] and Cotswold) is outlined in [Table 15.1](#).¹⁶

MANAGEMENT OF NEWLY DIAGNOSED CLASSICAL HODGKIN LYMPHOMA

The goal of therapy for CHL is cure while avoiding both early and late therapy-related toxicity. Most patients are treated with combination chemotherapy with or without radiation. Radiotherapy requires treatment directed at regions of known disease (especially if bulky) and adjacent nodal groups in many cases. The clinical decision to use radiation in HL is influenced by the age of the patient, prognostic factors, and interim PET scans. Importantly, physician-related factors such as expectations of long-term toxicities also influence the treatment decisions. Older physicians and those who practice at academic institutions are less likely to recommend radiation therapy than do younger physicians or those in community practices.¹⁷

After initial therapy is complete, the determination of complete response is critical for assessment of potential cure. Traditional criteria relied on anatomic imaging such as CT scans, but functional imaging with PET scans has been incorporated into the most recent consensus panel on response criteria.¹⁸ Current research is focused on the development of assays of the peripheral blood to monitor for minimal residual disease to further subdivide patients in complete remission, but these are not yet part of standard

response criteria.¹⁹

Table 15.1 Staging of Hodgkin Lymphoma

Stage I	Involvement of single lymph node region or lymphoid structure (spleen, thymus, Waldeyer's ring) or involvement of a single extralymphatic site (IE).
Stage II	Involvement of two or more lymph node regions on the same side of the diaphragm (II), which may be accompanied by localized contiguous involvement of an extralymphatic organ or site (IIE). The number of anatomic sites may be indicated by numeric subscript.
Stage III	Involvement of lymph node regions on both sides of the diaphragm (III), which may also be accompanied by localized involvement of an associated extralymphatic organ or site (IIIE), by involvement of the spleen (IIIS), or both (IIIE + S).
Stage IV	Disseminated involvement of one or more extralymphatic organs, with or without associated lymph node involvement, or isolated extralymphatic organ involvement with distant (nonregional) nodal involvement.

Each stage is divided into A and B categories: B for those with defined systemic symptoms, and A for those without. X, mass > 10 cm or a mediastinal mass larger than one-third the thoracic diameter; E, involvement of a single extranodal site contiguous to a known nodal site; CS, clinical staging; PS, pathologic staging.

Data from Lister TA, Crowther D, Sutcliffe SB, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol. 1989;7(11):1630–1636.

Patients are divided into two treatment groups:

- Early-stage CHL: Patients with stage I to II (non-bulky) HL are considered to be in early stage of the disease with favorable features. Patients with stage II disease and bulky tumors (large mediastinal mass) or those with systemic symptoms are treated similar to patients with advanced-stage HL. Because the cure rate for early-stage CHL is greater than 90%, combination chemotherapy is given with or without radiation therapy.
- Advanced-stage CHL: Patients with bulky tumors, systemic symptoms, or stage III to IV are considered to be in advanced stage of CHL. These patients are often cured with combination chemotherapy alone, but have a 25% to 30% chance of experiencing treatment failure or disease relapse after initial therapy. Attempts to intensify frontline chemotherapy regimens reduce the rate of early treatment failure but do not result in a higher cure rate overall.

Early-Stage Hodgkin Lymphoma

Most patients with early-stage HL are cured with chemotherapy alone, and yet the optimal treatment strategy is still unsettled. The main source of controversy in early-stage HL is consolidative radiation therapy. The rate of early treatment failure is decreased with the use of radiation at the cost of long-term toxicity. In the 1990s,

treatment for early-stage HL transitioned from extended-field radiation therapy to “combined modality therapy” that utilized both combination chemotherapy and involved-field radiation therapy (IFRT).

A landmark trial from the German Hodgkin Study Group (GHSG) tested lowering doses of both chemotherapy and radiation in 1,370 patients with early-stage HL.²⁰ This study randomized patients to one of four treatment groups: two cycles of ABVD (see Table 15.2) followed by 20 Gy of IFRT, four cycles of ABVD followed by 20 Gy of IFRT, two cycles of ABVD followed by 30 Gy of IFRT, or four cycles of ABVD followed by 30 Gy IFRT. The primary endpoint of the study was freedom from treatment failure and the patients with two cycles of ABVD and 20 Gy of IFRT demonstrated similar outcomes with less toxicity. There was not a treatment arm of chemotherapy alone.

In a study involving newly diagnosed, early-stage favorable or unfavorable CHL, ABVD alone was superior to RT with or without ABVD. They evaluated two cycles of ABVD followed by a subtotal nodal irradiation of 35 Gy for unfavorable early-stage or subtotal nodal irradiation alone (favorable risk patients) with an ABVD-only arm; patients in the ABVD arm received four or six cycles of chemotherapy depending on their remission status after two cycles of therapy. At a median of 12 years follow-up, the overall survival (OS) rates for chemotherapy alone were 94% with freedom from disease progression of 89%, a significantly higher OS rate ($P = .04$) than in the radiation therapy arms (87%), despite those arms having a higher freedom from disease progression rate (92%, $P = .05$).

Table 15.2 ABVD Regimen

ABVD

Doxorubicin, 25 mg/ m² per dose IV push for two doses, days 1 and 15 (total dose/ cycle, 50 mg/ m²)

Bleomycin, 10 U/ m² per dose IV push for two doses, days 1 and 15 (total dose/ cycle, 20 U/ m²)

Vinblastine, 6 mg/ m² per dose IV push for two doses, days 1 and 15 (total dose/ cycle, 12 mg/ m²)

Dacarbazine, 375 mg/ m² per dose IV infusion for two doses, days 1 and 15 (total dose/ cycle, 750 mg/ m²)

Treatment cycle repeats every 28 d.

ABVD, adriamycin, bleomycin, vinblastine, and dacarbazine; IV, intravenous.

The use of interim PET imaging in early-stage CHL is important for prognosis and further management. Multiple studies have shown that the predictive value of a negative PET scan after one to three cycles of chemotherapy is greater than 90% for progression-free survival (PFS). As such, several prospective studies were initiated to evaluate whether consolidative RT is necessary for patients with early metabolic response. In the

RAPID trial,²¹ 426 of 571 patients with early-stage CHL were PET negative after cycle 3 of ABVD and were subsequently randomized to consolidative RT (30-Gy IFRT) or no further therapy. The 3-year PFS and OS rates for the radiotherapy and no-further-therapy groups were 94.6% versus 90.8% and 97.1% versus 99%, respectively. Although this study did not meet its end point of noninferiority for PFS in the no-further treatment group, it does illustrate that most early-stage CHLs with negative interim PET imaging have excellent outcomes without radiation therapy.

Advanced-Stage Hodgkin Lymphoma

The treatment of advanced-stage HL underwent a remarkable transition with the institution of multi-agent chemotherapy regimens in 1964. Previously considered incurable with extensive radiation fields being the standard of care, Devita and colleagues at the National Cancer Institute pioneered the use of modern chemotherapy with a combination of mechlorethamine, vincristine (Oncovin), procarbazine, and prednisone (MOPP).¹ In advanced-stage patients who had received prior radiation therapy or who were previously untreated, this regimen achieved a CR rate of 84% and resulted in disease-specific OS of 63%.²²

Despite the transformative success of MOPP, the regimen was associated with high rates of infertility, toxicity, and secondary malignancies (myelodysplasia and acute myeloid leukemia). These toxicities led to trials of modifications of MOPP, such as omitting the alkylating agent, but they were inferior in terms of CR rates. The ABVD regimen was introduced by the Milan Institute to serve as a salvage regimen following MOPP failures, and subsequently a MOPP–ABVD alternating regimen for stage IV disease transiently became a standard of care. In a CALGB study, a prospective evaluation of MOPP versus ABVD versus MOPP–ABVD hybrid showed that doxorubicin-containing arms had significantly higher CR rates (82% to 83% in the doxorubicin-containing regimens versus 67% in the MOPP-only arm). Furthermore, the MOPP-containing arms had higher rates of hematologic toxicity and sterility, whereas the ABVD-containing arms had a higher incidence of pulmonary toxicity.²³ In a retrospective analysis, the 15-year actuarial risk of developing treatment-related AML with the MOPP regimen is higher than with ABVD (3.4% vs. 0.7%, $P < .05$).²⁴ The combination of superior CR rates and decreased overall rates of toxicity led to ABVD becoming the standard of care chemotherapeutic regimen in the United States.

In an attempt to increase dose intensity and thereby improve tumor control, the BEACOPP regimen (bleomycin, etoposide, doxorubicin [Adriamycin], cyclophosphamide, vincristine [Oncovin], procarbazine, and prednisone) was developed. In a trial conducted by the GSHG, patients with advanced-stage CHL randomized to initial therapy with COPP/ ABVD, BEACOPP, or increased-dose

BEACOPP, with consolidative radiation therapy to sites of initial bulky or residual disease. This showed a 5-year OS of 83% for COPP/ ABVD, 88% for BEACOPP, and 91% for increased-dose BEACOPP²⁵ ($P = .16$ for the comparison of the COPP–ABVD group with the BEACOPP group, $P = .06$ for the comparison of the BEACOPP group with the increased-dose BEACOPP group, and $P = .002$ for the comparison of the COPP–ABVD group with the increased-dose BEACOPP group). However, when salvage therapy including autologous stem cell transplantation (ASCT) is considered, there is no significant difference in OS between BEACOPP and ABVD.²⁶ As BEACOPP has significantly more acute and long-term toxicities, ABVD remains the standard of care for advanced-stage CHL.

Similar to early-stage CHL, interim PET imaging is recommended in advanced-stage CHL for its prognostic utility and to aid in further management. In a prospective study,²⁷ interim PET scan after two cycles (PET-2) of ABVD chemotherapy was shown to be a more accurate predictor of 2-year PFS than the International Prognostic Score (IPS) for advanced-stage disease; the 2-year PFS for a negative and positive PET-2 scan were 95% and 12.8%, respectively. Current clinical trials are evaluating whether therapy can be escalated or de-escalated based on interim PET results. In a phase II study by the Southwest Oncology Group,²⁸ patients with advanced-stage CHL that were PET-2 positive after ABVD were subsequently treated with escalated BEACOPP (eBEACOPP), and PET-negative patients continued treatment with ABVD for six cycles. Of 331 evaluable patients, 271 (82%) were PET-2 negative and 60 (18%) were PET-2 positive. The 2-year PFS was 79% for all patients and 64% for those that were PET-2 positive and treated with eBEACOPP, which is dramatically higher than 2-year PFS for PET-2 positive patients with standard of care therapy (12.8%). In a larger phase III study,²⁹ 937 of 1,119 patients with advanced-stage CHL had PET-2 negativity following ABVD and were randomized to ABVD or AVD (no bleomycin) for cycles 3 to 6. The 3-year PFS and OS rates were 85.7% and 97.2%, respectively for the ABVD group and 84.4% and 97.5%, respectively for the AVD group. This difference in PFS was not within the specified margin for noninferiority. The PET-2 positive patients were treated with either BEACOPP-14 or eBEACOPP and had a 3-year PFS of 67.5% and OS of 87.8%. Thus advanced-stage CHL patients with negative interim PET scans have excellent outcomes, and patients with positive interim PET scans may benefit from escalation to more dose intense regimens.

RELAPSED AND PRIMARY REFRACTORY CLASSICAL HODGKIN LYMPHOMA

Up to 8% of patients with early-stage HL and 30% of patients with advanced-stage HL relapse or are refractory to primary treatment. Three patterns of relapsed and primary

refractory classical Hodgkin lymphoma (RR-HL) are defined based on previous experience in the MOPP era and have prognostic significance:

- . Primary progressive CHL is defined by failure to achieve initial CR or relapse within 3 months of initial therapy. This accounts for approximately 6% of all cases of CHL and has a 5-year OS of 26%.³⁰
- . Early-relapsed CHL occurs within 12 months of complete remission. This accounts for about 3.5% of all cases of CHL and has a 5-year OS of 46%.
- . Relapse that occurs after 12 months following initial therapy is considered late relapse. This occurs for about 5.3% of all cases of CHL and has a 5-year OS of 71%.³¹

As noted earlier, time to relapse is an important factor in prognosis. Other important prognostic factors in RR-HL include poor performance status (Eastern Cooperative Oncology Group [ECOG] > 0), age >50 years, and failure to obtain an initial temporary remission.

Treatment of RR-HL includes radiation therapy, conventional chemotherapy, immunochemotherapy, and high-dose chemotherapy (HDCT) followed by ASCT, the choice depending on which modalities were employed in the initial therapy of the disease. Radiation therapy is indicated in patients whose initial therapy omitted this modality and in localized relapses outside of previously irradiated fields. For patients who have RR-HL after initial chemotherapy, the mainstay of treatment is HDCT followed by ASCT, which can provide long-term disease-free survival rates of 30% to 65%. Evidence for the superiority of HDCT compared with conventional salvage chemotherapy in RR-HL was seen in a study conducted by the British National Lymphoma Investigation. In this trial, RR-HL patients were randomized to HDCT with BEAM (carmustine [BCNU], etoposide, cytarabine [ara-C], and melphalan) followed by ASCT versus mini-BEAM chemotherapy alone (same chemotherapies at lower doses). Three-year event-free survival was 53% in the HDCT arm versus 10% in those receiving conventional chemotherapy.³² However, conventional salvage chemotherapy is still used before HDCT for tumor cytoreduction and to establish chemosensitivity prior to transplantation. There is no consensus on the optimal salvage chemotherapy regimen to be used, but most employ non-cross-resistant agents to those used in HDCT and contain a platinum-based backbone; examples include ICE (ifosfamide, carboplatin, and etoposide) and DHAP (dexamethasone, high-dose Ara-C, cisplatin).

Brentuximab vedotin, an anti-CD30 antibody drug conjugate, was initially approved for RR-HL patients having failed ASCT; brentuximab has demonstrated single-agent overall response rate (ORR) of 75% with 34% of patients achieving CR.³³ A study conducted at Memorial Sloan Kettering evaluated the use of PET-directed sequential salvage therapy with brentuximab and augmented ICE therapy prior to planned HDCT

and ASCT. In this non-randomized study, RR-HL patients were given brentuximab monotherapy followed by augmented ICE if they were PET positive after single-agent brentuximab. Twelve of 45 patients were able to achieve PET negativity with brentuximab alone, and 22 of 45 were able to subsequently achieve PET negativity with augmented ICE chemotherapy. In patients able to achieve PET negativity, 2-year event-free survival was greater than 90% versus 46% in those who remained PET positive before ASCT. Post-ASCT maintenance with brentuximab was evaluated in the AETHERA study. In this randomized, placebo-controlled trial patients with RR-HL were randomized to brentuximab (1.8 mg/ kg IV every 3 weeks) in a 1 : 1 ratio following ASCT. There was a significant increase in median PFS in the brentuximab arm versus placebo (42 vs. 24 months, respectively), but there was no significant difference in OS.³⁴

In RR-HL, cumulative toxicities of chemotherapy, including HDCT and ASCT, can preclude further treatment. Novel approaches with programmed cell death (PD-1 and its ligands) inhibitors have been developed and have different, yet favorable toxicities in contrast to conventional chemotherapy. Nivolumab (anti-PD-1 antibody) was approved as monotherapy for patients with RR-HL following ASCT and brentuximab failure. In a multicenter phase 2 study, nivolumab monotherapy (3 mg/ kg IV every 2 weeks) was well tolerated and had an ORR of 66% with 9% complete remissions;³⁵ follow-up is ongoing to determine the durability of responses.

SURVIVORSHIP

Most patients with CHL are cured, which makes the recognition and management of long-term effects of chemotherapy and radiation paramount to successful care of these patients.

Long-term effects of chemotherapy include gonadal and cardiac toxicity and the development of secondary malignancies. Cardiac as cardiomyopathy is rare with ABVD and BEACOPP due to the lower cumulative anthracycline dose, as compared to other regimens for other malignancies. Sterility can occur with BEACOPP, but only transient gonadal toxicity follows ABVD. Secondary hematologic malignancy, including acute myeloid leukemia and myelodysplastic syndrome, occurs infrequently (0.4% and 2.5% of cases with ABVD and BEACOPP, respectively).

Late manifestations of radiation therapy are increased risk of cardiovascular disease, secondary neoplasms (generally solid tumor), and thyroid dysfunction. The increased risk of cardiovascular disease is the leading nonmalignant cause of death in CHL survivors. Secondary malignancies include breast and lung cancer, which are the most common after radiation therapy, but they can also include non-Hodgkin lymphoma (NHL), soft tissue and bone tumors, gastrointestinal neoplasms, and thyroid cancer.

SPECIAL CONSIDERATIONS: NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

NLPHL is a distinct clinicopathologic entity from CHL. NLPHL lacks typical RS cells, and instead is characterized by a small population of neoplastic B cells with folded lobulated nuclei known as lymphocytic and histiocytic (L&H) cells. The immunophenotype is frequently CD20 positive and CD30 negative and other markers of normal B-cell differentiation are retained. EBV infection in neoplastic cells is rare. NLPHL is seen more in men and commonly presents with nodal-only disease. It should be approached similarly to other indolent lymphomas with a watch-and-wait strategy and treatment initiation for symptoms or organ compromise. Unlike CHL, NLPHL has a tendency to recur late and to transform to more aggressive histology (DLBCL); the actuarial risk of transformation is 7% and 30% at 10 and 20 years after diagnosis, respectively.³⁶

SPECIAL CONSIDERATIONS: DOSE INTENSITY AND TREATMENT OF THE ELDERLY PATIENT

In the initial trials comparing ABVD and MOPP, it was noted that the ability to adhere to dose intensity translated into meaningful differences in response rates and outcomes. For example, in the study evaluating MOPP versus ABVD and ABVD–MOPP hybrid, most patients were able to receive recommended doses of doxorubicin in the ABVD-containing arms in contrast to those receiving mechlorethamine in the MOPP-containing arms, in which less than 25% of patients received 85% of the dose intensity by the sixth cycle. The doxorubicin-containing arms had significantly higher CR rates, leading to the concept that dose reductions are not recommended and may compromise cure rates. In addition to adherence to recommended doses of chemotherapy, it is also recommended that delays in treatment be minimized. As such, dose delays for uncomplicated neutropenia on the day of treatment are to be avoided. There is an increased incidence of bleomycin-induced pulmonary toxicity with the concomitant use of growth factor support (such as granulocyte colony stimulating factor), and thus its routine use is not recommended.

In the trials listed in the aforementioned treatment sections, patients older than 60 years had a higher risk for treatment failure and experienced more treatment-related toxicity than did younger patients. Age is not a contraindication to aggressive treatment, but, compared with younger patients, fewer older adult patients receive intended chemotherapy doses, which influences the poorer outcomes seen in the elderly. The patient's physical and mental condition, disease history, and the presence of concurrent disorders influence the treatment strategy. ABVD is regarded as standard chemotherapy

for most older adult CHL patients, but there is an increased incidence of toxicity and mortality with this regimen. As such, the use of consolidative radiation therapy is more widely accepted in this population, which can limit the number of cycles of chemotherapy. Another possible approach is to omit bleomycin after the initial cycles of ABVD. This was evaluated in a prospective study by Johnson et al.²⁹ In patients with advanced CHL achieving PET negativity after two cycles of ABVD, omission of bleomycin for cycles 3 to 6 was similar to ABVD for all six cycles in terms of 3-year PFS or OS; this study did not meet its margin for noninferiority (5%), but the groups had almost identical OS rates.

CONCLUSIONS AND FUTURE DIRECTIONS

Most patients with early and advanced stage CHL should be cured of disease with current therapeutic approaches. Individualized treatment plans with the incorporation of interim PET scans have the ability to maintain curative efficacy while decreasing long-term toxicity by only using consolidative radiation in selected cases. However, there is still an unmet need for patients who have RR-HL despite ASCT. In addition, the role of immunotherapy with checkpoint inhibitors or anti-CD30 antibodies has yet to be fully defined; further large trials are needed to determine whether these agents should be incorporated into first-line therapies. The development of biomarkers, likely of circulating tumor DNA, could be helpful in assessing response, aiding in surveillance, and predicting the optimal use of consolidative radiation therapy.

References

1. Devita VT, Jr, Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med. 1970;73(6):881–895.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
3. Evens AM, Antillon M, Aschebrook-Kilfoy B, et al. Racial disparities in Hodgkin's lymphoma: a comprehensive population-based analysis. Ann Oncol. 2012;23(8):2128–2137.
4. Chetaigne B, Bertucci F, Finetti P, et al. Molecular profiling of classical Hodgkin lymphoma tissues uncovers variations in the tumor microenvironment and correlations with EBV infection and outcome. Blood. 2009;113(12):2765–3775.
5. Biggar RJ, Jaffe ES, Goedert JJ, et al. Hodgkin lymphoma and immunodeficiency in persons with HIV/ AIDS. Blood. 2006;108(12):3786–3791.
6. Saarinen S, Aavikko M, Aittomaki K, et al. Exome sequencing reveals germline NPAT mutation as a candidate risk factor for Hodgkin lymphoma. Blood. 2011;118(3):493–498.

7. Enciso-Mora V, Broderick P, Ma Y, et al. A genome-wide association study of Hodgkin's lymphoma identifies new susceptibility loci at 2p16.1 (REL), 8q24.21 and 10p14 (GATA3). *Nat Genet*. 2010;42(12):1126–1130.
8. Swerdlow SH, International Agency for Research on Cancer., World Health Organization. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: International Agency for Research on Cancer; 2008.
9. Jundt F, Acikgoz O, Kwon SH, et al. Aberrant expression of Notch1 interferes with the B-lymphoid phenotype of neoplastic B cells in classical Hodgkin lymphoma. *Leukemia*. 2008;22(8):1587–1594.
10. Mathas S, Janz M, Hummel F, et al. Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma. *Nat Immunol*. 2006;7(2):207–215.
11. Mathas S, Hartmann S, Kuppers R. Hodgkin lymphoma: pathology and biology. *Semin Hematol*. 2016;53(3):139–147.
12. Skinnider BF, Kapp U, Mak TW. The role of interleukin 13 in classical Hodgkin lymphoma. *Leuk Lymphoma*. 2002;43(6):1203–1210.
13. Juszczynski O, Ouyang J, Monti S, et al. The AP1-dependent secretion of galectin-1 by Reed-Sternberg cells fosters immune privilege in classical Hodgkin lymphoma. *PNAS*. 2007;104(32):13134–13139.
14. Roemer MG, Advani RH, Ligon AH, et al. PD-L1 and PD-L2 genetic alterations define classical Hodgkin lymphoma and predict outcome. *J Clin Oncol*. 2016;34(23):2690–2697.
15. Roemer MG, Advani RH, Ligon AH, et al. PD-L1 and PD-L2 genetic alterations define classical Hodgkin lymphoma and predict outcome. *J Clin Oncol*. 2016;34(23):2690–2697.
16. Lister TA, Crowther D, Sutcliffe SB, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. *J Clin Oncol*. 1989;7(11):1630–1636.
17. Ng AK, Li S, Neuberg D, et al. Factors influencing treatment recommendations in early-stage Hodgkin's disease: a survey of physicians. *Ann Oncol*. 2004;15(2):261–269.
18. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059–3068.
19. Vandenberghe P, Wlodarska I, Tousseyn T, et al. Non-invasive detection of genomic imbalances in Hodgkin/ Reed-Sternberg cells in early and advanced stage Hodgkin's lymphoma by sequencing of circulating cell-free DNA: a technical proof-of-principle study. *Lancet Haematol*. 2015;2(2):e55–65.
20. Engert A, Plutschow A, Eich HT, et al. Reduced treatment intensity in patients with early-stage Hodgkin's lymphoma. *N Engl J Med*. 2010;363(7):640–652.

21. Radford J, Illidge T, Counsell N, et al. Results of a trial of PET-directed therapy for early-stage Hodgkin's lymphoma. *N Engl J Med.* 2015;372(17):1598–1607.
22. Longo DL, Young RC, Wesley M, et al. Twenty years of MOPP therapy for Hodgkin's disease. *J Clin Oncol.* 1986;4(9):1295–1306.
23. Canellos GP, Anderson JR, Propert KJ, et al. Chemotherapy of advanced Hodgkin's disease with MOPP, ABVD, or MOPP alternating with ABVD. *N Engl J Med.* 1992;327(21):1478–1484.
24. Delwail V, Jais JP, Colonna P, et al. Fifteen-year secondary leukaemia risk observed in 761 patients with Hodgkin's disease prospectively treated by MOPP or ABVD chemotherapy plus high-dose irradiation. *Br J Haematol.* 2002;118(1):189–194.
25. Diehl V, Franklin J, Pfreundschuh M, et al. Standard and increased-dose BEACOPP chemotherapy compared with COPP-ABVD for advanced Hodgkin's disease. *N Engl J Med.* 2003;348(24):2386–2395.
26. Viviani S, Zinzani PL, Rambaldi A, et al. ABVD versus BEACOPP for Hodgkin's lymphoma when high-dose salvage is planned. *N Engl J Med.* 2011;365(3):203–212.
27. Gallamini A, Hutchings M, Rigacci L, et al. Early interim 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography is prognostically superior to international prognostic score in advanced-stage Hodgkin's lymphoma: a report from a joint Italian-Danish study. *J Clin Oncol.* 2007;25(24):3746–3752.
28. Press OW, Li H, Schoder H, et al. US intergroup trial of response-adapted therapy for stage III to IV Hodgkin lymphoma using early interim fluorodeoxyglucose-positron emission tomography imaging: Southwest Oncology Group S0816. *J Clin Oncol.* 2016;34(17):2020–2027.
29. Johnson P, Federico M, Kirkwood A, et al. Adapted treatment guided by interim PET-CT scan in advanced Hodgkin's lymphoma. *N Engl J Med.* 2016;374(25):2419–2429.
30. Josting A, Rueffer U, Franklin J, et al. Prognostic factors and treatment outcome in primary progressive Hodgkin lymphoma: a report from the German Hodgkin Lymphoma Study Group. *Blood.* 2000;96(4):1280–1286.
31. Josting A, Franklin J, May M, et al. New prognostic score based on treatment outcome of patients with relapsed Hodgkin's lymphoma registered in the database of the German Hodgkin's lymphoma study group. *J Clin Oncol.* 2002;20(1):221–230.
32. Linch DC, Winfield D, Goldstone AH, et al. Dose intensification with autologous bone-marrow transplantation in relapsed and resistant Hodgkin's disease: results of a BNLI randomised trial. *Lancet.* 1993;341(8852):1051–1054.
33. Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma.

J Clin Oncol. 2012;30(18):2183–2189.

34. Moskowitz CH, Nademanee A, Masszi T, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;385(9980):1853–1862.
35. Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol. 2016;17(9):1283–1294.
36. Al-Mansour M, Connors JM, Gascoyne RD, et al. Transformation to aggressive lymphoma in nodular lymphocyte-predominant Hodgkin's lymphoma. J Clin Oncol. 2010;28(5):793–799.

Non-Hodgkin Lymphomas

Richard F. Little

The non-Hodgkin lymphomas (NHLs) are a heterogeneous group of lymphoid tumors that have distinctive clinical and biologic behaviors. Modern tumor classification is based on the World Health Organization (WHO) 2008 classification that established specific designations within the various lymphomas based on pathologic and clinically relevant criteria. It was updated in 2016 and incorporates more molecular and genetic features relevant to biologically targeted therapeutics.¹ Prior to the WHO 2008 classification, clinical trials would enroll all comers without an appreciation of the separate entities with markedly different clinical behaviors. Comparing newer trial data to older “historic control” trial data is generally not valid in part for this reason. Current clinical trial design, inclusion criteria, and interpretation of findings must take these advances in classification into account so that the trial informs the treatment effects of the specific disease of interest.

This chapter focuses on the major disease entities of NHL while mentioning the spectrum of lymphoid diseases to emphasize the imprecision of the term NHL. This overview is intended to impress that accurate diagnosis of the specific NHL subtype is critical to interpretation of clinical trials data, to foster improved therapeutics in the future, and to understand individual patient management.

Refinement in diagnostic resolution is an evolving science. Recent advances have led to resolution of clinically relevant molecular-based distinctions among lymphomas. For example, gene transcription signatures enable distinction of multiple subtypes of diffuse large B-cell lymphoma (DLBCL). Advances that incorporate gene expression and tumor genetic mutational analysis are likely to become relevant for everyday clinical practice in the near future. Newer therapeutic agents that show activity according to the specific tumor biology require diagnostic tests so that physicians can select appropriate therapy.

EPIDEMIOLOGY

A steady increase in the age-adjusted incidence of NHLs per 100,000 persons has been documented with 11.1 cases in 1976, 19.0 in 2000, and 19.5 in 2009 to 2013. In 2013, there was an estimated 569,535 persons living with NHL in the United States. Approximately one-third of the increase may be attributed to a combination of iatrogenic immunosuppression and the AIDS epidemic. In more recent years, NHL cases attributable to HIV infection have decreased consequent to effective combination antiretroviral therapy (cART). Other potential causes include increased exposure to environmental carcinogens. Most, though not all, NHL types occur more commonly in males, and whites are affected more than blacks.

PATOPHYSIOLOGY

A major risk factor for NHL is an abnormality of immune function (either immune deficiency or dysregulation). Examples include HIV infection, iatrogenic immune suppression, autoimmune diseases, and congenital immune deficiencies (e.g., Wiskott–Aldrich, X-linked lymphoproliferative disorder). Also, oncogenic viruses have been implicated. The gamma herpes viruses are linked to certain NHL subtypes, especially lymphomas associated with immune deficiency states. These include Epstein–Barr virus (EBV), which is highly associated with African Burkitt lymphoma (BL) and AIDS-related DLBCL; the Kaposi sarcoma-associated herpes virus (KSHV; also known as human herpes virus-8 or HHV-8) is etiologically linked to primary effusion lymphomas (PELs) and multicentric Castleman disease, a rare lymphoproliferative disorder associated with increased risk of developing aggressive B-cell lymphoma. These latter two conditions are primarily seen in individuals with HIV infection. Other oncogenic viruses include the human retroviruses and RNA viruses. Human T-lymphotropic virus type 1 (HTLV-1) is causative of adult T-cell leukemia/ lymphoma (ATLL), and the hepatitis C virus (HCV) is associated with splenic marginal zone lymphoma. In addition to infectious agents, environmental and occupational exposures, especially organic compounds such as organophosphate insecticides have been linked to lymphoma risk.

CLASSIFICATION

The current WHO classification utilizes immunophenotypic, molecular, genetic, and clinical elements to distinguish NHL subtypes. New high-throughput technologies such as gene expression profiling (GEP), comparative genomic hybridization, single nucleotide polymorphism arrays, microRNAs, methylation, acetylation, and tissue microarrays foster mechanistic understanding of lymphoma biology that may eventually inform targeted therapeutics. Recent refinements in some of these technologies have

made them invaluable tools for clinical trials, and some may become relevant to routine clinical application. The expense can be lowered and complicated performance characteristics standardize in commercialized tests, such as lymphoma subtyping by GEP. The text of this chapter presents the lymphoid tumors in the order of the 2008 WHO classification of lymphoid tumors with the 2016 updates incorporated ([Table 16.1](#)). [Tables 16.2](#) to [16.5](#) provide summaries of molecular and immunophenotypic characteristics of selected tumors encountered in clinical practice.

NHLs are broadly classified as B-cell or T-cell lymphomas, depending on the lymphocyte lineage giving rise to the tumor. B-lymphocytes give rise to B-cell NHL, 88% of all NHL. T-lymphocytes give rise to T-cell NHL, 12% of NHL. Expression (or its lack thereof) of cell surface antigens and immunoglobulin proteins is dependent on the type of lymphocyte and its stage of differentiation. Analysis of these proteins in tumor cells is diagnostically useful as well as for determining tumor histogenesis. Importantly, no single immunophenotypic marker is specific for any tumor, and a constellation of features is required for specific diagnosis.

There is an increasing appreciation of the relationship between tumor tissue origin and clinical behavior. For example, those DLBCLs with an activated B-cell-like (ABC) gene signature (termed ABC-DLBCL) can be distinguished from those that have a germinal center-like signature (termed GCB-DLBCL), and the latter have a more favorable prognosis. In addition, the type may be predictive of therapeutic effect. Definitive clinical trials for ibrutinib and lenalidomide are in progress testing these agents in DLBCL ABC type.

The WHO recognizes five major categories of lymphoid neoplasms: (1) B-cell neoplasms, (2) T and natural killer (NK) cell neoplasms, (3) Hodgkin lymphoma, (4) posttransplant lymphoproliferative disorders (PTLDs), and (5) histiocytic and dendritic cell neoplasms. The latter are considered distinct diagnostic entities and are grouped together based on functional properties of their normal counterpart. A cell of origin is postulated for each neoplasm, although this may not necessarily be the cell in which the initial transforming event occurs. Thus, the designated cell of origin represents the state of differentiation of the tumor cells that are seen in the tissues.

It can also be helpful to consider lymphoid neoplasms broadly in terms of the arm of the immune system from which the tumor arises: The innate or adaptive immune response. The innate immune responses are a first-line of mucocutaneous defense not requiring antigen-presenting cells to initiate the immune response. Included in the innate immune systems are NK cells, CD3⁺ CD56⁺ T-cells or NK-like T-cells, and γδ T-cells. The innate immune system cells are mainly extranodal, and lymphomas arising from these cells thus tend to be extranodal. The adaptive immune response is more complex and specific for particular pathogens and capacity to develop memory. The complexity

of the adaptive immune response not only gives rise to the molecular heterogeneity of many B-cell lymphomas but also helps to determine the type of lymphoma as it relates to a particular B-cell stage of development. Most B-cell neoplasms tend to reflect the stages of normal B-cell differentiation, and the lymph node architecture is highly relevant to this process: Nodal neoplasms are more likely to be seen.

Table 16.1 2016 Revision in WHO Classification of Lymphoid Neoplasms

Precursor B-and T-Cell Neoplasms

- Precursor B lymphoblastic leukemia/ lymphoma
 - B lymphoblastic leukemia/ lymphoma, NOS
 - B lymphoblastic leukemia/ lymphoma with recurrent genetic abnormalities
 - B lymphoblastic leukemia/ lymphoma with T(9;22) (q34; q11.2; BCR-ABL1)
 - B lymphoblastic leukemia/ lymphoma with t(v;11Q23); KMT2A rearranged
 - B lymphoblastic leukemia/ lymphoma with t(12;21) (p13;22); TEL_AML1,(ETV 6-RUNX1)
 - B lymphoblastic leukemia/ lymphoma with hyperdiploidy
 - B lymphoblastic leukemia/ lymphoma with hypodiploidy (hypodiploid ALL)
 - B lymphoblastic leukemia/ lymphoma with t(5;14) (q31;q32); IL-3-IGH
 - B lymphoblastic leukemia/ lymphoma with t(1;19) (q23; p13.3);E2A-PBX1 (TCF3-PBX1)
 - Provisional entity: B-lymphoblastic leukemia/ lymphoma, BCR-ABL1-like
 - Provisional entity: B-lymphoblastic leukemia/ lymphoma with iAMP21
- Precursor T lymphoblastic leukemia/ lymphoma

Mature B-Cell Neoplasms

- Chronic lymphocytic leukemia/ small lymphocytic lymphoma
- Monoclonal B-cell lymphocytosis
- B-prolymphocytic leukemia
- Splenic marginal zone lymphoma
- Hairy cell leukemia variant
- Splenic B-cell lymphoma/ leukemia, unclassifiable
 - Splenic diffuse red pulp small B-cell lymphoma
 - Hairy cell leukemia variant
- Lymphoplasmacytic lymphoma/ Waldenström macroglobulinemia
- Monoclonal gammopathy of undetermined significance (MGUS), IgM
 - γ Heavy chain disease
 - μ Heavy chain disease
 - α Heavy chain disease
- Monoclonal gammopathy of undetermined significance (MGUS), IgA
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Monoclonal immunoglobulin deposition diseases
- Extranodal marginal zone lymphoma of mucosa-associated tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
 - Pediatric nodal marginal zone lymphoma
- Follicular lymphoma
 - In situ follicular neoplasia
 - Duodenal-type follicular lymphoma
- Pediatric-type follicular lymphoma

Large B-cell lymphoma with IRF4 rearrangement
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
 In situ mantle cell neoplasia
Diffuse large B-cell lymphoma (DLBCL), NOS
 Germinal center B-cell type
 Activated B-cell type
T-cell/ histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV⁺ DLBCL, NOS
EBV⁺ mucocutaneous ulcer
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK⁺ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
HHV- 8⁺ DLBCL, NOS
Burkitt lymphoma
Burkitt-like lymphoma with 11q aberration
High-grade B-cell lymphoma, with MYC and BCL2 and/ or BCL6 rearrangements
High-grade B-cell lymphoma, NOS
Hodgkin lymphoma

Mature T-Cell and NK-Cell Neoplasms

T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK-cell leukemia
Systemic EBV T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Adult T-cell leukemia/ lymphoma
Extranodal NK/ T-cell lymphoma nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30⁺ T-cell lymphoproliferative disorders
Primary cutaneous anaplastic T-cell lymphoma (ALCL)
Lymphomatoid papulosis
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8⁺ aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous CD4⁺ aggressive small to medium-size T-cell lymphoma
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, ALK⁺
Anaplastic large cell lymphoma, ALK⁻

Hodgkin Lymphoma

Nodular lymphocyte-predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

Immunodeficiency-Associated Lymphoproliferative Disorders

Lymphoproliferative diseases associated with primary immune disorders
Lymphomas associated with HIV infection
Posttransplant lymphoproliferative disorders (PTLD)
Early lesions
Plasmacytic hyperplasia
Infectious mononucleosis-like
PTLD Polymorphic
PTLD Monomorphic PTLD (B and T/ NK cell types)
Classical Hodgkin lymphoma type PTLD

ALCL, anaplastic large lymphoma; ALL, acute lymphoblastic leukemia; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; HHV-8, human herpes virus-8; Ig, immunoglobulin; MALT, mucosa-associated lymphoid tissue; NK, natural killer; NOS, not otherwise specified; WHO, World Health Organization.

Table 16.2 Molecular Characteristics of Selected B-Cell Lymphomas

Histology	Cytogenetics	Oncogene/ Protein	Immunoglobulin Gene Rearrangements	
			Heavy	$\kappa \lambda$
CLL/ SLL ^a	t(14; 19) Trisomy 12, 13q	Bcl-3	+	+
Lymphoplasmacytoid			+	+
Follicular center cell ^b grade I, II, or III	t(14; 18)	Bcl-2	+	
Marginal zone ^c	Trisomy 3 t(11; 18)		+	
Mantle cell lymphoma	t(11; 14)	Bcl-1/ Cyclin-D1	+	
Diffuse large B-cell ^d	t(3; 22)(q27;q11)	Bcl-6/ Bcl-2		+
Primary mediastinal (thymic) large B-cell ^e		MAL gene	+	+
Lymphoblastic lymphoma/ leukemia			+	+/-
Burkitt lymphoma	t(8; 14)(q24;q32) t(2; 8)(11p; q24) t(8; 22)(q24;q11)	C-MYC	+	$\lambda +$ $\kappa +$

^aTrisomy 12 is seen in 30% of cases and abnormalities in 13q are present in 25% of patients.

^bt(14; 18) is present in 75% to 95% of FCC NHL.

^cCytogenetic abnormalities have been seen in extranodal marginal zone NHL.

^dBcl-2 rearrangements up to 30% and Bcl-6 up to 45% of cases of DLBCL and C-MYC is uncommon.

CLL/ SLL, chronic lymphocytic leukemia/ small lymphocytic lymphoma.

Table 16.3 Molecular Characteristics of Selected T-Cell Lymphomas

Histology	Cytogenetics	Oncoprotein	TCR Gene Rearrangements
T-chronic lymphocytic leukemia/ T-prolymphocytic leukemia	Inv14(q11; q32), Trisomy 8q	Bcl-3	+
Mycosis fungoides			+
Peripheral T-cell lymphoma, unspecified			+/-
Extranodal NK/ T-cell	EBV ⁺		-
Angioimmunoblastic ^a T-cell lymphoma	Trisomy 3 or 5, EBV ⁺		+
Adult T-cell leukemia/ lymphoma	HTLV I integration +		+
Enteropathy T cell	EBV-		β+
Hepatosplenic T-cell lymphoma	Isochromosome 7q		γ+/ β+/-
Hepatosplenic γ/ δ T-cell lymphoma			δγ+
Systemic anaplastic large cell lymphoma ^{b,c}	T(2; 5)	Alk ⁺	+
Precursor T lymphoblastic lymphoma/ leukemia	Variable T(7; 9)	Tcl-4	Variable

^aTCR gene rearrangement is present in 75% and IgH in 10%.

^bTCR gene rearrangement in 60%⁺

^cAlk: Anaplastic lymphoma kinase gene.

EBV, Epstein–Barr virus; HTLV 1, human T-lymphotropic virus type 1; NK, natural killer.

Following are the specific disease entities in order of presentation of the 2016 revision of the WHO classification of lymphoid tumors.¹

Precursor B-and T-Cell Neoplasms

Specific entities are B lymphoblastic leukemia/ lymphoma that are (1) not otherwise specified, and (2) those with recurrently genetic abnormalities. Those with t(9;22) (q34;q11.2); BCR-ABL1 have improved prognosis in the era of imatinib and later-generation tyrosine kinase inhibitors. T lymphoblastic leukemia/ lymphoma is termed lymphoma when the disease is confined to nodal masses and does not involve the peripheral blood and marrow. T-acute lymphoblastic leukemia (ALL) comprises 25% of adult ALL. See Chapter 12 for discussion and treatment.

Mature B-Cell Neoplasms

These tumors range broadly in clinical behavior. The very indolent tumors have low curative potential but median survivals measured in years to decades. The aggressive tumors have varying curative potential but without treatment can have a rapidly fatal clinical course.

Table 16.4 B-Cell Immunophenotype

Histology	S Ig	C Ig	CD									
			5	10	11	15	20	23	30	34	43	45
Chronic lymphocytic leukemia/ small lymphocytic lymphoma ^a	+/-	-/+	+	-	-/+		Weak	+			+	
Lymphoplasmacytoid ^a	+	+	-	-	-/+		+	-			+/-	
Follicular center cell grade I-III ^{a,b}	+	-	-	+	-		+	-/+			-	
Marginal zone ^{a,c}	+	+	-	-	+/-		+	-			-/+	
Mantle cell lymphoma ^{a,d}	+	-	+	-	-/ (few)		+	-			+	
Diffuse large B-cell ^a	+/-	-/+	-/+	-/+			+	-			+/-	
Primary mediastinal large cell ^{a,d}	-	-	-/+	-/+		-	+	-	-/+		+/-	
Precursor B lymphoblastic lymphoma/ leukemia ^{a,e,f}	-	-/+		+/-			+				+/-	
Burkitt lymphoma ^a	+		-	+			+	-				
Burkitt-like lymphoma ^a	+/-	-/+	-	-/+			+					

^aPositive B-cell-associated antigens: CD19, CD20, CD22, and CD79.

^bS Ig⁺: IgM⁺ -, IgD > IgG > IgA.

^cS Ig M > G > A and IgD⁻; C Ig⁺ in 40%.

^dS IgM⁺ usually IgD⁺, κ > λ and CD11c⁻.

^eM > G > A and IgD⁻; C Ig⁺ in 40%.

^fTdT⁺, HLA-Dr⁺ and CD20^{-/+}.

CD, cluster designation; C Ig, cytoplasmic immunoglobulin; HLA, human leukocyte antigen; Ig, immunoglobulin; S Ig, surface immunoglobulin.

Table 16.5 T-Cell Immunophenotype

Histology	CD									
	1a	2	3	4	5	7	8	25	56	TdT
T-chronic lymphocytic	+	+	+	+	+	+	+	-		

leukemia/ T-prolymphocytic leukemia ^a							
Mycosis fungoides	+	+	+	+	-/+	-	-
Peripheral T-cell lymphoma ^b	+/-	+/-	+	+/-	-/+	+/-	
Angioimmunoblastic T-cell lymphoma	+	+	+	+			
Extranodal NK/ T	+	-	-	+/-	+/-	-	+
Enteropathy T cell ^c		+	-		+	+/-	
Adult T-cell lymphoma/ leukemia	+	+	+	+	-	-	+
Systemic anaplastic large cell lymphoma ^d			-/+				+/-
Hepatosplenic γ/δ			+	-		-	+
Precursor T lymphoblastic lymphoma/ leukemia	+/-	+/-	+	+	+/-	+	+

^aT-chronic lymphocytic leukemia:60% are CD4⁺ and 21% are CD4⁺CD8⁺; rare cases are CD4-8⁺ and CD25⁻.

^bPeripheral T-cells are most commonly CD4 > CD8. It can be CD4-8⁻; CD45RA may be + and CD45RA⁻.

^cIntestinal T cell is CD103⁺

^dALCL are CD30⁺, CD25[±], EMA⁺, and CD15⁺

ALCL, anaplastic large cell lymphoma; CD, cluster designation; NK, natural killer.

Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma

The term small lymphocytic lymphoma (SLL) is used for nonleukemic cases with tissue morphology and immunophenotype of chronic lymphocytic leukemia (CLL). There must be adenopathy and no cytopenias due to bone marrow (BM) infiltration. See [Chapter 14](#) for discussion and treatment.

Monoclonal B-Cell Lymphocytosis

It is important to recognize “low-count” (peripheral blood CLL count of $<0.5 \times 10^9/L$) has little if any change of progression and does not require routine follow-up outside of standard medical care. “High-count” monoclonal B-cell lymphocytosis requires yearly routine follow-up.

B-Prolymphocytic Leukemia

B-Prolymphocytic leukemia (B-PLL), formerly considered a variant of CLL, is now recognized as a distinct aggressive poor-prognosis tumor with a median survival of about 3 years. Purine analogues such as fludarabine, cladribine, and pentostatin are associated with approximately 50% response rates (RRs), including some complete responses (CRs). New B-cell receptor inhibitors, such as ibrutinib and idelalisib, may

have a role in the management. Allogeneic stem cell transplant may offer curative potential in selected patients.

Splenic Marginal Zone Lymphoma

Splenic marginal zone lymphoma comprises <2% lymphoid neoplasms, but may account for the majority of otherwise unclassifiable CLLs that are CD5-. It appears to be associated with hepatitis C infection and virus eradication may be effective lymphoma management. Indolent cases may be observed without therapy. Splenectomy can be effective. Systemic alkylating or purine analog therapy with rituximab may be used.

Hairy Cell Leukemia

Hairy cell leukemia (HCL) is a rare indolent lymphoid leukemia. Treatments include cladribine and pentostatin. Rituximab is also useful. Immunotherapy directed against CD22 is of interest. BRAF-V600E mutation is a disease-defining genetic event. Targeted therapy exploiting BRAF mutations in HCL may prove effective, although limited data currently exists.

Splenic B-Cell Lymphoma/ Leukemia, Unclassifiable

These include splenic diffuse red pulp small B-cell lymphoma, and HCL variant (HCLv). Both are indolent and may respond to splenectomy. HCLv may respond to immunotherapy directed at CD20 and/ or CD22.

Lymphoplasmacytic Lymphoma

This tumor is the most frequent cause of Waldenström macroglobulinemia and is associated with marrow and nodal involvement with paraproteinemia (usually immunoglobulin M [IgM]) that can cause hyperviscosity syndrome, which when present should be treated immediately with plasmapheresis. Subsequent treatment is guided in part by the level of paraproteinemia. Treatment with rituximab alone or combined with other agents such as cyclophosphamide, or bendamustine is very active. Other options include bortezomib-based therapy, carfilzomib, lenalidomide, and ibrutinib have important therapeutic roles. Assessment of mutational status of MYD88 and CXCR4 is important in selecting therapy. Some experts suggest autologous stem cell transplantation (ASCT), but this may not be suitable for some patients.

Monoclonal Gammopathy of Undetermined Significance, IgM

IgM monoclonal gammopathy of undetermined significance (MGUS) is characterized as μ -, γ -, or α heavy chains. The gamma form ranges from indolent to aggressive behavior; μ -heavy chain disease (HCD) is slowly progressive; α -HCD involves an immunoproliferative small intestinal disease that may remit with antibiotic therapy in

early stages. Transformation to DLBCL and a fatal outcome is frequent.

Monoclonal Gammopathy of Undetermined Significance, IgG/ IgA

It is defined by an M-protein <30 g/ L, BM plasma cell percentage <10%, and absence of signs or symptoms related to multiple myeloma (MM).

Plasma Cell Myeloma

MM diagnosis requires both clinical and biochemical features. For more details, see Chapter 17.

Solitary Plasmacytoma of the Bone

Localized bone tumor consisting of monoclonal plasma cells.

Extraosseous Plasmacytoma

Localized plasma cell neoplasms that arise in tissues other than the bone.

Monoclonal Immunoglobulin Deposition Diseases

Nonorganized immunoglobulin fragments are deposited along renal basement membranes with subsequent organ deterioration. Treatment is directed against the immunoglobulin-producing clone. Bortezomib-based therapy and other myeloma-type approaches are active.

Extranodal Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is an indolent extranodal tumor comprised of morphologically heterogeneous small B-cell and scattered immunoblasts and centroblast-like cells. It comprises up to 50% of primary gastric lymphoma and is associated with Helicobacter pylori. Antibiotic therapy eradication of H. pylori can induce gastric MALT lymphoma remission in cases demonstrated to be associated with this infection. Other localized sites and cases not associated with H. pylori can be treated with surgery or low-dose radiation.

Nodal Marginal Zone Lymphoma

Nodal marginal zone lymphoma (NMZL) is a primary nodal B-cell neoplasm morphologically similar to MALT lymphoma. It is essential to rule out MALT, Hashimoto thyroiditis, or Sjögren syndrome. The NMZL in children are predominately in males (ratio 20:1) and are usually asymptomatic stage 1 mainly in the head and neck lymph nodes. Studies for clonal immunoglobulin heavy locus (IGH) rearrangements are

necessary to help distinguish from reactive conditions.

Follicular Lymphoma

Follicular lymphoma (FL) arises from germinal center B-cells (GCB) and comprises about 20% of all lymphomas, primarily affecting adults in the sixth decade. Cells express BCL2 protein related to t(14;18)(q32;q21), the genetic hallmark of FL. Widespread disease at diagnosis and BM involvement is common. FL is graded by determining the content of centroblasts per high-power field (1 to 5 in grade 1, 5 to 15 in grade 2, and >15 in grades 3A and 3B. 3B reflects sheets of centroblasts without detectable centrocytes seen in 3A). This grading system has been criticized for lack of clinical relevance and poor interobserver reproducibility. Localized disease can be treated with radiation therapy alone. Asymptomatic disseminated disease may be watched. Symptomatic disease or disease-causing psychological distress can be managed in various ways (as discussed further). About 20% of treated patients have relapse within 2 years or fail to achieve a CR and appears to define a poor prognostic group. As yet, it is not possible to identify these patients at diagnosis.

In Situ Follicular Neoplasia

Formerly designated as in situ FL. These have a low rate of progression, but because of their frequent association with prior or synchronous lymphomas, they require additional evaluation to determine the risk of progression. If the assessment is not straightforward, expert hematopathological evaluation may be required. Circulating t(14:18)⁺ lymphocytes in excess of 10⁻⁴ of total cells indicate a higher risk of FL.

Duodenal-Type Follicular Lymphoma

This is distinct from other gastrointestinal tract lymphomas. Most patients have localized disease, and survival appears to be excellent even without treatment. The morphologic, immunophenotypic, and genetic features are similar to those of nodal FLs.

Pediatric-Type Follicular Lymphoma

Similar lymphomas may occur in adults. It is nodal with large highly proliferative follicles with blastoid follicular center cells rather than the classic centroblasts or centrocytes. BCL2 rearrangement must be absent, although BCL2 protein expression may be observed. Nearly all cases are localized and treatment may require only excision.

Large B-Cell Lymphoma with IRF4 Rearrangement

Occurs most commonly in children and young adults. It is typically low stage and found in Waldeyer ring and/ or cervical lymph nodes. Most cases have IG/ IRF4

rearrangements but lack BCL2 rearrangements. This lymphoma is more aggressive than the pediatric-type FL.

Primary Cutaneous Follicle Center Lymphoma

Primary cutaneous follicle center lymphoma (CFCL) generally presents on the head or trunk. This is distinguished from primary cutaneous DLBCL, leg type in that it has a variable number of centrocytes/ centroblasts as in FL. Rituximab and other immunotherapeutics are commonly used; disease control is variable.

Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) generally presents with stage III or IV disease and peripheral blood involvement is common. Cell cycle protein cyclin D1 overexpression is almost always present. MCL generally affects older adult males. Two types of clinically indolent variants are recognized. Classical MCL is generally associated with immunoglobulin heavy chain variable (IGHV)-unmutated or minimally mutated B cells expressing SOX11 involving both nodal and extranodal sites. When other mutations and cytogenetic abnormalities are present the course is generally more aggressive. MCL developing from IGHV-mutated SOX11-B cells lead to leukemic nonnodal MCL usually involving the peripheral blood, BM, and spleen. These are generally indolent, but if accompanied by other mutations such as TP53, the clinical behavior can be very aggressive.

In Situ Mantle Cell Neoplasia

This entity has a low rate of progression and is important to recognize to avoid overtreatment. The presence of cyclin D1⁺ cells typically in the inner mantle zones of follicles, in lymphoid tissue that otherwise do not suggest MCL.

Diffuse Large B-Cell Lymphoma

DLBCL is a constellation of heterogeneous disease entities each with distinct morphologic, biologic, and clinical characteristics. These differences are associated with molecular and immunophenotypic findings that help to segregate the various entities into various subgroups.

DLBCL, not otherwise specified comprises around 30% of adult NHL. It can arise de novo or consequent to the progression of other lymphoid malignancies such as CLL/SLL, FL, marginal zone lymphoma, or nodular lymphocyte-predominant Hodgkin lymphoma. Morphologic variants include centroblastic, immunoblastic, and anaplastic subtypes.

Rearrangements in the MYC oncogene are found in ~15% of DLBCL and is associated with BCL2 or BCL6 and termed as “double hit” lymphomas, or “triple hit”

when all three are present. These are now classified as a separate entity and are no longer part of DLBCL, not otherwise specified (NOS).

Two cell of origin subtypes are now included in the classification because the improved molecular pathogenesis of these has led to more targeted and specific therapies based on the differences in tumor biology. GEP may become commercially available as a U.S. Food and Drug Administration (FDA) approved classifier, but at present immunohistochemistry algorithms (Hans) are acceptable. However, they have poor reproducibility, fail to identify approximately 15% of unclassified cases by GEP, and do not capture prognostic effects in some studies.

Germinal Center B-Cell (GCB) Type Activated B-Cell (ABC) Type

Molecular subgroups include GCB and ABC. Adjusted for clinical features such as the international prognostic index (IPI— see later), the 5-year overall survival (OS) for the GCB-like subset is superior to that of the ABC-like subset, establishing these as distinct clinicobiologic entities. Most are CD20⁺, a therapeutic target exploited to benefit survival with the use of monoclonal antibodies, particularly rituximab. Definitive studies are in progress to determine the role of agents such as lenalidomide and ibrutinib that may have specific effectiveness in the ABC type.

Other Diffuse Large B-Cell Lymphoma Subtypes

- | **T-cell/ histiocyte-rich large B-cell lymphoma** accounts for <10% of DLBCL; it is found mainly in middle-aged males. The large B-cells are surrounded by abundant T-cells and histiocytes.
- | **Primary DLBCL of the central nervous system (CNS)** comprises <1% of NHL and approximately 2% of brain tumors; these occur mainly in older persons (median age 60 years) or those with immunosuppression. The latter are frequently associated with EBV in the tumor cells. Neurologic and psychiatric presentation is not uncommon. High-dose methotrexate forms the basis of therapy. Whole-brain radiation may prolong progression-free survival (PFS), although randomized study results show no survival advantage in comparison to high-dose methotrexate-based therapy alone. Radiotherapy induced late-term neurotoxicity can lead to severe cognitive deficit. Most of these are of ABC type, and ongoing studies may show agents such as ibrutinib to have a role in therapy.
- | **Primary cutaneous DLBCL, leg type** occurs mainly in women with median age in the 70s. Presentation is generally an ulcerative red or bluish tumor on the leg, although other sites can be involved. Five-year survival is approximately 50%. Rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is often used; relapse is common. Radiotherapy, immunotherapy, and stem cell transplant are

reported as being used in these patients.

- | **EBV⁺ DLBCL, NOS** generally occurs at age >50 years but is also seen in younger patients. Is unassociated with prior immunodeficiency and can have an aggressive course.
- | **EBV⁺ mucocutaneous ulcer**
- | **DLBCL associated with chronic inflammation**, as the name implies, arises in the setting of longstanding chronic inflammation, such as pyothorax or in chronic osteomyelitis. The inflammation appears to be etiologically related.
- | **Lymphomatoid granulomatosis** is a proliferation of EBV⁺ B-cells with reactive T-cells in the setting of a poorly defined T-cell immune-impaired state. Advanced disease can involve brain, kidneys, lung, liver, and skin. Early lower-grade disease may be cured with interferon therapy.
- | **Primary mediastinal (thymic) large B-cell lymphoma (PMBL)** arises in the mediastinum and is of putative thymic B-cell origin. PMBL has distinctive clinical, immunophenotypic, and genotypic characteristics. Presenting features include localized disease, and signs and symptoms related to a large anterior mediastinal mass. Dissemination to multiple organs can occur. CD19 and CD20 are present, while CD10 and CD5 are absent. GEP indicates PMBL is a molecular type of DLBCL distinct from ABC-and GCB-like tumors. Phase II data suggests favorable outcome with dose-adjusted EPOCH-R chemotherapy (see [table 16.11](#)).
- | **Intravascular large B-cell lymphoma (IVLBCL)** is a rare extranodal lymphoma. Growth within the lumina of capillaries and other smaller vessels, generally widely disseminated to virtually any organ and BM is characteristic. Lymph nodes are not usually involved. This is an aggressive tumor poorly responsive to chemotherapy. Rituximab may have activity.
- | **Anaplastic lymphoma kinase-positive (ALK⁺) large B-cell lymphoma** is also known as ALK⁺ plasmablastic B-cell lymphoma, and comprises <1% of lymphomas. Advanced-stage presentation is typical; so few cases have been reported and as such there is limited information on outcomes, although short median survival of less than 12 months is reported.
- | **Plasmablastic lymphoma (PBL)** is seen as diffuse proliferations of immunoblastic appearing cells and has the immunophenotype of plasma cells. Originally thought to involve predominantly the oral cavity, it is now appreciated to also involve other extranodal sites. It is uncommon and mainly seen in HIV-infected males. The tumor has a high proliferation rate. The presence of EBV by EBER can help establish the diagnosis. CD20 expression is generally weak. The clinical course is aggressive. Occasional patients have long-term survival. Some physicians have used abbreviated cycles of EPOCH chemotherapy with involved field radiotherapy for limited disease with encouraging results, although the data are anecdotal.
- | **PEL** is a viral associated tumor (HHV-8) and presents mainly as serous effusions in

the body cavities and is associated with HIV infection. All the tumor cells contain HHV-8 in 100% of the cases and 70% of the cases are also EBV+. Generally B-cell markers are absent, although they express CD45, and may even express CD30. Immunoglobulin genes are clonally rearranged and hypermutated. Occasional patients have good outcome, but in general the prognosis is very poor.

- | **HHV-8+ DLBCL, NOS** is a provisional diagnosis for those cases generally but not always associated with immunosuppression and/ or multicentric Castleman disease and are EBV unrelated.

Burkitt Lymphoma

BL is a rapidly growing lymphoma often involving extranodal sites and can present as an acute leukemia. The “starry sky” pattern on histopathology examination results from macrophages that have injected the apoptotic tumor cells. The endemic form found in equatorial Africa is generally EBV associated and is the most common childhood tumor there. The sporadic type represents 1% to 2% of lymphomas in developed countries. Immunodeficiency-associated BL is seen primarily in those with HIV infection, and can be the initial AIDS-defining condition. MYC translocations are a universal feature. Short course intensive chemotherapy with rituximab is generally recommended (for adults), and cure rates are quite high for standard-risk disease. Poor-risk features (involvement of BM, CNS, tumor mass >10 cm, and high-serum lactate dehydrogenase [LDH]) do not rule out curative intent, but this outcome may be achieved in only 60% of such patients. Preliminary data suggests that dose-adjusted EPOCH-R chemotherapy (given with additional rituximab doses) is very effective in BL and a national clinical trial is near completion to better define this regimen for BL.

Burkitt-Like Lymphoma With 11q Aberration

This is a provisional entity to address the finding of MYC nonrearranged tumors that resemble BL morphologically and to a large extend phenotypically and by GEP.

High-Grade B-Cell Lymphoma, With MYC and BCL2 and/ or BCL5 Rearrangements

This is a new category for all “double-/ triple-hit” lymphomas other than FL or lymphoblastic lymphomas. These are clinically aggressive tumors. The former classification “B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between Diffuse Large B-Cell Lymphoma and Burkitt Lymphoma” is being removed because of the distinction on that basis of DLBCL and BL was imprecise.

High-Grade B-Cell Lymphoma, Not Otherwise Specified

These cases have a blastoid appearance or appear intermediate between DLBCL and

BL, but lack MYC and BCL2 and/ or BCL6 rearrangements.

B-Cell Lymphoma, Unclassifiable, With Features Intermediate Between DLBCL and Classical Hodgkin Lymphoma

This overlap of clinical, morphological and/ or immunophenotypic features, especially with primary mediastinal large B-cell lymphoma has also been termed grey zone lymphoma and Hodgkin-like anaplastic large cell lymphoma.

Mature T-and Natural Killer-Cell Neoplasms

T-Cell Prolymphocytic Leukemia

The median age is 65 years and T-cell prolymphocytic leukemia (T-PLL) comprises <2% of mature lymphocytic leukemia. HTLV-1 is negative. Therapeutics includes alemtuzumab and allogeneic stem cell transplant. These are reported to benefit selected patients, but 3-year OSs are less than 30%.

T-Cell Large Granular Lymphocytic Leukemia

T-cell large granular lymphocytic leukemia (T-LGL) generally presents with a history of persistent, increased peripheral blood large granular lymphocytes of uncertain cause. Most cases are in those aged more than 40 years, but the disease is rare (<2% of mature lymphocytic leukemia). It is important to distinguish T-LGL from a restricted clonal proliferation that sometimes occurs following allogeneic hematopoietic cell transplant. An indolent course is typical. Severe neutropenia is frequent, although thrombocytopenia is not generally seen. Along with the association of inflammatory comorbid conditions, immunophenotype suggests a chronic antigen-driven process and informs the use of immunosuppressive therapy for T-LGL.

Chronic Lymphoproliferative Disorders of Natural Killer Cells

Characterized by >6 months increased peripheral blood NK cells without a clear etiology, this rare condition presents equally in both genders with a median age of 60 years. The course is indolent in most cases. Spontaneous remission or aggressive transformation can occur.

Aggressive Natural Killer-Cell Leukemia

Nearly always EBV associated, with a median age of 42 years, it predominantly affects the Asian ethnic groups. The immunophenotype is identical to that of extranodal NK/ T-cell lymphoma except that CD16 is frequently positive.

Systemic EBV+ T-Cell Lymphoma of Childhood

This is a life-threatening clonal proliferation of EBV-infected T-cells that occurs

shortly after primary acute EBV infection or in the setting of chronic active EBV infection. Rapid progression with multi-organ failure ensues over days to weeks.

Hydroa Vacciniforme-Like Lymphoproliferative Disorder

It is a cutaneous T-cell lymphoma (CTCL) occurring in children and associated with sun sensitivity. After a period of recurrent skin lesions over a period of 15 years, a more aggressive systemic progression may occur.

Adult T-Cell Leukemia/ Lymphoma

Caused by the human retrovirus HTLV-1, ATLL is endemic in Southwestern Japan, the Caribbean basin, and parts of Central Africa where HTLV-1 prevalence is high. There are several clinical variants: Acute, lymphomatous, chronic, and smoldering. The most common is the acute form with elevated white blood count, skin rash, lymphadenopathy, and hypercalcemia. Treatment with zidovudine and interferon- α (IFN- α) may prolong survival, but survival remains poor at a median of 9 months. Recent retrospective analysis supports using chemotherapy in sequence with zidovudine/ IFN- α . Of interest, HTLV-1 also causes non-hematologic disease: Tropical spastic paraparesis (TSP), also known as HTLV-associated myelopathy or chronic progressive myelopathy. HTLV-1 infection of the spinal cord results in paraparesis and weakness of the legs.

Extranodal Natural Killer/ T-Cell Lymphoma, Nasal Type

This extranodal, often EBV-associated lymphoma results in vascular damage and often shows a cytotoxic T-cell phenotype (hence the NK/ T designation). Upper aerodigestive involvement is characteristic, although it can involve other areas of the body. Nodular skin lesions, perforating intestinal lesions, or other sites are also seen. Prognosis is variable, but may be improved with upfront combination chemo-radiotherapy. BM transplantation may be curative in selected patients.

Enteropathy-Associated T-Cell Lymphoma

This is an intestinal lymphoma (mainly of the jejunum or ileum) composed of large lymphoid cells with an inflammatory background. It appears to be associated with celiac disease; early prevention with gluten-free diet appears to protect against lymphoma. There is a monomorphic variant occurring in regions where celiac disease is rare, and this is probably a different disease entity. Once lymphoma has developed, there is poor response to therapy and prognosis is unfavorable.

Monomorphic Epitheliotropic Intestinal T-Cell Lymphoma

Formerly type II enteropathy-associated T-cell lymphoma (EATL) it is now a specific entity without any association to celiac disease.

Indolent T-Cell Lymphoproliferative Disorder of the Gastrointestinal Tract

This is a new provisional entity with superficial monoclonal intestinal T-cell infiltrate. Some cases show progression.

Hepatosplenic T-Cell Lymphoma

The T cells of this extranodal and systemic lymphoma are generally derived from the $\gamma\delta$ T-cell receptor type. The peak incidence is in adolescents and young adults and accounts for <1% of all lymphomas. There is an association with long-term iatrogenic immunosuppression. Initial response to chemotherapy is followed by relapse and median survival is generally less than 2 years.

Subcutaneous Panniculitis-Like T-Cell Lymphoma

The median age with respect to subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is 35 years, and up to 20% may have associated autoimmune disease, such as systemic lupus erythematosus. Patients often present with multiple subcutaneous nodules, particularly in the extremities and trunk. Cytopenias and elevated liver function tests are common. The neoplastic cells are usually CD8+. They express β F1 and are negative for CD56: This finding helps distinguish from subcutaneous $\gamma\delta$ T-cell lymphoma (which has a worse prognosis). If hemophagocytic syndrome develops, prognosis is poor; otherwise, 80% survive 5 years or longer. Combination chemotherapy is used, but reports of immune suppressive therapy alone suggest activity and may help in treatment choices.

Mycosis Fungoides

Mycosis fungoides (MF) is a primary CTCL indicating the classical presentation of evolving skin patches, plaques, and tumors or the variants that show a similarly evolving clinical course. It accounts for 50% of CTCL. There is a wide age range, but most are older adults. In advanced disease, nodal, organ, and BM involvement may be seen. The indolent clinical course slowly progresses over years to decades. Clinically it is staged I to IV. Disease confined to the skin with no lymph nodes is stage I and when there is a high blood Sézary cell count of >10,000/ μ L and/ or extensive lymph node involvement it is stage IV. Stage II has some lymph node involvement and stage III has skin erythroderma with or without lymph node and/ or low Sézary cell count. Traditionally Sézary syndrome (SS) has referred to the leukemic form (see later). Early-stage disease does not benefit from multiagent chemotherapy, and dermatologic skin-directed therapy is generally most appropriate.

Sézary Syndrome

SS is defined by the triad of erythroderma, generalized lymphadenopathy, and the presence of clonally related neoplastic T cells with cerebriform nuclei (Sézary cells) in skin, lymph nodes, and peripheral blood. In addition, the absolute Sézary count must be $>1,000/\mu\text{L}$, there must be an expanded CD4 $^+$ T-cell population resulting in CD4/CD8 ratio of more than 10 and/ or loss of one or more T-cell antigens. This is an aggressive disease with less than 20% survival at 5 years. Patients often succumb to opportunistic infections.

Primary Cutaneous CD30 $^+$ T-Cell Lymphoproliferative Disorders

Primary cutaneous CD30 $^+$ T-cell lymphoproliferative disorders (PCALCL) account for 30% of CTCL; types are primary cutaneous anaplastic large lymphoma (C-ALCL), lymphoid papulosis (LyP), and borderline cases (where clear distinction between C-ALCL and LyP is not possible). Although prognosis is favorable, systemic lymphoma may develop, thus warranting an ongoing surveillance. In PCALCL, surgical excision and radiotherapy are most commonly used for solitary tumors, whereas chemotherapy is given for multifocal disease. Ultraviolet (UV) light phototherapy and low-dose methotrexate are commonly used therapies for LyP. There is an interest in using the CD30-directed monoclonal immunoconjugate brentuximab vedotin for these tumors.

Primary Cutaneous Peripheral T-Cell Lymphomas, Rare Subtypes

These include the primary cutaneous $\gamma\delta$ T-cell lymphoma with activated ($\gamma\delta$ T-cells having a cytotoxic phenotype) and the CD8 $^+$ aggressive epidermotropic cytotoxic T-cell lymphoma. Both have an aggressive course. Newly provisionally designated is the primary cutaneous acral CD8 $^+$ T-cell lymphoma originally described as originating in the ear. The CD4 $^+$ small/ medium T-cell types is now referred to as a lymphoproliferative disorder as they resemble clonal drug reactions and have limited clinical risk.

Peripheral T-Cell Lymphoma, Not Otherwise Specified

These account for about 30% of peripheral T-cell tumors in the Western world and are seen mainly in adults, with a male:female ratio of 2:1. Lymph node involvement is typical, but any site can be affected, including peripheral blood. Most patients present with advanced disease and B symptoms. CD30 expression is found in some cases. A variant, lymphoepithelioid (Lennert lymphoma) can be admixed with inflammatory and EBV $^+$ Reed–Sternberg-like cells. The follicular variant can appear similar to nodular lymphocyte-predominant Hodgkin lymphoma while the T-zone variant can be mistaken for benign hyperplasia. Owing to CD30 expression, there is interest in brentuximab vedotin therapeutically.

Angioimmunoblastic T-Cell Lymphoma

Angioimmunoblastic T-cell lymphoma (AITL) occurs in middle age and in the elderly, and accounts for 15% to 20% of peripheral T-cell lymphomas. It is nearly always EBV associated, although the neoplastic cells are EBV⁻. Generally, AITL presents with generalized lymphadenopathy, hepatosplenomegaly, systemic symptoms, and polyclonal hypergammaglobulinemia. Pruritic skin rash is common. Effusions, arthritis, circulating immune complexes, hemolytic anemia, and EBV⁺ B-cell expansion are common. Median survival is <3 years. The associated immune dysfunction renders aggressive chemotherapy administration infeasible in many cases. GEP suggests deregulation of vascular endothelial growth factor as part of the pathogenesis, and has created interest in targeting this pathway therapeutically.

Follicular T-Cell Lymphoma

Many of the genetic changes seen in PTCL, NOS manifest a T follicular helper phenotype to support this new designation.

Nodal Peripheral T-Cell Lymphoma with T Follicular Helper Phenotype

This category was created to highlight the spectrum of nodal lymphomas with a T follicular helper (Tfh) phenotype.

Anaplastic Large Cell Lymphoma, Anaplastic Lymphoma Kinase-Positive

Anaplastic large cell lymphoma (ALCL), ALK⁺, is a CD30⁺ T-cell lymphoma that has a translocation involving the ALK gene and expression of the ALK protein. It is important to distinguish this tumor from primary cutaneous ALCL and other lymphomas with anaplastic features. ALCL, ALK⁺ accounts for about 3% of adult and up to 20% of childhood lymphomas. Most cases present with advanced-stage disease with peripheral and/ or abdominal adenopathy and BM infiltration. High fevers are common. These tumors have a better prognosis than the ALCL, ALK⁻ counterpart. The overall 5-year survival is about 80% with standard CHOP chemotherapy. Brentuximab vedotin is approved for relapsed ALCL.

Anaplastic Large Cell Lymphoma, Anaplastic Lymphoma Kinase-Negative

Morphologically similar to ALCL, ALK⁺ and also CD30⁺, but lacking ALK protein expression, this entity was given a provisional categorization in the 2008 WHO classification system and is now a definite entity in the 2016 update with cytogenetic subsets that have prognostic significance. The ALK⁻ occurs mainly in adults aged more than 40 years, and has a poorer prognosis compared to the ALCL, ALK⁺. Less than 20%

OS at 5 years is expected. Brentuximab vedotin is approved for relapsed ALCL.

Breast Implant-Associated Anaplastic Large-Cell Lymphoma

This is a new provisional entity distinguished from other ALK-ALCL. It is noninvasive and has excellent outcome.

Hodgkin Lymphoma

Hodgkin lymphoma is covered in [Chapter 15](#).

Immunodeficiency-Associated Lymphoproliferative Disorders

Lymphoproliferative Disease Associated with Primary Immune Disorders

The most common lymphoproliferative disease (LPD) in this setting are ataxia telangiectasia, Wiskott–Aldrich syndrome, common variable immunodeficiency (CVID), severe combined immunodeficiency, X-linked lymphoproliferative disorder, Nijmegen breakage syndrome, hyper IgM syndrome, and autoimmune lymphoproliferative syndrome. In addition to LPD, other neoplasms occur at high rates in affected persons. Except for CVID, children are affected most. EBV is frequently involved.

Lymphomas Associated With HIV Infection

DLBCL (including primary brain), Burkitt, PEL, and PBL are AIDS-indicator conditions in those with HIV infection. Since the advent of cART, there has been a marked epidemiologic shift in the occurrence and outcomes of these tumors. Rituximab-based chemotherapy is the standard for DLBCL and Burkitt. Many experts recommend R-EPOCH in this setting. CHOP should not be used for HIV-Burkitt. Primary brain lymphoma (PCNSL) is rarely seen in the cART era. However, delays in diagnosis impair outcome. The standard diagnostic algorithm for HIV-PCNSL established early in the AIDS epidemic is no longer justified. Mass brain lesions should be approached in this population with the same degree of urgency and using the same diagnostic wherewithal as used in the HIV-unrelated setting, especially in patients likely to respond to cART. In particular, empiric antibiotic therapy to assess treatment failure as a means of establishing a malignant diagnosis should be relegated to the history books. When surgical biopsy is not feasible, the cerebrospinal fluid (CSF) should be assessed for EBV by polymerase chain reaction (PCR) in conjunction with fluorodeoxyglucose-positron emission tomography (FDG-PET) of the brain. If both tests are positive, the positive predictive value for lymphoma is 100%, and specific therapy can be commenced. Patients with advanced HIV disease not amenable to treatment because of resistant HIV are becoming rarer with improvement in HIV therapeutics. Thus, if palliative approaches are deemed most appropriate based on the underlying

HIV, it is essential to obtain expert HIV assessment in order to justify this decision.

Posttransplant Lymphoproliferative Disorders

These occur after solid organ or hematopoietic cell transplant. There is a range of pathology from those that are EBV-driven polyclonal proliferations (the majority of cases) to those that are EBV⁺ or EBV⁻ B-and T-cell lymphomas similar to those seen in the immunocompetent setting.

Other Iatrogenic Immunodeficiency-Associated Lymphoproliferative Disorders

These LPD arise in patients treated with immunosuppressive drugs and appear as a spectrum from polymorphic proliferations as seen in PTLD at one end, to DLBCL at the other end of the spectrum. EBV is variably associated depending on the underlying cause for iatrogenic immune suppression and the LPD presentation itself.

CLINICAL MANAGEMENT

Initial Evaluation

- I. Establish correct diagnosis
- II. Diagnostic confirmation by tissue biopsy
 - A. Sufficient material is critical to conduct the studies needed to ensure accurate diagnosis
 - B. Needle biopsies generally yield inadequate tissue for these studies and should be avoided for primary diagnosis
 - C. Important studies for diagnostic confirmation often include the following:
 1. Assessment of clonality
 2. Immunophenotypic, cytogenetic, and molecular studies
 3. Markers of histogenesis (B-versus T-cell origin; in DLBCL, determination of germinal center versus non-germinal center histogenesis are not yet part of standard care, but are increasingly important in developing novel therapeutics)
 4. Oncogene rearrangement can be diagnostically useful
 - MYC, BCL2, and/ or BCL6 in DLBCL
 - t(8;14) or MYC in BL
 - t(14;18) or BCL2 in FL
 - t(2;5) or ALK in ALCL
 - t(11;14) or BCL1 in MCL
 - Trisomy 3 or trisomy 18 (marginal zone lymphoma)
 - In the near future after this publication, tests to determine DLBCL cell of origin (ABC vs. GCB) may be needed for specific targeted therapeutics—

these tests may include IHC (immunohistochemistry) and/ or GEP.

- D. Some tumors (e.g., T-cell-rich B-cell lymphoma or lymphomatoid granulomatosis) have excess reactive T-cells that may obscure the minority of diagnostic malignant B-cells if inadequate tissue is obtained

III. History and physical examination

- Record disease-related symptoms, lymph nodes, and spleen size

IV. Viral testing if indicated by risk or lymphoma subtype

- A. HIV serology in all aggressive NHL
- B. HTLV-1 serology
- C. Hepatitis B and C serology

V. Clinical and laboratory assessment of organ function

- A. Complete blood count with differential
- B. Include CD4 cell count if HIV⁺
 - 1. Routine chemistries for renal and hepatic function
 - 2. Lactate dehydrogenase (LDH; indirect measure of tumor burden and prognosis)
 - 3. Uric acid
 - 4. Serum β_2 microglobulin
 - 5. Serum α -fetoprotein or β -human chorionic gonadotropin in young males with an isolated mediastinal mass where the differential diagnosis includes mediastinal germ cell tumor.

Staging

Pretreatment staging evaluation based on the Lugano recommendations² for systemic NHL follows.

Computed tomography (CT) scans of chest, abdomen, and pelvis, FDG-PET, or PET-CT for FDG-PET avid lymphomas (essentially all histologies except CLL/ SLL, lymphoplasmacytic lymphoma/ Waldenström macroglobulinemia, MF, and marginal zone NHLs, unless there is a suspicion of aggressive transformation)

CT scanning is preferred for non-FDG-PET avid NHLs.

VI. BM biopsy (a positive FDG-PET can designate advanced stage disease). BM biopsy can be considered in DLBCL with no PET evidence of BM involvement, if identification of discordant histology is relevant for patient management, or if the results would alter treatment. BM biopsy remains recommended for staging of other histologies, primarily if it impacts therapy.

VII. Lumbar puncture with cytology should be performed in patients at risk for CNS disease:

- A. DLBCL with elevated LDH and more than one extranodal site and/ or

lymphomatous involvement in the BM

B. All BL

C. Some investigators recommend that all AIDS-related lymphomas (ARL) cases (regardless of BM and extranodal sites or histologic subtype) be evaluated for CNS disease.

The Ann Arbor Staging System, initially developed for patients with Hodgkin lymphoma, is used in NHL. This system does not apply to lymphoblastic leukemia/lymphoma or to MF ([Table 16.6](#)). The Lugano classification uses a modified Ann Arbor Staging: Regardless of stage, general practice is to treat patients based on limited (stages I and II, nonbulky) or advanced (stages III or IV) disease, with stage II bulky disease considered limited or advanced, as determined by histology and a number of other prognostic factors.²

Response Evaluation Following Therapy

At the completion of therapy, repeat all restaging studies. Generally restaging after four cycles is indicated in aggressive lymphomas (repeat all abnormal tests). In indolent lymphomas, response to therapy may be slower; restaging can be performed less frequently. The rate of response may reflect tumor sensitivity to treatment and may have prognostic value. However, early PET in DLBCL has not consistently been shown to predict survival. Other novel imaging methods of interest include diffusion-weighted MRI scanning and may yield similar early response characteristics as FDG-PET.

Table 16.6 The Lugano Staging System for Primary Nodal Lymphomas

Stage	Description	Extranodal Status
Limited		
I	Single lymph node region	Single extranodal lesions without nodal involvement
II	Two or more lymph node regions on the same side of the diaphragm	Stage I or II by nodal extent with limited contiguous extranodal involvement
II Bulky	II as above with “bulky” disease	Not applicable
Advanced		
III	Nodal regions on both sides of the diaphragm or nodal involvement above the diaphragm with spleen involvement	Not applicable
IV	Diffuse or disseminated involvement of one or more extralymphatic organs: bone marrow, liver, brain involvement.	Not applicable

Extent of disease is determined by positron emission tomography–computed tomography for avid

lymphomas and computed tomography for nonavid histologies. Tonsils, Waldeyer's ring, and spleen are considered nodal tissue.

Whether stage II bulky disease is treated as limited or advanced disease may be determined by histology and a number of prognostic factors.

Adapted with permission from Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano Classification. *Journal of Clinical Oncology*. 2014;32(27):3059–3067. Reprinted with permission © 2014 American Society of Clinical Oncology. All rights reserved.

Disease progression or no response implies extremely poor prognosis. Biopsy of residual masses after therapy may be required to determine whether viable tumor is present. Routine PET surveillance is not recommended after completion of treatment restaging has been performed.

Response evaluation following novel biologic and immunologic therapeutics is an evolving science and consensus has not yet been reached. Owing to the sometimes observed inflammatory response after initiating therapy with certain types of treatment, such as checkpoint inhibitors, signs of worsening FDG-PET, or CT may not necessarily reflect disease progression in these settings and expert consultation is required.

PROGNOSTIC FEATURES

Prognostic features are related to disease and the individual patient. Disease-related features include tumor bulk, stage, number of extranodal disease sites, and histologic type and tumor histogenesis (or tumor biology). Patient-related factors include age and performance status, and whether there are comorbid conditions present that may affect the ability to administer therapy.

Prognostic assessment and modeling strategies have been developed to predict the outcome based on clinical presentation. The most commonly used model is the IPI ([Table 16.6](#)). It was initially developed for aggressive NHL, but is applicable or has been adapted to other NHL subtypes. For example ([Table 16.7](#)), the follicular IPI (FLIPI and potentially the m7-FLIPI incorporating assessment of seven gene mutations in the model) and the mantle cell IPI (MIPI) are adaptations of the IPI, and have prognostic value for the respective histologic NHL types ([Table 16.8](#)). In the IPI, 1 point is assigned for each of the following:

- Age
 - For IPI and FLIPI age more than 60 years, 1 point
 - For MIPI, 1 point for age 50 to 59 years, 2 points for age 60 to 69 years, 3 points for age ≥ 70 years

Table 16.7 Follicular and Mantle Cell Prognostic Index

Risk Category	FLIPI		MIPI	
	Score	5-Year OS (%)	Score	5-Year OS (%)
Low	0–1	90	≤3	>60
Intermediate	2	77	4–5	50
High	≥3	52	≥5	15

FLIPI, follicular international prognostic index; MIPI, mantle international prognostic index; OS, overall survival.

Table 16.8 International Prognostic Index for Diffuse Large B-Cell Lymphomas

International Prognostic Index Risk Category	Score	Patients in Risk Group (%)	Complete Responses (%)	5-Year Disease-Free Survival (%)	5-Year Survival (%)
Low	0 or 1	35	87	70	73
Low-intermediate	2	27	67	50	51
High-intermediate	3	22	55	49	43
High	4 or 5	16	44	40	26

- | Eastern Cooperative Oncology Group (ECOG) performance status 2 or more
 - For IPI, 1 point
 - For MIPI, 2 points
- | LDH above normal
 - For IPI and FLIPI, 1 point
 - For MIPI 0.67 to 0.99 1 point; 1.0 to 1.49, 2 points, ≥1.5, 3 points
- | Extranodal sites
 - For IPI, 2 or more, 1 point
 - For FLIPI, more than 4, 1 point
- | Stage III or IV disease
 - For IPI and FLIPI, 1 point
- | Hemoglobin level
 - For FLIPI < 120 g/L, 1 point
- | White blood cells (WBC), 10^6 /L
 - For MIPI 6.7 to 9.999, 1 point; 1.0 to 14,999, 2 points; ≥15,000, 3 points

Based on adding points from the various clinical characteristics, risk can be assigned. In age-adjusted IPI for patients younger than 60 years, 1 point each is assigned for

- | Performance status 2 or more
- | LDH above normal

Stage III or stage IV disease

In ARL, the primary prognostic determinant has traditionally been the CD4 cell count. However, among those whose HIV is sensitive to cART, the IPI and lymphoma-specific features appear to be of relatively greater prognostic importance. Low CD4 cell count alone does not confer poorer outcome to curative intent therapy. However, very low CD4 cell count increases the risk of death due to other AIDS-related causes and unless the patient has an increase in CD4 cells, this risk persists after the successful completion of lymphoma therapy.

TREATMENT PRINCIPLES

Treatment of NHL is guided by clinical behavior, and this is in large part dependent on the specific disease entity. Clinical behavior is generally described as indolent, aggressive, or highly aggressive. Conventional treatment now includes chemotherapy, radiotherapy, immunotherapy, or a combination of these modalities. Novel treatments, including immunoconjugates, immune modulators, and molecularly targeted agents are now in everyday practice or the subject of current investigations. Ongoing clinical research refines how newer treatments are used and combined to augment or supplant the current standards of care.

PRINCIPLES OF THERAPY: INDOLENT B-CELL AND T-CELL LYMPHOMAS

The natural history is one of a relatively slow-growing lymphoma with low potential for cure but with median survival measured in years to decades. However, approximately 20% of the patients fail to achieve CR or have progression of disease within 2 years following initial therapy, and this is associated with a poor prognosis (5-year survival 50%). For disseminated symptomatic disease, many experts recommend single agent rituximab or rituximab combined with CHOP or bendamustine. ASCT increases CRs but not cure. Maintenance rituximab improves PFS but not survival. Other approaches that may also be used in patients with disease progression include fludarabine (combined with rituximab and or mitoxantrone or cyclophosphamide) and yttrium 90 ibritumomab tiuxetan radioimmunotherapy. Novel agents such as lenalidomide with rituximab shows high RRs. Grade 3B lymphomas are often treated differently than the other grades, using therapy as in DLBCL. FL can progress with transformation to a DLBCL, and this nearly always requires treatment. Generally, the prognosis is poor after transformation. See the section on DLBCL for treatment of transformed disease.

Individual prognostic features must be considered in treatment planning. Those with the most favorable prognosis may benefit less from early therapy whereas those with

poorer prognosis may derive greater benefit from early therapy. It is important to consider whether the planned therapy will improve or detract from a patient's well-being. If the toxicity of therapy creates symptoms where there are none, watchful waiting may be a better strategy.

Rituximab as a single agent in previously untreated FL yields up to 75% RRs. Maintenance rituximab may prolong remission (at 3 years of median follow-up, duration of remission was 23 vs. 12 months, favoring rituximab maintenance group receiving 375 mg/ m² every 2 months for four doses post-induction). The RESORT study showed that for low tumor burden FL, after initial rituximab induction, a strategy of waiting until progression to retreat with four cycles of rituximab and then repeating this with each subsequent progression provided the same time to treatment failure (and was not associated with increased patient concern or anxiety), but with four times less rituximab compared with giving rituximab continuously as maintenance until progression.³

As a single agent in a previously treated FL, rituximab can yield responses in 50% to 60% of cases, with median response duration of 6 to 16 months. Rituximab with CHOP induces CRs in up to 95% of previously untreated FLs with a median response duration not reached at 50 months of follow-up. Rituximab combined with fludarabine yields results similar to CHOP plus rituximab (although fludarabine is profoundly immunosuppressive). Rituximab combined with bendamustine has in a randomized study showed superior RR, PFS, and less toxicity compared to CHOP combined with rituximab.⁴ Obinutuzumab provides improved PFS when combined with chemotherapy compared to rituximab with chemotherapy.⁵ Following initial therapy, 2 years of rituximab maintenance may improve PFS but not OS. In addition, some advocate the use of radioimmunotherapy for initial disease, utilizing ¹³¹I-tositumomab. Radioimmunotherapy for relapsed disease includes yttrium 90-ibritumomab tiuxetan (Zevalin), which is FDA-approved and well tolerated. In a randomized trial, Zevalin resulted in statistically and marginally clinically significant higher objective response rate (ORR) and CR but not response duration compared with rituximab alone in relapsed or refractory low-grade, follicular, or transformed B-cell NHL. Tositumomab and iodine-131 tositumomab (Bexxar) are approved by the FDA for the treatment of patients with CD20⁺, follicular NHL, in cases with and without transformation, when disease is refractory to rituximab and has relapsed after chemotherapy. Thus, there are a substantial number of therapeutic choices (Table 16.9).

Indolent stage I B-cell lymphomas may be curable with 10-year disease-free survival (DFS) of approximately 50% with radiation alone. Because of the long natural history, this is a difficult disease to study. For example, a large phase II trial of more than 100 patients was initiated in 1984 but was not completed and published until 2003. A 10-year DFS of 76% was reported, suggesting that combined radiation and chemotherapy

may be superior to radiotherapy alone in stage I and II disease. Large retrospective databases indicate that the observational strategy does not compromise survival compared to early intervention.

CLL/ SLL is discussed in [Chapter 14](#). Treatment options include bendamustine, fludarabine, and rituximab, given concurrently or sequentially. Alemtuzumab is approved for fludarabine-refractory disease, with RRs of approximately 30%. Cladribine may also be used. Novel therapeutics with agents such as PI3K δ inhibitors, lenalidomide plus rituximab, and Bruton tyrosine kinase inhibition are transforming therapeutics for this disease.

For lymphoplasmacytoid lymphoma/ Waldenström macroglobulinemia current initial treatment options include rituximab, ibrutinib, bortezomib, with rituximab, or conventional therapies with alkylating agents (especially chlorambucil), with or without corticosteroids. CHOP is sometimes used as well. Purine analogues such as fludarabine or cladribine are also active. RR to first-line therapy ranges from 38% to 85%. RR to fludarabine in previously treated patients ranges from 30% to 50%. Initial therapy with rituximab has produced overall RRs of 20% to 40%, with risk of IgM paraprotein flare. Preliminary data suggests a role for agents such as alemtuzumab, and bortezomib. There is an interest in thalidomide and analogs, particularly lenalidomide and pomalidomide.

For extranodal mucosal-associated marginal zone (MALT) lymphoma, effective eradication of *H. pylori* infection can result in lymphoma regression and likely cure, a finding that strongly supports this bacterial etiology of the tumor. When associated with autoimmune disease (such as Sjögren syndrome or Hashimoto thyroiditis), chemotherapy with or without rituximab may be useful. Local therapy such as surgery or regional irradiation may yield relatively long-term disease control. Splenectomy may be indicated for splenic marginal zone lymphoma. Cases associated with HCV infection may regress with effective HCV therapy.

Table 16.9 Indolent Lymphoma Treatment

Combination Chemotherapy	Treatment Description
CVP	Cyclophosphamide 400 mg/ m ² PO daily for 5 d, days 1–5 (total dose/ cycle = 2,000 mg/ m ²) Vincristine 1.4 mg/ m ² IV on day 1 (maximum dose/ cycle = 2 mg; total dose/ cycle = 1.4 mg/ m ²) Prednisone 100 mg/ m ² PO daily for 5 d, days 1–5 (total dose/ cycle = 500 mg/ m ²) ■ Treatment is repeated every 21 d
Single Agents	Treatment Description
Fludarabine	Fludarabine 25 mg/ m ² /d IV for 5 d, days 1–5 (total dose/ cycle = 125 mg/ m ²)

	■ Treatment is repeated every 28 d
Bendamustine	Bendamustine 50–60 mg/ m ² days 1–5 every 28 d; 120 mg/ m ² days 1,2 every 21 d
Rituximab (alone or combined with other agents)	Rituximab 375 mg/ m ² IV weekly (total dose/ wk = 375 mg/ m ²)
CVP, cyclophosphamide, vincristine, prednisone; IV, intravenous; PO, orally.	

Principles of Therapy: Aggressive B-Cell Lymphomas

Mantle Cell Lymphoma

Most patients with MCL present with advanced-stage disease. Unlike other aggressive lymphomas, it is incurable and has variable reported median survivals in studies ranging from 5 to more than 12 years. The blastic variant may be more aggressive with a propensity for CNS involvement (25%) and shorter survival. In general, patients younger than 60 years are treated differently from older patients. There may be a survival advantage in younger patients with stage IA or IIA treated with radiation therapy. There is no agreed upon single standard of care. In younger patients with advanced disease,. Induction therapy followed by ASCT has become a standard of care based on improved PFS, but without demonstrated survival benefit. Therapy with rituximab-based CHOP or more intensive regimens such as the hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone) or alternating courses of R-CHOP and R-DHAP (rituximab plus dexamethasone, high-dose cytarabine, and cisplatin) followed by a high-dose cytarabine-containing conditioning regimen and ASCT. Large cooperative group studies generally have not confirmed the more intensive therapy to benefit the general population with MCL as many subjects were unable to complete the planned therapy. Bortezomib is approved for the treatment of relapsed MCL. Molecular targeted therapies, including mTOR inhibitors, and immune modulator drugs (e.g., lenalidomide) are of interest.

Diffuse Large B-Cell Lymphoma

R-CHOP is the standard of care for curative intent in DLBCL (Table 16.10). For localized disease, many experts recommend limited cycles of chemotherapy combined with involved field radiotherapy. The long-awaited randomized phase III trial of R-CHOP versus DA-EPOCH-R failed to show an advantage of the DA-EPOCH-R regimen and since the dose adjustment strategy intentionally increases doses based on limits of tolerated toxicity, the regimen is not recommended as the initial therapy for most cases of DLBCL at the current time.⁶ Many experts continue to recommend DA-EPOCH-R for PMBCL. The version of DA-EPOCH-R for HIV-DLBCL remains the recommended choice in that setting: The doses are adjusted upward and downward by cycle to avoid excess toxicity as well as underdosing. In a meta-analysis utilizing

aggregated individual patient data from a number of clinical trials there was a survival advantage for HIV⁺ patients with DLBCL treated with the EPOCH regimen compared to other regimens.

Novel therapeutics are currently the focus of randomized phase III trials in ABC DLBCL. The addition of ibrutinib or lenalidomide to R-CHOP may become a standard of care in this disease depending on the results of the studies.

Salvage therapy following relapse is most effective for those whose disease remains chemotherapy sensitive. Following treatment with regimens such as rituximab, ifosfamide, carboplatin, and etoposide, ASCT offers curative potential for approximately 50% of patients. However, in the rituximab era, patients relapsing within 12 months after initial R-CHOP therapy appear to represent a particularly poor prognostic group. Median event-free survival following ASCT may be less than 12 months for this group.

Table 16.10 Standard Therapy for Aggressive Non-Hodgkin Lymphoma

Combination Chemotherapy ^a	Treatment Description
R-CHOP	<p>Rituximab 375 mg/ m² IV day 1</p> <p>Cyclophosphamide 750 mg/ m² IV day 1 (total dose/ cycle = 750 mg/ m²)</p> <p>Doxorubicin 50 mg/ m² IV day 1 (total dose/ cycle = 50 mg/ m²)</p> <p>Vincristine 1.4 mg/ m² IV day 1 (maximum dose/ cycle = 2 mg; total dose/ cycle = 1.4 mg/ m²)</p> <p>Prednisone 50 mg/ m²/d PO for 5 d, days 1–5 (total dose/ cycle = 250 mg/ m²)</p> <ul style="list-style-type: none">■ Treatment is repeated every 21 d

^aSee Table 16.11 for possible alternatives.

IV, intravenously; PO, orally; R-CHOP, rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone.

Probability of cure with initial therapy can be estimated using prognostic models, such as the IPI.

For stage I to II disease, three cycles of CHOP plus involved-field radiotherapy results in 5-year PFS of 77% and OS of 82%, better than with eight cycles of CHOP alone (64% and 72%, respectively). However, R-CHOP now is commonly used in early-stage disease without radiation.

In advanced-stage disease, the OS and PFS are approximately 50% and 32%, respectively, at 5 years with CHOP. Randomized trials show that addition of rituximab on day 1 of each cycle of CHOP or CHOP-like cycle resulted in improved event-free survival, PFS, and OS in all patient groups. Maintenance rituximab therapy in DLBCL

shows no clear advantage when rituximab is administered concomitantly with chemotherapy. Possible alternatives to R-CHOP as front-line therapy, or as salvage therapy are listed in [Table 16.11](#).

For HIV-associated DLBCL, an underpowered randomized trial of CHOP versus R-CHOP was reported; there was no rituximab benefit in these patients owing to increased infection-related death. However, excess infection was restricted to those with CD4 cells under 50/ μm^3 ³. Most experts recommend all HIV⁺ patients receive rituximab if curative intent therapy is planned. Antibiotic prophylaxis is recommended for those with low CD4 cell counts. As mentioned earlier, the DA-EPOCH-R regimen that is based on adjusting the dose each cycle to ensure adequate dosing and also avoiding repeated episodes of prolonged neutropenia is the recommended regimen for HIV-related aggressive lymphomas.

TREATMENT PRINCIPLES: HIGHLY AGGRESSIVE B-CELL LYMPHOMA

Burkitt Lymphoma/ B-Cell Acute Lymphoblastic Leukemia

Intensive short duration therapy forms the basic treatment strategy for curative intent ([Table 16.12](#)). Treatment may provoke tumor lysis syndrome and prophylaxis should be used: Alkalinize the urine with D5W plus 100 mEq sodium acetate at 100 to 150 mL/ hr, add allopurinol, 600 mg, orally daily for 2 days, then 300 mg/ day orally until resolution of the tumor lysis syndrome. Aggressive chemotherapy is potentially curative in these tumors. For adults, rituximab is now considered a standard part of the treatment. This has not yet been shown to benefit children. For adults, randomized trials have not informed therapy. The CODOX-M/ IVAC risk-stratified regimen is based on phase II data.^{5,6} The regimen consists of three cycles of CODOX-M for low-risk disease ([Tables 16.13 to 16.15](#)). (All of the following: normal LDH, WHO performance status 0 or 1, Ann Arbor stage I to II, and no tumor mass 10 cm or larger). For high-risk disease (e.g., do not meet criteria for low risk) four cycles of alternating CODOX-M and IVAC are used. An alternative approach is the hyper-CVAD regimen with the addition of rituximab.⁷ In addition, multicenter phase II data support DA-EPOCH-R as effective in BL, but without the more intensive toxicity of the other regimens.⁸ The favorable toxicity profile may make it more preferable to use if a given patient cannot tolerate more established aggressive dose-intensive therapy. However, some experts recommend it for all, especially the low risk patients as they appear to have superior outcome with the DA-EPOCH-R regimen. The regimen is the focus of a nearly completed national clinical trial to define its efficacy in Burkitt and C-MYC-positive DLBCL.

Treatment of Recurrent and Refractory B-Cell Lymphoma

Many patients with NHL require additional therapy because of disease recurrence or refractoriness to therapy. Although grades I and II FL are not curable, high-dose chemotherapy followed by autologous transplant improves PFS. In aggressive NHL, approximately 40% to 50% of patients fail to achieve remission with conventional chemotherapy. Among those who do achieve a CR, 30% to 40% will relapse. These patients may benefit from salvage therapy (see [Table 16.11](#)).³

Table 16.11 Alternative or Salvage Regimens for Aggressive Non-Hodgkin Lymphoma

Combination Chemotherapy	Treatment Description
R-EPOCH (dose adjusted) ^a (See Table 16.16 for HIV ⁺ patients)	Rituximab 375 mg/ m ² IV day 1 Etoposide 50 mg/ m ² /d by continuous IV infusion for 4 d, days 1–4 (total dose/ cycle = 250 mg/ m ²) Oxorubicin 10 mg/ m ² /d by continuous IV infusion for 4 d, days 1–4 (total dose/ cycle = 40 mg/ m ²) Vincristine 4 mg/ m ² /d by continuous IV infusion for 4 d, days 1–4 (total dose/ cycle = 1.6 mg/ m ² [no cap]) Prednisone 60 mg/ m ² per dose PO every 12 hrs for 5 d, days 1–5 (total dose/ cycle = 600 mg/ m ²) Cyclophosphamide 750 mg/ m ² IV day 5 (total dose/ cycle = 750 mg/ m ²) ■ Filgrastim 5 µg/ kg/d SC starting day 6; continues until ANC > 5,000 cells/ mm ³ ■ Treatment is dose adjusted based on neutrophil nadirs and repeated every 21 d
CHOEP ^b	CHOP with etoposide 100 mg/ m ² IV days 1, 2, and 3
R-ICE	Rituximab 375 mg/ m ² IV 48 hrs prior to cycle 1 and on day 1 of cycles one to three Etoposide 100 mg/ m ² IV days 3, 4, and 5 Carboplatin AUC 5: dose = 5 × (25 + creatinine clearance) capped at 800 mg IV on day 4 Ifosfamide 5,000 mg/ m ² mixed with an equal amount of MESNA CIV for 24 hrs on day 4
R-DHAP	Rituximab 375 mg/ m ² IV day 1 Cisplatin 100 mg/ m ² by continuous IV infusion for 24 hrs on day 1 (total dose/ cycle = 100 mg/ m ²) Cytarabine 2,000 mg/ m ² per dose IV over 3 hrs every 12 hrs for 2 doses on day 2 (total dose/ cycle = 4,000 mg/ m ²) Dexamethasone 40 mg/d PO or IV for 4 d, days 1–4 (total dose/ cycle = 160 mg/ m ²) ■ Treatment is repeated every 21–28 d
R-ESHAP	Rituximab 375 mg/ m ² IV day 1 Etoposide 40 mg/ m ² /d over 1 hr IV for 4 d, days 1–4 (total dose/ cycle = 160 mg/ m ²) Methylprednisolone 250–500 mg/d IV for 5 d, days 1–5 (total dose/ cycle = 1,250–

	2,500 mg)
	Cytarabine 2,000 mg/ m ² IV over 2 hrs on day 5 (total dose/ cycle = 2,000 mg/ m ²)
	Cisplatin 25 mg/ m ² /d by continuous IV infusion for 4 d, days 1–4 (total dose/ cycle = 100 mg/ m ²)
	■ Treatment is repeated every 21–28 d
R-ACVBP	Rituximab 375 mg/ m ² IV day 1 Doxorubicin 75 mg/ m ² IV day 1 Cyclophosphamide 1,200 mg/ m ² per dose IV day 1 Vindesine 2 mg/ m ² IV days 1 and 5 Bleomycin 10 mg IV days 1 and 5 Prednisone 60 mg/ m ² /d PO days 1–5 ■ Treatment is repeated every 14 days

^aEtoposide, cyclophosphamide, and doxorubicin dosages may be increased by 20% from the previous cycle's dosage if there is no evidence of absolute neutropenia (ANC < 500/ mm³) or thrombocytopenia (platelet count < 25,000/ mm³).

^bVarious dose escalation schemes reported

ANC, absolute neutrophil count; IV, intravenously; MESNA, 2-mercaptoethane sulfonate; PO, orally; SC, subcutaneously.

Table 16.12 Outcome in Adults Burkitt Lymphoma Patients Treated with DA-EPOCH-R Regimen

Number	PFS (%)	OS (%)
HIV ⁺ : 20	84	83
HIV ⁻ : 57	88	90
Total: 77	87	88

Median follow-up 25 months.

PFS, progression-free survival; OS overall survival.

Adapted from Dunleavy K, Noy A, Abramson JS, et al. Risk-adapted therapy in adults with Burkitt Lymphoma: preliminary report of a multicenter prospective phase II study of DA-EPOCH-R. Blood. 2015;126(23):342–342.

Table 16.13 Estimate of 1-Year Event-Free Survival for Subgroups of High-Risk Patients Treated with CODOX-M/ IVAC Regime for HIV-Unrelated Burkitt Lymphoma

Variable	n	1-Year EFS (%)	(95% CI)	Log-Rank P-Value
International Prognostic Index Score				
0–1	6	83.3	(53.5–99.0)	
2	19	63.2	(41.5–84.9)	
3	14	57.1	(31.2–83.1)	0.8852

CI, confidence interval; EFS, event-free survival.

Data from Mead GM, Sydes MR, Walewski J, et al. An international evaluation of CODOX-M and CODOX-M alternating with IVAC in adult Burkitt's lymphoma: results of United Kingdom Lymphoma Group LY06

study. Ann Oncol. 2002;13:1264–1274.

Table 16.14 CODOX-M Regimen

Day	Drug	Dose	Route	Time
1	Cyclophosphamide	800 mg/ m ²	IV	
	Vincristine	1.5 mg/ m ² (max 2 mg)	IV	
	Doxorubicin	40 mg/ m ²	IV	
	Cytarabine	70 mg	IT	
2–5	Cyclophosphamide	200 mg/ m ²	IV	Daily
3	Cytarabine	70 mg	IT	
8	Vincristine	1.5 mg/ m ² (max 2 mg)	IV	
10	Methotrexate	1,200 mg/ 240 mg/ m ²	IV	Over 1 hr Each hour over 23 hrs
11	Leucovorin	192 mg/ m ² 12 mg/ m ²	IV	At hour 36 Every 6 hrs until MTX level <5 × 10 ⁻⁸ M
13	G-CSF	5 µg/ kg	SC	Daily until AGC > 10 ⁹ / L
15	Methotrexate	12 mg	IT	
16	Leucovorin	15 mg	PO	24 hrs after IT methotrexate

Begin next cycle on the day that unsupported ANC is >1.0 × 10⁹/ L, and unsupported platelet >75 × 10⁹/ L. AGC, absolute granulocyte count; ANC, absolute neutrophil count; G-CSF, granulocyte-colony-stimulating factor; IT, intrathecally; IV, intravenously; MTX, methotrexate; PO, orally; SC, subcutaneously.

Table 16.15 IVAC Regimen

Day	Drug	Dose	Method	Time
1–5	Etoposide	60 mg/ m ²	IV	Daily over 1 hr
	Ifosfamide	1,500 mg/ m ²	IV	Daily over 1 hr
	Mesna	360 mg/ m ² (mixed with ifosfamide) then 360 mg/ m ²	IV	3 hourly (7 doses/ 24 hrs period)
1, 2	Cytarabine	2 g/ m ²	IV	Over 3 hrs, 12 hourly (total of 4 doses)
5	Methotrexate	12 mg	IT	
6	Leucovorin	15 mg	PO	24 hrs after IT MTX
7	G-CSF	5 µ/ kg	SC	Daily until AGC > 1.0 × 10 ⁹ / L

Begin next cycle (CODOX-M) on the day the unsupported ANC is >1.0 × 10⁹/ L, and unsupported platelet >75 × 10⁹/ L

AGC, absolute granulocyte count; ANC, absolute neutrophil count; G-CSF, granulocyte-colony-stimulating factor; IT, intrathecally; IV, intravenously; IVAC, ifosfamide, cytarabine, methtrexate, mesna; MESNA, 2-mercaptopethane sulfonate; MTX, methotrexate; PO, orally; SC, subcutaneously.

Principles of Salvage Therapy

Salvage therapy to induce response following relapse can successfully reduce tumor burden sufficiently so that subsequent high-dose chemotherapy and ASCT can confer substantial clinical benefit. Salvage regimens such as R-DHAP, R-EHAP (rituximab, etoposide, methyl prednisolone, cytarabine, and cisplatin), and R-ICE (ifosfamide, carboplatin, and etoposide) are all effective and the choice is made based on individual patient features. Addition of targeted agents such as ibrutinib for (for ABC type DLBCL) to such regimens may render these salvage regimens more effective and thus, benefit more patients with relapsed disease.

High-dose chemotherapy and ASCT may confer curative advantage in some patients whose disease is responsive to salvage chemotherapy. ASCT achieves long-term survival in up to 50% of patients with chemotherapy-sensitive relapsed DLBCL, and some prospective randomized studies have documented the superiority of ASCT over salvage chemotherapy for relapsed DLBCL. Patients with low-risk IPI are most likely to benefit. However, in the rituximab era, those that do relapse following rituximab-inclusive therapy appear to be selected for the poorest-prognosis patients, and salvage is less successful in many cases owing to this.

Allogeneic transplantation remains investigational. Nonmyeloablative or reduced intensity stem cell transplantation (RIST) attempts to exert immunologic effects against the tumor without the risk of high-dose chemotherapy. High-dose chemotherapy does not appear to overcome tumor resistance in the majority of cases. Graft engineering to enhance graft-versus-lymphoma benefit and to decrease graft-versus-host complications remains an active area of investigation. Studies have not consistently shown strong graft-versus-lymphoma effects in the majority of patients.

Patients with well-controlled HIV with relapsed NHL should not be routinely excluded from consideration for ASCT.

AIDS-Related Lymphoma (Systemic) Treatment Considerations

In the current era of effective cART for HIV infection, most HIV-infected patients with lymphoma should be treated similar to their HIV-unrelated counterparts. One exception is that some experts recommend CNS prophylaxis for all systemic ARL ([Table 16.16](#)).⁹ It is preferable to continue cART during chemotherapy unless toxicity management requires temporary suspension of concomitant medications (e.g., in the setting of severe liver toxicity). Preferred cART regimens when administering lymphoma therapy are those without substantial drug-drug interactions or overlapping clinical toxicities. It is

best to once-daily combination pills because they contain pharmacokinetic boosters. Avoiding protease-inhibitor-containing regimens is also advised. Current regimens of choice include integrase strand transfer inhibitors such as and a combination nucleoside analogs such as dolutegravir/ abacavir/ lamivudine (only for patients who are HLA-B*5701 negative), or dolutegravir plus either tenofovir disoproxil fumarate/ emtricitabine or tenofovir alafenamide/ emtricitabine. These regimens are well tolerated and tend to have less drug-drug interactions than others.

Table 16.16 Dose-Adjusted EPOCH for ARL

Etoposide	50 mg/ m ² /d CIV days 1–4 (total dose/ cycle = 250 mg/ m ²)		
Doxorubicin	10 mg/ m ² /d CIV days 1–4 (total dose/ cycle = 40 mg/ m ²)		
Vincristine)	4 mg/ m ² /d CIV days 1–4 (total dose/ cycle = 1.6 mg/ m ² (no cap))		
Prednisone	60 mg/ m ² PO daily, days 1–5		
Cyclophosphamide	Cycle 1 dependent on CD4 cell count		
	CD4/ mm ³	<100	187 mg/ m ² IV on day 5 (Note: the authors recommend starting at 375 mg/ m ² if no other AIDS complications and good PFS)
		≥100	375 mg/ m ² IV on day 5
	Cycles 2 and beyond dependent on ANC nadir		
	ANC nadir	<500	Decrease dose by 187 mg/ m ²
		≥500	Increase dose by 187 mg/ m ² (maximum dose is 750 mg/ m ²)
Filgrastim	300 µg/d SC starting day 6; continues until ANC > 5,000 cells/ mm ³ Treatment is repeated every 21 d		
Rituximab 375 mg/ m ² given on day 1 before EPOCH infusion may increase efficacy cART suspended until completion of all EPOCH cycles in original phase II trial; cART continued in randomized multicenter phase II trial. PCP prophylaxis for all patients and continues until CD4 > 200 cells/ mm ³ MAC prophylaxis for all patients with CD4 < 100 cells/ mm ³			

ANC, absolute neutrophil count; ARL, AIDS-related lymphomas; CD, cluster designation; CIV, continuous intravenous infusion; IV, intravenous; MAC, mycobacterium complex; PCP, pneumocystis jiroveci pneumonia; PO, orally; SC, subcutaneous.

Adapted from Little RF, Pittaluga S, Grant N, et al. Highly effective treatment of acquired immunodeficiency syndrome related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. *Blood*. 2003;101:4653–4659.

In specific circumstances a case can be made to temporarily suspend cART. For example, a patient with Burkitt and tumor lysis syndrome should be started on definitive Burkitt therapy and cART can wait until the patient's physiological condition permits

consideration of administering lifelong chronic disease HIV therapy.

T-Cell Lymphoma: Treatment Principles

T-cell lymphomas tend to have a poorer PFS and OS than aggressive B-cell lymphomas. Systemic ALCL is an exception and is among the most curable subtypes with doxorubicin-based treatment. Brentuximab vedotin is approved by the FDA for ALCL that has relapsed following a prior multiagent treatment regimen. Some T-cell subtypes have no potential for cure and should be approached palliatively, as in ATL and primary cutaneous anaplastic lymphoma. Other T-cell subtypes, including angioimmunoblastic and PTL, have low curative potential with conventional dose treatment and should be considered for trials targeting high-risk patients.

Corticosteroids, alkylating agents, and bexarotene are available for topical use and are frequently utilized. In addition to topical therapies, UV radiation, either UVA or UVB, and total skin electron beam therapy (TSEBT) are utilized in limited-stage CTCL. Psoralen combined with UVA (PUVA) is associated with a CR rate exceeding 90%, and a prolonged disease-free interval, in early-stage disease. For advanced-stage disease, bexarotene, denileukin difitox, and more recently histone deacetylase inhibitors (vorinostat and romidepsin are FDA approved for this indication in previously treated patients) all have activity. Systemic multiagent chemotherapy is of limited use and reserved primarily for patients with advanced disease which has recurred and are not responding following other interventions.

Approval of histone deacetylase inhibitors for previously treated CTCL has advanced systemic therapeutic approaches. Both vorinostat and romidepsin are agents in this class with activity in CTCL.

There are current early clinical trials findings that checkpoint inhibitors are active at least in some subsets of T-cell lymphomas and this is an area of active investigation.

Adult T-Cell Leukemia/ Lymphoma

ATLL cannot be cured. Initial therapy with CHOP-like regimens is recommended. Sequential combination chemotherapy followed by zidovudine with IFN- α may improve survival.

References

1. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375–2390.
2. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano Classification. *Journal of Clinical Oncology*.

2014;32(27):3059–3067.

3. Kahl BS, Hong F, Williams ME, et al. Rituximab extended schedule or re-treatment trial for low-tumor burden follicular lymphoma: eastern cooperative oncology group protocol e4402. *J Clin Oncol*. 2014;32(28):3096–3102.
4. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. *Lancet*. 2013;381(9873):1203–1210.
5. Marcus RE, Davies AJ, Ando K, et al. Obinutuzumab-based induction and maintenance prolongs progression-free survival (PFS) in patients with previously untreated follicular lymphoma: primary results of the randomized phase 3 GALLIUM study. *Blood*. 2016;128(22):6.
6. Wilson WH, sin-Ho J, Pitcher BN, et al. Phase III randomized study of R-CHOP versus DA-EPOCH-R and molecular analysis of untreated diffuse large B-Cell lymphoma: CALGB/ Alliance 50303. *Blood*. 2016;128(22):469.
7. Mead GM, Sydes MR, Walewski J, et al. An international evaluation of CODOX-M and CODOX-M alternating with IVAC in adult Burkitt's lymphoma: results of United Kingdom Lymphoma Group LY06 study. *Ann Oncol*. 2002;13(8):1264–1274.
8. Dunleavy K, Noy A, Abramson JS, et al. Risk-adapted therapy in adults with Burkitt Lymphoma: preliminary report of a multicenter prospective phase II study of DA-EPOCH-R. *Blood*. 2015;126(23):342.
9. Little RF, Pittaluga S, Grant N, et al. Highly effective treatment of acquired immunodeficiency syndrome related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. *Blood*. 2003;101:4653–4659.

Multiple Myeloma

Neha Korde, Sham Mailankody, Dickran Kazandjian, and Ola Landgren

EPIDEMIOLOGY

In the United States, an estimated 30,000 patients were diagnosed with multiple myeloma (MM) in 2016.¹ According to the Surveillance, Epidemiology, and Ends Results program (SEER), the US prevalence count was 51,930 in 2003² and increased to 95,688 in 2013.¹ The increasing prevalence is likely due to improved diagnostic laboratory, imaging-based testing, and available therapeutics.³ Accordingly, death rates from MM have declined at a rate of 0.8% per year from 2004 to 2013 largely attributable to the availability of novel therapeutics.¹

Etiologic risk factors include older age, male gender, a family history of MM, and non-Hispanic African origin.^{4,5} The median age at diagnosis is 69 years with the highest incidence between 65 and 74 years.¹ MM is extremely rare in those <30 years of age with a reported frequency of 0.02% to 0.3%.^{6,7} Compared to European Americans, African Americans have over a twofold increased risk of MM, earlier onset of disease, and improved survival.^{8–10} The exact putative cause of MM remains elusive, but environmental risk factors, such as pesticides, including agent orange and post–World Trade Center exposures, have been linked to an increased incidence of MM.^{11–14} Despite complex disease biology, survival rates in patients with MM have improved during the past 15 years across multiple age groups with a 5-year survival estimate of 48.5% (2006 to 2012).^{1,15}

DIAGNOSTIC CRITERIA OF MULTIPLE MYELOMA AND RELATED PLASMA CELL DISORDERS

Before 2014, consensus guidelines stipulated that end-organ damage in the form of

“CRAB” criteria (hypercalcemia, renal insufficiency, anemia, and bone disease) should be present to fulfill MM diagnosis and warrant initiation of systemic therapy. In 2014, the International Myeloma Working Group (IMWG) recognized the clinical benefit in treating MM patients before symptoms occurred, and updated the MM diagnostic criteria beyond CRAB criteria to include three additional biomarkers to the category of myeloma-defining events: bone marrow plasmacytosis $\geq 60\%$, involved/ unininvolved serum-free light chain ratio ≥ 100 with an involved serum-free light chain $>100 \text{ mg/L}$, or >1 focal lesion identified on magnetic resonance imaging (MRI).¹⁶ Each of these markers confers approximately an 80% risk of progression to symptomatic MM within 2 years.

MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA

MM is consistently preceded by asymptomatic precursor disease states, either monoclonal gammopathy of unknown significance (MGUS) or smoldering MM (SMM) (Table 17.1).¹⁷ The annual risk of transformation from MGUS to MM is 0.5% to 1%^{18–20} per year and from SMM to MM is 10% for the first year, declining thereafter.²¹ Most of the MGUS and SMM patients do not progress to MM, and clinical management relies on routine monitoring with history, physical examination, and laboratory testing (Table 17.2).²²

In risk models using available biomarkers, high-risk SMM patients have an increased risk of progression, 72% to 76% at 5 years.^{23,24} A randomized phase III clinical trial by Mateos and colleagues compared treatment with lenalidomide/ dexamethasone versus no treatment in 119 high-risk SMM patients and found that patients receiving lenalidomide/ dexamethasone had a delayed time to progression (median not reached vs. 21 months; hazard ratio [HR] for progression = 0.18 [$P < .001$]) and improved 3-year overall survival (OS) rates (94% vs. 80%; HR for death = 0.31 [$P = .03$]).²⁵ The ongoing clinical trials are confirming these results, and current consensus recommendations are to offer high-risk SMM patients treatment solely on clinical interventional trials.

MULTIPLE MYELOMA

Pathophysiology and Genetics

Based on key landmark studies, a number of recurrent genetic mutations have been identified in MM, including KRAS, NRAS, TP53, BRAF, and others.^{26,27} Importantly, an individual’s MM disease can be clonally heterogeneous and made up of different clonal subset populations with varying compositions of mutations.²⁸ As stated earlier,

MM arises from MGUS and SMM; however, based on smaller studies, there are no single identifiable mutations associated with the progression of early precursor disease to symptomatic MM.^{29,30} In a prospective study, in which high-risk SMM patients received carfilzomib-based treatment, Mailankody and colleagues performed whole exome sequencing on baseline pretreatment CD138 bone marrow plasma cell samples from high-risk SMM and newly diagnosed MM patients; they found no difference in the frequency of mutations but different types of mutations or patterns involved.³¹ Further studies need to be performed to confirm these findings.

Initial Evaluation

- History and physical examination
- Complete blood count with differential
- Serum blood urea nitrogen (BUN)/ creatinine, electrolytes, albumin, and calcium
- Serum lactate dehydrogenase (LDH) and beta-2-microglobulin
- Serum quantitative immunoglobulins, protein electrophoresis with immunofixation, and free light chain assay
- Twenty 4-hour urine for total protein and protein electrophoresis with immunofixation
- Skeletal survey or fluorodeoxyglucose (FDG)-positron emission tomography (PET) computed tomography (CT) scan or whole-body MRI or whole-body low-dose CT scan
- Unilateral bone marrow aspirate and biopsy with immunohistochemistry, cytogenetics, or fluorescence in situ hybridization (FISH) (evaluating for hyperdiploidy of odd-numbered chromosomes, del 13, del 17p, t(4;14), t(14;16), t(11;14), and 1q21 amplification), and flow cytometry if available (evaluating aberrant plasma cell markers)
- Possibly useful: Bone densitometry, tissue biopsy of plasmacytoma, serum viscosity, plasma cell proliferation, Congo red and amyloidosis workup, heavy and/ or light chain rearrangement for B-cell clonality, human leukocyte antigen (HLA) typing.

Table 17.1 2014 IMWG Diagnostic Criteria for MM and Related Plasma Cell Disorders

Disease	Definition	Clinical Notes
MGUS— Non-IgM	<ul style="list-style-type: none"> ▪ IgG or IgA M-protein < 30 g/ L, and ▪ BMPC < 10%, and ▪ Absence of MDE or amyloidosis 	<ul style="list-style-type: none"> ▪ Progression risk to MM, amyloid, or plasmacytoma is 0.5%–1.0%/ y
MGUS— IgM	<ul style="list-style-type: none"> ▪ IgM M-protein < 30 g/ L, and ▪ Bone marrow lymphoplasmacytic cells <10%, and ▪ Absence of end-organ damage (anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, hepatosplenomegaly) or MDE or amyloidosis 	<ul style="list-style-type: none"> ▪ Progression risk to Waldenstrom's or lymphoproliferative disorder is 1.5%/ y

MGUS— light chain	<ul style="list-style-type: none"> ■ Abnormal FLC ratio (<0.26 or >1.65) with an increased involved corresponding serum FLC, and ■ No immunoglobulin heavy chain expression, and ■ Urinary monoclonal protein < 500 mg/ 24 hr, and ■ BMPC < 10%, and ■ Absence of MDE or amyloidosis 	<ul style="list-style-type: none"> ■ Progression risk to MM, amyloid, or plasmacytoma is 0.3/ y
Smoldering multiple myeloma	<ul style="list-style-type: none"> ■ IgG or IgA M-protein \geq 30 g/ L^a, or ■ BMPC \geq 10%, and ■ Absence of MDE or amyloidosis 	<ul style="list-style-type: none"> ■ Progression risk to MM, amyloid, or plasmacytoma is 10%/ year \times 5 years, then 3%/ year \times next 5 years, and 1%/ year thereafter
Multiple myeloma	<ul style="list-style-type: none"> ■ Bone marrow or biopsy proven plasmacytoma consisting of \geq10% plasma cells, and ■ MDE attributed to plasma cell disorder: <ul style="list-style-type: none"> ■ Hypercalcemia— calcium > 0.25 mmol/ L (>1 mg/ dL) higher than upper normal limit or >2.75 mmol/ L (11 mg/ dL) ■ Anemia— hemoglobin > 20 g/ L below normal limit or < 100 g/ L ■ Bone lesions— \geq1 or more osteolytic lesions on X-rays, CT, PET-CT ■ BMPC \geq 60% ■ Involved/ unininvolved serum FLC ratio \geq 100 with involved serum-free light \geq 100 mg/ L ■ \geq2 focal lesions on MRI of spine 	<ul style="list-style-type: none"> ■ Must fulfill at least 1 MDE and should be related to plasma cell disorder
Solitary plasmacytoma	<ul style="list-style-type: none"> ■ Biopsy proven bone or extra-medullary lesion with clonal plasma cells, and ■ BMPC < 10%, and ■ Normal skeletal survey, MRI, or CT (except for the solitary lesion), and ■ Absence of MDE or amyloidosis or lymphoproliferative disorder 	<ul style="list-style-type: none"> ■ Three-year risk of MM progression— bone 60% and extramedullary 20%
POEMS syndrome	<ul style="list-style-type: none"> ■ Polyneuropathy, and ■ Monoclonal plasma cell disorder (usually lambda), and ■ Major criteria (fulfill at least 1): <ul style="list-style-type: none"> ■ Sclerotic bone lesions ■ Castleman's disease ■ Elevated levels of VEGF ■ Minor criteria (fulfill at least 1): <ul style="list-style-type: none"> ■ Organomegaly ■ Extravascular volume overload ■ Endocrinopathy ■ Skin changes ■ Papilledema ■ Thrombocytosis/ polycythemia 	

Systemic AL amyloidosis	<ul style="list-style-type: none"> ■ Organ involvement— renal, liver, GI, heart or peripheral nerve, and ■ Positive amyloid staining by Congo red in any tissue, and ■ Amyloid is light-chain related (established by electron microscopy or mass spectrometry), and ■ Monoclonal plasma cell disorder (either by serum or urine electrophoresis, BMPC, or abnormal serum FLCs)
-------------------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

^aExtremely rare, MM has been associated with an IgD, IgE, or IgM monoclonal protein isotype.

BMPC, bone marrow plasma cells; CT, computed tomography; FLC, free light chain; Ig, immunoglobulin; IMWG, International Myeloma Working Group; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; MDE, myeloma-defining events; M-protein, monoclonal protein; VEGF, vascular endothelial growth factor.

From Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15(12):e538–e548.

Table 17.2 MGUS and SMM Management

Disease	General Risk of Progression	Risk Models	Clinical Management
MGUS	0.5–1.0%/ y	Mayo 20-y progression risk Low (0/ 3 RF)— 5% Intermediate (1–2/ 3 RF)— 21%–37% High (3/ 3 RF)— 58% Spanish PETHEMA 5-y progression risk Low (0/ 2 RF)— 2% Intermediate (1/ 2 RF)— 10% High (2/ 2 RF)— 46%	H&P with labs every 6 mo × 1 y, and then consider follow-up every 1–2 y after
SMM	10%/ y first 5 y, and 3%/ y next 5 y, and 1%/ y thereafter	Mayo 5-y progression risk Low (1/ 3 RF)— 25% Intermediate (2/ 3 RF)— 51% High (3/ 3 RF)— 76% PETHEMA 5-y progression risk Low (0/ 2 RF)— 4% Intermediate (1/ 2 RF)— 46% High (2/ 2 RF)— 72%	H&P with labs every 3 mo × 1 y, and then consider follow-up every 4–6 mo Offer clinical trials to high-risk SMM

Mayo MGUS— risk factors: M-protein ≥ 1.5 g/ dL, non-IgG MGUS, free light chain ratio < 0.26 or > 1.65 ; Spanish PETHEMA (Programa Español de Tratamientos en Hematología)— risk factors: $\geq 95\%$ abnormal/ normal plasma cell ratio, DNA aneuploidy; Mayo SMM— risk factors: 3 g/ dL, BMPC $\geq 10\%$, FLC ratio < 0.125 or > 8 ; Spanish PETHEMA— risk factors: $\geq 95\%$ abnormal/ normal plasma cell ratio, immunoparesis.

MGUS, monoclonal gammopathy of unknown significance; SMM, smoldering multiple myeloma.

Staging

In 2005, the International Staging System (ISS) defined three stages of MM: stage I, $\beta 2$ -microglobulin < 3.5 mg/ L and albumin ≥ 3.5 g/ dL; stage II, neither stage I/ III; and

stage III, β 2-microglobulin $> 5.5 \text{ mg/L}$ and albumin any level.³² The ISS was updated in 2015 to formulate the “Revised International Staging System (R-ISS),” after data analysis from more than 4,000 patients and 11 trials, including mostly novel agent regimens (Table 17.3).³³ The R-ISS incorporated FISH abnormalities and LDH parameters into the already-existing ISS stages.

Response Criteria

Over the past two decades, the emergence of highly effective novel antimyeloma therapies has resulted in higher rates of deep responses, defined as complete response (CR)/ stringent CR (sCR) and minimal residual disease (MRD) negativity.³⁴ Standard International Myeloma Workshop Consensus Panel³⁵ response categories include progressive disease (PD), stable disease (SD), partial response (PR), very good partial response (VGPR), CR, and sCR. However, the limits of conventional disease markers, such as serum and urine immunofixation/ electrophoresis, have challenged the ability to precisely quantify the depth of response. An effort to further characterize MRD in MM has yielded two main methodological approaches, multiparametric flow cytometry (MFC) and next-generation molecular sequencing (NGS; Table 17.4).³⁶ The MFC method (two 8-color tubes or one 10-color tube) uses a gating strategy on bone marrow mononuclear cells to identify a clonal aberrant plasma cell population (minimum requirement of >5 million cells to achieve a sensitivity detection of ≥ 1 in 10^5 cells).³⁶ The NGS technique relies on baseline sequencing (before treatment) of the clonal plasma cell immunoglobulin gene locus of interest (immunoglobulin heavy chain gene [IGH] and immunoglobulin k light chain gene [IGK] rearrangements), and detection of the tumor-specific immunoglobulin gene sequence (the unique VDJ recombination) of interest in subsequent treated samples (a validated assay with a sensitivity detection of at least ≥ 1 in 10^5 cells is required; commercially available assays from Adaptive Biotechnologies and Invivoscribe provide a sensitivity detection of ≥ 1 in 10^6 cells). It is anticipated that NGS platforms will continue to evolve and perhaps become the standard of care for monitoring. Currently, both are accepted practices at evaluating MRD, and both techniques have been validated across multiple treatment platforms where reaching MRD negativity has been associated with significant improvement in clinical outcomes.^{37–40} Two independent meta-analysis studies demonstrated that MRD negativity was associated with favorable progression-free survival (PFS) (HR = 0.41–0.35) and OS (0.57 to 0.48).^{41,42}

Table 17.3 Revised International Staging System (R-ISS)

R-ISS	Features	5-Y Overall Survival Rates (%)
R-ISS	ISS stage I ^a	82

Stage I	No high-risk CA ^b Normal LDH	
R-ISS Stage II	Not R-ISS stage I or III ^a	62
R-ISS Stage II	ISS stage III ^a , and either of the following: High-risk CA ^b or Elevated LDH ^c	40

^aISS: stage I, β2-microglobulin < 3.5 mg/ L and albumin ≥ 3.5 g/ dL; stage II, neither stage I/ III; and stage III, β2-microglobulin > 5.5 mg/ L and albumin any level.

^bHigh risk defined as del (17p), t(4;14), or t(14;16).

^cGreater than the laboratory upper limit of normal.

CA, chromosomal abnormalities using interphase fluorescence in situ hybridization; ISS, International Staging System; LDH, lactate dehydrogenase.

From Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised international staging system for multiple myeloma: a report from international myeloma working group. J Clin Oncol. 2015;33(26):2863–2869.

Approach to Newly Diagnosed Multiple Myeloma

Once a diagnosis of MM has been established, the general approach to management involves considering medical and psychosocial factors when selecting an appropriate treatment regimen for the individual patient. All patients should be assessed for autologous stem cell transplant (ASCT) candidacy in the early stages of treatment regimen selection. In the United States, age is not a sole determinant of transplant eligibility, but rather fitness, frailty, comorbidities, desire to undergo transplant, and support networks are important factors. For transplant-eligible patients, it is generally recommended that stem cell collection occur after four cycles of initial therapy due to the potential stem cell toxicity that may be associated with continuous exposure to certain classes of therapy (lenalidomide).⁴³ A number of primary regimens from the National Comprehensive Cancer Network (NCCN) have been established for newly diagnosed MM ([Table 17.5](#)) in both transplant and nontransplant settings.⁴⁴ Importantly, several studies have shown the benefit of three-drug regimens compared with two-drug regimens when considering improved responses, clinical end points, and lack of toxicity in both treatment-naïve and relapsed/ refractory patients.^{45–47} For unfit and frail patients, clinicians can consider dose reductions of the three-or two-drug regimens if deemed clinically appropriate. When selecting an initial regimen, the number of drugs is not the only critical decision; rather, current comorbidities, ease of administration, availability in the country, and the balance between efficacy and toxicity must be taken into consideration. For initial treatment, the NCCN panel recommends novel agents, including bortezomib, lenalidomide, and cyclophosphamide over older drug choices (melphalan, thalidomide, vincristine, doxorubicin) where data is outdated.⁴⁴ For

instance, in the Frontline Investigation of Revlimid and Dexamethasone versus Standard Thalidomide (FIRST) trial ($n = 1,623$ patients), continuous lenalidomide/dexamethasone compared to lenalidomide/ dexamethasone $\times 18$ cycles and melphalan/thalidomide/ prednisone showed superiority in PFS (25.5 months vs. 20.7 months vs. 21.2 months, HR = 0.72 and 0.70, respectively) and 4-year OS(59% vs. 56% vs. 51%, respectively).⁴⁸

Table 17.4 IMWG Response Criteria

Standard Response Categories

Clinical relapse	<ul style="list-style-type: none"> ▪ Any one or more of following criteria: <ul style="list-style-type: none"> ▪ Worsening disease and/ or end-organ dysfunction related to plasma cell neoplasm ▪ Hypercalcemia (11 mg/ dL) ▪ Decrease in hemoglobin ≥ 2 g/ dL ▪ Rise in serum creatinine by 2 mg/ dL or more from the start of the therapy ▪ Hyperviscosity
Relapse from complete response	<ul style="list-style-type: none"> ▪ Any one or more of following criteria: <ul style="list-style-type: none"> ▪ Reappearance of serum or urine M-protein by immunofixation or electrophoresis ▪ Development of $\geq 5\%$ clonal plasma cells ▪ Signs of clinical relapse
Relapse from MRD negative	<ul style="list-style-type: none"> ▪ Any one or more of following criteria: <ul style="list-style-type: none"> ▪ Loss of MRD negative state (evidence of clonal plasma cells on MFC or NGS, or positive imaging study) ▪ Reappearance of serum or urine M-protein by immunofixation or electrophoresis ▪ Development of $\geq 5\%$ clonal plasma cells ▪ Signs of clinical relapse
Progressive disease	<ul style="list-style-type: none"> ▪ Any one or more of following criteria: <ul style="list-style-type: none"> ▪ Increase of 25% from lowest confirmed response value in one or more of the following: <ul style="list-style-type: none"> ▪ Serum M-protein (absolute increase must be ≥ 0.5 g/ dL) ▪ Serum M-protein increase ≥ 1 g/ dL (if the lowest M component was ≥ 5 g/ dL) ▪ Urine M-protein (absolute increase must be ≥ 200 mg/ 24 hr) ▪ Patients without M-protein: <ul style="list-style-type: none"> ▪ Difference between involved and unininvolved FLC levels (absolute increase must be ≥ 10 mg/ dL) ▪ Patients without M-protein and involved FLC levels: <ul style="list-style-type: none"> ▪ Bone marrow cell percentage irrespective of baseline status (absolute increase must be $\geq 10\%$) ▪ Appearance of new lesion ▪ $\geq 50\%$ increase from nadir SPD of >1 lesion ▪ $\geq 50\%$ increase in the longest diameter of a previous lesion in >1 cm in short axis ▪ $\geq 50\%$ increase in 50% circulating plasma cells (minimum 200 μL) if this is the only measurable disease
Stable disease	<ul style="list-style-type: none"> ▪ Not meeting criteria for complete response, very good partial response, minimal response, partial response, or progressive disease
Minimal response	<ul style="list-style-type: none"> ▪ Serum: $\geq 25\%$ but $\leq 49\%$ reduction of serum M-protein ▪ Urine: $\geq 50\%$ but $\leq 89\%$ reduction of urine M-protein ▪ If present at baseline, $\geq 50\%$ reduction of soft tissue plasmacytomas

Partial response	<ul style="list-style-type: none"> ■ Serum: ≥50% but ≤89% reduction of serum M-protein ■ Urine: ≥90% reduction of urine M-protein or <200 mg/ 24 hr ■ If serum or urine are unmeasurable, ≥50% reduction in difference of involved and uninvolved FLC ■ If serum, urine, and FLC are unmeasurable, ≥50% reduction in bone marrow plasma cell percentage (if baseline ≥30%) ■ If present at baseline, ≥50% reduction of soft tissue plasmacytomas
Very good partial response	<ul style="list-style-type: none"> ■ Serum and urine detectable by immunofixation but not electrophoresis or ≥90% reduction in serum M-protein and urine M-protein <100 mg/ 24 hr
Complete response	<ul style="list-style-type: none"> ■ Negative immunofixation of serum and urine ■ Disappearance of any soft tissue plasmacytomas ■ <5% bone marrow plasma cells
Stringent complete response	<ul style="list-style-type: none"> ■ Complete aforementioned response definition, and ■ Normal FLC ratio ■ Absence of clonal plasma cells by immunohistochemistry or flow cytometry (K/ L ratio ≤4:1 or ≥1:2 for kappa and lambda patients, respectively, after counting 100 cells)

Minimal Residual Disease Categories

Flow "MFC" MRD negative	<ul style="list-style-type: none"> ■ Absence of detectable phenotypic abnormal clonal plasma cells by NGF on bone marrow aspirate using EuroFlow or equivalent standard with minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing "NGS" MRD negative	<ul style="list-style-type: none"> ■ Absence of plasma cell clonal signature in which presence of clone is <2 identical sequencing reads obtained after sequencing bone marrow aspirate with a platform (commercial or otherwise) minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging plus MRD negative	<ul style="list-style-type: none"> ■ MRD negativity by MFC or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/ CT or decrease to less than mediastinal blood pool SUV or decrease less than surrounding normal tissue
Sustained MRD-negative	<ul style="list-style-type: none"> ■ MRD negativity in marrow (by NGS and/ or MFC) and by imaging, confirmed minimum of 1 y apart

FLC, free light chain; IMWG, International Myeloma Working Group; M-protein, monoclonal protein; MFC, multiparametric flow cytometry; MRD, minimal residual disease; NGS, next-generation sequencing; SPD, sum of perpendicular diameters; NGF, next generation flow cytometry.

Data from Rajkumar SV, Harousseau JL, Durie B, et al.; International Myeloma Workshop Consensus Panel 1. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. *Blood*. 2011;117(18):4691–4695 and Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016;17(8):e328–e346.

Bortezomib

Bortezomib is a first-generation reversible proteasome inhibitor that interrupts the cell's ability to degrade protein products, resulting in myeloma tumor death. Bortezomib can be administered weekly (starting dose = 1.5 mg/ m^2) or twice-weekly (starting dose = 1.3 mg/ m^2) dosing schedules as intravenous or subcutaneous injections. Common side effects include peripheral neuropathy, gastrointestinal toxicity, shingles reactivation, and thrombocytopenia. Studies demonstrate that weekly and subcutaneous dosing of

bortezomib yield lower peripheral neuropathy rates, allowing for increased tolerability and noninferior efficacy results.^{49,50} The inclusion of bortezomib in combination with other agents for newly diagnosed MM has been well established and particularly favored in patients with high-risk genetic mutations and disease-associated renal insufficiency.^{51–54} More recently, the triplet regimen of bortezomib with lenalidomide and dexamethasone demonstrated a significant improvement in clinical benefit in a randomized phase III trial (Southwest Oncology Group [SWOG] S0777) when compared to lenalidomide and dexamethasone alone (13-and 11-month improvements in PFS and OS, respectively).

Carfilzomib

Carfilzomib is an irreversible proteasome inhibitor, acting on the chymotrypsin domain of the proteasome complex. It is approved by the Food and Drug Administration (FDA) for treatment of relapsed/ refractory MM for patients receiving one or more prior treatments and administered as twice-weekly intravenous infusions with doses ranging from 20/ 27 mg/ m² to 20/ 56 mg/ m². Common side effects include fatigue, decreased blood counts, and gastrointestinal (GI)/ hepatic toxicity; while serious but rare side effects include cardiac toxicities and acute renal failure. In the phase III ASPIRE study (n = 792), carfilzomib, lenalidomide, dexamethasone (CRd) showed improved PFS and quality of life when compared to bortezomib, lenalidomide, dexamethasone (RVd) in relapsed MM patients (median PFS = 26.3 vs. 17.6 months, P = .0001).^{55,56} Building upon prior phase I/ II CRd studies with newly diagnosed MM achieving high MRD negativity rates (37% to 62%), an ongoing Eastern Cooperative Oncology Group phase III study is comparing CRd to RVd in treatment-naïve patients (NCT01863550).^{57,58}

Lenalidomide

Lenalidomide is an immunomodulatory agent (IMiD), whose mechanism of action involves binding cereblon, a protein that regulates the degradation of two members of the Ikaros family important for lymphoid cell development and differentiation— Ikaros (IKZF1) and Aiolos (IKZF3).⁵⁹ It is administered orally with daily doses ranging from 5 to 25 mg. Side effects include fatigue, cytopenias, rash, diarrhea, and increased risk of thromboembolism. Lenalidomide has been combined with dexamethasone alone⁶⁰ or with other proteasome inhibitors and dexamethasone for initial treatment of MM ([Table 17.5](#)).

Transplantation

As stated earlier, for those patients eligible for ASCT, stem cell collection should occur after four cycles of primary induction chemotherapy. Given the efficacy of modern

combination therapy, the timing of early versus delayed ASCT approach remains controversial and is currently being evaluated by the Intergroupe Francophone du Myelome/ Dana-Farber Cancer Institute (IFM/ DFCI) trial. In an updated analysis of the IFM study from the American Society of Hematology (ASH) 2015 meeting, patients receiving early ASCT after RVd therapy achieved higher CRs (58% vs. 46%, $P < .01$) and 3-year PFS (61% vs. 48%, $P < .0002$) with no difference in 3-year OS compared to the continuous RVd chemotherapy arm and higher rate of deaths due to toxicity.⁶⁷ Importantly, the DFCI study results have yet to be reported. Other randomized studies attempting to establish the timing of ASCT have not used the typical novel-therapy regimens used in the United States and may not be relevant to the population. The role of tandem ASCT is unclear and remains to be further investigated. Past studies have demonstrated that a subset of patients not achieving a CR or VGPR after the first transplant may derive benefit from a second ASCT.^{68,69} However, recent results from the StAMINA study were presented at the 2016 ASH annual meeting and demonstrated no differences in PFS and OS between second ASCT, RVD consolidation or lenalidomide maintenance.⁷⁰ A second randomized phase III study, EMN02/ HO95 MM Trial, showed that tandem ASCT improved PFS over single ASCT after bortezomib, cyclophosphamide, and dexamethasone induction.⁷¹

Maintenance

After induction/ consolidation therapy or ASCT, maintenance therapy may be considered and initiated with a goal of prolonging durability of response. Although a number of different therapeutics have been tested in the maintenance setting, the most successful agent to show clinical benefit is oral lenalidomide. Three phase III randomized studies (two post-ASCT, one post nontransplant induction with median follow-up of 30 to 34 months) demonstrated an improvement in median PFS among patients receiving lenalidomide maintenance compared to those with no maintenance (IFM 2005-02: 41 vs. 21 months,⁷² $P < .001$; Cancer and Leukemia Group B (CALGB) 1001104: 46 vs. 27 months, $P < .001$ ⁷³; MM-015: 31 vs. 14 months, $P < .001$ ⁷⁴). Of note, there was increased risk of secondary primary malignancies in patients receiving lenalidomide maintenance. Although the mechanism remains poorly understood, subsequent analysis suggests risk of secondary malignancy seems to be increased in alkylator therapy exposed patients, as either melphalan induction or ASCT.⁷⁵ Finally, bortezomib has also been established in the maintenance setting and some studies suggest that patients with high-risk chromosomal abnormalities exquisitely benefit over other maintenance strategies.^{52,76}

Table 17.5 Primary Regimens for Treatment of MM

Regimen	Side Effects (SE)	References
Transplant		
Bortezomib Lenalidomide Dexamethasone	SE: neuropathy, cytopenias, VTE risk, hyperglycemia, fatigue, insomnia	45,53,61,78
Bortezomib Cyclophosphamide Dexamethasone	SE: neuropathy, neutropenia, hyperglycemia, fatigue, insomnia	61,62,78,79
Bortezomib Doxorubicin Dexamethasone	SE: neuropathy, cytopenias, hyperglycemia, fatigue, insomnia	52
Carfilzomib Lenalidomide Dexamethasone	SE: cytopenias, cardiac toxicity, constipation, fatigue, hyperglycemia, rash	57,58
Ixazomib Lenalidomide Dexamethasone	SE: skin disorders, neutropenia, thrombocytopenia, neuropathy	80
Bortezomib Thalidomide Dexamethasone	SE: neuropathy, cytopenias, hyperglycemia, fatigue	46,63,64,81,82
Bortezomib Dexamethasone	SE: neuropathy, cytopenias, hyperglycemia, fatigue, insomnia	51
Lenalidomide Dexamethasone	SE: rash, neutropenia, thrombocytopenia, fatigue, VTE risk	48,60
Bortezomib/ Thalidomide/ Cisplatin/ Doxorubicin/ Cyclophosphamide/ Etoposide/ Dexamethasone (VTD-PACE)	SE: infections, mucositis, decreased blood counts, hair loss	
Non-Transplant		
Bortezomib Lenalidomide Dexamethasone	SE: neuropathy, cytopenias, VTE risk, hyperglycemia, fatigue, insomnia	45,53,78
Lenalidomide Dexamethasone	SE: rash, neutropenia, thrombocytopenia, fatigue, VTE risk Notes: Include VTE prophylaxis. Try to include 3d agent	
Bortezomib Cyclophosphamide Dexamethasone	SE: neuropathy, neutropenia, hyperglycemia, fatigue, insomnia	78,79
Carfilzomib Lenalidomide Dexamethasone	SE: cytopenias, cardiac toxicity, constipation, fatigue, hyperglycemia, rash	57,58
Carfilzomib Cyclophosphamide Dexamethasone	SE: neutropenia, cardiopulmonary toxicity	66,83

Ixazomib	SE: skin disorders, neutropenia, thrombocytopenia,	65
Lenalidomide	neuropathy	
Dexamethasone		
Bortezomib	SE: neuropathy, cytopenias, hyperglycemia, fatigue, insomnia	
Dexamethasone		

NCCN, National Comprehensive Cancer Network; SE, side effects; TE, transplant eligible; TI, transplant ineligible; VTE, venous thromboembolism.

Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Guideline Name V.3.2018. © 2017 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of NCCN. To view the most recent and complete version of the NCCN Guidelines, go online to NCCN.org. The NCCN Guidelines are a work in progress that may be refined as often as new significant data becomes available. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

Salvage Therapy

Over the last 5 to 10 years, several new drugs have been developed and approved to expand the therapeutic arsenal against MM. Many of these novel agents have unique mechanisms of actions compared to more traditional cytotoxic therapies and proteasome inhibitor and IMiD classes. The diverse array of mechanistic actions allow for synergistic combinations that have nonoverlapping toxicity profiles ([Table 17.6](#)). When selecting a salvage regimen, clinicians must consider the degree of aggressiveness a patient is relapsing, preexisting conditions, and toxicities. Relapsed/ refractory MM patients may also be suitable candidates for a clinical trial. Previously used agents or a second ASCT can be considered again, especially if the duration of remission from the original regimen was optimal and >1 year.

Pomalidomide

Pomalidomide is a thalidomide analog that is administered daily with oral doses ranging from 1 to 4 mg and approved for patients who have received two or more prior lines of therapy (including lenalidomide and dexamethasone) and disease progression within completing 60 days of last therapy. In a multicenter randomized phase II study (n = 221), pomalidomide and dexamethasone (Pd) were compared to pomalidomide alone with 29% demonstrating a ≥PR in the Pd arm.⁷⁷ Of note, the population treated had a median of 5 prior lines of therapy, and lenalidomide refractoriness or lenalidomide/bortezomib resistance did not impact the clinical outcome. In the MM-003 phase III study, Pd was compared to dexamethasone alone, and PFS was 4.0 versus 1.9 months (P < .0001), respectively.⁷⁸ Several other studies have investigated pomalidomide in combination with bortezomib, carfilzomib, ixazomib, cyclophosphamide, and monoclonal antibodies.

Ixazomib

Ixazomib is a first-in-class oral reversible proteasome inhibitor approved by the FDA in 2015 for use in relapsed/ refractory MM in patients with at least one other prior therapy. It is administered at starting doses of 4 mg weekly and combined with lenalidomide and dexamethasone. In the Tourmaline-MM1 study ($n = 722$), median PFS was longer in the ixazomib, lenalidomide, and dexamethasone arm compared to the placebo, lenalidomide, and dexamethasone arm (20.6 vs. 14.7 months, $P = .01$).⁷⁹

Panobinostat

Panobinostat is a pan-deacetylase inhibitor that is approved for MM patients with at least two prior lines of therapy, including bortezomib and IMiD. It is administered orally with starting doses at 20 mg three times per week. Common side effects include diarrhea, vomiting, decreased appetite, and cytopenias. In a single-arm phase II study, PANORAMA-2, patients relapsed and refractory to bortezomib ($n = 55$) were treated with panobinostat, bortezomib, and dexamethasone, and yielded a 34.5% response rate $\geq PR$.⁸⁰ However, with significant toxicity and black box warning, treatment with panobinostat should be reserved for patients who are refractory to IMiDs and proteasome inhibitors and who may not be good candidates for treatment with the newer monoclonal proteins.

Daratumumab

Daratumumab is an anti-CD38 monoclonal antibody approved for the treatment of MM as monotherapy in patients who have received three or more prior lines of therapy or those double refractory to PI and IMiD; in addition, it is approved in combination with lenalidomide or bortezomib for the treatment of MM patients who have received one prior therapy. Therapy begins with weekly infusions at 16 mg/ kg. The most common side effects include infusion reactions, fatigue, thrombocytopenia, and anemia. Importantly, two practical considerations must be made when performing laboratory testing on patients receiving daratumumab. CD38 antigen is expressed on red blood cells, and for those patients receiving red blood cell transfusions, daratumumab may interfere with blood compatibility testing and falsely yield a positive indirect Coomb's test. Additionally, daratumumab can comigrate with the monoclonal band on serum protein electrophoresis, resulting in a small overestimation of the m-protein. In the Pollux study ($n = 569$), daratumumab was combined with lenalidomide and dexamethasone (DRd) and compared to lenalidomide and dexamethasone. The DRd group yielded a 12-month PFS rate of 83.2% compared to 60.1% in the control arm (HR for progression or death = 0.37 [$P < .001$]). Of note, the DRd group achieved a CR rate or higher of 43% with close to 23% yielding MRD negativity.⁸¹ The combination of

daratumumab, bortezomib, and dexamethasone was evaluated in the phase III Castor study ($n = 498$) and compared to bortezomib and dexamethasone. The daratumumab arm resulted in higher CR rates (19.2% vs. 9.0%, $P = .001$) and 12-month PFS (60.7% vs. 26.9%).⁸²

Table 17.6 Regimens for Previously Treated MM

Preferred Regimens

- Repeat induction therapy (if relapse >6 mo)
- Bortezomib/ lenalidomide/ dexamethasone
- Carfilzomib/ dexamethasone
- Carfilzomib/ lenalidomide/ dexamethasone
- Daratumumab/ lenalidomide/ dexamethasone
- Daratumumab/ bortezomib/ dexamethasone
- Elotuzumab/ lenalidomide/ dexamethasone
- Ixazomib/ lenalidomide/ dexamethasone

Other Recommended Regimens

- Bendamustine/ bortezomib/ dexamethasone
- Bendamustine/ lenalidomide/ dexamethasone
- Bortezomib/ liposomal doxorubicin
- Bortezomib/ cyclophosphamide/ dexamethasone
- Carfilzomib/ cyclophosphamide/ dexamethasone
- Carfilzomib/ dexamethasone (weekly)
- Lenalidomide/ cyclophosphamide/ dexamethasone
- Bortezomib/ dexamethasone
- Daratumumab
- Daratumumab/ pomalidomide/ dexamethasone
- Elotuzumab/ bortezomib/ dexamethasone
- Ixazomib/ dexamethasone
- Ixazomib/ pomalidomide/ dexamethasone
- Lenalidomide/ dexamethasone
- Panobinostat/ carfilzomib
- Panobinostat/ bortezomib/ dexamethasone
- Pomalidomide/ dexamethasone
- Pomalidomide/ bortezomib/ dexamethasone
- Pomalidomide/ carfilzomib/ dexamethasone
- Pomalidomide/ cyclophosphamide/ dexamethasone

Useful in Certain Circumstances

- Dexamethasone/ cyclophosphamide/ etoposide/ cisplatin (DCEP)
- Bortezomib/ thalidomide/ cisplatin/ doxorubicin/ cyclophosphamide/ etoposide/ dexamethasone (VTD-PACE)
- High-dose cyclophosphamide

MM, multiple myeloma.

Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Guideline Name V.3.2018. © 2017 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of NCCN. To view the most recent and complete version of the NCCN Guidelines, go online to NCCN.org. The NCCN Guidelines are a work in progress that may be refined as often as new significant data becomes available. NCCN makes no warranties of any kind whatsoever.

regarding their content, use or application and disclaims any responsibility for their application or use in any way.

Elotuzumab

Elotuzumab is a monoclonal antibody targeting SLAMF7 or CS-1, a glycoprotein antigen expressed on NK cells and plasma cells. It is approved for the treatment of MM patients who have received one to three lines of therapy. Initial doses start at 10 mg/ kg on a weekly basis for the first two cycles, and then every 2 weeks thereafter. The drug is administered with lenalidomide and dexamethasone, because single-agent activity is minimal. Similar to daratumumab, elotuzumab may interfere with results of protein electrophoresis. The ELOQUENT-2 (n = 646) study demonstrated improved median PFS in the lenalidomide and dexamethasone arm combined with elotuzumab compared to lenalidomide and dexamethasone alone (19.4 vs. 14.9 months, P < .001).⁸³

SUMMARY

Over the course of the past two decades, advances in diagnostics and therapeutics have greatly improved clinical outcomes for MM patients, leading to a paradigm shift in the management of the disease. In the past, older rules suggested that patients should wait to develop symptomatic CRAB end-organ damage before considering therapy. The MM diagnostic criteria have undergone a “re-structuring of its classification” that acknowledges that early MM with defined high-risk biomarker features may benefit from treatment (before actual symptom development). Other frontiers of research have focused efforts on characterizing the natural history of MM based on genetic mutations and advancing diagnostic tools by measuring low levels of MRD disease. In the therapeutic arena, different mechanistic classes of drugs have been approved and these are expanding the arsenal beyond proteasome inhibitors and IMiDs to other pathways. Elotuzumab and daratumumab are the first monoclonal antibodies approved for MM, giving rise to a new manner in which MM may be targeted, while ongoing clinical studies are investigating immune checkpoint blockade and CAR-T-cells to further illicit an immune-based response against MM. A number of different questions remain unanswered, including the development of new pathway inhibitors, the role of transplant in modern therapeutics, and the sequencing or timing of approved regimens. Ongoing research clinical trials are addressing these questions.

References

1. SEER Stat Fact Sheets: Myeloma. 2016.
<http://seer.cancer.gov/statfacts/html/mulmy.html>
2. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review,

- 1975–2010. Bethesda, MD: National Cancer Institute; 2013.
3. Turesson I, Velez R, Kristinsson SY, et al. Patterns of improved survival in patients with multiple myeloma in the twenty-first century: a population-based study. *J Clin Oncol*. 2010;28(5):830–834.
 4. Landgren O, Kristinsson SY, Goldin LR, et al. Risk of plasma cell and lymphoproliferative disorders among 14621 first-degree relatives of 4458 patients with monoclonal gammopathy of undetermined significance in Sweden. *Blood*. 2009;114(4):791–795.
 5. Landgren O, Weiss BM. Patterns of monoclonal gammopathy of undetermined significance and multiple myeloma in various ethnic/ racial groups: support for genetic factors in pathogenesis. *Leukemia*. 2009;23(10):1691–1697.
 6. Bladé J, Kyle RA, Greipp PR. Multiple myeloma in patients younger than 30 years. Report of 10 cases and review of the literature. *Arch Intern Med*. 1996;156(13):1463–1468.
 7. Kazandjian D. Multiple myeloma epidemiology and survival: a unique malignancy. *Semin Oncol*. 2016;43(6):676–681.
 8. Waxman AJ, Mink PJ, Devesa SS, et al. Racial disparities in incidence and outcome in multiple myeloma: a population-based study. *Blood*. 2010;116(25):5501–5506.
 9. Wiernik PH, Lossos IS, Tuscano JM, et al. Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. *J Clin Oncol*. 2008;26(30):4952–4957.
 10. Waxman AJ, Mink PJ, Devesa SS, et al. Racial disparities in incidence and outcome in multiple myeloma: a population-based study. *Blood*. 2010;116(25):5501–5506.
 11. Landgren O, Shim YK, Michalek J, et al. Agent orange exposure and monoclonal gammopathy of undetermined significance: an operation Ranch Hand Veteran Cohort Study. *JAMA Oncol*. 2015;1(8):1061–1068.
 12. Kachuri L, Demers PA, Blair A, et al. Multiple pesticide exposures and the risk of multiple myeloma in Canadian men. *Int J Cancer*. 2013;133(8):1846–1858.
 13. Moline JM, Herbert R, Crowley L, et al. Multiple myeloma in World Trade Center responders: a case series. *J Occup Environ Med*. 2009;51(8):896–902.
 14. Wu K, Li L, Thakur C, et al. Proteomic Characterization of the World Trade Center dust-activated mdig and c-myc signaling circuit linked to multiple myeloma. *Sci Rep*. 2016;6:36305.
 15. Kristinsson SY, Anderson WF, Landgren O. Improved long-term survival in multiple myeloma up to the age of 80 years. *Leukemia*. 2014;28(6):1346–1348.
 16. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538–e548.

17. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood*. 2009;113(22):5412–5417.
18. Kyle RA, Therneau TM, Rajkumar SV, et al. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. *Blood*. 2003;102(10):3759–3764.
19. Turesson I, Kovalchik SA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance and risk of lymphoid and myeloid malignancies: 728 cases followed up to 30 years in Sweden. *Blood*. 2014;123(3):338–345.
20. Kyle RA, Therneau TM, Rajkumar SV, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2002;346(8):564–569.
21. Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. *N Engl J Med*. 2007;356(25):2582–2590.
22. Kyle RA, Durie BG, Rajkumar SV, et al.; International Myeloma Working Group. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia*. 2010;24(6):1121–1127.
23. Dispenzieri A, Kyle RA, Katzmann JA, et al. Immunoglobulin free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. *Blood*. 2008;111(2):785–789.
24. Pérez-Persona E, Vidriales MB, Mateo G, et al. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. *Blood*. 2007;110(7):2586–2592.
25. Mateos MV, Hernández MT, Giraldo P, et al. Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. *N Engl J Med*. 2013;369(5):438–447.
26. Lohr JG, Stojanov P, Carter SL, et al.; Multiple Myeloma Research Consortium. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. *Cancer Cell*. 2014;25(1):91–101.
27. Bolli N, Avet-Loiseau H, Wedge DC, et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nat Commun*. 2014;5:2997.
28. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. *Nat Rev Cancer*. 2012;12(5):335–348.
29. Zhao S, Choi M, Heuck C, et al. Serial exome analysis of disease progression in premalignant gammopathies. *Leukemia*. 2014;28(7):1548–1552.
30. Walker BA, Wardell CP, Melchor L, et al. Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of

- clinical symptoms. *Leukemia*. 2014;28(2):384–390.
31. Mailankody S. Genetic plasma cell signatures in high-risk smoldering myeloma versus multiple myeloma patients. Paper presented at: 2016 ASCO Annual Meeting; June 3, 2016; Chicago, IL.
 32. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. *J Clin Oncol*. 2005;23(15):3412–3420.
 33. Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised international staging system for multiple myeloma: a report from international myeloma working group. *J Clin Oncol*. 2015;33(26):2863–2869.
 34. Mailankody S, Korde N, Lesokhin AM, et al. Minimal residual disease in multiple myeloma: bringing the bench to the bedside. *Nat Rev Clin Oncol*. 2015;12(5):286–295.
 35. Rajkumar SV, Harousseau JL, Durie B, et al.; International Myeloma Workshop Consensus Panel 1. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. *Blood*. 2011;117(18):4691–4695.
 36. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016;17(8):e328–e346.
 37. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. *Blood*. 2014;123(20):3073–3079.
 38. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. *J Clin Oncol*. 2013;31(20):2540–2547.
 39. Korthals M, Sehnke N, Kronenwett R, et al. The level of minimal residual disease in the bone marrow of patients with multiple myeloma before high-dose therapy and autologous blood stem cell transplantation is an independent predictive parameter. *Biol Blood Marrow Transplant*. 2012;18(3):423–431.e3.
 40. Paiva B, Gutiérrez NC, Rosiñol L, et al.; PETHEMA/ GEM (Programa para el Estudio de la Terapéutica en Hemopatías Malignas/ Grupo Español de Mieloma) Cooperative Study Groups. High-risk cytogenetics and persistent minimal residual disease by multiparameter flow cytometry predict unsustained complete response after autologous stem cell transplantation in multiple myeloma. *Blood*. 2012;119(3):687–691.
 41. Munshi NC, Avet-Loiseau H, Rawstron AC, et al. Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: a meta-analysis. *JAMA Oncol*. 2017;3(1):28–35.
 42. Landgren O, Devlin S, Boulad M, et al. Role of MRD status in relation to clinical

- outcomes in newly diagnosed multiple myeloma patients: a meta-analysis. *Bone Marrow Transplant.* 2016;51(12):1565–1568.
43. Kumar S, Dispenzieri A, Lacy MQ, et al. Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma. *Leukemia.* 2007;21(9):2035–2042.
44. Kumar S, Callander NS, Alsina M, et al. National Comprehensive Cancer Network Guidelines Multiple Myeloma Version 3.2018. https://www.nccn.org/professionals/physician_gls/pdf/myeloma.pdf. Accessed November 28, 2016.
45. Durie BG, Hoering A, Abidi MH, et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. *Lancet.* 2017;389(10068):519–527.
46. Cavo M, Tacchetti P, Patriarca F, et al.; GIMEMA Italian Myeloma Network. Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: a randomised phase 3 study. *Lancet.* 2010;376(9758):2075–2085.
47. Palumbo A, Bringhen S, Caravita T, et al.; Italian Multiple Myeloma Network, GIMEMA. Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial. *Lancet.* 2006;367(9513):825–831.
48. Benboubker L, Dimopoulos MA, Dispenzieri A, et al.; FIRST Trial Team. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. *N Engl J Med.* 2014;371(10):906–917.
49. Bringhen S, Larocca A, Rossi D, et al. Efficacy and safety of once-weekly bortezomib in multiple myeloma patients. *Blood.* 2010;116(23):4745–4753.
50. Moreau P, Pylypenko H, Grosicki S, et al. Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study. *Lancet Oncol.* 2011;12(5):431–440.
51. Harousseau JL, Attal M, Avet-Loiseau H, et al. Bortezomib plus dexamethasone is superior to vincristine plus doxorubicin plus dexamethasone as induction treatment prior to autologous stem-cell transplantation in newly diagnosed multiple myeloma: results of the IFM 2005-01 phase III trial. *J Clin Oncol.* 2010;28(30):4621–4629.
52. Sonneveld P, Schmidt-Wolf IG, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial. *J Clin Oncol.* 2012;30(24):2946–2955.

53. Roussel M, Lauwers-Cances V, Robillard N, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myélome. *J Clin Oncol.* 2014;32(25):2712–2717.
54. Avet-Loiseau H, Leleu X, Roussel M, et al. Bortezomib plus dexamethasone induction improves outcome of patients with t(4;14) myeloma but not outcome of patients with del(17p). *J Clin Oncol.* 2010;28(30):4630–4634.
55. Stewart AK, Rajkumar SV, Dimopoulos MA, et al.; ASPIRE Investigators. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. *N Engl J Med.* 2015;372(2):142–152.
56. Stewart AK, Dimopoulos MA, Masszi T, et al. Health-related quality of life results from the open-label, randomized, phase III ASPIRE trial evaluating carfilzomib, lenalidomide, and dexamethasone versus lenalidomide and dexamethasone in patients with relapsed multiple myeloma. *J Clin Oncol.* 2016;34(32):3921–3930.
57. Korde N, Roschewski M, Zingone A, et al. Treatment with carfilzomib-lenalidomide-dexamethasone with lenalidomide extension in patients with smoldering or newly diagnosed multiple myeloma. *JAMA Oncol.* 2015;1(6):746–754.
58. Jakubowiak AJ, Dytfield D, Griffith KA, et al. A phase ½ study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. *Blood.* 2012;120(9):1801–1809.
59. Lu G, Middleton RE, Sun H, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science.* 2014;343(6168):305–309.
60. Rajkumar SV, Jacobus S, Callander NS, et al.; Eastern Cooperative Oncology Group. Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial. *Lancet Oncol.* 2010;11(1):29–37.
61. Kumar S, Flinn I, Richardson PG, et al. Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. *Blood.* 2012;119(19):4375–4382.
62. Reeder CB, Reece DE, Kukreti V, et al. Cyclophosphamide, bortezomib and dexamethasone induction for newly diagnosed multiple myeloma: high response rates in a phase II clinical trial. *Leukemia.* 2009;23(7):1337–1341.
63. Moreau P, Hulin C, Macro M, et al. VTD is superior to VCD prior to intensive therapy in multiple myeloma: results of the prospective IFM2013-04 trial. *Blood.* 2016;127(21):2569–2574.

64. Rosiñol L, Oriol A, Teruel AI, et al.; Programa para el Estudio y la Terapéutica de las Hemopatías Malignas/ Grupo Español de Mieloma (PETHEMA/ GEM) group. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/ GEM study. *Blood*. 2012;120(8):1589–1596.
65. Kumar SK, Berdeja JG, Niesvizky R, et al. Safety and tolerability of ixazomib, an oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma: an open-label phase ½ study. *Lancet Oncol*. 2014;15(13):1503–1512.
66. Bringhen S, Petrucci MT, Larocca A, et al. Carfilzomib, cyclophosphamide, and dexamethasone in patients with newly diagnosed multiple myeloma: a multicenter, phase 2 study. *Blood*. 2014;124(1):63–69.
67. Attal M, Lauwers-Cances V, Hulin C, eds. Autologous transplantation for multiple myeloma in the era of new drugs: a phase III study of the Intergroupe Francophone Du Myeloma (IFM/ DFCI 2009). Paper presented at: ASH Annual Meeting; December 5–8, 2015; Orlando, FL.
68. Attal M, Harousseau JL, Facon T, et al.; InterGroupe Francophone du Myélome. Single versus double autologous stem-cell transplantation for multiple myeloma. *N Engl J Med*. 2003;349(26):2495–2502.
69. Cavo M, Tosi P, Zamagni E, et al. Prospective, randomized study of single compared with double autologous stem-cell transplantation for multiple myeloma: Bologna 96 clinical study. *J Clin Oncol*. 2007;25(17):2434–2441.
70. Stadtmauer E. Comparison of autologous hematopoietic cell transplant (autoHCT), Bortezomib, Lenalidomide (Len) and Dexamethasone (RVD) Consolidation with Len Maintenance (ACM), Tandem Autohtct with Len Maintenance (TAM) and Auto Hct with Len Maintenance (AM) for up-front treatment of patients with multiple myeloma (MM): primary results from the randomized phase III trial of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN 0702— StaMINA Trial). Paper presented at: ASH 58th Annual Meeting and Exposition; December 6, 2016; San Diego, CA.
71. Cavo M. Upfront single versus double autologous stem cell transplantation for newly diagnosed multiple myeloma: an intergroup, multicenter, phase III study of the European Myeloma Network (EMN02/ HO95 MM Trial). Paper presented at: ASH 58th Annual Meeting and Exposition; December 6, 2016; San Diego, CA.
72. Attal M, Lauwers-Cances V, Marit G, et al.; IFM Investigators. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. *N Engl J Med*. 2012;366(19):1782–1791.
73. McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. *N Engl J Med*. 2012;366(19):1770–1781.
74. Palumbo A, Hajek R, Delforge M, et al.; MM-015 Investigators. Continuous

- lenalidomide treatment for newly diagnosed multiple myeloma. *N Engl J Med.* 2012;366(19):1759–1769.
- 75. Palumbo A, Bringhen S, Kumar SK, et al. Second primary malignancies with lenalidomide therapy for newly diagnosed myeloma: a meta-analysis of individual patient data. *Lancet Oncol.* 2014;15(3):333–342.
 - 76. Nooka AK, Kaufman JL, Muppudi S, et al. Consolidation and maintenance therapy with lenalidomide, bortezomib and dexamethasone (RVD) in high-risk myeloma patients. *Leukemia.* 2014;28(3):690–693.
 - 77. Richardson PG, Siegel DS, Vij R, et al. Pomalidomide alone or in combination with low-dose dexamethasone in relapsed and refractory multiple myeloma: a randomized phase 2 study. *Blood.* 2014;123(12):1826–1832.
 - 78. San Miguel J, Weisel K, Moreau P, et al. Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2013;14(11):1055–1066.
 - 79. Moreau P, Masszi T, Grzasko N, et al.; TOURMALINE-MM1 Study Group. Oral ixazomib, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med.* 2016;374(17):1621–1634.
 - 80. Richardson PG, Schlossman RL, Alsina M, et al. PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma. *Blood.* 2013;122(14):2331–2337.
 - 81. Dimopoulos MA, Oriol A, Nahm H, et al.; POLLUX Investigators. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med.* 2016;375(14):1319–1331.
 - 82. Palumbo A, Chanan-Khan A, Weisel K, et al.; CASTOR Investigators. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. *N Engl J Med.* 2016;375(8):754–766.
 - 83. Lonial S, Dimopoulos M, Palumbo A, et al.; ELOQUENT-2 Investigators. Elotuzumab therapy for relapsed or refractory multiple myeloma. *N Engl J Med.* 2015;373(7):621–631.

Hematopoietic Stem Cell Transplantation

**Phuong T. Vo, Ramaprasad Srinivasan,
and Richard W. Childs**

Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapeutic modality widely used in the management of some hematologic malignancies and a variety of nonmalignant disorders. Autologous HSCT (auto-HSCT) typically involves administration of high-dose chemotherapy followed by infusion of hematopoietic stem cells procured from the recipient before ablative therapy. In contrast, allogeneic HSCT involves infusion of hematopoietic stem cells from a related or unrelated human leukocyte antigen (HLA)-matched or partially matched donor, following myeloablative or reduced intensity conditioning (RIC) of the recipient.

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

Auto-HSCT was developed to overcome the lethal hematopoietic toxicity associated with high-dose chemotherapy used to treat dose-responsive malignancies.^{1,2} A role for auto-HSCT has been clearly established in the management of multiple myeloma and aggressive non-Hodgkin lymphoma (NHL). Initial enthusiasm for this approach in solid tumors such as metastatic breast, ovarian, and lung cancer has been tempered by the failure of prospective randomized trials to demonstrate benefit over conventional treatments. The role of auto-HSCT continues to be explored in neuroblastoma and Ewing's sarcoma.

General Considerations

Most auto-HSCTs are performed using peripheral blood stem cells (PBSCs) collected after mobilization with granulocyte-colony-stimulating factor (G-CSF), with or without chemotherapy priming or plerixafor. Curative potential resides solely in the ability of

high-dose chemotherapy to eradicate the underlying malignancy; in contrast to allogeneic transplantation, no immune-mediated graft-versus-tumor (GVT) effects are generated with auto-HSCT. The high-dose therapeutic regimen utilized is tailored to the malignancy being treated based on its chemosensitivity profile; for instance, melphalan (200 mg/ m²) is the most widely used high-dose conditioning agent in patients with multiple myeloma undergoing auto-HSCT.

Infections related to chemotherapy-induced neutropenia and immunosuppression, as well as extramedullary toxicities from high-dose therapeutic agents, account for the majority of complications occurring after auto-HSCT. There is a lower risk of treatment-related mortality (TRM) compared with allogeneic HSCT, typically less than 5% in most series.

Contamination of the stem cell product by malignant cells may limit the beneficial effects of high-dose chemotherapy. Efforts to purge tumor cells contaminating hematopoietic grafts by CD34⁺ cell selection or by in vitro incubation of the stem cell graft with cytotoxic drugs remain investigational and are of questionable benefit.

Results of Autologous Hematopoietic Stem Cell Transplantation

Autologous Hematopoietic Stem Cell Transplantation in Hematologic Malignancies

Multiple Myeloma Large phase II trials have demonstrated high response rates (complete response [CR] = 30% to 50%) and impressive disease-free survival (DFS) and overall survival (OS) rates (median, more than 5 years). Randomized phase III trials in relatively young patients (less than 65 years of age) have shown superior response rates, DFS, and OS for auto-HSCT versus conventional chemotherapy.³

Consecutive or tandem auto-HSCTs have been compared with single auto-HSCT in several randomized prospective studies. Although some studies suggest improved DFS (but not OS) with the tandem approach, other large multicenter trials also indicated a survival benefit for tandem auto-HSCT. Subgroup analyses suggest that the survival benefit is most pronounced in patients who do not achieve a CR or a very good partial response following the first auto-HSCT.

Tandem auto-HSCT has also been compared with auto-HSCT followed by reduced intensity or nonmyeloablative allogeneic HSCT, with one randomized study demonstrating superior OS and progression-free survival (PFS) with the latter approach. However, a randomized trial conducted through the Clinical Trials Network (CTN) that enrolled more than 700 subjects reported no difference in 3-year PFS and OS between patients receiving a tandem auto-HSCT versus a tandem auto/allotransplant, with significantly higher TRM being reported in the latter group.⁴

Auto-HSCT is currently considered standard first-line therapy for younger patients

(up to the age of 65 years) with myeloma and results in long-term CR rates of 5% to 10%. Melphalan (200 mg/ m²) is the most commonly used preparative regimen.^{5,6} The role of tandem auto-HSCT is not clearly defined, although some subgroups of patients appear to benefit from this approach. Due to recent data showing no clear survival benefit with an upfront auto/ allogeneic transplant approach, as well as the advent of newer agents that are highly active against myeloma such as lenalidomide, pomalidomide, bortezomib, carfilzomib, daratumumab, and elotuzumab, the role of allogeneic transplant in this disease remains increasingly controversial. Although auto-HSCT remains a standard of care for myeloma patients, the strong antitumor activity of these newer agents has led to a reevaluation of the role of auto-HSCT in the management of this disease. Several ongoing studies are attempting to determine whether

- | Combinations of newer anti-myeloma agents as up-front therapy can supplant auto-HSCT as the standard of care in most patients^{7,8}
- | Integration of these agents in the management algorithm can potentiate or extend the benefits derived from auto-HSCT

Emerging data suggest that bortezomib-based pretransplant induction therapy as well as posttransplant consolidation using a variety of strategies might improve the overall outcome associated with auto-HSCTs.⁹

Lymphoma Lymphomas are among the most common indications for auto-HSCT. The benefit of auto-HSCT has been most clearly observed in chemosensitive Hodgkin's disease and in intermediate-and high-grade NHL. Auto-HSCT results in improved event-free survival (EFS) and OS in patients with relapsed chemosensitive intermediate-/ high-grade NHL, compared with conventional salvage therapy.¹⁰ Results from a randomized study suggest that initial treatment with auto-HSCT may benefit some patients with intermediate-/ high-grade NHL, compared to standard chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP).

Among Hodgkin's disease patients, those with chemosensitive disease on the first relapse have an improved EFS with auto-HSCT compared with standard salvage chemotherapy. Patients with primary progressive disease (those who progress on first-line chemotherapy) may also benefit from auto-HSCT as part of a salvage regimen.

Incorporation of therapeutic antibodies such as rituximab (anti-CD20) in first-line treatment regimens has significantly improved our ability to treat several subsets of NHL. The role of auto-HSCT in the era of B-cell–targeted monoclonal antibodies remains to be determined.

Acute Myeloid Leukemia Auto-HSCT has been used both as post-remission therapy in acute

myeloid leukemia (AML) in first complete remission 1 (CR1) and as therapy after relapse. Phase III studies in patients in CR1 suggest an improvement in DFS but not in OS, compared with conventional post-remission therapy. Further studies are required to clarify that, if any, prognostic subgroups are likely to benefit from auto-HSCT.

Autologous Hematopoietic Stem Cell Transplantation in Solid Tumors

The knowledge that some malignancies exhibited dose-dependent responses to chemotherapy led to the investigation of high-dose chemotherapy followed by auto-HSCT in the treatment of solid tumors. Based on the negative results from phase III trials, auto-HSCT has been largely abandoned in the management of some malignancies (particularly metastatic breast cancer) but remains under investigation in several other solid tumor types such as rhabdomyosarcoma.

Breast Cancer While promising results from phase II studies in patients with metastatic breast cancer paved the way for randomized phase III studies of auto-HSCT, these later larger studies failed to demonstrate an unequivocal benefit. At least seven large trials have compared auto-HSCT with standard chemotherapy in patients with metastatic breast cancer. Six demonstrated superior EFS with auto-HSCT, but none showed an OS advantage.¹¹ Similar results were obtained in patients with high-risk breast cancer undergoing adjuvant auto-HSCT. Because of a lack of survival benefit and higher toxicity, there is little enthusiasm for further investigation of auto-HSCT in breast cancer.

Germ Cell Tumors Phase II trials of auto-HSCT in relapsed or refractory germ cell tumors have yielded response rates of 40% to 65% and long-term survival rates of 15% to 40%. Patients with progressive disease or human chorionic gonadotropin levels greater than 1,000 IU/ L at transplantation, mediastinal primaries, and those refractory to cisplatin-based therapy have a worse outcome and may not benefit from auto-HSCT. An interim report from the European Group for Blood and Marrow Transplantation study suggested no advantage for auto-HSCT over standard salvage chemotherapy in patients failing cisplatin-based chemotherapy.

Auto-HSCT is considered an option for salvage of selected patients in first or subsequent relapse, although randomized phase III studies have failed to demonstrate unequivocal benefit for high dose over standard chemotherapy in this setting.¹²

There is no role for auto-HSCT in the initial management of germ cell tumors; several randomized phase III studies have demonstrated similar outcomes for conventional chemotherapy and auto-HSCT in patients with germ cell tumors with high-risk features.¹³

Other Tumors Available data do not suggest a clear benefit for auto-HSCT in ovarian and lung cancers. When administered after an initial course of standard induction chemotherapy, auto-HSCT appears to improve short-term DFS in high-risk neuroblastoma compared with conventional dose maintenance chemotherapy. However, a large randomized study was unable to demonstrate a survival advantage for patients undergoing auto-HSCT.

Some patients with Ewing's sarcoma/ primitive neuroectodermal tumor and other soft tissue sarcomas may benefit from high-dose chemotherapy. In the absence of evidence to support a survival benefit, patients should be treated with auto-HSCT only in the setting of a clinical trial.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Allogeneic HSCT can cure patients with advanced chemotherapy-resistant hematologic malignancies.^{14–16} The first successful allogeneic HSCTs in humans were reported in 1968. These transplants were performed in children with congenital immune deficiencies, using donor stem cells from HLA-compatible sibling donors. In the early years after the advent of allogeneic HSCT, immune deficiency syndromes and disorders of hematopoiesis constituted the major indications for the procedure. With a better understanding of the graft-versus-leukemia (GVL) effect and its curative potential, hematologic malignancies have now become the most common indication for an allogeneic HSCT. The number of transplants performed worldwide continues to grow, with more than 25,000 allogeneic HSCTs performed annually for a wide array of both malignant and nonmalignant disorders.

Indications for Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic HSCT has been used as a potentially curative treatment modality in both malignant and nonmalignant diseases (Table 18.1). Currently, the most common indication for an allogeneic HSCT (more than 75%) is an underlying hematologic malignancy (acute myelogenous leukemia, acute lymphocytic leukemia, myelodysplastic syndrome [MDS], and NHL being the most common). Nonmalignant conditions that are potentially curable by HSCT include disorders of hematopoiesis (e.g., aplastic anemia [AA]), immunodeficiency syndromes (e.g., Chediak–Higashi disease and severe combined immunodeficiency syndrome), congenital disorders of erythropoiesis (e.g., thalassemias and sickle cell anemia), and inborn errors of metabolism (e.g., mucopolysaccharidoses).^{17,18} The advent of new and highly effective therapies for certain malignant diseases (such as the tyrosine kinase inhibitors) and improvements in the toxicity profile associated with HSCT have altered the role allogeneic

transplantation plays for diseases such as chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL).

Antileukemic Potential of Allogeneic Hematopoietic Stem Cell Transplantation: Underlying Principles

In the 1960s and 1970s, allogeneic HSCT was largely viewed as a means of ensuring immuno-hematopoietic reconstitution or replacement after administration of high doses of chemotherapy with or without radiation. This premise still applies to the treatment of nonmalignant conditions, for which the major goal is to provide normal cellular components to replace or rectify an underlying deficiency. For hematologic malignancies, the dose-intensive preparative or conditioning regimen was initially considered to be critical for the eradication of the underlying malignancy, with HLA-matched donor stem cells used merely to reverse the accompanying fatal bone marrow (BM) ablation. However, it subsequently became clear that the ability to generate a donor immune-mediated antimalignancy effect, termed GVL or GVT, is critical for the successful eradication of malignancy after allogeneic HSCT. The following clinical observations have provided incontrovertible evidence for the existence of GVL and highlight the role of donor T lymphocytes in mediating this effect^{14–16,19–21}:

- | There is a decreased risk of leukemia relapse in patients experiencing chronic graft-versus-host disease (GVHD).
- | There is an increased risk of leukemia relapse in patients undergoing T-cell–depleted transplants.
- | There is an increased risk of leukemia relapse in recipients of syngeneic as opposed to non-twin sibling donor allografts.
- | The observation that immunosuppression withdrawal and/ or donor lymphocyte infusions (DLIs) can induce sustained remission in patients with some malignancies relapsing after transplantation.

Recognition of GVL led to the development of RIC or nonmyeloablative conditioning regimens, in which long-term DFS is largely dependent on the generation of donor immune-mediated antitumor responses.

Planning Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic HSCT is a complex procedure requiring careful planning and a multidisciplinary approach to patient management. Factors including patient age, performance status, underlying disease and disease status, and donor availability need to be considered before decisions regarding the type of transplantation to be performed (e.g., conventional myeloablative vs. nonmyeloablative) and GVHD prophylaxis regimen to be used are made.

Table 18.1 Indications for Hematopoietic Stem Cell Transplantation**Acute Leukemias**

Acute lymphoblastic leukemia (ALL)
Acute myelogenous leukemia (AML)

Chronic Leukemias

Chronic myelogenous leukemia (CML)
Chronic lymphocytic leukemia (CLL)
Juvenile chronic myelogenous leukemia (JCML)
Juvenile myelomonocytic leukemia (JMML)

Myelodysplastic Syndromes

Refractory anemia (RA)
Refractory anemia with ringed sideroblasts (RARS)
Refractory anemia with excess blasts (RAEB)
Refractory anemia with excess blasts in transformation
(RAEB-T)
Chronic myelomonocytic leukemia (CMML)

Stem Cell Disorders

Aplastic anemia (severe)
Fanconi anemia
Paroxysmal nocturnal hemoglobinuria
Pure red cell aplasia

Myeloproliferative Disorders

Acute myelofibrosis
Agnogenic myeloid metaplasia (myelofibrosis)
Polycythemia vera
Essential thrombocythemia

Lymphoproliferative Disorders

Non-Hodgkin lymphoma
Hodgkin disease

Phagocyte Disorders

Chediak–Higashi syndrome
Chronic granulomatous disease
Neutrophil actin deficiency
Reticular dysgenesis

Inherited Metabolic Disorders

Mucopolysaccharidoses (MPS)
Hurler syndrome (MPS-IH)
Scheie syndrome (MPS-IS)
Hunter syndrome (MPS-II)
Sanfilippo syndrome (MPS-III)
Morquio syndrome (MPS-IV)
Maroteaux–Lamy syndrome (MPS-VI)
Sly syndrome, β -C glucuronidase deficiency (MPS-VII)
Adrenoleukodystrophy
Mucolipidosis II (I-cell disease)
Krabbe disease
Gaucher disease

Histiocytic Disorders

Familial erythrophagocytic
lymphohistiocytosis
Histiocytosis-X
Hemophagocytosis

Inherited Erythrocyte Abnormalities

β -Thalassemia major
Sickle cell disease

Inherited Immune System Disorders

Ataxia-telangiectasia
Kostmann syndrome
Leukocyte adhesion deficiency
DiGeorge syndrome
Bare lymphocyte syndrome
Ommen syndrome
Severe combined immunodeficiency
(SCID)
SCID with adenosine deaminase
Absence of T & B cells SCID
Absence of T cells, normal B cell SCID
Common variable immunodeficiency
Wiskott–Aldrich syndrome
X-linked lymphoproliferative disorder

Other Inherited Disorders

Lesch–Nyhan syndrome
Cartilage-hair hypoplasia
Glanzmann thrombasthenia
Osteopetrosis

Inherited Platelet Abnormalities

Amegakaryocytosis/ congenital
thrombocytopenia

Plasma Cell Disorders

Multiple myeloma
Plasma cell leukemia
Waldenstrom macroglobulinemia

Other Malignancies

Breast cancer
Ewing sarcoma
Neuroblastoma
Renal cell carcinoma

Niemann–Pick disease
Wolman disease
Metachromatic leukodystrophy

Adapted from the list of transplant indications provided by the National Marrow Donor Program (NMDP).

Evaluation of Transplant Recipients

The degree of donor–host HLA compatibility is one of the most important factors affecting outcome after HSCT. Evaluation of the transplant recipient begins with HLA testing and a search for an appropriate donor. The initial donor search focuses on identifying a sibling matched at the allele level for HLA-A, HLA-B, and HLA-DR loci. If a suitable sibling donor is not available, a search for an HLA haploidentical related donor, or an HLA compatible matched unrelated donor (MUD), or HLA compatible unrelated cord blood unit can be made through the National Marrow Donor Program (NMDP).

A thorough history and physical examination, with emphasis on the underlying diagnosis and its treatment, concomitant medical problems, performance status, transfusion history, and any history of opportunistic (particularly fungal) infections should be performed. Assessment of major organ function, including pulmonary function testing and full cardiac evaluation, should be undertaken. Serologic testing to detect prior exposure to cytomegalovirus (CMV), herpes virus, Epstein–Barr virus (EBV), adenovirus, hepatitis viruses, HIV, toxoplasmosis, and varicella is also needed. Counseling is required to focus on the potential benefits and risks of transplantation, need for a dedicated caregiver, and, when appropriate, fertility preservation.

Identification of a Suitable Donor

- Matched related donor: Approximately one-third of the patients screened will have a suitable HLA–compatible sibling donor. While donors with a less than complete (10/10) HLA match can be used, greater HLA disparity increases the risk of both graft rejection and GVHD.
- Syngeneic donor: Identical twin transplants are rarely performed because of the rareness of their availability. It is also important to consider that high degrees of histocompatibility (including for minor histocompatibility antigens) in this setting minimize clinically meaningful GVL effects. Syngeneic donors are most desirable in the transplantation of acquired nonmalignant diseases (such as severe AA [SAA]) where GVL is not required.
- Matched unrelated donor: There are more than 25 million donors in worldwide registries. Searches conducted through the NMDP, in general, identifies a suitable donor for approximately two-thirds of Caucasians, but for some minority groups, due to increased HLA diversity, it is much harder to find a match. The process should be

initiated as early as possible because the time to transplant once a search is initiated is typically 2 to 4 months. For a given degree of donor–host HLA disparity, the risk of GVHD and graft rejection is higher with unrelated donors compared to related donors.

Haploidentical donor: Most patients have a sibling, parent, or child with one matched HLA haplotype who could serve as a donor. Historically, transplants from haploidentical donors were associated with a higher incidence of GVHD, necessitating T-cell depletion to prevent life-threatening GVHD. Impaired immune reconstitution is common and leads to a high incidence of opportunistic infections in recipients of haploidentical transplants. Recent studies utilizing posttransplant cyclophosphamide as a method to induce *in vivo* T-cell depletion following the transplantation of either haploidentical BM or PBSCs have shown encouraging results, with remarkably low incidences of both acute and chronic GVHD. Several recent retrospective studies have reported comparable transplant outcomes, including DFS with haploidentical BM utilizing posttransplant cyclophosphamide as observed with MUD transplants and cord blood transplants.^{22,23} Based on these promising results, a randomized trial comparing cord blood transplantation with haploidentical BM transplantation with posttransplant cyclophosphamide is being conducted through the CTN.

Umbilical cord cells: Blood collected from the placenta at the time of childbirth can be used as a source of hematopoietic stem cells. Although umbilical cord transplants are associated with a lower incidence of GVHD (despite HLA mismatching), their widespread use is limited by an increased risk of graft failure due to the small numbers of stem cells harvested. Umbilical cord transplants were largely restricted to children and young adolescents. However, recent studies have established that umbilical cord transplants in adults are feasible. The use of dual cord units and cord transplants using *ex vivo* expanded cord progenitor cells are some of the strategies being explored to boost the stem cell dose available for transplant. As of 2016, it is estimated that more than 730,000 cord blood units are available in the worldwide registry for public use.

Procurement of Hematopoietic Stem Cells

The majority of hematopoietic stem cells reside within the BM, which traditionally served as the source of the allograft. However, the availability of G-CSF, which mobilizes hematopoietic stem cells into the circulation, and the ease of collection have led to the widespread use of PBSC allografts.

Obtaining stem cells from the BM involves multiple aspirations from the iliac crests, a relatively safe procedure performed under general anesthesia. Mobilized stem cells are typically collected from the peripheral blood by apheresis after administration of G-CSF (10 to 15 µg/ kg/ day) for 4 to 6 days. G-CSF mobilized PBSC grafts usually contain higher numbers of CD34⁺ progenitor cells as well as T cells (CD3⁺ cells)

compared with BM grafts. A recent study reported high-dose plerixafor as a single agent can also be used to rapidly mobilize high numbers of CD34⁺ cells into the circulation, which may offer an alternative to GCSF for PBSC allograft mobilization.²⁴

Compared with BM stem cells, transplants using PBSCs are associated with faster neutrophil and platelet engraftment, a reduction in transfusion requirements, and a similar incidence of acute GVHD, although chronic GVHD occurs with higher frequency with the use of PBSC compared with BM transplants.²⁵ A recent multicenter trial conducted through the CTN that randomized more than 500 patients with hematologic malignancies to either a BM or a PBSC transplant from an unrelated donor reported no difference in relapse, DFS, or OS between the two approaches with recipients of PBSC transplant having a lower rejection rate but a significantly higher incidence of extensive chronic GVHD.²⁶ These data suggest that for patients with hematologic malignancies undergoing a MUD transplant, BM may be the preferred stem cell source.

Nevertheless, despite this limitation, the ease of stem cell collection from both the practitioner and the donor perspectives, the higher progenitor cell yield, earlier engraftment, and a lower risk of graft failure, the majority of allogeneic HSCTs worldwide in adults currently employ mobilized peripheral blood as a source of hematopoietic stem cells.

Conditioning Regimen

Various conditioning regimens have been used in allogeneic HSCT. The choice of conditioning regimen for a given patient is dictated by the underlying disease, the age of the patient, the presence of medical comorbidity, and donor characteristics (especially the degree of HLA compatibility). Table 18.2 lists some commonly used conditioning regimens.

Conventional or Myeloablative Conditioning

Myeloablative conditioning regimens serve a dual purpose:

- High doses of chemotherapy with or without radiation provide tumor cytoreduction, usually accompanied by eradication or ablation of host hematopoietic function.
- Suppression of the host's immune system, a prerequisite for preventing rejection of the transplant.

The burden of tumor eradication in conventional transplants rests on both the transient cytoreductive properties of the conditioning agents and on more durable GVL effects mediated by donor immune cells.

The two most commonly used regimens are cyclophosphamide in combination with either total-body irradiation (TBI) or busulfan. TBI-based regimens have a higher

incidence of secondary malignancies, growth retardation, thyroid dysfunction, and cataracts, while non-TBI regimens, particularly those containing busulfan (oral or IV), are associated with more veno-occlusive disease (VOD) and mucositis. The advent of intravenous (IV) busulfan, with its more predictable pharmacokinetic profile, has allowed more consistent exposure to busulfan and a reduction in the incidence of VOD. The underlying condition often dictates the optimal conditioning regimen. For example, patients with acute lymphoblastic leukemia (ALL) appear to have a lower risk of relapse with TBI-based regimens, which are therefore preferentially used for this indication.

Reduced Intensity Conditioning Reduced intensity preparative regimens were devised in an effort to minimize conditioning-related morbidity associated with conventional transplants, while retaining the host immunosuppression necessary to ensure engraftment. Reduced intensity preparative regimens that do not eradicate host hematopoiesis are also referred to as nonmyeloablative conditioning regimens.

Table 18.2 Preparative Regimens Commonly Used in Allogeneic Stem Cell Transplantation

Myeloablative Regimens

Cy/ TBI

Cyclophosphamide	120 mg/ kg IV
TBI	1,000–1,575 cGy

Bu/ Cy

Busulfan	16 mg/ kg PO or 12.8 mg/ kg
Cyclophosphamide	120–200 mg/ kg IV

Reduced-Intensity Regimens

Flu/ low-dose TBI

Fludarabine	90 mg/ m ² IV
TBI	200 cGy

Flu/ Mel

Fludarabine	125 mg/ m ² IV
Melphalan	180 mg/ m ² IV

Flu/ Bu/ ATG

Fludarabine	180 mg/ m ² IV
Busulfan	8 mg/ kg PO or 6.4 mg/ kg IV
ATG	40 mg/ kg IV

Cy/ Flu

Cyclophosphamide	120 mg/ kg IV
Fludarabine	125 mg/ m ² IV

ATG, antithymocyte globulin; Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; IV, intravenous; Mel, melphalan; PO, orally; TBI, total body irradiation.

The burden of achieving sustained tumor eradication following reduced intensity transplants rests more on the donor immune-mediated GVL effect. These regimens are associated with a lower incidence of some conditioning-related toxicities (VOD, mucositis, prolonged neutropenia, etc.).

Reduced intensity regimens are better tolerated by older patients (up to the age of 70 years) and by those with medical comorbidities and have allowed allogeneic HSCT to be extended to these populations. Several transplant centers are currently evaluating reduced intensity transplantation in patients with hematologic malignancies, solid tumors, and nonmalignant hematologic disorders. Although the risk of TRM appears lower with RIC regimens, several retrospective studies and a recent prospective study²⁷ has shown that the risk of disease relapse in malignant diseases such as myeloma, MDS and AML may be higher with this approach compared with conventional myeloablative transplants.

Results of Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic HSCT is the only curative option for many patients with hematologic malignancies: 85% to 90% of all allogeneic transplants in the United States are undertaken for this indication.

Chronic Myeloid Leukemia

CML is a myeloproliferative disorder characterized by the presence of a characteristic t(9;22) (q34;q11) translocation, the Philadelphia chromosome. The natural history consists of a relatively indolent chronic phase with progression to the more aggressive accelerated phase and blast crisis. Although allogeneic HSCT is the only proven curative therapy for this condition, the introduction of targeted agents with remarkable efficacy (such as imatinib mesylate, dasatinib, and nilotinib) has led to the acceptance of these agents as standard initial therapy for patients with chronic-phase CML. Consequently, allogeneic HSCT is usually reserved for patients with accelerated phase or blast crises CML and for chronic-phase patients who have failed agents targeting the Abl kinase.

Among chronic-phase patients undergoing transplantation from an HLA-compatible sibling, 65% to 80% are cured; similar results are now being reported in patients undergoing MUD transplantation. Early results in patients receiving RIC are promising, but prospective studies are needed to determine if this approach is equivalent to conventional allogeneic HSCT. Transplantation is much less effective in accelerated phase or blast crisis (where cure rates are 10% to 20%).

Younger patients and patients who undergo transplantation within a year of diagnosis have the best outcomes. Chronic-phase CML is sensitive to GVL effects and a single DLI can reinduce remission in 70% of patients who relapse after transplantation, as can withdrawal of immunosuppression (such as cyclosporine) used for GVHD prophylaxis/treatment. Chronic-phase CML patients who fail to achieve a cytogenetic remission with imatinib or other TKIs can be successfully salvaged with allogeneic HSCT.

Acute Myeloid Leukemia

The indication and timing for transplantation in AML and outcome after allogeneic HSCT depends on the risk category.

- | Patients with intermediate or poor prognosis AML as determined by cytogenetics are at a high risk of relapse after chemotherapy and should be evaluated for allogeneic HSCT in CR1 when an HLA-matched sibling donor is available. Recent studies have also shown that patients aged more than 45 years and/ or with normal cytogenetics who have an FLT3-ITD, or a wild-type NPM1 or CEBPA without an FLT3-ITD, are also at an increased risk for relapse and may benefit from an allogeneic transplant in CR1.²⁸
- | Patients transplanted in CR1 have a 45% to 60% probability of long-term DFS.
- | Patients transplanted in first relapse or after induction of second complete remission 2 (CR2) have a lower chance of long-term DFS.
- | Allogeneic HSCT in good-prognosis AML with a favorable karyotype is usually reserved for CR2 or first relapse, because the risk of TRM outweighs the benefits from early transplantation (CR1) in this group.
- | Less than 20% of patients who fail to respond to chemotherapy induction, either at first diagnosis or at relapse, as well as those patients transplanted beyond CR2 have durable leukemia remission after allogeneic HSCT.

Acute Lymphoblastic Leukemia

Although a significant proportion of childhood ALL is curable with chemotherapy, the majority (60% to 70%) of adults with this disease relapse following initial chemotherapy. Patients older than 60 years of age, those with a leukocyte count higher than 30,000/ μ L, or with high-risk cytogenetics (t[4;11], t[1;19], t[8;14], or t[9;22]) have a particularly poor prognosis. Traditionally, allogeneic HSCT in CR1 has been recommended for adult patients with ALL with poor prognostic features (DFS rates in the range of 40% to 60%), reserving transplant for patients without adverse factors for CR2 (DFS rates of approximately 40%). However, a randomized trial showed patients with standard-risk ALL who received an upfront allogeneic transplant in CR1 had a survival advantage compared with patients who received consolidative chemotherapy reserving transplant for CR2.²⁹ Therefore, allogeneic transplant in CR1 is a reasonable

strategy to prevent disease relapse in adults with either standard-risk or high-risk ALL.

Myelodysplastic Syndrome

Allogeneic HSCT offers approximately a 40% probability of long-term DFS in patients with MDS. The two most important factors predicting outcome after transplantation are blast percentage and cytogenetic risk group. Accordingly, patients with few blasts (refractory anemia or refractory anemia with ringed sideroblasts) have 50% to 75% long-term DFS, while more advanced stages (e.g., refractory anemia with excess blasts) are associated with a 30% DFS. Similarly, patients with good-risk cytogenetics have an approximately 50% probability of DFS compared with 10% or less for those with poor-risk cytogenetics. Nonetheless, allogeneic HSCT remains the only curative therapy for MDS and should be considered a potential definitive therapy. Reduced intensity HSCT is associated with a higher risk of relapse in MDS patients than in conventional HSCT and should be reserved for patients who are not candidates for myeloablative HSCT or performed as a part of well-designed trials.²⁷

Non-Hodgkin's Lymphoma

Low-Grade Non-Hodgkin Lymphoma and Chronic Lymphocytic Leukemia Experience with allogeneic HSCT in low-grade lymphomas and CLL is largely restricted to patients undergoing the procedure late in the course of their disease after multiple chemotherapeutic options have been exhausted; 50% to 65% of patients will achieve long-term DFS. The typically indolent disease course and profound susceptibility to GVL makes low-grade lymphomas and CLL amenable to management and cure using nonmyeloablative conditioning approaches. Remarkably, several studies have shown that more than 40% of patients with 17p deletion CLL, who have the worst prognosis with conventional chemotherapy, can obtain long-term DFS with a reduced intensity transplant.³⁰ However, with the recent US Food and Drug Administration (FDA) approval of novel kinase inhibitors to BTK and PI3K as well as venetoclax, a BCL-2 inhibitor, it is anticipated transplants for CLL will likely decline substantially.^{31,32}

Aggressive Non-Hodgkin Lymphoma The role of allogeneic HSCT in patients with intermediate-and high-grade lymphomas is unclear. Most studies have reported a high incidence of TRM with myeloablative transplantation in this group. As a consequence, allogeneic HSCT that uses RIC is generally reserved for those patients in whom potentially curative autologous HSCT has failed or for those unlikely to benefit from an autologous transplant (patients with chemotherapy-resistant disease).

Multiple Myeloma

TRM rates in the range of 50% have discouraged the use of conventional myeloablative

transplantation for multiple myeloma. Nevertheless, there is evidence that donor immune-mediated graft-versus-myeloma effects can be curative. RIC has been explored as a safer transplant approach to treat multiple myeloma. TRM has been reported to be significantly lower (less than 25%) compared with historic myeloma cohorts undergoing myeloablative conditioning; importantly, graft-versus-myeloma effects resulting in durable disease remission can be induced after reduced-intensity transplants. Autologous transplantation as myeloma cytoreduction followed by nonmyeloablative allogeneic transplantation as immunotherapy to eradicate minimal residual disease has been associated with DFS of more than 50% in some studies. However, a randomized trial conducted through the CTN group, which enrolled more than 700 subjects reported no difference in 3-year PFS and OS between patients receiving a tandem autotransplant versus a tandem auto/ allotransplant with significantly higher TRM being reported in the latter group.⁴ Based on the results of this trial, as well as the advent of monoclonal antibodies and numerous new drugs that are highly active against this malignancy, enthusiasm for the use of allogeneic transplantation for multiple myeloma has decreased substantially.

Aplastic Anemia

Allogeneic HSCT can cure SAA.³³ Early studies of allogeneic HSCT in patients with SAA showed a high incidence of graft rejection (up to 35% in some early series) and GVHD. Sensitization to histocompatibility antigens as a result of multiple transfusions and the use of cyclophosphamide alone as pretransplant conditioning accounted for these high rejection rates. Subsequent approaches added antithymocyte globulin (ATG) to cyclophosphamide to minimize graft rejection while preventing severe and potentially lethal GVHD. Additionally, the routine use of leukocyte-depleted and irradiated blood products has decreased the risk of graft rejection to less than 5% in most studies when transplantation is used as an upfront therapy. A combination of cyclosporine A (CSA) and methotrexate is generally used as GVHD prophylaxis with delayed and gradual withdrawal of immunosuppression to minimize the risk of GVHD. Patients under the age of 40 years receiving an allogeneic HSCT from an HLA-matched sibling have an excellent chance for cure, with long-term survival rates approaching 90% in children. More recent data suggest that the use of MUD donors for those lacking an HLA matched sibling can result in long-term survival rates of >80%.³⁴ Several studies indicate that transplant outcomes are better in patients with AA when BM is used as the primary graft source, rather than PBSCs, because the latter is associated with an increased incidence of chronic GVHD.

Complications of Allogeneic Hematopoietic Stem Cell Transplantation

Complications of allogeneic HSCT are most commonly related to preparative regimen toxicities, infections occurring as a consequence of immunosuppression, or acute or chronic GVHD.

Conditioning-Related Toxicities

Conditioning-related toxicities vary depending on the type and dosage of agents used in the preparative regimen. Nausea, vomiting, and mucositis occur commonly with myeloablative preparative regimens. Busulfan tends to be associated with more severe mucositis.

Hemorrhagic cystitis occurring early in the course of transplantation is usually associated with preparative regimens containing high-dose cyclophosphamide. In contrast, hemorrhagic cystitis more than 72 hours after conditioning is typically viral (polyoma virus BK or adenovirus). Attention to hydration and the routine use of 2-mercaptopethanesulfonate (mesna) has virtually eliminated cyclophosphamide-associated hemorrhagic cystitis.

Opportunistic infections occur with conditioning-related neutropenia. Bacteria and fungi that are normally present in the skin, gastrointestinal (GI) tract, or respiratory tract cause the majority of these infections.

Damage to gut mucosa and indwelling venous catheters serve as the portal of entry for most life-threatening gram-negative or aerobic gram-positive organisms. The use of prophylactic oral antibiotics such as quinolones for gut decontamination has decreased the incidence of gram-negative bacteremia without impacting survival. Routine use of these agents should be weighed against the increased risk of gram-positive bacteremia and emergence of resistant gram-negative strains.

Candida and Aspergillus fungal infections occur commonly during conditioning-induced neutropenia. Prophylactic fluconazole appears to protect against sensitive Candida. In a phase III randomized trial, prophylactic voriconazole was associated with a trend toward a lower incidence of invasive fungal infection compared with fluconazole, although OS was similar in the two groups.³⁵

VOD is characterized by the triad of jaundice, tender hepatomegaly, and ascites occurring early posttransplant. The risk factors include

- | Advanced age
- | Conditioning with busulfan, with up to 30% of patients receiving oral busulfan developing this complication. The advent and preferential use of IV busulfan appears to have led to a decrease in the incidence of VOD
- | Preexisting liver disease
- | Development of acute GVHD
- | Transplants from matched, unrelated, and haploidentical donors.

Prophylaxis with oral ursodiol may protect against this complication. VOD can be severe and life-threatening in approximately 25% of patients developing this complication. In 2016, the FDA has approved defibrotide, for the treatment of adults and children with hepatic VOD and renal or pulmonary dysfunction following HSCT based on the results of two open-label studies^{36,37}; and an unpublished study (summarized in the package insert). It is the first drug to be approved by the FDA for the treatment of severe hepatic VOD. Furthermore, in a randomized phase III study in pediatric patients undergoing HSCT, defibrotide prophylaxis was associated with a lower incidence of VOD.³⁸

Graft-Versus-Host Disease

GVHD is one of the most common complications of allogeneic HSCT. GVHD is a consequence of allogeneic donor T-cells damaging normal recipient tissues. Based on the time of onset, clinical features, and pathophysiology, GVHD is classified as either acute or chronic.³⁹

Acute Graft-Versus-Host Disease Acute GVHD typically commences during the first 100 days after transplantation. Of HLA-matched sibling donor transplant recipients, 20% to 50% experience acute GVHD; the incidence is higher in transplants utilizing unrelated or partial HLA-matched related donors. The extent of donor–host HLA disparity, recipient age, T-cell content of the graft, intensity of the conditioning regimen, and the type of GVHD prophylaxis regimen utilized all influence the incidence and severity of GVHD.

The skin, GI tract, and liver are the most common targets of alloreactive donor T-cells causing GVHD. The following clinical and laboratory features should arouse suspicion of GVHD:

- | Skin: Erythematous maculopapular rash frequently involving the palms and soles. Severe cases can present with skin desquamation.
- | GI: Crampy abdominal pain, and large-volume watery diarrhea characterize GVHD of the colon and distal small bowel. In severe cases, bloody diarrhea or ileus may occur. Anorexia, dyspepsia, weight loss, and nausea and vomiting are characteristic of upper GI GVHD.
- | Hepatic: Elevated alkaline phosphatase and direct bilirubin with or without elevations in transaminases or elevations in transaminases alone characterize acute GVHD of the liver.

Definitive diagnosis can be difficult because a variety of other conditions (such as drug-induced skin rash, viral colitis, or hepatitis) can present with similar features. Biopsy and histopathologic examination of involved tissue is considered the gold

standard for diagnosing GVHD.

GVHD is a major contributor to TRM; strategies directed at preventing this complication are an important aspect of transplant planning. Pharmacologic prophylaxis and graft T-cell depletion are established methods that effectively reduce the incidence and severity of GVHD. CSA or tacrolimus combined with methotrexate or mycophenolate mofetil are commonly used for GVHD prophylaxis. Effective T-cell depletion of the allograft can be achieved *in vitro* by CD34⁺ cell selection or *in vivo* pharmacologically (alemtuzumab or posttransplant cyclophosphamide). However, in general, T-cell-depleted transplants are associated with a higher risk of graft failure, leukemia relapse, and opportunistic viral infections. Selective depletion of alloreactive T-cells and partial T-cell depletion have been shown to reduce the incidence of GVHD without compromising engraftment.⁴⁰

Treatment of established GVHD depends on the type and severity of involved organs (for grading of acute GVHD, see Table 18.3).⁴¹ While mild (grade I) skin GVHD can be managed effectively with topical corticosteroids, visceral GVHD and more severe forms of cutaneous GVHD require systemic immunosuppressive therapy. Glucocorticoids (methylprednisolone, typically at doses of 1 to 3 mg/ kg/ day) are the mainstay of therapy and are given in conjunction with cyclosporine or tacrolimus, with doses titrated to maintain therapeutic serum levels. Although most patients with mild grade I/ II GVHD will have complete resolution of GVHD with corticosteroid therapy, only about 50% of patients with \geq grade III will achieve durable responses to this form of therapy (for the treatment of established acute GVHD, see Table 18.4).

Table 18.3 Grading of Acute Graft-Versus-Host Disease

	Organ Involvement		
	Skin	Liver	Gastrointestinal
Stage			
1	Rash < 25% of skin	Bilirubin 2–3 mg/ dL	Diarrhea > 500 mL/ day or persistent nausea with histologic evidence of upper GI GVHD
2	Rash 25%–50% of skin	Bilirubin 3–6 mg/ dL	Diarrhea > 1,000 mL/ day
3	Rash > 50% of skin	Bilirubin 6–15 mg/ dL	Diarrhea > 1,500 mL/ day
4	Generalized erythroderma	Bilirubin >15 mg/ dL	Severe abdominal pain with or with bullae without ileus
Grade			
I	Stage 1–2	0	0
II	Stage 3 or	Stage 1 or	Stage 1

III	Stage 2–3 or	Stage 2–4
IV	Stage 4 or	Stage 4

GI, gastrointestinal; GVHD, graft-versus-host disease.

Data from Przepiorka D, Weisdorf D, Martin P, et al. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825–828.

Nonresponding or steroid-refractory patients have a poor outcome, with mortality rates more than 80%. The majority developing steroid-refractory GVHD die from infectious complications or organ damage related to relentless immune attack. Comprehensive management of steroid-refractory GVHD patients with immunosuppressive agents such as daclizumab or basilizimab with or without infliximab accompanied by targeted infectious prophylaxis against enteric bacteria and *Aspergillus* appears to be a promising strategy that deserves further study (see [Table 18.4](#)).⁴²

Chronic Graft-Versus-Host Disease The onset of chronic GVHD is usually between 100 days and 2 years after transplantation. It affects 20% to 50% of recipients of allogeneic BM transplants and up to 80% receiving an allogeneic PBSC transplant. Risk is increased by:

- Prior history of acute GVHD
- Older patient age
- Use of HLA-mismatched or unrelated donors
- DLI administration
- Use of PBSC (as opposed to BM) allografts.

Table 18.4 Treatment of Acute Graft-Versus-Host Disease: The National Heart, Lung, and Blood Institute Approach

Initial Management

Grade I GVHD (Stage 1–2 Skin)

- Topical corticosteroid therapy

Grade II–IV GVHD

- High-dose methylprednisolone 1–10 mg/ kg/ day up to a maximum of 500 mg/ day IV 3–6 days and
- IV cyclosporine or IV tacrolimus
- Steroids tapered once response is evident over 10–14 days
- All patients receiving \geq 1 mg/ kg of methylprednisolone undergo routine surveillance blood cultures every 3 d
- All patients with \geq grade III GI GVHD receive prophylactic antibiotic therapy against enteric organisms (e.g., ampicillin–sulbactam, piperacillin/ tazobactam or meropenem)

Management of Steroid-Refractory GVHD

(GVHD not responsive to 6 or more days of continuous therapy with \geq 1 mg/ kg methylprednisolone)

(A) Treatment

- Rapid taper of methylprednisolone to ≤ 1 mg/ kg
- Basiliximab (monoclonal antibody to interleukin-2 receptor- α) 20 mg IV on days 1, 4, 8, 15, 22
- Infliximab (monoclonal antibody to tumor necrosis factor- α) 10 mg/ kg on days 1, 8, 15, 22

(B) Supportive care

- All patients with GI GVHD are maintained NPO
- All patients with \geq grade III GI GVHD receive prophylactic antibiotic therapy against enteric organisms as above
- All patients with steroid-refractory GVHD and those patients who receive ≥ 1 mg/ kg methylprednisolone for more than 6d receive prophylaxis against Aspergillus (e.g., voriconazole, posaconazole, etc.)
- All patients receiving ≥ 1 mg/ kg of methylprednisolone undergo routine surveillance blood cultures every 3 d

GI, gastrointestinal; GVHD, graft-versus-host disease; IV, intravenous; NPO, nothing by mouth.

Patients may present with a myriad of clinical features including lichenoid or sclerodermatosus skin changes, elevated liver function tests, xerostomia, dry eyes, diarrhea, weight loss, bronchiolitis obliterans, and thrombocytopenia with or without pancytopenia.

Most clinicians use a two-stage staging system: limited GVHD, representing localized skin involvement and extensive GVHD, which includes patients with more diffuse skin involvement or involvement of other target organs. A more recent National Institute of Health (NIH) consensus criteria for the diagnosis and classification of chronic GVHD is based on histopathologic, clinical, laboratory, and radiologic features, rating chronic GVHD as mild, moderate, or severe.⁴³

Therapy typically consists of cyclosporine or tacrolimus given in conjunction with low-dose corticosteroids. Other treatments which have activity against chronic GVHD include extracorporeal photopheresis or drugs including mycophenolate mofetil, thalidomide, imatinib, ruxolitinib, ibrutinib or monoclonal antibodies directed against T or B lymphocytes or cytokines implicated in pathogenesis.

A high risk of bacterial infections in patients with chronic GVHD warrants routine use of antibiotic prophylaxis against encapsulated bacteria and opportunistic pathogens.

Pulmonary Complications

Pulmonary complications may occur both early and late after transplantation, and they may be infectious or noninfectious in etiology.

Pulmonary Complications Attributable to Infections Fungi (Aspergillus and other agents) as well as viruses (CMV, respiratory syncytial virus, influenza, parainfluenza, etc.) can cause life-threatening pneumonia in the posttransplant setting. Early diagnosis, prophylactic or preemptive therapy (e.g., ganciclovir or foscarnet for CMV antigenemia), and prompt institution of definitive therapy when available are the major principles guiding management of these complications. The risk of *Pneumocystis jiroveci* pneumonia is

greatest in the first 6 months after transplantation, particularly in patients receiving T-cell-depleted grafts or those suffering from chronic GVHD; prophylaxis with sulfa/trimethoprim or inhaled pentamidine virtually eliminates this complication.

Idiopathic interstitial pneumonitis usually occurs early after transplantation and is characterized by fever, hypoxia, and diffuse pulmonary infiltrates. TBI or drugs with pulmonary toxicity (i.e., busulfan) in the preparative regimen increase the risk of this complication. An infectious etiology as well as diffuse alveolar hemorrhage should be excluded before a diagnosis of idiopathic interstitial pneumonitis can be rendered. Corticosteroids and tumor necrosis factor- α antagonists have been used to treat this condition with modest results.

Diffuse alveolar hemorrhage is a relatively infrequent, but often fatal complication of allogeneic HSCT. It is characterized by the rapid onset of dyspnea, cough, and hypoxia with diffuse bilateral infiltrates on radiography. High-dose corticosteroids and recombinant activated factor VII (Novo7) may be of therapeutic benefit, although the condition is lethal in 40% to 80% of cases.

Infectious Complications

Recipients of allogeneic HSCT continue to be at risk for infections beyond the period of conditioning-related neutropenia, with viral and fungal pathogens and encapsulated bacteria posing the greatest hazard.⁴⁴ Factors influencing infectious risk include the presence of acute or chronic GVHD, the extent of immunosuppressive pharmacotherapy in the posttransplant period, T-cell depletion of the graft, and the use of cord blood transplants or partial HLA-mismatched or unrelated donors.

Bacterial Infections

Gram-negative bacteremia associated with GI GVHD and venous catheter-related infections (predominantly gram-positive pathogens) occur with greatest frequency during the first 3 to 4 months after transplantation.

Recurrent sinus and pulmonary infections are associated with chronic GVHD. Antibiotic prophylaxis against encapsulated organisms, using penicillin or an appropriate alternative, reduces the risk of these infections.

Patients with recurrent infections and low serum immunoglobulin levels may benefit from prophylactic IV immunoglobulin (IVIg) infusions.

Fungal Infections

Fungal infections constitute a major cause of mortality after allogeneic HSCT: 60% to 70% of patients developing invasive fungal infections die despite antifungal therapy.

Yeast (*Candida* species) and molds (*Aspergillus*) account for the majority of opportunistic fungal infections in the posttransplant period.

Candida infections typically occur early in the course of transplantation, often near the end of the neutropenic phase. Candidal infections can manifest as mucocutaneous candidiasis, candidemia, or with visceral involvement (the liver and spleen are most commonly involved). Routine prophylaxis with fluconazole or echinocandins offers protection against sensitive strains of Candida.

Invasive Aspergillus infections typically involve the lungs, paranasal sinuses, and the central nervous system (CNS), although dissemination to other visceral organs has been described. Predisposing factors are

- | Corticosteroids
- | Severe GVHD
- | The use of non-HLA-identical and unrelated stem cell donors.

Invasive fungal infections are difficult to eradicate. Fluconazole or echinocandins may be effective against sensitive Candida strains (like *Candida albicans*). Ambisome, echinocandins (caspofungin, micafungin, etc.), voriconazole, and posaconazole have demonstrated efficacy against Aspergillus and a wide spectrum of Candida species.

The diligent application of preventive measures such as avoiding the indiscriminate use of corticosteroids or the prophylactic use of antifungal drugs in patients receiving corticosteroid therapy is the most effective strategy for minimizing mortality related to invasive fungal infections.

Viral Infections

Cytomegalovirus Although advances in screening and preventive therapy have reduced CMV-related mortality, CMV infection continues to be a major contributor to posttransplant morbidity. CMV is a DNA virus belonging to the herpes virus family. Posttransplant CMV infection is most often a consequence of viral reactivation in patients with prior exposure to CMV and is observed in 50% to 70% of CMV seropositive recipients. Reactivation typically occurs in the first 100 days after transplantation. CMV immunity can be transferred from the donor to the patient following transplantation as evidenced by CMV-seropositive patients receiving transplants from CMV seronegative donors having a higher risk of CMV reactivation than seropositive patients receiving a transplant from seropositive donors. Acquisition of primary infection from CMV-positive transfusion products has been all but eliminated with the routine use of leukocyte-depleted or CMV-negative blood products.

GVHD, the use of T-cell-depleted allografts, cord blood transplants, and the use of immunosuppressive agents such as alemtuzumab, corticosteroids, or calcineurin inhibitors and transplants from CMV seronegative donors into seropositive patients all increase the risk of CMV reactivation.

Interstitial pneumonitis is the most common and serious manifestation of CMV

disease, followed by enteritis/ colitis. The other manifestations include febrile episodes and marrow suppression resulting in thrombocytopenia with or without neutropenia. Mortality rates with CMV pneumonitis range from 65% to 85%. Ganciclovir or foscarnet given in conjunction with IVIg improves outcome associated with CMV disease. Early detection methods utilize polymerase chain reaction (PCR) for viral DNA in the blood that predicts the subsequent development of CMV disease.

Preemptive therapy with ganciclovir or foscarnet began when CMV reactivation is first detected (by PCR) has dramatically reduced the incidence of CMV pneumonitis/ enteritis and consequently CMV-related mortality. Newer approaches to the treatment of or prophylaxis against CMV disease include adoptive transfer of ex vivo expanded CMV-specific cytotoxic T lymphocytes from partially HLA matched third-party donors.

Epstein–Barr Virus–Associated Lymphoproliferative Disorder Epstein–Barr Virus infection is a part Viral infections (like CMV or other viral infections.)

EBV-related lymphoproliferative disorder is a B-cell malignancy arising as a consequence of impaired T-cell immunity against EBV. It is a relatively rare complication, affecting approximately 1% of all allogeneic transplant recipients, although certain transplants (especially T-cell depleted or umbilical cord blood) are associated with a significantly higher risk. The natural course of untreated EBV-related lymphoproliferative disorder is rapid progression, culminating in death. Allograft T-cell depletion, transplants from HLA-mismatched and unrelated donors or cord blood transplants, and the use of immunosuppressive agents such as ATG predispose to the development of this malignancy.

Treatment with a monoclonal antibody to CD20 (rituximab), DLIs, withdrawal of posttransplant immunosuppression, or adoptive infusion of EBV-specific cytotoxic T-cells from the stem cell donor or from a partially HLA-matched third party donor are all effective in eradicating this disease, particularly when combined with the withdrawal of immunosuppression.

Other Viral Infections Patients undergoing allogeneic HSCT are at risk for infectious complications associated with the herpes viruses, varicella zoster, and various respiratory viruses (respiratory syncytial virus, influenza, and parainfluenza). Adenoviruses or polyomavirus BK may appear clinically as hemorrhagic cystitis. Because cellular immunity is impaired in the posttransplant setting, otherwise self-limiting viral infections can have fatal sequelae. Recently, the investigational agent brincidofovir has shown promising activity in patients with systemic adenoviral infection.

Graft Failure

Graft failure is the inability to achieve (primary) or maintain (secondary) persistent donor hematopoiesis. Graft failure mediated by the recipient immune system is referred to as graft rejection. Graft failure is relatively uncommon in patients undergoing transplantation from an HLA-identical sibling donor (less than 2%). T-cell depletion, the use of HLA-mismatched or unrelated donor grafts, cord blood transplants, and pretransplant HLA alloimmunization caused by repeated transfusions are factors that increase the risk of graft rejection.

In myeloablative HSCT using BM or PBSC, primary graft failure presents as persistent pancytopenia (more than 3 to 4 weeks) after conditioning and is associated with a high mortality rate. Secondary graft failure is characterized by the initial recovery of blood counts followed by a later loss of donor hematopoiesis.

Up to 50% of patients with graft rejection can be salvaged by repeat conditioning or immunosuppression (e.g., OKT3 plus corticosteroids) followed by reinfusion of a T-cell–replete allograft. Graft failure can also result from infections, drugs, or chronic GVHD. Patients can sometimes be salvaged with hematopoietic growth factors (e.g., G-CSF, eltrombopag) with or without additional stem cells.

Late Sequelae of Transplantation

Secondary Malignancies In addition to EBV-related lymphomas, leukemias and solid tumors can complicate allogeneic HSCT. The risk of solid tumors in transplant survivors at 10 years increases eightfold compared to age-matched controls. Melanomas, tumors of the oral cavity, bone, liver, CNS, and thyroid are some commonly encountered secondary malignancies. Younger patient age at transplantation and TBI-based conditioning regimens predispose to the development of secondary solid tumors.

Other Late Complications Growth retardation, infertility, restrictive pulmonary disease, cataracts, endocrine dysfunction, avascular necrosis of bones, osteopenia, and neurocognitive defects are other delayed sequelae of allogeneic HSCT.

Reduced Intensity Conditioning Hematopoietic Stem Cell Transplantation

The high risk of TRM with conventional transplants and the appreciation that GVL effects can cure some hematologic malignancies provided the impetus for the development of reduced intensity and nonmyeloablative conditioning regimens.^{45,46} The basic principles underlying these regimens include the following:

- RIC to induce adequate host immunosuppression for donor allograft “take” while minimizing toxicities related to dose-intensive conditioning.

- Manipulation of posttransplant immunosuppression and administration of DLIs to promote rapid transition to complete donor immunohematopoiesis.
- Reliance on the GVL effect for eradication of the underlying malignancy and prevention of disease relapse.

A variety of different conditioning regimens have been used, and the cumulative experience from various transplant centers has led to the following observations:

- Toxicities such as VOD and mucositis are absent or mild compared to myeloablative transplants.
- TRM is substantially lower (7% to 20%) than the 15% to 25% mortality associated with standard or myeloablative transplants.
- The improved toxicity profile has expanded the eligibility of allogeneic transplantation to older patients (up to 70 years of age) and patients with comorbid medical conditions.

GVL effects against several hematologic malignancies including AML, CML, ALL, CLL, NHL, and myeloma have been observed. However, at least in some malignancies (e.g., MDS and myeloma), the risk of relapse following RIC appears to be higher than that following myeloablative transplantation.²⁷

Pilot trials of nonmyeloablative HCT in solid tumors demonstrated for the first time the ability of the GVT effect to induce disease regression in treatment-refractory metastatic solid tumors. Renal cell carcinoma provides the best example of a tumor that may be susceptible to GVT effects⁴⁷; GVT effects have also been described in other solid tumors including breast, pancreatic, colon, and ovarian carcinoma.⁴⁸

Alternative Donor Transplantation

Transplantation from Matched Unrelated Donors

An HLA-matched sibling donor can be identified for less than a third of patients evaluated for an allogeneic HSCT. A 10/ 10 HLA-matched (at the HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ loci) volunteer donors registered with the NMDP can be identified for many patients who do not have a matched sibling donor, but are otherwise considered candidates for an allogeneic HSCT. It is estimated that up to 70% of Caucasians will have at least one HLA-matched donor available. It may be more difficult to find appropriately matched donors for patients belonging to some racial/ethnic subgroups.

Outcome following matched unrelated HSCT has improved significantly with the introduction of routine molecular typing of HLA loci (as opposed to serologic typing) to identify donor–host compatibility. Transplantation using donors who are HLA-identical (10/ 10 HLA match) by high-resolution molecular typing leads to outcomes that are only

slightly inferior to that following matched sibling transplants; nevertheless, acute GVHD rates remain higher in unrelated donor transplants.⁴⁹

Transplantation from Mismatched Related Donors

Siblings with mismatches at one or more HLA loci can be used as donors for allogeneic HSCT. However, the incidence of both graft failure and GVHD is higher in recipients of partially matched sibling transplants.

Haploidentical transplants utilize parents, siblings, or children who share one haplotype with the recipient as donors. The high risk of lethal GVHD accompanying haploidentical transplantation mandates extensive T-cell depletion of grafts (either ex vivo of the allograft itself before infusion or in vivo using T-cell-depleting agents such as posttransplant cyclophosphamide or alemtuzumab). In particular, the implementation of high-dose posttransplantation cyclophosphamide (PTCy) has greatly reduced GVHD, graft failure, and non-relapse mortality, and has led to the increasing utilization of haploidentical donors over the past 5 years. Remarkably, the majority studies published to date utilizing haploidentical transplantation with PTCy have shown that the incidence of acute and chronic GVHD was either similar or lower than that observed with a fully HLA-matched BMT.^{23,50} However, some studies have shown that relapse (as high as 45%) may be problematic with this approach.²² Taken altogether, recent data have established that haploidentical transplantation represents a viable alternative to HLA-matched transplantation, although further studies are needed to elucidate the exact clinical scenarios where haploidentical BMT with PTCy may be a preferred transplant strategy.

In addition, strategies such as the use of high CD34⁺ cell dose and/ or nonmyeloablative conditioning have been utilized in attempts to improve outcome in transplants using HLA mismatched related donors. Donor–host killer immunoglobulin-like receptor (KIR) incompatibility may affect outcome following haploidentical transplantation. Specifically, transplants in which recipient cells do not express HLA molecules that can inhibit donor KIR are associated with a lower risk of GVHD and disease relapse, notably in patients with myeloid malignancies.⁵¹ Current evidence suggests that natural killer cell alloreactivity may mediate both the heightened GVL effects and reduced GVHD incidence in KIR-incompatible haploidentical transplants.

Umbilical Cord Transplants

Cord blood, collected from peripartum placenta, contains stem cells with remarkable proliferative capability and is being used increasingly as an alternative source of stem cells for allogeneic HSCT. Both their proliferative potential and reduced and relatively immature lymphocyte content (which would be predicted to lead to a lower incidence of GVHD) are viewed as advantages over other alternative stem cell sources such as

haploidentical and mismatched unrelated donors. Furthermore, since umbilical cord allografts are derived from previously collected and stored cord blood, they are more readily available than unrelated donor grafts, which involve donor preparation and collection of stem cells once a suitable donor is identified. The major limitation of cord blood as a source of hematopoietic stem cells (particularly in adults) is the relatively small number of hematopoietic stem cells that can be obtained from single cord blood units. Most studies have shown that a minimum of 2.5×10^7 total nucleated cells/ kg (TNC) and/ or $\geq 1.2 \times 10^5$ CD34⁺ cells/ kg that are HLA matched at least 4/ 6 loci (serologically at HLA A and B loci and molecularly at the HLA-DR loci) are required to obtain acceptable engraftment rates and survival.

Early studies of cord blood transplants in children established the feasibility of this procedure, with acceptable engraftment rates (85% in one study) and low rates of acute GVHD (<10% in matched cord blood transplants).⁵² Retrospective analyses comparing cord blood transplants to unrelated donor marrow transplants have since been undertaken and indicate the following^{53,54}:

- | Cord blood transplants are typically associated with delayed hematopoietic recovery (median neutrophil recovery time of 24 to 27 days and median platelet recovery time of 60 days in one study), leading to a higher risk of infectious complications.
- | A lower incidence of acute GVHD as well as chronic GVHD is encountered following cord blood transplants.
- | TRM, disease relapse rates, and DFS following cord blood transplants are comparable to that seen in transplants using mismatched unrelated donor marrow.

These results, therefore, support the use of CB transplantation when a cord unit is unavailable that is HLA matched for $\geq 4/ 6$ loci and contains adequate TNC and CD34 number as previously defined.

Recently, a retrospective study on behalf of Eurocord, and the Acute Leukemia Working party of EBMT has reported comparable outcomes after CBT and haploidentical transplant in adults with hematologic malignancies.⁵⁵ Prospective data comparing outcomes of haploidentical transplants using PTCy versus cord transplants are ongoing and yet to be published. However, because most patients have a haploidentical donor that is readily available and because cord transplants are more expensive than the costs for collecting a haploidentical graft, haploidentical transplants may have economic advantages over cord transplants.

The simultaneous use of multiple cord blood units (from different donors), the co-infusion of cord units with CD34⁺ selected haploidentical stem cells, and ex vivo expansion of cord blood stem cells are being explored as means of overcoming the limitations imposed by low stem cell dose, and it is hoped that these approaches will allow more adults to undergo this procedure.^{56–58}

References

1. Blume KG, Thomas ED. A review of autologous hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2000;6:1–12.
2. Thomas ED. Bone marrow transplantation: a review. *Semin Hematol.* 1999;36:95–103.
3. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. *N Engl J Med.* 1996;335:91–97.
4. Krishnan A, Pasquini MC, Logan B, et al. Autologous haemopoietic stem-cell transplantation followed by allogeneic or autologous haemopoietic stem-cell transplantation in patients with multiple myeloma (BMT CTN 0102): a phase 3 biological assignment trial. *Lancet Oncol.* 2011;12:1195–1203.
5. Gay F, Oliva S, Petrucci MT, et al. Chemotherapy plus lenalidomide versus autologous transplantation, followed by lenalidomide plus prednisone versus lenalidomide maintenance, in patients with multiple myeloma: a randomised, multicentre, phase 3 trial. *Lancet.* 2015;16(16):1617–1629. doi:10.1016/S1470-2045(15)00389-7
6. Palumbo A, Cavallo F, Gay F, et al. Autologous transplantation and maintenance therapy in multiple myeloma. *NEJM.* 2014;371(10):895–905. doi:10.1056/NEJMoa1402888.
7. Jakubowiak AJ, Dytfield D, Griffith KA, et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. *Blood.* 2012 Aug 30;120(9):1801–1809. doi:10.1182/blood-2012-04-422683.
8. Korde N, Zingone A, Kwok ML, et al. Phase II clinical and correlative study of carfilzomib, lenalidomide, and dexamethasone followed by lenalidomide extended dosing (CRD-R) induces high rates of MRD negativity in newly diagnosed multiple myeloma (MM) patients [abstract]. *Blood.* 2013;122(21);538.
9. Cavo M, Rajkumar SV, Palumbo A, et al. International Myeloma Working Group consensus approach to the treatment of multiple myeloma patients who are candidates for autologous stem cell transplantation. *Blood.* 2011;117:6063–6073.
10. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med.* 1995;333:1540–1545.
11. Antman KH. Randomized trials of high dose chemotherapy for breast cancer. *Biochim Biophys Acta.* 2001;1471:M89–M98.
12. Pico JL, Rosti G, Kramar A, et al. A randomised trial of high-dose chemotherapy in the salvage treatment of patients failing first-line platinum chemotherapy for advanced germ cell tumours. *Ann Oncol.* 2005;16:1152–1159.

13. Voss MH, Feldman DR, Motzer RJ. High-dose chemotherapy and stem cell transplantation for advanced testicular cancer. *Expert Rev Anticancer Ther.* 2011;11:1091–1103.
14. Childs RW. Allogeneic hematopoietic cell transplantation. In: De Vita VJ, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. London, UK: Lippincott Williams and Wilkins; 2011:2244–2261.
15. Mathe G, Amiel JL, Schwarzenberg L, et al. Adoptive immunotherapy of acute leukemia: experimental and clinical results. *Cancer Res.* 1965;25:1525–1531.
16. Storb R. Allogeneic hematopoietic stem cell transplantation— yesterday, today, and tomorrow. *Exp Hematol.* 2003;31:1–10.
17. Boelens JJ, Prasad VK, Tolar J, et al. Current international perspectives on hematopoietic stem cell transplantation for inherited metabolic disorders. *Pediatr Clin North Am.* 2010;57:123–145.
18. Smith AR, Gross TG, Baker KS. Transplant outcomes for primary immunodeficiency disease. *Semin Hematol.* 2010;47:79–85.
19. Antin JH. Stem cell transplantation-harnessing of graft-versus-malignancy. *Curr Opin Hematol.* 2003;10:440–444.
20. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood.* 1990;75:555–562.
21. Riddell SR, Berger C, Murata M, et al. The graft versus leukemia response after allogeneic hematopoietic stem cell transplantation. *Blood Rev.* 2003;17:153–162.
22. Brunstein CG, Fuchs EJ, Carter SL, et al. Alternative donor transplantation after intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood.* 2011;118:282–288.
23. Ciurea SO, Zhang MA, Bacigalupo AA, et al. Haploidentical transplant with post-transplant cyclophosphamide versus matched unrelated donor transplant for acute myeloid leukemia. *Blood.* 2015;126(8):1033–1040. doi:10.1182/blood-2015-04-639831
24. Pantin J, Purev E, Tain X, et al. Effect of high-dose plerixafor on CD34⁺ cells mobilization in healthy stem-cell donors: results of a randomized crossover trial. *Hematologica.* 2016;102:600–609. doi:10.3324/haematol.2016.147132.
25. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med.* 2001;344:175–181.
26. Claudio Anasetti BRL, Lee SJ, Waller EK, et al. Increased incidence of chronic graft-versus-host disease (GVHD) and no survival advantage with filgrastim-mobilized peripheral blood stem cells (PBSC) compared to bone marrow (bm) transplants from unrelated donors: results of blood and marrow transplant clinical trials network (BMT CTN) protocol 0201, a phase III, prospective, randomized

- trial. *Blood*. (ASH Annual Meeting Abstracts) 2011;118:1.
- 27. Scott BL, Pasquini MC, Logan B, et al. Results of a phase III randomized, multicenter study of allogeneic stem cell transplantation after high versus reduced intensity conditioning in patients with myelodysplastic syndrome or acute myeloid leukemia: Blood and Marrow Transplant Clinical Trials Network 0901. Paper presented at: 2015 ASH Annual Meeting. Abstract LBA-8; December 8, 2015 in Orlando, Florida, USA.
 - 28. Stelljes M, Krug U, Beelen DW, et al. Allogeneic transplantation versus chemotherapy as postremission therapy for acute myeloid leukemia: a prospective matched pairs analysis. *JCO*. 2013;32(4):288–296. doi:10.1200/JCO.2013.50.5768.
 - 29. Goldstone AH, Richards SM, Lazarus HM, et al. In adults with standard-risk ALL, the greatest benefit is achieved from a matched sibling allogeneic transplantation in CR1, and an autologous transplantation is less effective than conventional consolidation/ maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ ECOG E2993). *Blood*. 2008;111:1827–1833.
 - 30. Dreger P, Dohner H, Ritgen M, et al. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. *Blood*. 2010;116:2438–2447.
 - 31. Burger JA, Keating MJ, Wierda WG, et al. Ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. *Lancet Oncol*. 2014;15(10):1090–1099. doi:10.1016/S1470-2045(14)70335-3.
 - 32. Stilgenbauer S, Eichhorst BF, Schetelig JS, et al. Venetoclax (ABT-199/ GDC-0199) monotherapy induces deep remissions, including complete remission and undetectable MRD, in ultra-high risk relapsed/ refractory chronic lymphocytic leukemia with 17p deletion: results of the pivotal international phase II study. Paper presented at: 57th American Society of Hematology Annual Meeting; December 5–8, 2015; Orlando, Florida. Abstract LBA6.
 - 33. Doney K, Leisenring W, Storb R, et al. Primary treatment of acquired aplastic anemia: outcomes with bone marrow transplantation and immunosuppressive therapy. Seattle Bone Marrow Transplant Team. *Ann Intern Med*. 1997;126:107–115.
 - 34. A. Bacigalupo, G. Socié, R.M. Hamladji, et al. Current outcome of HLA identical sibling vs. unrelated donor transplants in severe aplastic anemia: an EBMT analysis. *Haematologica*. 2015;100(5):696–702.
 - 35. Wingard JR, Carter SL, Walsh TJ, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood*. 2010;116:5111–5118.

36. Richardson PG, Riches ML, Kernan NA, et al. Phase 3 trial of defibrotide for the treatment of severe veno-occlusive disease and multi-organ failure. *Blood*. 2016;127:1656.
37. Richardson PG, Soiffer RJ, Antin JH, et al. Defibrotide for the treatment of severe hepatic veno-occlusive disease and multiorgan failure after stem cell transplantation: a multicenter, randomized, dose-finding trial. *Biol Blood Marrow Transplant*. 2010;16:1005.
38. Corbacioglu S, Cesaro S, Faraci M, et al. Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial. *Lancet*. 2012;379:1301–1309.
39. Vogelsang GB, Lee L, Bensen-Kennedy DM. Pathogenesis and treatment of graft-versus-host disease after bone marrow transplant. *Annu Rev Med*. 2003;54:29–52.
40. Purev E, Tian X, Aue G, et al. Allogeneic transplantation using CD34⁺ selected peripheral blood progenitor cells combined with non-mobilized donor T-cells for refractory severe aplastic anemia. *Brit J Heme*. 2016;176(6):950–960.
41. Przepiorka D, Weisdorf D, Martin P, et al. 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825–828.
42. Srinivasan R, Chakrabarti S, Walsh T, et al. Improved survival in steroid-refractory acute graft versus host disease after non-myeloablative allogeneic transplantation using a daclizumab-based strategy with comprehensive infection prophylaxis. *Br J Haematol*. 2004;124:777–786.
43. Shulman HM, Kleiner D, Lee SJ, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. *Biol Blood Marrow Transplant*. 2006;12:31–47.
44. Leather HL, Wingard JR. Infections following hematopoietic stem cell transplantation. *Infect Dis Clin North Am*. 2001;15:483–520.
45. Anagnostopoulos A, Giralt S. Critical review on non-myeloablative stem cell transplantation (NST). *Crit Rev Oncol Hematol*. 2002;44:175–190.
46. Storb RF, Champlin R, Riddell SR, et al. Non-myeloablative transplants for malignant disease. *Hematology Am Soc Hematol Educ Program*. 2001:375–391.
47. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med*. 2000;343:750–758.
48. Childs RW, Srinivasan R. Allogeneic hematopoietic cell transplantation for solid tumors. In: Blume KG, Forman S, Appelbaum FR, eds. *Thomas' Hematopoietic Cell Transplantation*. 4th ed. Malden, MA: Blackwell Science;2009:958–969.
49. Ringden O, Labopin M, Schidmid C, et al. Sequential chemotherapy followed by reduced-intensity conditioning and allogeneic haematopoietic stem cell transplantation in adult patients with relapse or refractory acute myeloid

- leukaemia: a survey from the Acute Leukaemia Working Party of EBMT. *Br J Haematol.* 2017 Feb;176(3):431–439.
50. Bashey A, Zhang X, Jackson K, et al. Comparison of outcomes of hematopoietic cell transplants from T-replete haploidentical donors using post-transplantation cyclophosphamide with 10 of 10 HLA-A, -B, -C, -DRB1, and -DQB1 allele-matched unrelated donors and HLA-identical sibling donors: a multivariable analysis including disease risk index. *Biology of Blood and Bone Marrow Transplant.* 2016 January;22(1):125–133.
 51. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science.* 2002;295:2097–2100.
 52. Wagner JE, Kernan NA, Steinbuch M, et al. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. *Lancet.* 1995;346:214–219.
 53. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004;351:2265–2275.
 54. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med.* 2004;351:2276–2285.
 55. Ruggeri A, Labopin M, Sanz G, et al. Comparison of outcomes after unrelated cord blood and unmanipulated haploidentical stem cell transplantation in adults with acute leukemia. *Leukemia.* 2015 Sep;29(9):1891–1900. doi: 10.1038/leu.2015.98.
 56. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood.* 2010;116:4693–4699.
 57. Brunstein CG, Miller JS, Cao Q, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. *Blood.* 2011;117:1061–1070.
 58. Horwitz ME, Chao NJ, Rizzieri DA, et al. Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. *J Clin Invest.* 2014 Jul;124(7):3121–3128. doi:10.1172/JCI74556.

Thrombocytopenia

Karolyn A. Oetjen and Cynthia E.
Dunbar

PLATELET BIOLOGY

- Platelets are anucleate blood cells that participate in primary hemostasis, the formation of a platelet plug at sites of vascular injury.
- Platelets are produced from megakaryocytes, multinucleate hematopoietic cells located in the bone marrow. Cytokines such as thrombopoietin are necessary for normal platelet maturation and release.
- Once released into the circulation, the average life span of a platelet is 7 to 10 days. Platelets are removed from circulation as they are activated and utilized at sites of vascular injury, or as they become senescent.
- At any given time, up to one-third of the platelet mass is stored in the spleen, providing a reserve of platelets that may be released during periods of physiologic stress.
- The normal platelet concentration in the blood is 150,000 to 400,000/ μL as measured in most hospital laboratories.

ETIOLOGY AND CLINICAL FEATURES OF THROMBOCYTOPENIA

Thrombocytopenia may occur due to

- Decreased production of platelets
- Increased consumption of platelets
- Increased sequestration of platelets
- Any combination of these mechanisms ([Table 19.1](#)).

Regardless of the cause of thrombocytopenia, “platelet-type” bleeding is typically

mucocutaneous and is characterized by petechiae, ecchymoses, epistaxis, and gingival and conjunctival hemorrhages. Less commonly, severe thrombocytopenia may lead to gastrointestinal, genitourinary, or central nervous system bleeding.

Spontaneous bleeding or bruising normally does not occur until the platelet count has fallen below 10,000 to 20,000/ μ L. The rate of decline of the platelet count may also correlate directly with the likelihood of unprovoked bleeding, likely due to compensatory processes that may occur over time with persistent thrombocytopenia, resulting in hyperfunctional platelets. However, it is important to remember that patients with dysfunctional platelets, for instance in the setting of myelodysplastic syndromes or leukemia, may bleed with higher platelet counts. Patients without bleeding and with platelet counts greater than 20,000 to 30,000/ μ L usually do not require immediate treatment to increase the platelet count. A platelet count of 50,000 to 100,000/ μ L is generally adequate for hemostasis during most invasive procedures, including surgery (Table 19.2).

Table 19.1 Causes of Thrombocytopenia

Disorders Characterized by Decreased Production of Platelets

Bone marrow failure syndromes

Congenital (amegakaryocytic thrombocytopenia, Fanconi anemia, dyskeratosis congenita, Schwachmann–Diamond syndrome, thrombocytopenia–absent radii syndrome)

Acquired (aplastic anemia, amegakaryocytic thrombocytopenia)

Myelodysplasia

Marrow infiltration (neoplastic, infectious)

Chemotherapy-induced

Irradiation-induced

Cyclic thrombocytopenia (some cases)

Immune thrombocytopenia

Folate, B₁₂, or iron (advanced cases) deficiency

Ethanolism

Disorders or Conditions Characterized by Increased Clearance of Platelets

Immune thrombocytopenia

Heparin-induced thrombocytopenia

Thrombotic thrombocytopenic purpura/ hemolytic-uremic syndrome

Disseminated intravascular coagulation (HELLP syndrome)

Posttransfusion purpura

Neonatal alloimmune thrombocytopenia

Von Willebrand disease, type IIB

Cyclic thrombocytopenia (most cases)

Mechanical destruction (aortic valvular dysfunction; extracorporeal bypass)

Disorders Characterized by Increased Sequestration of Platelets

Hypersplenism (see [Table 19.5](#))

Other Conditions

Artifactual (pseudothrombocytopenia)

Drug induced (see [Table 19.7](#))

Gestational thrombocytopenia

HIV-associated thrombocytopenia

Infection-and sepsis-related thrombocytopenia

Hemophagocytosis

Qualitative platelet disorder-related (Bernard–Soulier disease, gray platelet syndrome, May–Hegglin anomaly)

Wiskott–Aldrich syndrome

HELLP, hemolysis, elevated liver enzymes, and low platelets syndrome.

DISORDERS CHARACTERIZED BY DECREASED PRODUCTION OF PLATELETS

Bone Marrow Failure

- Congenital disorders, such as Fanconi anemia or dyskeratosis congenita, typically present early in life; these syndromes often cause depression of other blood cell lineages (i.e., white cells and red cells) in addition to platelets.
- Other congenital disorders such as congenital amegakaryocytic thrombocytopenia and the thrombocytopenia with absent radius (TAR) syndrome are characterized by isolated thrombocytopenia.
- Adult patients with acquired amegakaryocytic thrombocytopenia initially may appear to have immune thrombocytopenia (ITP; see the following section), but the bone marrow reveals markedly reduced or absent megakaryocytes. The disorder may progress to aplastic anemia.
- Patients with acquired aplastic anemia rarely present with isolated thrombocytopenia, although more typically all blood cell lineages are depressed at the time of diagnosis. Marked bone marrow hypocellularity with decreased megakaryocytes would suggest this diagnosis (see [Chapter 6](#)).

Table 19.2 Target Platelet Count Values for Commonly Encountered Clinical Scenarios^a

Goal or Intervention	Desired Platelet Count (per μL)
Prevention of spontaneous intracranial bleeding	>5,000–10,000
Prevention of spontaneous mucocutaneous bleeding	>10,000–30,000

Placement of central vascular catheters	>20,000–30,000 (compressible site) >40,000–50,000 (noncompressible site or tunneled catheter)
Use of anticoagulant medications in therapeutic doses	>40,000–50,000
Invasive procedures	
Endoscopy with biopsy	>60,000
Liver biopsy	>80,000
Major surgery	>80,000–100,000

^aValues are approximate and reflect target ranges for patients with otherwise intact hemostasis. Patients with thrombocytopenia and bleeding may benefit from augmentation of the platelet count (e.g., by platelet transfusion) irrespective of the platelet value.

Myelodysplasia

- Mild thrombocytopenia with macrocytosis, with or without anemia or neutropenia, in an older individual is a typical presentation of myelodysplasia (MDS). Isolated severe thrombocytopenia (<20,000/ μ L) in MDS, without any other blood count abnormalities, is not typical but can occasionally be observed.
- Bone marrow aspirate may show megakaryocytic dysplasia (including small and mononuclear “micromegakaryocyte” forms) and maturation abnormalities of erythrocytic and granulocytic precursor cells. Concurrent cytogenetic abnormalities may be present. For further discussion of diagnosis and treatment of the myelodysplastic syndromes, please refer to [Chapter 7](#).

Marrow Infiltration

- Infiltration of the bone marrow by malignancy may cause thrombocytopenia, but usually only after massive replacement of the marrow space by malignant cells has occurred. Examination of the bone marrow biopsy and aspirate is required.
- Acute and chronic leukemias, myeloma, and lymphoma are the most common malignancies resulting in cytopenias due to neoplastic marrow infiltration and direct suppression of normal hematopoiesis by tumor cells. Solid tumors are much less likely to cause extensive marrow infiltration except in cases of very advanced disease. Isolated thrombocytopenia is unusual.
- Certain infections, such as tuberculosis and ehrlichiosis, can result in formation of granulomas in the bone marrow that supplants the normal marrow architecture and results in cytopenias.
- Effective treatment of the underlying condition should be expected to restore a low platelet count to the normal range, but platelet transfusions may be required initially if bleeding is present or an invasive procedure is planned.

Irradiation and Chemotherapy

- | Irradiation and/ or myelotoxic chemotherapy induce thrombocytopenia via direct toxicity to megakaryocytes or more immature hematopoietic stem and progenitor cells. The degree and duration of thrombocytopenia depend on the intensity and the type of the myelotoxic regimen.
- | Chemotherapy-induced thrombocytopenia typically resolves more slowly than does neutropenia or anemia, especially following repetitive cycles of treatment.
- | Platelet transfusions may be given if required. To date, no specific therapies, including thrombopoietin analogs, have been shown to specifically accelerate platelet recovery following chemotherapy, but trials are ongoing.

Cyclic Thrombocytopenia

- | This exceedingly rare disorder is characterized by episodes of thrombocytopenia that occur cyclically, typically every 3 to 6 weeks.
- | Thrombocytopenia is frequently severe and may be associated with significant bleeding.
- | Treatment with oral contraceptives for female patients, androgens, immunosuppressive agents such as azathioprine, or thrombopoietic growth factor has resulted in responses in some cases.

Nutritional Deficiencies

- | Folate deficiency and vitamin B₁₂ deficiency may cause decreased megakaryocytopoiesis and thrombocytopenia, usually in conjunction with anemia.
- | Alcoholism is commonly associated with nutritional deficiencies including folate deficiency; in addition, alcoholism may cause cytopenias via direct toxicity to the bone marrow and/ or via hypersplenism secondary to cirrhosis.
- | In contrast, iron deficiency typically results in thrombocytosis. However, in very severe iron deficiency, thrombocytopenia may occur.
- | In any of these situations, replacement of the deficient vitamin or mineral corrects the thrombocytopenia.

DISORDERS CHARACTERIZED BY INCREASED CLEARANCE OF PLATELETS

Immune Thrombocytopenia

ITP is an acquired autoimmune disorder characterized by increased platelet destruction and, if severe thrombocytopenia results, an increased risk of bleeding.

- | Epidemiology. The annual incidence of ITP in adults has been estimated to be about 2

to 4 cases per 100,000 persons and increases with age.¹

| Pathophysiology. Pathogenic antiplatelet antibodies can be identified in approximately 75% of patients with ITP and are directed against the platelet glycoprotein complexes IIb/ IIIa and/ or Ib/ IX. The antibody-coated platelets are cleared by reticuloendothelial macrophages in the liver or spleen, decreasing the platelet life span from approximately 7 days to less than 2 days.² Platelet production does not increase sufficiently to normalize platelet numbers in ITP, possibly because of antiplatelet antibody binding to bone marrow megakaryocytes, and lack of appropriate compensatory increases in thrombopoietin.

- Primary adult-onset ITP is generally idiopathic and becomes chronic.
- Secondary ITP occurs in association with lymphoproliferative disorders (lymphoma or chronic lymphocytic leukemia [CLL]) or immune dysregulation (systemic lupus erythematosus, HIV infection).³
- In children, ITP typically follows a viral infection and frequently resolves spontaneously without specific therapy.

| Presentation. Typically, new-onset severe ITP (platelets < 30,000/ μ L) manifests with petechial bruising and bleeding from mucous membranes, including conjunctival hemorrhages, gingival bleeding, and epistaxis. Milder disease (platelet count > 50,000/ μ L) often presents as an asymptotically low platelet count on routine blood work.

| Diagnosis. ITP is a diagnosis of exclusion. New-onset, isolated thrombocytopenia with no other readily apparent cause, including medications, in an otherwise asymptomatic adult generally may be regarded as sufficient for the diagnosis of ITP.⁴

- Evaluation of the blood smear should be performed to rule out microangiopathy or changes consistent with marrow infiltration by tumor or infection.
- Direct antiglobulin test (Coombs test) and blood type for Rh status should be obtained.
- The presence of other cytopenias, red cell macrocytosis or other abnormalities on peripheral blood smear, or failure of initial therapy (corticosteroids for 1 week or a single course of intravenous immunoglobulin) should prompt bone marrow examination. The presence of morphologically abnormal or decreased numbers of megakaryocytes, abnormal marrow cellularity, or marrow infiltration should redirect the diagnostic evaluation away from ITP.

- All patients should be screened for hepatitis B, hepatitis C, and HIV infections.
- Testing for Helicobacter pylori, antiphospholipid antibodies, and antinuclear antibodies may be indicated in selected patients.

| Treatment. Individuals with mild or moderate thrombocytopenia (platelets > 30,000/ μ L) who are not actively bleeding or undergoing surgery should not receive treatment. Rather, they may be observed at regular intervals for bleeding symptoms or worsening thrombocytopenia. Adults with platelet counts of less than 20,000 to 30,000/ μ L or

those with significant bleeding generally should be treated.⁴

- **Initial treatment** ([Table 19.3](#)) generally consists of a short course of corticosteroids (prednisone 1 mg/ kg/ day for 7 to 10 days followed by tapering, or alternatively “pulse” dexamethasone cycles of 40 mg daily for 4 days). A significant increase in the platelet count should be seen within 3 to 7 days. In the event of a platelet response, prednisone may be tapered rapidly to a dose of 20 mg/ day; thereafter, tapering should proceed more slowly (by dose decrements of no more than 5 mg/ adjustment, no more frequently than once every 2 to 3 weeks). Dexamethasone cycles may be given every other week for four cycles or monthly for up to 6 months.
- For patients with serious active bleeding and/ or very severe thrombocytopenia (<5,000 to 10,000/ μ L), intravenous immune globulin (IVIg; 1 g/ kg/ day for 1 or 2 days) can be given in addition to corticosteroids in order to decrease the clearance of antibody-coated platelets. Patients with contraindications to steroids can also be treated with IVIg alone. Alternatively, anti-D (WinRho, 75 μ g/ kg/ dose) can be considered but is only appropriate for Rh blood type–positive patients without anemia. Anti-D is more effective in patients with intact spleens, however, responses have been observed in some patients postsplenectomy. Responses are generally seen within 3 to 5 days of IVIg or anti-D administration.
- Platelet transfusions may be administered if the presentation is complicated by serious (i.e., intracranial or massive gastrointestinal) bleeding. Transfused platelets are expected to be cleared very rapidly in the presence of antiplatelet antibodies, but they may improve hemostasis temporarily.
- Immediately immunize against encapsulated bacterial organisms (pneumococcus, *Haemophilus influenzae*, meningococcus) before prolonged immunosuppressive therapy, in preparation for splenectomy at a later time point.
- **Second-line treatment** ([Table 19.3](#)). Despite a high initial response rate (60% to 75%), the majority of adults with ITP experience relapse and develop chronic thrombocytopenia once initial treatments are reduced or discontinued. Treatment is appropriate for patients with platelet counts <20,000 to 30,000/ μ L or clinically significant bleeding.⁴ Whether splenectomy or other medical therapies should be pursued is currently unsettled, due to the introduction of new effective medical therapies, and thus is up to patient and provider preference. Some individuals prefer sequential medical therapies prior to undergoing splenectomy,³ but splenectomy may be preferable in cases of very severe thrombocytopenia associated with bleeding because of a typically rapid postoperative increase in the platelet count in most responding patients.
- IVIg and anti-D (see earlier discussion) typically must be readministered every 1 to 3 weeks in most instances; therefore, these agents are not generally utilized as a chronic second-line therapy.
- The thrombopoietin receptor agonists eltrombopag (starting dose 50 mg orally daily;

25 mg daily in individuals of Asian descent) or romiplostim (starting dose 1 µg/ kg subcutaneous [SC] weekly) produce platelet responses ($\geq 50,000/\mu\text{L}$) in approximately 70% of patients with chronic ITP, and are generally well tolerated.^{5,6} Potential complications include reticulin deposition in the bone marrow, thrombocytosis, and thrombosis. Abrupt discontinuation of thrombopoietin receptor agonists may transiently worsen thrombocytopenia.

- The monoclonal anti-B cell (anti-CD20) antibody rituximab (dose: 375 mg/ m² weekly for 4 weeks), induces initial and long-term responses in about 50% and 25%, respectively, of adults with severe, chronic ITP.⁷
- Splenectomy yields an immediate response rate of 70% to 75% and durable response rates of 60% to 70%. All patients must receive immunization against encapsulated bacterial organisms (pneumococcus, H. influenzae, meningococcus) several weeks before splenectomy if possible.⁴ Laparoscopic splenectomy should be feasible in most patients.

Secondary ITP. A variety of autoimmune, infectious, inflammatory, or malignant conditions may underlie a presentation of ITP.³ Treatment of the underlying predisposing condition may be required in some cases, in addition to management of the thrombocytopenia using accepted interventions (Table 19.3).

Pregnancy-associated ITP. Pregnant women with platelet counts $< 30,000/\mu\text{L}$ during the second or third trimester, or with platelet counts $< 10,000/\mu\text{L}$, or bleeding in any trimester, should be treated. Intermittent infusions of IVIg or moderate-dose oral prednisone (commonly given on an every-other-day schedule) are standard. Splenectomy during the first or second trimester may be considered for women whose ITP has failed treatment with IVIg and corticosteroids and who have platelet counts $< 10,000/\mu\text{L}$ with associated bleeding. Platelets may be administered prophylactically prior to cesarean section in women who have platelet counts $< 10,000/\mu\text{L}$ or mucocutaneous bleeding near the time of delivery. A platelet count of $> 50,000/\mu\text{L}$ generally is regarded as adequate prior to cesarean section or vaginal delivery.

Table 19.3 Therapy of Immune Thrombocytopenia

Phase of Treatment	Suggested Interventions ^a
Initial	Prednisone/ dexamethasone; anti-D, IVIg
Second-line (preferred) ^{b,c,d}	Anti-D (serial infusions), IVIg (serial infusions), rituximab, splenectomy, thrombopoietin receptor agonist (eltrombopag, romiplostim)
Second-line (non-preferred) ^{b,e}	Anti-CD52 antibody, azathioprine, combination chemotherapy, cyclosporine A, cyclophosphamide, danazol, dapsone, hematopoietic progenitor cell transplant, mycophenolate mofetil, vinca alkaloids

^aAgents with a Food and Drug Administration (FDA)-approved indication for immune thrombocytopenia

(ITP) are presented in **bold**.

^bOrder of listed interventions is alphabetical and does not indicate selection preference.

^cMay be offered after failure of initial treatment with corticosteroids and/ or IVIg/ anti-D.

^dPreferred second-line therapies may be better tolerated, more easily administered, and/ or more likely to produce a lasting remission.

^eNon-preferred second-line therapies tend to be less tolerated and may be less likely to yield a lasting remission (~30% in most cases).

IVIg, intravenous immune globulin.

Heparin-Induced Thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is an antibody-mediated disorder that results in platelet activation and clearance. Patients with HIT paradoxically are at high risk of thrombosis due to platelet activation, including catastrophic venous or arterial thrombosis, with mortality up to 5% to 10%. If HIT is suspected, all forms of heparin should be discontinued immediately, and, if appropriate, alternative anticoagulation administered.⁸

- Epidemiology. HIT occurs in approximately 3% and <1% of patients who are exposed to unfractionated heparin or low-molecular-weight heparin, respectively. As many as half of these individuals will develop thrombosis.⁸
- Pathophysiology. The pathogenesis of HIT begins with binding of the heparin molecule to platelet factor 4 (PF4), a platelet alpha-granule chemokine. The heparin–PF4 complex stimulates the formation of an immunoglobulin (IgG) antibody (HIT antibody) that binds both to the heparin–PF4 complex (via its Fab portion) and to platelet Fc receptors (via its Fc portion). Binding of the HIT antibody to platelets activates them, resulting in the release of procoagulant microparticles, platelet clearance, and subsequent thrombocytopenia. PF4 also binds to polysaccharides (heparan sulfate) on the endothelial surface; recognition of these PF4–polysaccharide complexes by HIT antibodies may lead to endothelial damage, expression of tissue factor, and a prothrombotic state.
- Presentation. The typical presentation of HIT involves a hospitalized patient who develops thrombocytopenia within 5 to 10 days of receiving heparin.
 - A decline of 50% or more from the baseline value in a heparin-treated patient may signify HIT. Platelet counts generally do not fall below 20,000/ µL.
 - Spontaneous bleeding and petechial rash are not typical.
 - Venous (upper or lower limb, dural sinus) or arterial (lower limb, cerebrovascular accident [CVA], myocardial infarction [MI], other locations) thromboses may occur in up to 50% of untreated cases. In a minority of patients with HIT, thrombosis is the presenting clinical sign. The risk of HIT-related thrombosis persists for at least 30 days after the discontinuation of heparin if alternative anticoagulation is not administered.

- Rapid-onset HIT occurs in patients with preexisting HIT antibodies due to heparin administration within the preceding 30 days. Thrombocytopenia develops within 1 to 3 days of reexposure to heparin and is often accompanied by an acute systemic reaction characterized by fever, chills, hypotension, or cardiovascular compromise.
- Delayed-onset HIT describes new thrombocytopenia and venous or arterial thrombosis that occurs up to 14 days after completion of an uneventful course of heparin therapy. Laboratory markers of disseminated intravascular coagulation (DIC) may be positive. Thrombocytopenia and thrombosis typically worsen if heparin is administered.
- Diagnosis. Theoretically, the diagnosis of HIT requires both an appropriate clinical context and confirmatory laboratory testing (e.g., demonstration of HIT antibodies). Due to the limited immediate availability of HIT-specific laboratory assays, however, any patient in whom the clinical probability for the disorder is intermediate or high should be managed for HIT, even if the results of diagnostic tests are pending or not immediately available.
- Clinical probability. A variety of clinical prediction models, such as the 4Ts,⁹ have been developed to assist in determination of pretest probability, but have not undergone rigorous external validation and may overestimate HIT diagnoses.⁸ Detailed review of the medical chart including nursing notes and medication administration records may be necessary to document the extent and duration of exposure to heparin, particularly if its use was transient (heparin flushes) or covert (heparin-impregnated catheters).
- Laboratory diagnosis. All patients with suspected HIT ideally should undergo testing with two types of assays: immunologic and functional. The immunologic testing via enzyme-linked immunosorbent assay (ELISA) for PF4-heparin-associated antibody has a sensitivity of >90% but a limited specificity. Functional testing with platelet activation assays measures activation of donor platelets in the presence of the patient's serum and heparin. These assays are much more specific for HIT than the immunologic testing but require specialized laboratory techniques that are not widely available and may take several days to complete. A positive result on both tests or the immunologic assay alone indicates high and intermediate probabilities for HIT, respectively.
- Doppler ultrasound of the lower extremities should be performed to evaluate for subclinical deep vein thrombosis.
- Treatment. All forms of heparin, including low-molecular-weight preparations, must be discontinued immediately. In patients in whom laboratory testing for HIT eventually proves negative or in whom an alternative explanation for thrombocytopenia has been found, heparin may be subsequently restarted, with very careful laboratory and clinical monitoring.
- Initial treatment. In all cases of suspected HIT with intermediate or high clinical

probability, an alternative anticoagulant such as a direct thrombin inhibitor (DTI) should be administered due to the high rate of serious thrombosis in the setting of HIT¹⁰ (Table 19.4 and Chapter 23). The alternative anticoagulant should be continued until the platelet count has recovered to within normal limits or for at least 5 days, whichever is longer. Warfarin is contraindicated as initial treatment of clinically proven or suspected HIT, due to its propensity to exacerbate hypercoagulability by reduction of plasma levels of proteins C and S (see Chapter 23). Reversal of warfarin with vitamin K may be considered in patients who were receiving warfarin prior to the diagnosis or suspicion of HIT.

- Longer-term anticoagulation. The increased risk of thrombosis continues up to 1 month following a diagnosis of HIT. Patients without concurrent thrombosis require at least 30 days of anticoagulation. Patients with HIT and thrombosis should receive anticoagulation for a total duration of 3 to 6 months.¹⁰ Warfarin therapy is appropriate in most patients beginning after recovery of platelet counts, and starting in low dose with adjustment to a goal international normalized ratio (INR) of 2.0 to 3.0. When transitioning from a DTI to warfarin, the DTI must be continued for a minimum of 5 days and until therapeutic anticoagulation with warfarin has been achieved. Note that argatroban will prolong the prothrombin time (PT), requiring a special approach for monitoring when administered concurrently with warfarin (see Chapter 23).
- Thrombolysis/ thromboembolectomy. Low-dose or very-low-dose thrombolytic agents may be indicated in acute limb ischemia or life-threatening pulmonary embolism caused by HIT-associated thrombi. Surgical removal of large-vessel arterial thrombi may be required if the limb is threatened and other treatments have failed. Patients managed by either medical or surgical means require concomitant use of an alternative anticoagulant, regardless of the degree of thrombocytopenia.
- Retreatment with heparin. Even in a patient with a prior diagnosis of HIT, once HIT antibodies have disappeared, as documented by both immunologic and functional assays, very brief treatment with heparin may be used when necessary, such as during cardiac or vascular surgery. HIT antibodies generally do not persist beyond 100 days from the initial episode of HIT.⁸ If the functional assay remains positive, delaying the surgery is preferred until the functional assay becomes negative. In the event that surgery is urgently indicated, a DTI can be used.¹⁰
- Platelet transfusions are rarely indicated and should be avoided unless there is active bleeding or a planned invasive procedure. Bleeding is uncommon in patients with HIT and transfused platelets may worsen the increased thrombotic risk by providing substrate for HIT antibodies.

Thrombotic Microangiopathies

The thrombotic microangiopathies (TMAs) result in hemolytic anemia and

thrombocytopenia due to the formation of platelet-rich thrombi in the arterial and capillary microvasculature and comprise thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS). These disorders can be classified into acquired (or “spontaneous” or “classical”) and congenital forms of TTP, and endemic (or “typical”) and atypical forms of HUS. In addition, some forms of TMA have been recognized in association with surgery, pregnancy, malignant hypertension, exposure to certain medications (most notably calcineurin inhibitors), and bone marrow or solid organ transplantation (Table 19.5). Importantly, early aggressive intervention with plasma exchange is crucial in cases of classical TTP due to an extremely high mortality rate.

Table 19.4 Alternative Anticoagulants in the Treatment of Heparin-Induced Thrombocytopenia

Agent	Description	Indication	Dosing	Comment
Argatroban	Synthetic direct thrombin inhibitor	Prophylaxis or treatment of HIT, including post-percutaneous coronary intervention	Obtain baseline aPTT. Start continuous infusion at 2 µg/kg/min. Titrate to achieve aPTT 1.5–3.0 times the baseline value (check aPTT every 4 hr initially)	For hepatic insufficiency, decrease initial infusion rate to 0.5 µg/kg/min. INR will be increased. If warfarin is used concurrently, follow special protocols for monitoring
Bivalirudin (Angiomax)	Direct thrombin inhibitor; semi-synthetic derivative of hirudin	Treatment of HIT with/ without thrombosis; percutaneous coronary intervention	Obtain baseline aPTT. Start continuous infusion at 0.15 mg/kg/hr. Titrate to achieve aPTT of 1.5–2.5 times the baseline value (check every 4 hr initially)	For renal and/ or hepatic insufficiency, the infusion rate should be decreased. INR will be increased. If warfarin is used concurrently, follow special protocols for monitoring
Danaparoid sodium (Orgaran) ^a	Mixture of negatively charged glycosaminoglycans	Treatment of HIT; cardiopulmonary bypass surgery	N/A	Monitoring requires anti-Xa levels and danaparoid calibration curve 10% cross-reactivity with HIT antibodies

^aNot available in the United States.

aPTT, activated partial thromboplastin time; HIT, heparin-induced thrombocytopenia.

Table 19.5 Features of Selected Thrombotic Microangiopathies

Parameter	Classical TTP	Typical HUS	Atypical HUS	Therapy-Related TTP–HUS
Patient-specific factors	Most cases in previously healthy adults	Most cases in previously healthy children. Bloody diarrhea within prior 2 weeks in 90%	Presents in early childhood; some cases in older adults	Recent (<200 d) HSCT, or use of TMA-associated drugs (especially cyclosporine)
Causative factors	Antibody to ADAMTS13	E. coli 0157:H7, Shiga toxin	Inherited defects in complement regulatory proteins in some patients	Endothelial cell damage from direct toxicity or antibody mediated
Thrombocytopenia	Most patients, typically moderate to severe	Most patients, but mild or absent in 30%	Variable	Most patients
Fever	Present in 75%	Typically absent	Variable	Variable
Renal insufficiency	Maybe mild	All patients	All patients	Most patients
Neurologic impairment	Most patients	<50% of patients	Minority of patients	Most patients
Corroborative specialized laboratory findings	Decreased activity of ADAMTS13 <10% of normal	Positive stool culture for E. coli 0157:H7; positive (antibody to) Shiga(-like) toxin. ADAMTS13 activity usually normal	Defects in complement regulatory proteins	In some cases, decreased activity of ADAMTS13
Treatment	Immediate plasma exchange. Supportive care. Few patients require dialysis. Usually, platelet transfusions contraindicated	Supportive care. Most require (temporary) dialysis. Adults may benefit from plasma exchange	Immediate plasma exchange in most cases. Supportive care. Some require temporary dialysis. Eculizumab helpful in some cases	Discontinue offending drugs. Plasma exchange probably not helpful in HSCT-associated TMA. Supportive care. Usually, platelet transfusions contraindicated

ADAMTS13, von Willebrand factor-cleaving protease; HSCT, hematopoietic stem cell transplantation;

HUS, hemolytic-uremic syndrome; MAHA, microangiopathic hemolytic anemia; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura.

- **Epidemiology.** The incidence of classical TTP is approximately 3 to 4 cases/ 100,000 persons, and there is a slight female predominance. Most cases of endemic or “typical” HUS occur in young children and are related to infection with enteropathogenic bacteria. TMA occurs at an increased rate during pregnancy and in the peripartum period.
- **Pathophysiology.** The TMAs are thought to arise from factors that directly or indirectly cause platelet aggregation and/ or endothelial cell damage, leading to the formation of microvascular thrombi and ischemia in involved organs. These factors include toxins, cytokines, drugs, or deficiencies in the function of the von Willebrand factor (VWF) cleaving protease (VWFCP, also called ADAMTS13). Red cells are sheared as they negotiate thrombotic obstructions and fibrin strands in the microvasculature, leading to hemolytic anemia. Consumption of platelets results in thrombocytopenia and bleeding.
- **Classical TTP** is an acquired deficiency of ADAMTS13 resulting from an autoantibody against ADAMTS13,¹¹ leading to an accumulation of ultra large VWF (ULVWF) in the plasma. ADAMTS13 is a metalloproteinase whose normal function is to cleave newly synthesized ULVWF multimers released in the plasma into multimers of smaller size. ULVWF multimers bind to platelets more avidly than smaller VWF molecules and may incite platelet aggregation.
- **Congenital TTP** is a rare inherited deficiency in ADAMTS13 resulting in decreased activity.
- **Endemic HUS** is associated with Shiga toxin from *Escherichia coli* type 0157:H7, which is thought to promote platelet aggregation by damaging endothelial cells or by other mechanisms.
- **Atypical HUS** may result from genetic defects in proteins that regulate complement activity in the alternative pathway, such as factor H,¹² or other very rare inherited disorders of metabolism or coagulation. Reference laboratory testing is needed.
- **Pregnancy-associated TTP–HUS** may stem from decreased levels of ADAMTS13 that naturally occur in the second and third trimesters; in some cases, an antibody to ADAMTS13 is present.¹³ Refer to [Chapter 26](#) and [Table 26.1](#) for a detailed discussion of TMA in pregnancy, including preeclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets syndrome), TTP, and postpartum HUS.
- **Drugs** such as cyclosporine, quinine, mitomycin C, and bleomycin may cause TMA by endothelial cell injury and/ or pro-aggregatory effects on platelets ([Table 19.6](#)). Antibodies inhibiting ADAMTS13 have been described in patients who received some of these medications.
- **TMA in the setting of cancer, hematopoietic stem cell or solid organ**

transplantation, or **HIV infection** has not been linked to abnormalities of ADAMTS13, but effects on endothelial cells or platelets may be responsible. TMA in transplant recipients may be difficult to distinguish from renal insufficiency due to calcineurin inhibitors or renal allograft rejection in kidney transplant recipients.

- **Presentation.** All patients with TMA have microangiopathic hemolytic anemia (MAHA). Varying degrees of neurologic impairment (more typical of classical TTP) or symptoms related to renal failure (predominant in HUS) may also be present ([Table 19.5](#)). The classic pentad of TTP with MAHA, thrombocytopenia, fever, renal insufficiency, and neurologic system abnormalities occurs in only about 25% of patients. Most patients with typical HUS have a recent or current diarrheal illness.
 - In adults, TTP and HUS are often difficult to distinguish due to the overlap of symptoms, but if renal dysfunction predominates, the syndrome usually is classified as HUS.
 - Manifestations of renal insufficiency may include elevated creatinine, azotemia, proteinuria, hematuria, and/ or oliguria.
 - Neurologic impairment from microthrombi in the cerebral vasculature occurs in about 75% and 30% of patients with TTP and HUS, respectively, and includes headache, somnolence, confusion, seizures, and less commonly paresis and coma.
- **Diagnosis.** The presence of new-onset MAHA and thrombocytopenia (and/ or renal failure) in the absence of any other plausible explanation will suffice for the diagnosis.
 - MAHA is essential to the diagnosis of TTP–HUS and is defined by anemia with positive markers of intravascular hemolysis (elevated lactate dehydrogenase [LDH], elevated indirect bilirubin, decreased haptoglobin, and reticulocytosis) and negative direct antiglobulin test (DAT) (Coombs test). The blood smear shows more than three schistocytes per high-power microscopic field, although fewer schistocytes may be present if the disorder is caught early.
 - Depending on the type of TMA, the degree or even presence of thrombocytopenia may be variable.
 - Clinical features such as an antecedent bloody diarrheal illness or renal insufficiency (more typically associated with HUS); neurologic abnormalities or fever (more typically associated with TTP); recent or current pregnancy, associated medications, cancer, or recent hematopoietic stem cell transplantation are diagnostically corroborative. Screening for pregnancy, and for HIV, hepatitis C and hepatitis B viruses is appropriate.

Table 19.6 Drugs Associated With Thrombocytopenia

Medication	D-ITP	DI-TMA	Myelosuppression	Pseudo ^a
Abciximab	Rapid			X
Acetaminophen		X		

Amiodarone	X		
Beta-lactams (e.g., piperacillin)	X		
Carbamazepine			X
Daptomycin		X	
Eptifibatide	Rapid		
Ethambutol	X		
Gold	Primary ^b		X
Haloperidol	X		
Heparin	HIT		
Ibuprofen	X		
Linezolid			X
Mirtazapine	X		
Naproxen	X		
Phenytoin	X		
Quinidine	X		
Quinine	X	X	
Ranitidine	X		
Rifampin	X		
Simvastatin	X		
Sulfisoxazole	X		
Suramin	X		
Tirofiban	Rapid		
Trimethoprim–sulfamethoxazole	X		
Valproic acid	X		X
Vancomycin	X		
Chemotherapy/immunosuppression			
Alemtuzumab	Primary ^b		
Bevacizumab		X	
Bleomycin		X	
Cyclosporine		X	
Gemcitabine		X	X
Irinotecan	X		X
Interferon		X	X
Mitomycin		X	X
Oxaliplatin	X	X	X
Pentostatin		X	
Sirolimus		X	
Sunitinib		X	

Tacrolimus
Most chemotherapeutic drugs

X

X

^aPseudothrombocytopenia. ^bPrimary ITP-like thrombocytopenia.

D-ITP, drug-induced thrombocytopenia; DI-TMA, drug-induced thrombotic microangiopathy; HIT, heparin-induced thrombocytopenia.

Adapted from Reese JA, Li X, Hauben M, et al. Identifying drugs that cause acute thrombocytopenia: an analysis using 3 distinct methods. *Blood*. 2010; 116(12):2127–2133 and Al-Nouri ZL, Reese JA, Terrell DR, et al. Drug-induced thrombotic microangiopathy: a systemic review of published reports. *Blood*. 2015;125(4):616–618.

- **Stool culture for *E. coli* 0157:H7 or assays for antibody against Shiga or Shiga-like toxins, or against specific bacterial lipopolysaccharide** may be positive in patients with endemic HUS.
- **Assays for ADAMTS13 activity** are abnormal in congenital and classical TTP, with activity typically less than 5 to 10%. Inhibitors for ADAMTS13 are detectable in classical TTP.
- Coagulation studies including PT, activated partial thromboplastin time (aPTT), and fibrinogen are typically within the normal range in TMA.
- Treatment. Without plasma exchange, the mortality rate of classical TTP exceeds 90%. Plasma exchange must be instituted expeditiously, and improves mortality of acute TTP to 10% to 20%.¹⁴ An important exception is children or adults with endemic (*E. coli* diarrhea-associated) HUS, who generally recover with supportive care within 3 weeks, without plasma exchange.
- Plasma exchange should begin as soon as appropriate vascular access has been obtained. It should be performed once daily until the LDH has normalized and the platelet count has returned to the preexisting baseline (if known) for at least 2 or 3 days. Failure to respond to once-daily therapy requires twice-daily treatments; once the LDH and platelet count indicate response, once-daily treatments can be resumed until these parameters have normalized for 2 or 3 days. Whether treatments then may be discontinued or continued for a number of weeks in a tapering fashion is controversial, but many clinicians prefer to gradually increase the interval between treatments instead of abrupt termination of therapy.
- Steroids are often used in combination with plasma exchange during initial treatment. Typical adult dosing is methylprednisolone intravenously 1g daily for 3 days or prednisone orally 1 mg/ kg/ day.¹⁴
- Selected patients with atypical HUS may respond to treatment with the monoclonal anti-C5 inhibitor eculizumab.¹⁵
- Platelet transfusions generally are contraindicated in the treatment of TMA due to possible propagation or new formation of platelet-rich microthrombi. If computed tomography (CT)-or magnetic resonance imaging (MRI)-documented intracranial bleeding or other life-threatening bleeding is present, however, platelets may be

- transfused slowly, ideally after plasma exchange is initiated.
- Packed red cells may be transfused commensurate with the pace of the MAHA and degree of bleeding.
 - In the event that plasma exchange is unavoidably delayed, infusion of fresh frozen plasma (FFP) may be helpful as a temporizing measure. Plasma infusion as a sole treatment of TMA, however, generally is regarded as substandard due to (1) the possible role of plasma exchange in removing offending drugs, cytokines, bacterial proteins, ULVWF multimers, or antibodies to the ADAMTS13 and (2) the volume overload frequently incurred when the required large volumes of FFP are infused. An exception is familial relapsing TMA, wherein a congenital deficiency in ADAMTS13 can be corrected merely by infusion of smaller volumes of plasma.
 - Refractory and relapsing TMA. If remission is not achieved with aggressive plasma exchange, second-line treatments should be considered. These include the addition of steroids, IVIg, vincristine, cyclophosphamide, cyclosporine (in select cases of sporadic TTP), and splenectomy. The monoclonal antibody rituximab has also achieved responses in a limited number of refractory cases.¹⁶ Up to one-third of patients with classical TTP relapse after discontinuation of plasma exchange. In these cases, plasma exchange should be reinitiated according to the guidelines mentioned previously, and, if ineffective, immunosuppressive treatments should be considered.
 - Hemodialysis. More than half of all patients with HUS and a minority with TTP require hemodialysis. Approximately half of these patients will attain durable restoration of renal function, while 25% develop chronic renal failure. The remainder experience variable degrees of permanent renal insufficiency.

Disseminated Intravascular Coagulation

Thrombocytopenia in DIC occurs as a result of uncontrolled activation of coagulation in the circulation. Platelets participate in these reactions, leading to their consumption. If bleeding is present, platelet transfusions may be administered to reach a platelet count target of 20,000 to 30,000/ μ L (most cases) or >50,000/ μ L (if there is intracranial or life-threatening hemorrhage). Thrombocytopenia and the other clinical and laboratory manifestations of DIC are expected to resolve with effective treatment of the underlying, inciting disorder. For a full discussion of DIC (see [Chapter 21](#)).

Posttransfusion Purpura

- Posttransfusion purpura (PTP) is characterized by the unexplained, sudden appearance of thrombocytopenia in an otherwise asymptomatic individual who recently has received a blood transfusion (red cells, platelets, or plasma) within 1 week before development of thrombocytopenia. The precipitating events are unknown, but more than 90% of individuals with PTP display antibodies against the human platelet

antigen (HPA) Pl^{A1}.

- | Most patients with PTP are postmenopausal females who are either multiparous or who have received prior transfusions; they commonly present with severe thrombocytopenia and bleeding.
- | Treatment. If untreated, thrombocytopenia typically persists for up to 2 to 3 weeks, concurrent with a mortality rate of 10% from bleeding; hence, IVIg (1 g/ kg/ day for 2 days) should be administered as soon as the diagnosis is suspected. Most patients will respond, but in the case of relapse, IVIg may be administered as a second course. Plasma exchange, adjunctive corticosteroids, and splenectomy are alternative treatments for refractory cases. Because transfused platelets are thought to be as susceptible to binding by antigen–antibody complexes as the patient’s own platelets, platelet transfusion is generally not performed unless severe bleeding is present; in this case, HLA antigen-matched platelets are preferred. Future transfusions should be administered judiciously, with washed or Pl^{A1}-negative blood products. Refer to [Chapter 24](#) for further details.

Neonatal Alloimmune Thrombocytopenia

- | Neonatal alloITP (NAIT) is a cause of severe thrombocytopenia in neonates. It occurs when fetal platelet antigens cross the placenta and trigger formation of maternal alloantibodies that can then enter the fetal circulation, bind platelets, and induce thrombocytopenia. Antibodies commonly have specificity for HPA-1a, also known as Pl^{A1}. The presence of certain maternal platelet phenotypes (such as the homozygous HPA-1b state) appears to influence the risk of the disorder, especially if the fetus inherits a different, paternal platelet phenotype. Refer to [Chapter 24](#) for further details.
- | The thrombocytopenia is typically severe and associated with a high prevalence of intracranial hemorrhage during or following delivery and 5% neonatal mortality. Thrombocytopenia typically resolves by 2 to 3 weeks of age.
- | IVIg, with or without corticosteroids, is recommended for any neonate with platelet counts <20,000 to 25,000/ µL. Ideally, irradiated maternally antigen-matched platelets are administered in cases of intracranial hemorrhage, or alternatively, random donor platelets may be utilized. Subsequent pregnancies are regarded as high risk for recurrent NAIT.

Von Willebrand Disease, Type 2B

This type of von Willebrand disease (VWD) is characterized by an abnormal VWF that has increased affinity for its platelet receptor, glycoprotein Ib. Due to the bridging action of VWF, platelets aggregate in vivo and are cleared, typically resulting in a mild thrombocytopenia with giant platelets. VWD is discussed in detail in [Chapter 21](#).

Extracorporeal Circulation-Related Thrombocytopenia

Passage of the blood for prolonged periods outside the body in an artificial circuit (such as used for cardiac bypass surgery) typically results in platelet activation and clearance. Thrombocytopenia generally is not severe and resolves within a few days. Other common causes of thrombocytopenia in the postsurgical patient (such as HIT, DIC, and sepsis-related and drug-induced thrombocytopenia) concomitantly must be considered.

Table 19.7 Selected Causes of Splenomegaly

Lymphoproliferation

- Lymphoma
- Chronic lymphocytic leukemia
- Collagen vascular disease (Felty syndrome, systemic lupus)
- Autoimmune lymphoproliferative disorder

Myeloproliferation

- Myeloid leukemia
- Polycythemia vera
- Essential thrombocythemia

Inborn Errors of Metabolism

- Gaucher disease
- Niemann–Pick disease

Congestion

- Cirrhosis
- Heart failure

Hemolysis

- Hereditary spherocytosis
- Paroxysmal nocturnal hemoglobinuria
- Thalassemia

Infection

- Viral (CMV, EBV, hepatitis)
- Parasitic (malaria, babesiosis)

Immunodeficiency

- Common variable immunodeficiency
- CMV, cytomegalovirus; EBV, Epstein–Barr virus.

DISORDERS CHARACTERIZED BY INCREASED SEQUESTRATION OF PLATELETS

- Hypersplenism results in sequestration of blood cells (including platelets) in an

enlarged or abnormal spleen. Mild to moderate thrombocytopenia is most commonly observed, but if the bulk of the platelet mass is contained within a massively enlarged spleen, thrombocytopenia can be severe.

- Splenomegaly with hypersplenism is almost always an acquired condition, and there are many possible underlying disorders ([Table 19.7](#)).
- If adequate production of platelets can be documented and significant splenomegaly with thrombocytopenia is present, splenectomy may be considered in some cases. Splenic embolization and splenic irradiation are alternatives to removal of the spleen that generally do not result in maximal platelet responses. They may be considered, however, in patients with significant hypersplenism and disorders such as CLL or lymphoma who cannot tolerate surgery.

OTHER CAUSES OF THROMBOCYTOPENIA

Pseudothrombocytopenia

- Pseudothrombocytopenia is a laboratory artifact observed in certain patients as a result of calcium chelation by the anticoagulant ethylenediaminetetraacetic acid (EDTA) in blood collection tubes. For reasons that are unclear, calcium chelation causes changes on the platelet membranes of certain patients, which expose cryptic antigens for binding by preformed, otherwise nonpathogenic agglutinating antibodies. The peripheral blood smear will reveal platelet clumps, particularly along the edges. Automated cell counters used in most clinical laboratories will report a falsely low platelet count in this situation.
- A blood smear prepared from a fingerstick source will be unclumped.
- Collection of the blood specimen in citrate anticoagulant instead of EDTA will result in a normal platelet count as measured on an automated cell counter.

Drug-Induced Thrombocytopenia

- By definition, drug-induced thrombocytopenia develops after initiation of a given drug, resolves when the offending medication is discontinued and may recur if the agent is reintroduced. It may be triggered by the medication itself or a metabolite.
- Myelosuppression is the primary cause of thrombocytopenia resulting from chemotherapeutic agents.
- Drug-induced ITP (DITP) results from antibody-mediated destruction of platelets after exposure to a given drug. The drug or its metabolite may bind to the platelet surface or to an antibody to cause recognition of platelet antigens as foreign. Patients with DITP present with severe thrombocytopenia (<20,000/ μ L) and mucocutaneous bleeding, including purpura and ecchymoses.¹⁷ Thrombocytopenia should resolve within days to weeks of discontinuing the agent. In cases of severe bleeding, IVIg and platelet

transfusions appear to be more effective than steroids.

- | Drug-induced TMA manifests with thrombocytopenia and MAHA, which may be immune mediated or result from direct endothelial cell toxicity.
- | Medications most commonly associated with thrombocytopenia are listed in [Table 19.6](#). Comprehensive medication lists for DITP^{17–19} and drug-induced TMA^{20,21} are available in the references.
- | A careful dietary history may identify foods, beverages, or nutritional supplements containing quinine, which is one of the most common causes of both DITP and drug-induced TMA.^{17,20}

Gestational Thrombocytopenia

- | The blood volume increases by as much as 40% to 45% during pregnancy, causing a progressive hemodilution. The increase in plasma volume results in relative cytopenias, although production of blood cells, including platelets, is normal or increased.
- | Approximately 10% and <1% of pregnant women experience platelet counts <100,000/ μL and <50,000/ μL by the third trimester, respectively. The incidence of true immune-mediated platelet destruction, that is, ITP, is thought to be even lower.
- | Severe thrombocytopenia in pregnancy (<50,000/ μL) should prompt investigation for preexisting conditions, preeclampsia, or a pregnancy-related TMA. For a detailed discussion of pregnancy-related conditions, please refer to [Chapter 26](#).
- | If alternative etiologies are excluded, the diagnosis may be presumed to be ITP and treated accordingly (see Immune Thrombocytopenia section).

HIV-Related Thrombocytopenia

- | Screening for HIV infection is appropriate in all patients with thrombocytopenia.
- | Thrombocytopenia in HIV infection may result both from immune-mediated phenomena leading to increased clearance of platelets, as well as ineffective platelet production, possibly due to direct infection of megakaryocytes with HIV. Improvement or resolution of thrombocytopenia after initiation of highly active antiretroviral therapy is commonly observed.
- | Concurrent hepatitis C virus infection, hypersplenism due to cirrhosis, marrow suppression from uncontrolled opportunistic infection, malignancy, or HIV-associated TMA may also contribute to thrombocytopenia in some patients.
- | If the thrombocytopenia proves refractory and no other causative factors are identified, therapies commonly used in the treatment of ITP (e.g., IVIg, anti-D, steroids, splenectomy) are employed, but the potential immunosuppressive effects of some of these approaches need to be taken into consideration.

Infection-and Sepsis-Related Thrombocytopenia

- Thrombocytopenia in the setting of infection or sepsis is common. DIC is often implicated in critically ill patients, but other causes, such as megakaryocyte-specific effects or increased clearance due to fever or splenic enlargement, may be responsible.
- Transient thrombocytopenia is commonly observed in the setting of many viral infections; certain bacterial infections, such as ehrlichiosis, Rickettsial disease, and dengue characteristically produce thrombocytopenia. A corroborative travel history and directed microbiologic testing are usually necessary to make the diagnosis.
- If the platelet count does not return to baseline with effective antimicrobial treatment or after resolution of the infection, an alternative etiology should be sought.

Hemophagocytosis

- Hemophagocytosis is a process in which bone marrow macrophages (histiocytes) engulf cellular components of the marrow. The phenomenon is considered to be nonspecific if it is found only sporadically within an aspirate smear, but the observation of abundant histiocytes with intracytoplasmic white cells, red cells, or platelets in the setting of peripheral cytopenias indicates a pathogenic process.
- In adults, sepsis or Epstein–Barr virus (EBV)-related infection or malignancy can drive T cells to produce cytokines that mediate hemophagocytosis, leading to thrombocytopenia. In these cases, the treatment is principally immunosuppressive, but the disorder often is aggressive and unresponsive to treatment.
- Familial hemophagocytic lymphohistiocytosis is a rare inherited disorder featuring cytopenias, hemophagocytosis, fever, organomegaly, and hypertriglyceridemia or hypofibrinogenemia. It most commonly presents at a young age, and the only curative treatment is allogeneic hematopoietic stem cell transplantation

Qualitative Platelet Disorders

Several heritable platelet anomalies of structure or function, including the MYH9-related disorders (also known as May–Hegglin anomaly) or Bernard–Soulier syndrome, are typically associated with mild thrombocytopenia and giant platelet size. These are discussed in more detail in [Chapter 21](#).

Wiskott–Aldrich Syndrome

Wiskott–Aldrich syndrome (WAS) is an X-linked recessive disorder featuring thrombocytopenia, eczema, and immunodeficiency resulting from mutations that disrupt the WAS gene, critical for control of the intracellular actin cytoskeleton. Some mutations in the WAS gene may have milder manifestations of X-linked thrombocytopenia without associated immunodeficiency or eczema. The abnormal WAS

platelets are cleared more rapidly by the spleen and have a characteristically small platelet size. Cases of severe thrombocytopenia (<20,000/ uL) with associated bleeding have been reported. Splenomegaly is not observed, but thrombocytopenia responds well to splenectomy. Allogeneic hematopoietic stem cell transplantation is the only curative treatment option for the associated immunodeficiency and eczema.

References

1. Abrahamson PE, Hall SA, Feudjo-Tepie M, et al. The incidence of idiopathic thrombocytopenic purpura among adults: a population-based study and literature review. *Eur J Haematol.* 2009;83(2):83–89.
2. Semple JW, Provan D, Garvey MB, et al. Recent progress in understanding the pathogenesis of immune thrombocytopenia. *Curr Opin Hematol.* 2010;17(6):590–595.
3. Neunart C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood.* 2011;117(16):4190–4207.
4. Provan D, Stasi R, Newland AC, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood.* 2010;115(2):168–186.
5. Cheng G, Saleh MN, Marcher C, et al. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. *Lancet.* 2011;377(9763):393–402.
6. Kuter DJ, Rummel M, Boccia R, et al. Romiplostim or standard of care in patients with immune thrombocytopenia. *N Engl J Med.* 2010;363(20):1889–1899.
7. Godeau B, Porcher R, Fain O, et al. Rituximab efficacy and safety in adult splenectomy candidates with chronic immune thrombocytopenic purpura: results of a prospective multicenter phase 2 study. *Blood.* 2008;112(4):999–1004.
8. Cuker A. Heparin-induced thrombocytopenia: present and future. *J Thromb Thrombol.* 2011;31(3):353–366.
9. Crowther MA, Cook DJ, Albert M, et al. The 4Ts scoring system for heparin-induced thrombocytopenia in medical-surgical intensive care unit patients. *J Crit Care.* 2010;25(2):287–293.
10. Linkins L-A, Dans AL, Moores LK, et al. Treatment and prevention of heparin-induced thrombocytopenia: antithrombotic therapy and prevention of thrombosis, 9th ed.: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* 2012;141(2 suppl):e495S–e530S.
11. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med.* 1998;339(22):1585–1594.
12. Dragon-Durey MA, Blanc C, Garnier A, et al. Anti-factor H autoantibody-

- associated hemolytic uremic syndrome: review of literature of the autoimmune form of HUS. *Semin Thromb Hemost*. 2010;36(6):633–640.
- 13. Lattuada A, Rossi E, Calzarossa C, et al. Mild to moderate reduction of a von Willebrand factor cleaving protease (ADAMTS-13) in pregnant women with HELLP microangiopathic syndrome. *Haematologica*. 2003;88(9):1029–1034.
 - 14. Scully M, Hunt BJ, Benjamin S, et al. Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. *Br J Haematol*. 2012;158(3):323–335.
 - 15. Nurnberger J, Philipp T, Witzke O, et al. Eculizumab for atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;360(5):542–544.
 - 16. Caramazza D, Quintini G, Abbene I, et al. Relapsing or refractory idiopathic thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: the role of rituximab. *Transfusion*. 2010;50(12):2753–2760.
 - 17. Arnold DM, Nazi I, Warkentin TE, et al. Approach to the diagnosis and management of drug-induced immune thrombocytopenia. *Transfus Med Rev*. 2013;27(3):137–145.
 - 18. Reese JA, Li X, Hauben M, et al. Identifying drugs that cause acute thrombocytopenia: an analysis using 3 distinct methods. *Blood*. 2010; 116(12):2127–2133.
 - 19. Arnold DM, Kukaswadia S, Nazi I, et al. A systematic evaluation of laboratory testing for drug-induced immune thrombocytopenia. *J Thromb Haemost*. 2013;11(1):169–176.
 - 20. Reese JA, Bougie DW, Curtis BR, et al. Drug-induced thrombotic microangiopathy: experience of the Oklahoma registry and the BloodCenter of Wisconsin. *Am J Hematol*. 2015;90(5):406–410.
 - 21. Al-Nouri ZL, Reese JA, Terrell DR, et al. Drug-induced thrombotic microangiopathy: a systemic review of published reports. *Blood*. 2015;125(4):616–618.

Disorders of Hemostasis I: Coagulation

Angela C. Weyand and Patrick F. Fogarty

APPROACH TO THE BLEEDING PATIENT

Abnormalities of the activity of coagulation proteins and related molecules, decreased platelet function, or disruption of the vasculature (e.g., by surgery or trauma) can lead to bleeding. Careful assessment of both the clinical history and laboratory testing is necessary to establish the reason for bleeding.

- Initial laboratory studies in a patient with new onset or recent bleeding include a platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT), and fibrinogen. If the bleeding is moderate to severe, a hemoglobin level and specimen for red cell cross-matching should also be sent.
- The character, timing, and location of the bleeding should be considered. Is the bleeding spontaneous, or associated only with invasive procedures or trauma? If periprocedural, is the bleeding immediate or delayed? Mucocutaneous bleeding (epistaxis, gingival hemorrhage, petechiae/ ecchymoses, gastrointestinal, and urinary tract bleeding) are more characteristic of a defect in the activity of platelets, von Willebrand factor (VWF) or the vasculature, whereas soft-tissue bleeding or hemarthrosis suggests a deficiency in the activity of coagulation factors.
- The clinical context is very important in determining the reason for bleeding. Hemorrhage in a patient who has been receiving heparin or warfarin may indicate excess anticoagulation or presence of a previously undetected lesion. A lifelong history of excessive bleeding may occur because of an inherited disorder of hemostasis. Bleeding in an individual who is in septic shock may point to disseminated intravascular coagulation (DIC). New-onset, diffuse bleeding in a patient who is pregnant or postpartum may signify the hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome or other entities. Postsurgical bleeding may stem

from a number of causes, but an initial consideration should be deficient hemostasis due to a traumatized, bleeding vessel, as well as a coagulation factor defect or deficits.

- | A positive family history of bleeding raises the clinical suspicion for heritable disorders such as hemophilia A or B (X-linked recessive inheritance) or von Willebrand disease (VWD; autosomal dominant inheritance in most cases). However, a negative family history does not rule out an inherited coagulation disorder as up to 30% to 40% of patients with Hemophilia A have a negative family history.¹
- | Importantly, bleeding does not necessarily indicate an intrinsic abnormality of hemostasis. Individuals with perfectly normal coagulation and platelet function will bleed, given a sufficient hemostatic challenge (trauma, surgery, invasive malignancy, etc).

THE COAGULATION SYSTEM

Coagulation Factors: Background

- | Coagulation factors (clotting factors) are synthesized in the liver.
- | Factors II, VII, IX, X, XI, and XII are serine proteases that are inactive as synthesized and acquire enzymatic capability when cleaved (activated) by other proteins. A postsynthetic step in the production of factors II, VII, IX, and X and the natural anticoagulant proteins C and S require the activity of a vitamin K-dependent carboxylase that modifies the amino-terminus of each factor, enabling it to function.
- | Tissue factor (TF) and factors V and VIII serve as cofactors for coagulation reactions.
- | The activity of all clotting factors culminates in a principal event: the generation of thrombin at sites of vascular injury. Thrombin activates platelets (primary hemostasis) and cleaves fibrinogen to form fibrin (secondary hemostasis) at sites of blood vessel compromise.
- | The normal laboratory range of factor activity levels is approximately 50% to 150% and is derived from plasma activity as observed in a reference pool of normal donors. The hemostatic level of a given clotting factor (i.e., the level of factor necessary to maintain normal hemostasis) typically is much lower. For instance, 5% activity of factor VIII (FVIII) is well below the laboratory reference range but usually is sufficient to prevent spontaneous bleeding.

The Coagulation Cascade

The coagulation cascade illustrates the activation of coagulation factors in the formation of a fibrin clot. It comprises the tissue injury (also known as extrinsic), contact (also known as intrinsic), and common pathways of coagulation (Fig. 20.1). The pathways within the coagulation cascade probably best reflect the activity of clotting factors in

vitro, whereas, in vivo, the pathways not only interact at multiple points but also function in concert with the activation and aggregation of platelets to achieve hemostasis.

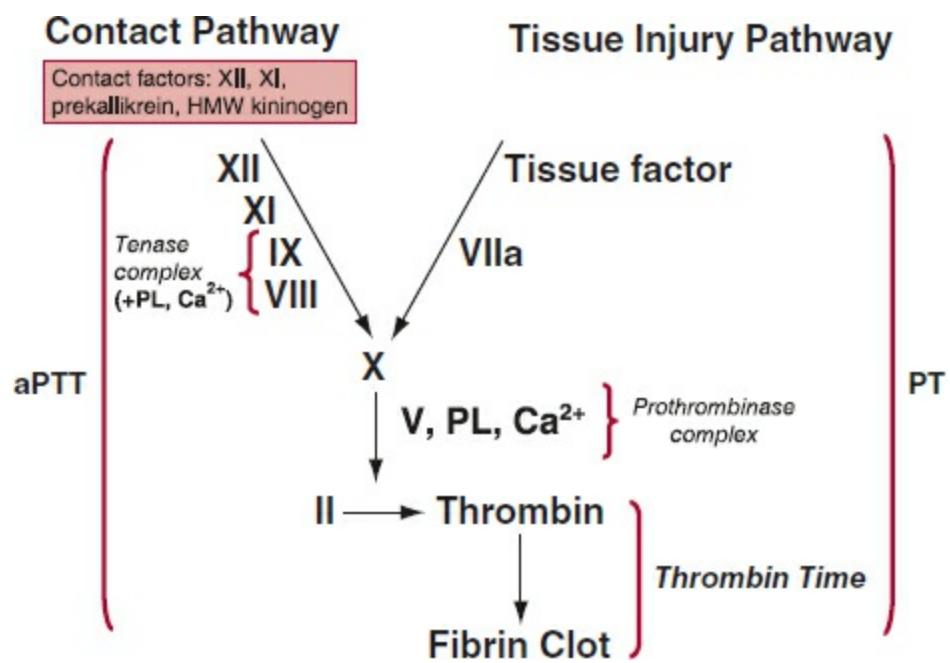


FIGURE 20.1 The coagulation cascade. The tissue injury pathway of coagulation begins with the binding of activated factor VII (VIIa) to tissue factor (TF), which is provided by cell membranes. VIIa converts X to Xa. The prothrombinase complex, formed by the binding of Xa to Va in the presence of phospholipid and Ca^{2+} , converts II (prothrombin) to IIa (thrombin). The contact pathway of coagulation begins with the activation of factor XII to XIIa by kallikrein. XIIa cleaves XI to XIa; XIa cleaves IX to IXa. IXa forms a complex with VIIIa in the presence of phospholipid and Ca^{2+} (tenase complex) and converts X to Xa. Xa, in the presence of Va, PL, and Ca^{2+} , then cleaves II (prothrombin) to IIa (thrombin). The common pathway involves the cleavage of II by prothrombinase to yield thrombin, and thrombin cleavage of fibrinogen to form fibrin, which is then cross-linked via the action of XIIIa. Activation of coagulation usually begins with the tissue injury system, which provides feedback to the contact system by IIa-mediated activation of factor XI. There are additional points of interaction between the pathways (not indicated).

aPTT, activated partial thromboplastin time; HMW, high molecular weight; PL, phospholipid; PT, prothrombin time.

- Tissue injury pathway. The tissue injury pathway of coagulation begins with the binding of activated factor VII (VIIa) to TF. The TF–VIIa complex mediates the conversion of X to Xa. The prothrombinase complex, formed by the binding of Xa to Va on a phospholipid (PL) surface (usually platelet membranes) in the presence of Ca^{2+} , converts II (prothrombin) to IIa (thrombin).
- Contact pathway. Activation of contact factors at the site of vascular injury leads to the conversion of factor XII to XIIa and the sequential conversion of XI to XIa and IX to IXa. IXa complexes with VIIIa, PL, and Ca^{2+} , forming the tenase complex, which converts X to Xa. Xa, in a complex with Va, PL, and Ca^{2+} then cleaves II (prothrombin) to IIa (thrombin). The TF–VIIa complex can also activate IX leading to the subsequent formation of the tenase complex.
- Common pathway. The tissue injury and contact pathways converge in the common pathway, where X is converted to Xa, and prothrombin (II) is cleaved to form thrombin. Thrombin then cleaves fibrinogen to form fibrin, which is then cross-linked via the action of XIII.

Common Coagulation Tests

An understanding of the basic laboratory tests for coagulation assists in the evaluation of bleeding disorders.

- The PT measures the time it takes for plasma to clot when exposed to TF. It is performed by adding thromboplastin (TP), composed of crude or recombinant TF plus Ca^{2+} , to plasma that has been anticoagulated with citrate, and the time to formation of a fibrin clot is measured. Because the PT comprises reactions of coagulation that occurs in the tissue injury and common pathways of coagulation, deficiencies in the activity of II, V, VII, X, or fibrinogen may prolong the PT.
- The international normalized ratio (INR) was developed to standardize the reporting of PT values in warfarin-anticoagulated patients. Standardization is necessary because commercially available TP reagents have varying potencies that directly impact the PT; one TP may yield a different PT result than another when the same sample is tested. The potency of a given TP is expressed in terms of the International Sensitivity Index (ISI).
- Because the INR was developed to report factors measured by the PT that are decreased by warfarin impairment of vitamin K-mediated synthesis (i.e., it is not standardized for abnormalities of factor V and fibrinogen), the INR should be used only to describe anticoagulation in patients who are receiving warfarin. In all other patients (e.g., patients with liver disease), the PT should be referenced.
- The formula for the INR is $(\text{PT}_{\text{patient}} / \text{PT}_{\text{mean normal}})^{\text{ISI}}$
- The aPTT measures the time it takes for plasma to clot when exposed to reagents that activate the contact factors. It begins with the addition of a contact activating agent to

citrate-anticoagulated plasma. PL and Ca^{2+} are added, and the time to formation of a fibrin clot is measured. Because the aPTT reflects reactions of coagulation that occur in the contact and common pathways of coagulation, deficiencies in the activity of factors II, V, VIII, IX, X, XI, or XII may prolong the aPTT. A deficiency of other contact factors, such as prekallikrein or high-molecular-weight kininogen (HMWK), may also prolong the aPTT. Isolated abnormalities of fibrinogen rarely impact the aPTT.

- The long-incubation aPTT is performed by incubating the sample with activating agents for 10 minutes before the addition of PL and Ca^{2+} . If the contact factor prekallikrein is deficient, this extra incubation time allows activation of factor XII and correction of the aPTT.
- Mixing studies are performed using a mixture of 50% patient plasma and 50% normal control plasma; the PT or aPTT is then performed as usual. Correction of a prolonged PT or aPTT with mixing generally implies a qualitative or quantitative abnormality of one or more clotting factors in the patient plasma. In contrast, failure of the PT or aPTT to correct completely on mixing suggests the presence of an inhibitor in the patient plasma that neutralizes a component of the patient and normal plasma. Both lupus anticoagulants (LAs; see the following) and inhibitors to specific clotting factors can result in a prolonged aPTT or PTT that does not correct on mixing.
- When evaluating a prolonged aPTT, an aPTT is performed on the mixture, then the mixture is allowed to incubate for an hour and the aPTT is repeated; some inhibitors of FVIII are maximally inhibitory at an hour or more post-mix. For instance, the aPTT on a 1:1 mixture of normal and patient plasma containing a FVIII inhibitor may show correction initially but demonstrate a prolongation at 1 hour.
- Occasionally, a weak LA may produce a prolonged aPTT or PT that corrects on mixing.
- The bleeding time (BT) involves making a controlled incision in soft tissue (usually at a site on the forearm) and measuring the time to cessation of bleeding. Anemia and abnormalities of coagulation factors, platelets, or the vasculature may prolong the BT. The BT does not correlate with the risk of surgical bleeding in most patients,² and is no longer widely used.
- The thrombin time (TT) measures the final step of coagulation: the conversion of fibrinogen to fibrin. It involves the addition of exogenous thrombin to patient plasma, inducing cleavage of fibrinogen to fibrin and the formation of a fibrin clot.
- The most common cause of a prolonged TT is the presence of heparin in the sample, which can be confirmed by documentation of a normalization of the TT when the test is repeated using a heparin-binding agent such as protamine or Heparisorb.
- Abnormalities of fibrinogen and circulating heparin-like anticoagulants also cause a prolonged TT.
- The reptilase time is also used to assess abnormalities of fibrinogen (reptilase

cleaves fibrinogen to fibrin). Unlike thrombin, however, reptilase is not inhibited by the presence of heparin. Thus, a prolonged TT in conjunction with a normal reptilase time usually indicates heparin contamination, whereas prolongation of both tests indicates a qualitative abnormality of fibrinogen.

- | The functional fibrinogen assay assesses fibrinogen concentration by addition of an excess of thrombin to a sample of diluted plasma. The Clauss method is most commonly used.

Specialized Coagulation Tests

- | The anti-Xa assay provides information about the degree of anticoagulation that has occurred in the patient plasma due to the effect of certain anticoagulants (unfractionated or low-molecular-weight heparin, fondaparinux, rivaroxaban, apixaban, and edoxaban) on factor Xa in the sample. By convention, peak levels should be drawn 4 to 6 hours after low molecular-weight heparin (LMWH) administration to estimate anticoagulation.
- | Tests for LAs distinguish an inhibitor of a specific clotting factor from an LA as the cause of a prolongation in that the aPTT that does not correct with mixing. Most tests for LAs involve addition of excess PLs to the reaction system to neutralize the LA and result in a correction of a prolonged clotting time. One such test is Russell's viper venom time, which takes advantage of the ability of viper venom to activate factor X directly; other systems for the diagnosis of LAs are available.³
- | The Bethesda assay is a special type of mixing study that involves incubation of dilutions of patient plasma with normal (control) plasma to assess the potency of an inhibitor (generally, to FVIII) in the patient plasma. After a 2-hour incubation phase at 37°C, a FVIII assay (or other appropriate factor assay, as indicated) is performed on each dilution (and on samples used to create a control curve); as the proportion of patient plasma in the mixture decreases, the effect of the inhibitor decreases, and the factor assay clotting time shortens. The Nijmegen modification to the Bethesda assay includes slightly different buffers to stabilize the proteins during the incubation period.⁴
- | The potency of the inhibitor is expressed in Bethesda units (BUs). The reciprocal of the dilution of the mixture of patient and normal control plasma that contains ~50% of normal FVIII activity is the inhibitor titer in BU. For instance, if 50% inhibition of normal FVIII activity occurred at a 1 : 40 dilution, the inhibitor titer would be said to be 40 BU.
- | Assays for specific clotting factors. Factor activity levels can be assessed by clot-based reactions (that use modifications of the aPTT or PT), and some factors by chromogenic systems, where the amount of factor is measured by its action on a highly specific chromogenic substrate. The intensity of color produced is directly proportional to the amount of factor present so the level can be calculated from the

absorbance of the sample at a specific wavelength.

- Factor activity levels are generally reported as percentages (of “normal” activity) or in units per milliliter (U/ mL), with 1 U/ mL corresponding to 100% of the factor found in 1 mL of normal plasma.
- Usually, levels of 25% to 40% are necessary to prolong the PT or aPTT. Mild or moderate deficiencies of a given clotting factor may lead to an elevated PT or aPTT, but may be adequate for hemostasis.
- The euglobulin clot lysis time (ECLT) measures time to dissolution of a fibrin clot; a shortened ECLT indicates activation of the fibrinolytic system. The most common cause of a shortened ECLT is DIC, in which fibrinolysis is activated in response to an activation of coagulation. Deficiencies in the activity of plasminogen activator inhibitor (PAI) or alpha-2-antiplasmin also shorten the ECLT (see the following). A shortened ELT in the absence of thrombocytopenia and schistocytes is indicative of a primary hyperfibrinolysis.
- The major distinctive of thromboelastography (TEG) is a measurement of various parameters of clot formation dynamically in whole blood, thus capturing functional data on the activity of both platelets and coagulation factors.⁵ It is used clinically mostly in cardiovascular surgical settings although other applications have been explored.^{6a} The rotational thromboelastography (ROTEM) is an adaptation of the TEG.

DIFFERENTIAL DIAGNOSIS OF ABNORMAL COAGULATION TESTS

In this section, conditions that predispose to bleeding are presented according to which coagulation test results are characteristically affected. Entities that prolong the aPTT, PT, or both (Table 20.1 and Figs. 20.2–20.4) are discussed.

Table 20.1 Causes of Abnormal Routine Coagulation Studies

Isolated Prolonged aPTT	Isolated Prolonged PT	Prolonged aPTT and PT
Lupus anticoagulant ^a	Warfarin use	DIC
Heparin in sample (at clinically relevant concentrations)	Vitamin K deficiency	Vitamin K deficiency ^d
Dabigatran in sample (variably, rivaroxaban/ apixaban)	Liver disease	Liver disease ^d
Deficiency of, or inhibitor to, factors VIII, IX, X ^{b,c}	Variably, dabigatran, rivaroxaban or apixaban in sample	Variably, dabigatran, rivaroxaban or apixaban in sample
Deficiency of, or inhibitor to, factor XII prekallikrein or HMWK ^c (not	Lupus anticoagulant ^a	Warfarin use ^e
	Deficiency of, or inhibitor to, factor VII	Deficiency of, or inhibitor to, fibrinogen, factors II, V, or X

associated with bleeding)		
Traumatic venipuncture	Hypo- or dysfibrinogenemia	Lupus anticoagulant ^a
Severe Von Willebrand disease		Anticoagulants ^f

^aAn isolated prolongation of aPTT is the most common laboratory manifestation of a lupus anticoagulant. Lupus anticoagulants are not associated with increased bleeding risk.

^bFVIII deficiency may occur in hemophilia A (congenital or acquired) or von Willebrand disease.

^cDeficiencies of FXII, HMWK, and pre-kallikrein are not associated with bleeding.

^dAdvanced cases.

^eTypically, supratherapeutic doses.

^fIncluding direct thrombin inhibitors (especially argatroban), very high concentrations of heparin, superwarfarins.

aPTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; HMWK, high-molecular-weight kininogen; PT, prothrombin time.

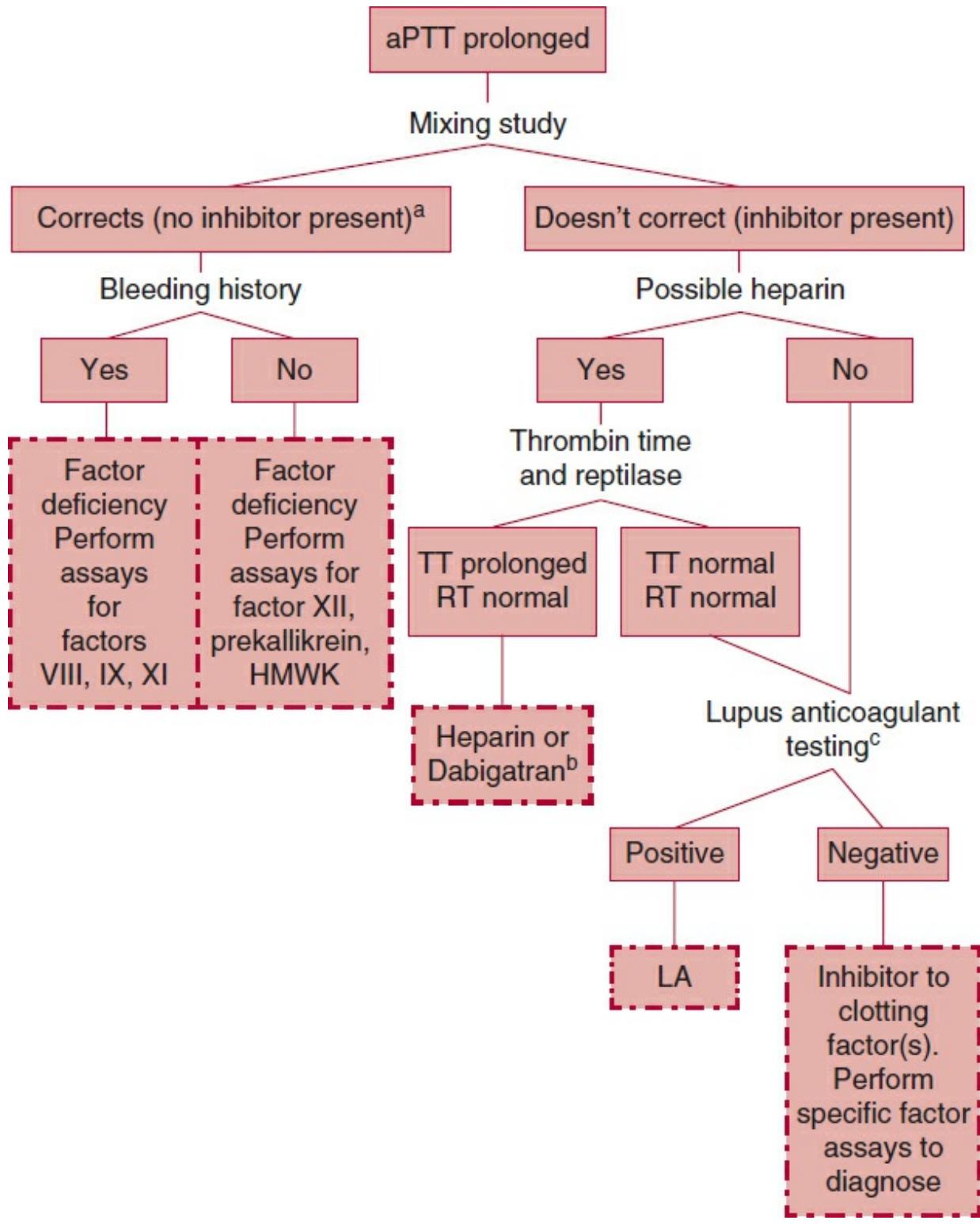


FIGURE 20.2 Laboratory diagnostic algorithm for a prolonged aPTT and normal PT.

^aOccasionally, weak LAs can cause a prolongation in the aPTT that corrects completely on mixing. In this scenario, factor assays may be indicated in addition to LA testing, especially if demonstration of hemostatic factor levels is regarded as important (e.g., in a preoperative patient).

^bFurther evaluation based on the clinical scenario. If suspect dabigatran, perform HEMOCLOT

assay (provides dabigatran level).

^cSome LAs escape detection, even after performing two tests. If all relevant coagulation factor activities are demonstrated to be hemostatic, an LA may be the explanation for the aPTT prolongation.

aPTT, activated partial thromboplastin time; HMWK, high-molecular weight kininogen; LA, lupus anticoagulant; RT, reptilase time; TT, thrombin time.

Conditions Associated With a Prolonged aPTT

- LAs³ are a very common cause of a prolongation in the aPTT that does not correct completely on mixing with normal plasma but does correct with addition of excess PL.
- LAs were so named due to their frequent presence in patients with systemic lupus erythematosus and tendency to prolong coagulation tests by interacting with PL in the test sample. In contradistinction to their name, however, LAs are not physiologic anticoagulants and the phenotype is usually increased risk of thrombosis; additionally, more than one-half of patients with LAs do not have connective tissue disease (see [Chapter 22](#)).
- LAs are diagnosed using the methods described previously.

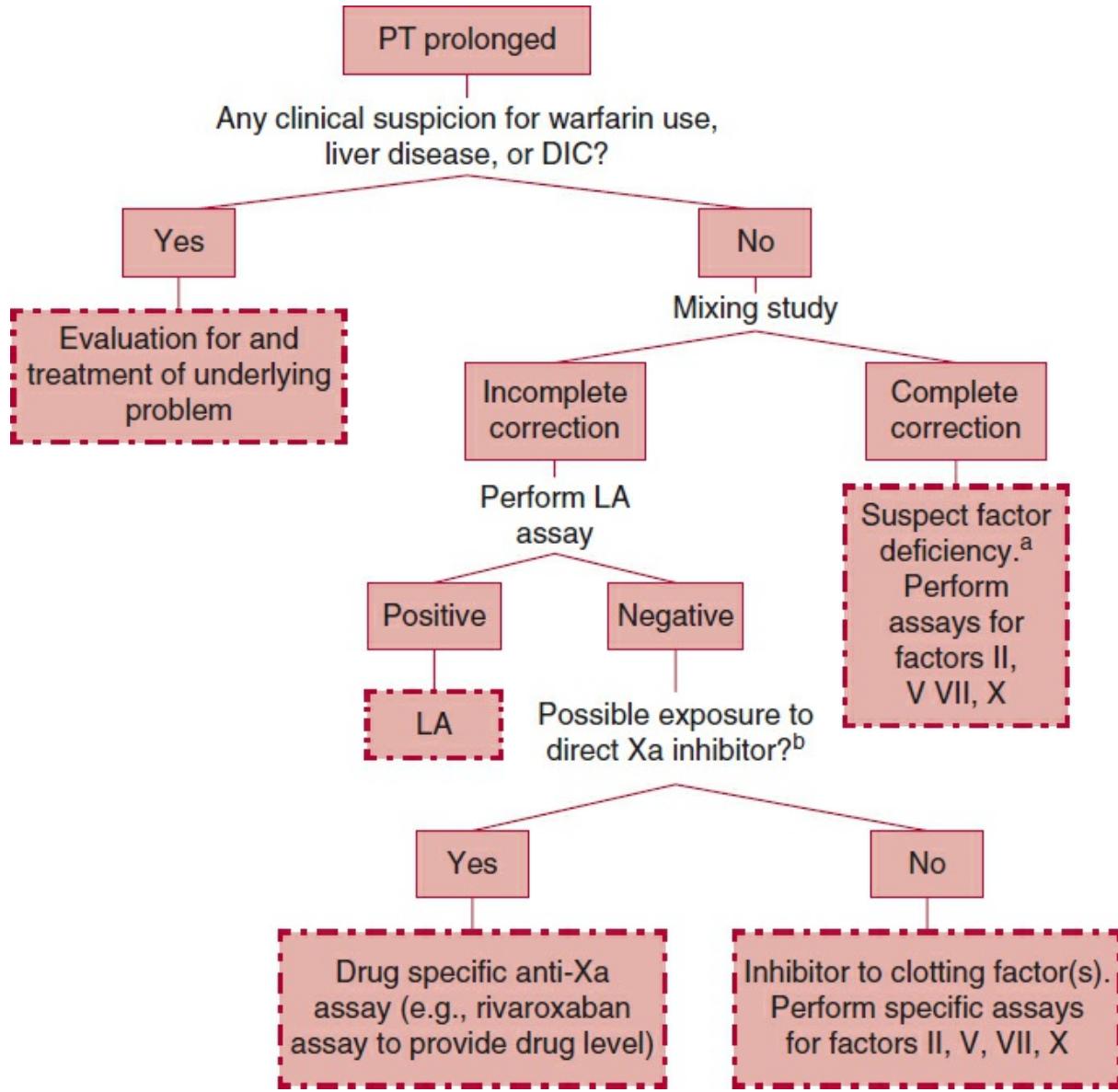


FIGURE 20.3 Laboratory diagnostic algorithm for a prolonged PT and normal aPTT.

^aOccasionally, LAs can cause a prolongation in the PT that corrects on mixing.

^bPT prolongation to a greater degree than a concomitant aPTT prolongation may be observed when rivaroxaban or edoxaban is in the test sample. Apixaban may have little effect on PT or aPTT.^{6b}

aPTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; LA, lupus anticoagulant; PT, prothrombin time; TT, thrombin time.

- Hemophilia A and B. A deficiency of coagulation FVIII or factor IX (FIX) causes a prolongation in the aPTT that corrects completely on mixing. Congenital FVIII deficiency is referred to as hemophilia A. (FVIII is also decreased in moderate and severe VWD; see the following) Congenital deficiency of FIX is referred to as hemophilia B (Christmas disease).

- Hemophilia A is estimated to occur in 1 per 5,000 to 10,000 live male births; hemophilia B is about a fifth as common. The disorders are inherited in an X-linked recessive fashion: Males are affected, whereas females are carriers and are generally not affected unless significant lyonization has occurred favoring the X chromosome bearing the abnormal copy of the FVIII gene. There is no race predilection.
- The presentation of the disease relates to the level of residual factor activity in the plasma. Severe hemophilia (<1% factor activity seen in approximately one-half of patients with hemophilia A and approximately one-third of patients with hemophilia B) typically presents in infancy with bleeding at circumcision or in early childhood with spontaneous bleeding into soft tissues (muscles) or joints, and intracranial, gastrointestinal, or urinary bleeding. Moderate hemophilia (1% to 5% factor activity) is typified by less severe bleeding than that observed in severe disease, whereas individuals with mild hemophilia (>5% activity) usually do not experience spontaneous bleeding but may bleed on significant hemostatic challenges such as trauma or surgery. Coagulation factor replacement products are the mainstay of treatment; both plasma-derived and recombinant products are commercially available. Dosing depends on the severity of hemophilia and the product used ([Table 20.2](#)).⁷ Patients with mild hemophilia A may respond to infusion of desmopressin (DDAVP) (0.3 mcg/ kg/ dose),⁸ but a trial should be done in the non-bleeding state to document a rise of the FVIII activity level into the hemostatic range.

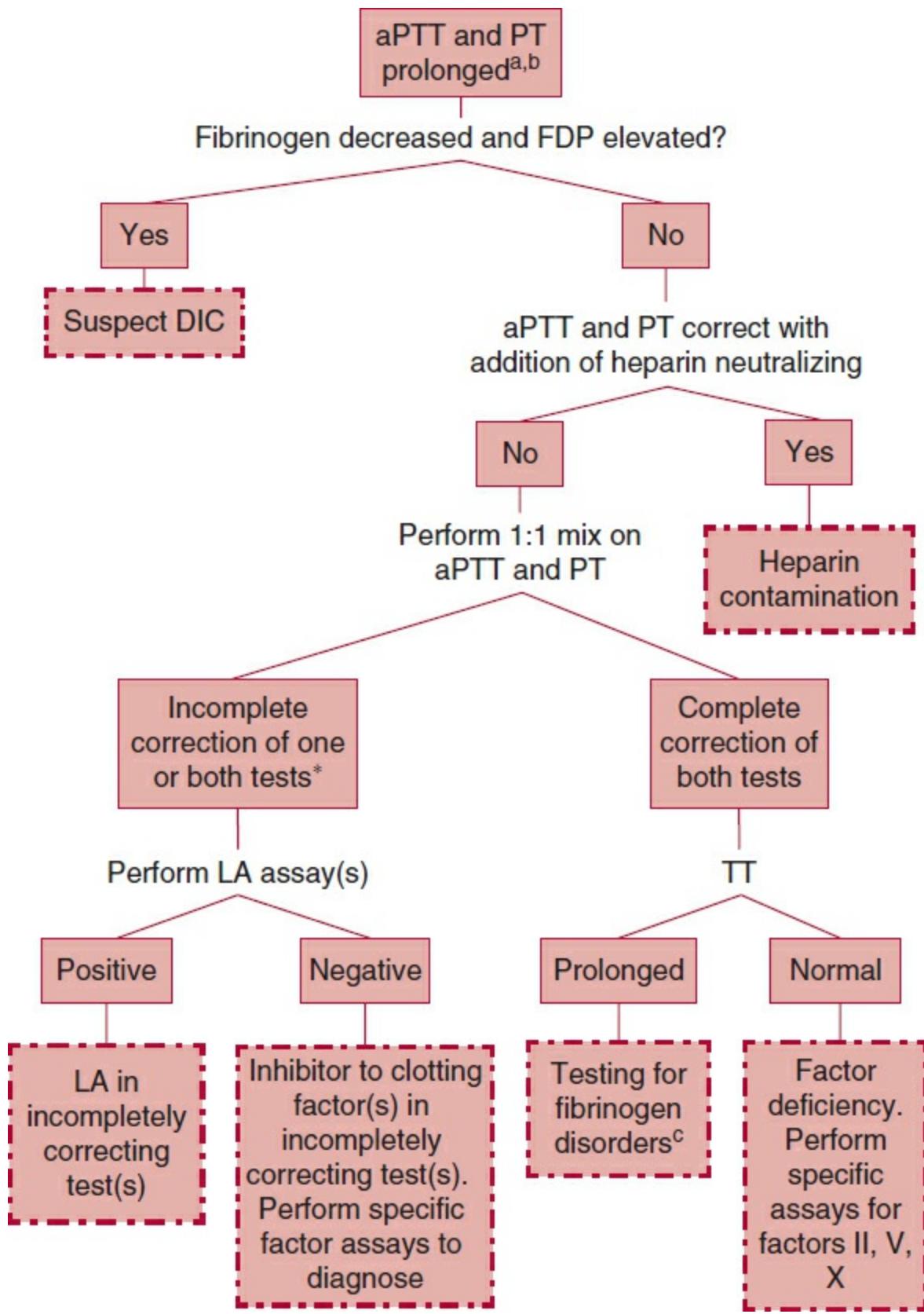


FIGURE 20.4 Laboratory diagnostic algorithm for a prolonged aPTT and PT.

^aCoexisting conditions, such as vitamin K deficiency (leading to a prolonged PT) and a

concomitant LA (leading to an elevated aPTT), are possible.

^bRarely, coinheritance of deficiencies in multiple coagulation factors (such as factors V and VIII) can occur.

^cDecreased functional fibrinogen in conjunction with a normal immunologic fibrinogen indicates an abnormal fibrinogen (dysfibrinogenemia), whereas decreased functional and immunologic assays are typical of hypofibrinogenemia.

aPTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; FDP, fibrin degradation products; LA, lupus anticoagulant; PT, prothrombin time; TT, thrombin time.

- The oral antifibrinolytic agent aminocaproic acid (Amicar), given at a dose of 1 to 2g every 4 to 6 hours, may be useful for patients with mucosal or oral bleeding, or bleeding associated with dental procedures.
- Prophylactic factor replacement (scheduled, every-other-day to weekly infusions, depending on the product used) is used as a means of preventing the morbidity incurred from recurrent joint bleeding.⁹ The practice ideally is begun by the age of 2 years, before joint disease develops.
- Inhibitors. Patients with congenital hemophilia (usually with severe disease) who have received factor concentrates as a treatment for bleeding are at risk for inhibitor formation. More than 25% of patients with hemophilia A and less than 5% of patients with hemophilia B will develop inhibitors to FVIII or FIX, respectively. The potency of the inhibitor is expressed in BU. Low-titer inhibitors (<5 BU) often may be overcome by increasing the dose or frequency of infused factor concentrate. It is usually not possible to overcome high-titer inhibitors (>5 BU) using this approach, however, and administration of activated prothrombin complex concentrates, recombinant factor VIIa, or recombinant porcine FVIII is necessary (Table 20.2).¹⁰ Inhibitor eradication via immune tolerance induction usually is attempted in hemophilia A, with approximately 70% of cases achieving tolerance.¹¹

Table 20.2 Acute Bleeding Episodes in Hemophilia: Initial Treatment

Disorder	Severity	Major Bleeding^a	Minor/Moderate Bleeding^b
Hemophilia A	Mild	FVIII concentrate, 40–50 U/kg IV ^c	DDAVP ^d or FVIII concentrate
	Moderate or severe	FVIII concentrate, 40–50 U/kg IV ^c	FVIII concentrate: 20–40 U/kg IV ^c
Hemophilia B	N/A	FIX concentrate, 80–120 U/kg IV ^e	FIX concentrate, 30–60 U/kg IV ^f
Hemophilia + inhibitor ^g	N/A	aPCC 50–100 IU/kg IV ^h or rVIIa 90–270 mcg/kg IV ⁱ or	aPCC 50–100 IU/kg IV ^h or rVIIa 90–270 mcg/kg IV ⁱ or

Susoctocog alfa 200 U/ kg IV (hemophilia A only)	Susoctocog alfa 200 U/ kg IV (hemophilia A only)
-----------------------------------------------------	-----------------------------------------------------

^aBleeding requiring hospitalization or posing a threat to life/ limb, including intracranial, retroperitoneal, and gastrointestinal bleeding, or compartment syndrome.

^bJoint bleeding or muscle/ soft tissue bleeding.

^cFor major bleeding, maintain factor activity $\geq 50\%$ for 3 to 10 days or as long as bleeding is present (may require longer treatment, i.e., up to 4 weeks, in cases of intracranial bleeding). Minor bleeding may respond to a single dose. Consider adjunctive Amicar for mucosal bleeding.

^dDDAVP dose is 0.3 mcg/ kg in 50 mL NS IV administered over at least 20 minutes; may repeat in 12 to 24 hours, max two to three doses; intranasal formulation (Stimate) also available; dose for adults weighing >50 kg is 150 mcg (one spray) in each nostril; fluid restrict (≤ 750 mL in 24 hours after dosing) and limit doses to reduce likelihood of hyponatremia; tachyphylaxis occurs after two to three doses.

^eDosing depends on factor replacement product used; see product information. Consider adjunctive Amicar® for mucosal bleeding.

^fDosing depends on factor replacement product used; see product information. Consider adjunctive Amicar® for mucosal bleeding.

^gAssumes high-titer inhibitor (i.e., >5 Bethesda units). Lower-titer inhibitors may respond to higher doses of factor concentrate.

^haPCC, activated prothrombin complex concentrate (plasma-derived, such as FEIBA); give every 8 to 12-hour IV initially for major bleeding; minor bleeding may require a single dose. Consider adjunctive Amicar® for mucosal bleeding.

ⁱrVIIa, activated factor VII (recombinant, NovoSeven); give 90 mcg/ kg every 2 hours IV initially, give 270 mcg/ kg less frequently; minor bleeding may require a single dose. Consider adjunctive Amicar for mucosal bleeding.

^jSusoctocog alfa (recombinant porcine Factor VIII, Obizur); indicated for acquired hemophilia A. An initial dose of 200 U/ kg IV has been recommended; however, in the authors' experience, a somewhat reduced initial dose of ≤ 140 U/ kg IV may be appropriate to avoid a supratherapeutic FVIII level. Titrate subsequent doses to maintain recommended factor VIII trough levels (50–100 IU/ dL in minor or moderate bleeding, or in major bleed after bleed is controlled; approximately 100 IU/ dl to treat major acute bleed) and account for individual clinical response.

DDAVP, desmopressin; FVIII, factor VIII; FIX, factor IX.

Table 20.3 Non-Coagulation Factor-Based Strategies for Hemophilia in Clinical Trials

Mechanism	Molecule(s)	Type of Hemophilia ^a
siRNA knockdown of antithrombin	Fitusiran	A, B; inhibitor
Inhibition of TFPI	Concizumab, BAY-1093884, PF 06741086	A, B; inhibitor
Bispecific antibody to factors IXa and X	Emicizumab	A; inhibitor

^aPotential clinical target is indicated.

siRNA, small interfering RNA; TFPI, tissue factor pathway inhibitor.

- New treatments. The first recombinant factor replacement products became available in the 1990s, and feature defined terminal half-lives. A variety of bioengineering

strategies, such as PEGylation or fusion to recombinant albumin or the Fc portion of an immunoglobulin type 1 molecule now have resulted in half-life extension of recombinant FVIII and FIX product.¹² Other approaches that are under clinical investigation include gene therapy¹³; antagonism of natural inhibitors of anticoagulation¹⁴; and mimicry of VIII's cofactor activity by a bispecific antibody to factors IXa and X¹⁵ (Table 20.3). The former strategy may allow for the sustained endogenous production of the necessary coagulation protein,¹⁶ while the latter strategies have the additional potential benefit of use in hemophilia that has been complicated by an inhibitor.

- | Acquired hemophilia A occurs at an incidence of approximately 1.5 per million per year,¹⁷ typically in elderly persons or in those with underlying lymphoproliferative conditions, cancer, autoimmunity, or prior pregnancy. Anti-FVIII immunoglobulin G (IgG) antibodies neutralize FVIII, leading to a prolonged aPTT that does not correct on mixing. The clinical presentation commonly features extensive ecchymoses and soft-tissue hematomas. Bypassing agents (see Table 20.2), as well as recombinant porcine factor VII¹⁸ can be used to treat acute bleeding, whereas a variety of immunosuppressive agents, typically incorporating corticosteroids initially, may produce a response. The mortality of this condition is considerable.¹⁹
- | Moderate or severe VWD, due to the lack of adequate VWF to bind and protect circulating FVIII from clearance, can lead to low FVIII levels and a prolongation in the aPTT (see Chapter 21). The prolongation in the aPTT corrects on mixing.
- | Factor XI deficiency (sometimes called hemophilia C or Rosenthal syndrome) typically results in a prolonged aPTT that corrects on mixing. It is inherited in an autosomal recessive manner and is most prevalent among individuals of Ashkenazi Jewish descent.²⁰ It typically causes a mild bleeding tendency that is worsened by trauma or surgery although the phenotype is heterogeneous. Levels of factor XI do not correlate well with bleeding symptoms.²¹ Fresh frozen plasma (FFP) may be used prophylactically or for treatment of bleeding, and adjunctive aminocaproic acid decreases unmitigated fibrinolysis, making it useful for chronic prophylaxis, oral bleeding, dental work, or minor surgical procedures. In some locales, a plasma-derived FXI concentrate is available (not currently available in the United States).
- | Factor XII deficiency and deficiencies of prekallikrein and HMWK. Though they may lead to a prolonged aPTT, these conditions do not cause bleeding.
- | Acquired inhibitors to coagulation proteins. Occasionally, adults without a prior history of hemophilia develop high-titer inhibitors to FVIII; not infrequently, a concomitant lymphoproliferative or immune disorder is present. Immunosuppressive treatment with steroids or chemotherapy usually is effective.²² Autoantibodies to a variety of other clotting factors (prothrombin, thrombin, factors V, VII, IX, X, XI, XIII) have also been described.
- | Heparin contamination. The presence of heparin in the sample used for the aPTT

determination may be verified by documenting normalization of the aPTT after the test is repeated using a heparin-binding agent.

- Warfarin (usually at supratherapeutic doses) may mildly prolong the aPTT due to depletion of factors II, XI, or X, and will also increase the sensitivity of the aPTT to heparin effect.
- Direct thrombin and direct Xa inhibitors. Dabigatran in the test sample reliably prolongs the aPTT, whereas rivaroxaban and apixaban may prolong it or have no effect.²³
- Other medications (such as the glycopeptide oritavancin) may bind to PL and prolong the aPTT.
- Occasionally, traumatic venipuncture may cause the aPTT to be prolonged, due to the direct activation of coagulation at the site of venipuncture, leading to depletion of crucial coagulation proteins in the collected specimen. The blood should be redrawn with careful technique during phlebotomy, and the aPTT repeated to document normalization. (Traumatic venipuncture may also lead to a shortening of the aPTT due to small amounts of thrombin generation.)

Conditions Associated With a Prolonged PT

- Vitamin K deficiency can cause an elevated PT that typically corrects completely on mixing. The vitamin K-dependent factors that are measured by the PT are II, VII, and X.
- Malabsorption or deficient dietary intake of vitamin K (from green leafy vegetables such as cabbage, cauliflower, and spinach, cereals, soybeans, and other foods) or decreased production by intestinal bacteria (which may be destroyed by antibiotics) may lead to vitamin K deficiency. Vitamin K deficiency is quite rare in otherwise healthy patients due to the wide distribution in plants, production by micro-flora in the gut, and recyclability in cells. A deficiency may be associated with long-term antibiotic use, total parenteral nutrition, or conditions leading to malabsorption of fat-soluble vitamins (e.g., cystic fibrosis).
- For treatment, vitamin K (phytonadione) may be administered via parenteral or oral routes. Intravenous administration (1 mg/ day) results in faster normalization of a prolonged PT than subcutaneous dosing, but occasionally has been associated with anaphylaxis; therefore, intravenous doses should be administered slowly (more than 30 minutes) while the patient is monitored. Subcutaneous administration should be avoided due to erratic absorption.²⁴
- At least partial correction of the PT is expected within 24 hours after parenteral administration of vitamin K if vitamin K deficiency is the only reason for the prolongation in the PT.
- Coagulopathy of liver disease. Hepatic insufficiency leads to decreased synthesis of

clotting factors, such as the vitamin K-dependent factors and, with more severe disease, factors V, VIII, XI, XII, and fibrinogen, resulting in a prolonged PT (and with severe disease, aPTT) that correct(s) on mixing.

- In contradistinction to coagulopathy due to isolated vitamin K deficiency, liver disease may feature a reduced factor V level in addition to decreased levels of factors II, VII, IX, and X.
- FVIII and VWF may be elevated.
- Patients with prolonged clotting times due to liver disease paradoxically may be prone to thrombosis.²⁵
- In addition to the coagulation factor defects, patients with liver disease also often have thrombocytopenia, platelet function defects, and hyperfibrinolysis.
- Warfarin. Warfarin inhibits the vitamin K-dependent carboxylase that is important for the synthesis of factors II, VII, IX, and X. Decreased functional levels of factors II, VII, and X may prolong the PT and produce an elevated INR. INR is affected by many factors including comorbidities, acute illnesses, day-to-day variation in vitamin K intake, and polypharmacy.
 - Supratherapeutic INRs that are not associated with bleeding generally are managed by temporary withholding warfarin to allow the INR to descend into the desired range, and restarting the warfarin at a lower dose.
 - Critically elevated INRs (>9) may be addressed with temporary discontinuation of warfarin, plus administration of vitamin K²⁴ or FFP if the patient is felt to be at very high risk of bleeding.
 - For treatment of warfarin-associated major bleeding,²⁶ the warfarin should be discontinued and FFP (4 U), prothrombin complex concentrate (such as Bebulin, 35 U/ kg/ dose), or rhVIIa (NovoSeven, 15 to 90 µg/ kg/ dose) may be administered along with intravenous or oral vitamin K.
- LAs can cause a mild prolongation in the PT (see mentioned previously).
- Hypo/ dysfibrinogenemia. Quantitative or qualitative abnormalities of fibrinogen typically produce a long TT and reptilase time (mentioned previously), but the PT also may be prolonged (Fig. 20.3). The PT is much more sensitive to hypo/ dysfibrinogenemia than is the aPTT.
- Defects in the function of fibrinogen are more commonly acquired (e.g., cirrhosis or active liver disease) than congenital. For instance, DIC characteristically produces a consumptive hypofibrinogenemia.
- Replacement of fibrinogen in a bleeding patient with hypo/ dysfibrinogenemia may be accomplished by administration of cryoprecipitate, or a plasma-derived fibrinogen concentrate (where available); for major surgery or bleeding, a target plasma fibrinogen level of at least 80 to 100 mg/ dL may be used.²⁷
- Deficiencies of individual clotting factors. Congenital deficiencies of isolated

coagulation factors (e.g., VII) leading to a prolonged PT are extremely rare and typically are inherited in autosomal recessive pattern.

- | Antibodies to bovine factor V and thrombin may develop after exposure to topical bovine thrombin (used in orthopedic, neurologic, and vascular surgery). The antibodies cross-react with human factor V and/ or thrombin, resulting in prolongation of the PT and bleeding in some patients.²⁸
- | Direct thrombin inhibitors and direct Xa inhibitors in the test sample may prolong the PT, or have no effect.²³

Conditions Associated With Prolonged aPTT and PT

- | Coagulopathy of liver disease. If hepatic insufficiency is extreme, multiple factor deficiencies can result in a prolonged PT and aPTT.
- | Deficiencies of individual clotting factors. Isolated deficiencies of factors II, V, or X are rare,²⁹ but may prolong both the PT and aPTT.
- | DIC. Depletion of coagulation factors via diffuse activation of coagulation may cause a prolongation in both the PT and aPTT ([Chapter 21](#)).
- | LAs can prolong both the aPTT and PT (as mentioned previously).
- | Direct thrombin inhibitors and direct Xa inhibitors in the test sample (as mentioned previously).
- | Severe vitamin K deficiency can result in prolongation of the aPTT in addition to the PT.

Conditions Associated With Bleeding and Normal Coagulation Tests

- | Factor XIII deficiency. Activated factor XIII cross-links fibrin strands, stabilizing the fibrin clot. Individuals with a deficiency of factor XIII characteristically develop delayed bleeding several hours to days following surgery or trauma. Umbilical stump bleeding, traumatic soft tissue and joint bleeds, recurrent pregnancy loss, and spontaneous intracranial hemorrhages also have been described.¹² The deficiency is usually transmitted as an autosomal recessive trait; severely affected individuals carry homozygous or compound heterozygous mutations.
 - Clot lysis or enzymatic assays and sequencing of either of the two genes that encode the molecule may be diagnostic.
 - Treatment of bleeding consists of infusion of cryoprecipitate, or pasteurized plasma concentrates. A new recombinant product is available in Europe and Canada.
- | Alpha-2-antiplasmin deficiency or PAI-1 deficiency leads to accelerated digestion of fibrinogen and fibrin clots and (in some patients) increased bleeding.³⁰ Antifibrinolytics or infusion of FFP may be clinically useful.
- | Congenital and acquired abnormalities of the vasculature and integument can cause

increased fragility of blood vessels and bruising or bleeding, despite normal coagulation, fibrinolysis, and platelet function.³¹ Such conditions include hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease), heritable defects of collagen (Ehlers–Danlos syndrome, osteogenesis imperfecta), acquired collagen-associated conditions (scurvy, prolonged glucocorticoid administration, the normal aging skin) and other anomalies (Marfan syndrome, amyloidosis, vasculitis). There is no effective treatment for bruising associated with the congenital disorders; preventative measures to reduce the risk of trauma should be followed. Repletion of vitamin C (scurvy) and reduction of corticosteroids (glucocorticoid excess) ameliorate bruising associated with those acquired processes.

References

1. Carcao MD. The diagnosis and management of congenital hemophilia. *Semin Thromb Hemost.* 2012;38(7):727–734.
2. Thomas S, Katbab H, abu Fanas SH. Do preoperative cutaneous bleeding time tests predict the outcome of intraoral surgical bleeding? *Int Dent J.* 2010;60(4):305–310.
3. Bonar R, Favaloro E, Zebeljan D, et al. Evaluating laboratory approaches to the identification of lupus anticoagulants: a diagnostic challenge from the RCPA Haematology QAP. *Pathology.* 2012 Apr;44(3):240–247.
4. Duncan E, Collecutt M, Street A. Nijmegen-Bethesda assay to measure factor VIII inhibitors. *Methods Mol Biol.* 2013;992:321–333.
5. Chitlur M, Sorensen B, Rivard GE, et al. Standardization of thromboelastography: a report from the TEG-ROTEM working group. *Haemophilia.* 2011;17(3):532–537.
- 6a. Nogami K. The utility of thromboelastography in inherited and acquired bleeding disorders. *Br J Haematol.* 2016;174(4):503–514.
- 6b. Gosselin R, Grant RB, Adcock DM, et al. Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. *Int J Laborat Hematol.* 2016;38(5):505–513.
7. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia.* 2013;19(1):e1–e47.
8. Svensson PJ, Bergqvist PB, Juul KV, et al. Desmopressin in treatment of haematological disorders and in prevention of surgical bleeding. *Blood Rev.* 2014;28(3):95–102.
9. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med.* 2007;357(6):535–544.
10. Berntorp E. Importance of rapid bleeding control in haemophilia complicated by inhibitors. *Haemophilia.* 2011;17(1):11–16.

11. Hay CR, DiMichele DM. The principal results of the International Immune Tolerance Study: a randomized dose comparison. *Blood*. 2012;119(6):1335–1344.
12. Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. *Lancet* 2016;388(10040):187–197.
13. George LA, Fogarty PF. Gene therapy for hemophilia: past, present and future. *Semin Hematol*. 2016;53(1):46–54.
14. Chowdary P, Lethagen S, Friedrich U, et al. Safety and pharmacokinetics of anti-TFPI antibody (concizumab) in healthy volunteers and patients with hemophilia: a randomized first human dose trial. *J Thromb Haemost*. 2015;13(5):743–754.
15. Shima M, Hanabusa H, Taki M, et al. Factor VIII-mimetic function of humanized bispecific antibody in hemophilia A. *N Eng J Med*. 2016;374(21):2044–2053.
16. Nathwani AC, Reiss UM, Tuddenham EG, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Eng J Med*. 2014;371(21):1994–2004.
17. Collins PW, Hirsch S, Baglin TP, et al. Acquired hemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. *Blood*. 2007;109(5):1870–1877.
18. Tarantino MD, Cuker A, Hardesty B, et al. Recombinant porcine sequence factor VIII (rpFVIII) for acquired haemophilia A: practical clinical experience of its use in seven patients. *Haemophilia*. 2017;23(1):25–32.
19. Kessler CM, Knobl P. Acquired haemophilia: an overview for clinical practice. *Eur J Haematol*. 2015;95(suppl 81):36–44.
20. Duga S, Salomon O. Congenital factor XI deficiency: an update. *Semin Thromb Hemost*. 2013;39(6):621–631.
21. Gueguen P, Galinat H, Blouch MT, et al. Biological determinants of bleeding in patients with heterozygous factor XI deficiency. *Br J Haematol*. 2012;156(2):245–251.
22. Shetty S, Bhave M, Ghosh K. Acquired hemophilia a: diagnosis, aetiology, clinical spectrum and treatment options. *Autoimmun Rev*. 2011;10(6):311–316.
23. Siegal DM, Garcia DA, Crowther MA. How I treat target-specific oral anticoagulant-associated bleeding. *Blood*. 2014;123(8):1152–1158.
24. Crowther MA, Douketis JD, Schnurr T, et al. Oral vitamin K lowers the international normalized ratio more rapidly than subcutaneous vitamin K in the treatment of warfarin-associated coagulopathy. A randomized, controlled trial. *Ann Intern Med*. 2002;137(4):251–254.
25. Lippi G, Targher G, Favaloro EJ, et al. Venous thromboembolism in chronic liver disease. *Semin Thromb Hemost*. 2011;37(1):66–76.
26. Chai-Adisaksopha C, Hillis C, Siegal DM, et al. Prothrombin complex concentrates versus fresh frozen plasma for warfarin reversal. A systematic review and meta-analysis. *Thromb Haemost*. 2016;116(5):879–890.
27. Bornikova L, Peyvandi F, Allen G, et al. Fibrinogen replacement therapy for

- congenital fibrinogen deficiency. *J Thromb Haemost*. 2011;9(9):1687–1704.
28. Hervent AS, Schepens M, Bourgeois M, et al. Acquired thrombin and FV inhibitors upon aortic bioprosthetic valve replacement. *Haemophilia*. 2014;20(4):e354–e356.
29. Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. *Blood*. 2015;125(13):2052–2061.
30. Iwaki T, Tanaka A, Miyawaki Y, et al. Life-threatening hemorrhage and prolonged wound healing are remarkable phenotypes manifested by complete plasminogen activator inhibitor-1 deficiency in humans. *J Thromb Haemost*. 2011;9(6):1200–1206.
31. De Paepe A, Malfait F. Bleeding and bruising in patients with Ehlers-Danlos syndrome and other collagen vascular disorders. *Br J Haematol*. 2004;127(5):491–500.

Disorders of Hemostasis II

Angela C. Weyand and Patrick F. Fogarty

In addition to thrombocytopenia ([Chapter 19](#)) and primary deficiencies in the activity of coagulation proteins ([Chapter 20](#)), disseminated intravascular coagulation (DIC), von Willebrand disease (VWD), and qualitative abnormalities of platelets can also result in bleeding.

DISSEMINATED INTRAVASCULAR COAGULATION

Although it frequently manifests as bleeding, DIC begins as a result of an uncontrolled local or systemic activation of coagulation due to an underlying disorder. DIC may be acute or chronic, limited or diffuse, and accompanied by hemorrhage or (less commonly) thrombosis.

Pathophysiology

The inciting events are numerous, but generally involve either overwhelming release of tissue factor (see [Chapter 20](#)) by cellular, vascular, or hypoxic injury, or the presence of endogenously or exogenously derived procoagulant molecules (e.g., bacterial lipopolysaccharide proteins produced by neoplastic cells).¹ As coagulation is inappropriately and systemically activated, clotting factors and platelets are consumed, leading to bleeding. If activation of coagulation is chronic and low grade, however, clotting factors and platelets may be replenished and hypercoagulability may occur, manifesting as thrombosis (e.g., Trousseau syndrome). End-organ damage may occur from bleeding, thrombosis, or decreased perfusion.

Presentation

The appearance of DIC always indicates a serious underlying condition. A typical

presentation of DIC involves a patient who has been hospitalized due to another disorder (Table 21.1) when unexplained bleeding and/ or abnormalities in routine coagulation are observed.

- The hemorrhage of DIC is typically diffuse and may involve bleeding at sites of surgical incisions or vascular access catheters, as well as urinary, gastrointestinal, pulmonary, central nervous system, or cutaneous hemorrhage. Acral cyanosis and petechial and ecchymotic lesions may also occur. Widespread DIC-associated truncal and extremity bruising (purpura fulminans), characterized by skin infarction and necrosis, usually is limited to children and may be associated with meningococcal disease or a viral infection.²
- Severe systemic DIC may lead to widespread tissue hypoxia and multiorgan dysfunction; hepatic, neurologic, cardiac, renal impairment may occur. The development of multiorgan dysfunction is associated with a high mortality rate.

Table 21.1 Conditions Associated With DIC

Condition	Example
Severe toxic or immunological reactions	Snake bites, recreational drugs, severe transfusion reactions, transplant rejection
Tissue damage	Trauma, burns, head injury
Sepsis	Gram-negative or -positive bacterial infection; rickettsial, protozoal or viral infection
Shock	Cardiogenic, septic
Pregnancy related	Toxemia, placental abnormalities (abruption or previa), amniotic fluid embolism, retained uterine placental or fetal tissue (HELLP syndrome), fatty liver of pregnancy
Vascular stasis	Cavernous hemangiomas (Kasabach–Merritt syndrome), abdominal aortic aneurysm
Fat embolism	Fracture of long bones, sickle cell crisis
Organ destruction	Severe pancreatitis
Malignancy	Acute promyelocytic leukemia, adenocarcinoma (Trousseau syndrome), brain tumors
Neonatal	NEC, hereditary protein C and S deficiencies, birth asphyxia, metabolic conditions (galactosemia)

DIC, disseminated intravascular coagulation; HELLP, hemolysis, elevated liver enzymes, low platelet count; NEC, necrotizing enterocolitis.

Table 21.2 ISTH Diagnostic Scoring System for Overt DIC

Laboratory Parameter	0 Point	1 Point	2 Points	3 Points
Platelets (K/ µL)	>100	<100	<50	
Elevated fibrin-related marker	No increase		Moderate	Strong increase

			increase
Prolonged PT	<3 s	>3 but <6 s	>6 s
Fibrinogen level	>1 g/ dL	<1 g/ dL	

If total score ≥ 5 ; compatible with overt DIC, repeat score daily. If total score < 5 ; suggestive (not affirmative) for non-overt DIC; repeat in 1 to 2 days.

DIC, disseminated intravascular coagulation; ISTH, International Society on Thrombosis and Haemostasis; PT, prothrombin time.

Data from Taylor FB, Jr, Toh CH, Hoots WK, et al. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb Haemost*. 2001;86(5):1327–1330.

Diagnosis

No single laboratory test is sufficient to diagnosis or rule out DIC; a scoring system is available ([Table 21.2](#)). DIC is a dynamic condition, especially in the acutely ill patient; considerable variation in laboratory markers from timepoint to timepoint is possible, and analysis of trends rather than isolated values is crucial. Laboratory parameters may show:

- | Increased (prolonged) activated partial thromboplastin time (aPTT), prothrombin time (PT), or thrombin time (TT)— due to consumption of clotting factors and/ or fibrinogen (most patients).
- | Decreased fibrinogen (compared to baseline)— due to consumption of fibrinogen.
- | Increased products of fibrinogen and fibrin degradation (FDPs; D-dimer assay)— due to plasmin-mediated cleavage of fibrinogen and fibrin. The D-dimer assay measures fibrin products that have been cross-linked by activated factor XIII.
- | Decreased platelet count (compared to baseline)— due to clearance resulting from activation and aggregation at the sites of local prothrombotic reactions (most patients). DIC rarely produces a platelet count less than 20,000/ μ L. Patients with thrombosis and chronic DIC due to malignancy may have normal or even elevated platelet counts.
- | Fragmented red cells (schistocytes) on peripheral blood smear— due to microvascular hemolysis (25% to 50% of patients with DIC).
- | Decreased factor V and factor VIII may help us to differentiate between DIC and coagulopathy of liver disease where factor VIII is elevated.

Treatment

The clinical and laboratory manifestations of DIC are expected to resolve with correction of the inciting disorder. This might entail effective administration of antimicrobials to a patient with sepsis, treatment of malignancy, surgery to repair an aneurysmal dilatation, removal of conceptus and placenta, or another intervention as dictated by the clinical scenario. If the DIC is severe enough to have eventuated in multiorgan dysfunction, management in an intensive care unit is required.

- | Blood products should not be administered to patients with acute DIC unless clinically significant bleeding is present or if the risk of bleeding is felt to be high (e.g., thrombocytopenia in patient who has sustained major trauma); there is, however, no reason to withhold blood products for fear of “fueling the fire.”
- | If bleeding is present, platelet transfusions may be administered to stop clinical bleeding; a target platelet count of 20 to 30,000/ uL (most cases) or >50,000/ uL (intracranial or life-threatening hemorrhage) is reasonable. Higher target ranges may be desired for patients who are to undergo invasive procedures such as major surgery, but the consumptive process may make achieving the goal difficult.
- | Cryoprecipitate may be administered for bleeding in the setting of fibrinogen levels that are consistently less than 80 to 100 mg/ dL. Fresh frozen plasma (FFP) should be given only to patients with significant bleeding and a prolonged PT and aPTT.
- | Due to its potential to exacerbate hemorrhage, heparin should be considered in acute DIC only in cases of bleeding when DIC is ongoing despite appropriate treatment (i.e., blood product infusion). Due to its short half-life and reversibility, heparin may be preferred over low-molecular-weight heparin (LMWH). It should not be given unless the platelet count can be supported to 50,000/ uL or higher and there is no central nervous system or diffuse gastrointestinal bleeding. If heparin is to be used, a low-dose infusion (6 to 10 unit/ kg/ hr) with no bolus dose is recommended. An improving platelet count and fibrinogen concentration signifies that the treatment is effective. Heparin is contraindicated in patients with placental abruption or other obstetrical conditions that will require surgical management because the anticoagulation is likely to complicate the curative treatment.
- | Fibrinolysis inhibitors may have a role in patients with profuse bleeding who have failed to respond to other management, in whom FDPs are felt to be inhibiting platelets. However, blockade of the fibrinolytic system may increase the risk of thrombosis.
- | Recombinant factor VIIa: May be considered for persistent bleeding despite blood product transfusions.⁴ Typical dose of 20 µg/ kg/ dose. Increased risk of stroke and thrombosis. Acidosis, thrombocytopenia, and hypothermia should be corrected to ensure optimal effect.
- | Due to questionable efficacy and worsened bleeding in some subjects, activated protein C concentrate (APC, drotregoggin alfa) is no longer recommended for patients with severe sepsis and DIC⁵ although it may be considered for patients with purpura fulminans due to homozygous or acquired protein C deficiency.⁶
- | Use of antithrombin concentrates is controversial.⁷
- | Laboratory parameters (PT, PTT, fibrinogen, and platelet count) should be monitored at least every 6 hours in the acutely ill patient with DIC, and clinical bleeding should be followed to assess efficacy of therapeutic measures.

HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) affects

women in the peripartum period that produces clinically significant hemolytic anemia, hepatocellular injury, and low platelets. Diagnostic criteria include microangiopathic hemolytic anemia with characteristic schistocytes on blood smear, platelet count \leq 100,000 cells/ μ L, total bilirubin \geq 1.2 mg/ dL, and serum aspartate transaminase (AST) $>$ two times the upper limit of normal (Tennessee classification).⁸ Hepatic dysfunction (leading to elevated transaminases) may in some cases distinguish the diagnosis from thrombotic thrombocytopenic purpura, which may also complicate pregnancy (see Chapter 19). Introduction of placental proteins into the maternal circulation has been thought to be etiologic; potential biomarkers have been identified.⁹ Gross hemoglobinuria with renal dysfunction and hypotension are common; the mortality rate is high. Management must include evacuation of the uterus, either by delivery of a term or near-term infant, or by dilatation and curettage to remove retained placental or fetal tissue.

Acute promyelocytic leukemia (APL) is frequently associated with DIC, potentially due to procoagulant molecules (tissue factor and others) contained within circulating promyelocytes. Bleeding commonly occurs in the lungs and brain and is frequently fatal. In addition to the appropriate use of blood products (e.g., FFP, cryoprecipitate, platelets) on detection of APL-associated DIC, emergent initiation of treatment with all-trans retinoic acid (i.e., ATRA) is recommended (see Chapter 11).¹⁰

Trousseau syndrome is a form of chronic DIC in which recurrent episodes of venous thromboembolism (VTE) complicate an underlying malignancy, especially adenocarcinomas. Experience in the management of the disorder has suggested that anticoagulation with warfarin is not effective in preventing further VTE; instead, subcutaneous low molecular weight heparin in therapeutic doses is usually necessary to prevent recurrence of thromboembolism (see Chapter 23).¹¹

VON WILLEBRAND DISEASE

Epidemiology

VWD is the most common inherited bleeding disorder; an estimated 1% of the population is believed to have activity of von Willebrand factor (VWF) that is below the laboratory reference, although symptoms are only present in 1 in 1,000 persons.¹²

Pathophysiology and Classification

VWF is an extremely large multimeric glycoprotein (GP) that is synthesized in endothelial cells and megakaryocytes. Binding of VWF to its receptor, platelet glycoprotein Ib (GPIb), tethers platelets to one another and to the subendothelial collagen matrix, localizing them to the site of injury. This interaction is especially

important to assure primary hemostasis in vessels such as arterioles, where a “high shear” state is present ([Fig. 21.1](#)). VWF also binds factor VIII (FVIII) in the circulation, protecting it from clearance.

- Type 1 (quantitative defect in VWF) includes approximately 75% to 80% of patients, the majority of which do not have an identified causal mutation in the VWF gene, which is located on chromosome 12. Patients may have mild or moderate bleeding. Autosomal dominant inheritance is typical.
- Type 1C (increased clearance of VWF) accounts for about 15% of type 1. These patients demonstrate an initial response to desmopressin acetate (DDAVP) challenge with marked decrease seen 4 hours post-DDAVP.¹³
- Type 2 (qualitative defect in VWF) includes four subtypes; patients usually have moderate bleeding symptoms and present before adulthood. Type 2A (10% to 15% of VWD) involves mutations in VWF that cause either a defect in intracellular transport (2A, type 1) or render the molecule more susceptible to proteolysis (2A, type 2). Laboratory testing ([Table 21.2](#)) typically shows a marked decrease in VWF activity relative to antigen (a ratio of ≤ 0.6 is typical). Type 2B (5% of VWD) mutations result in an abnormal structure in the binding site for platelet GPIb (A1 domain of VWF), and are responsible for a “gain-of-function” defect that allows spontaneous binding of the abnormal VWF to platelets in the circulation. Patients typically have thrombocytopenia due to removal of VWF-bound platelet aggregates. The ristocetin-induced platelet aggregation (RIPA; [Table 21.3](#)) shows an increase in platelet aggregation to low concentrations of ristocetin.¹ Type 2N (uncommon) features mutations in VWF that decrease its ability to bind and protect FVIII from clearance, resulting in decreased FVIII levels in the plasma and a phenotype similar to hemophilia A. Soft tissue and joint bleeding are common. The presence of affected females in the family is an important clue to consider this diagnosis. Laboratory studies show decreased FVIII (2% to 10%), and normal VWF function and antigen. Type 2M (very uncommon) results from mutations affecting the A1 domain in a different area from those mutations in type 2B. These result in decreased binding of platelets to VWF.

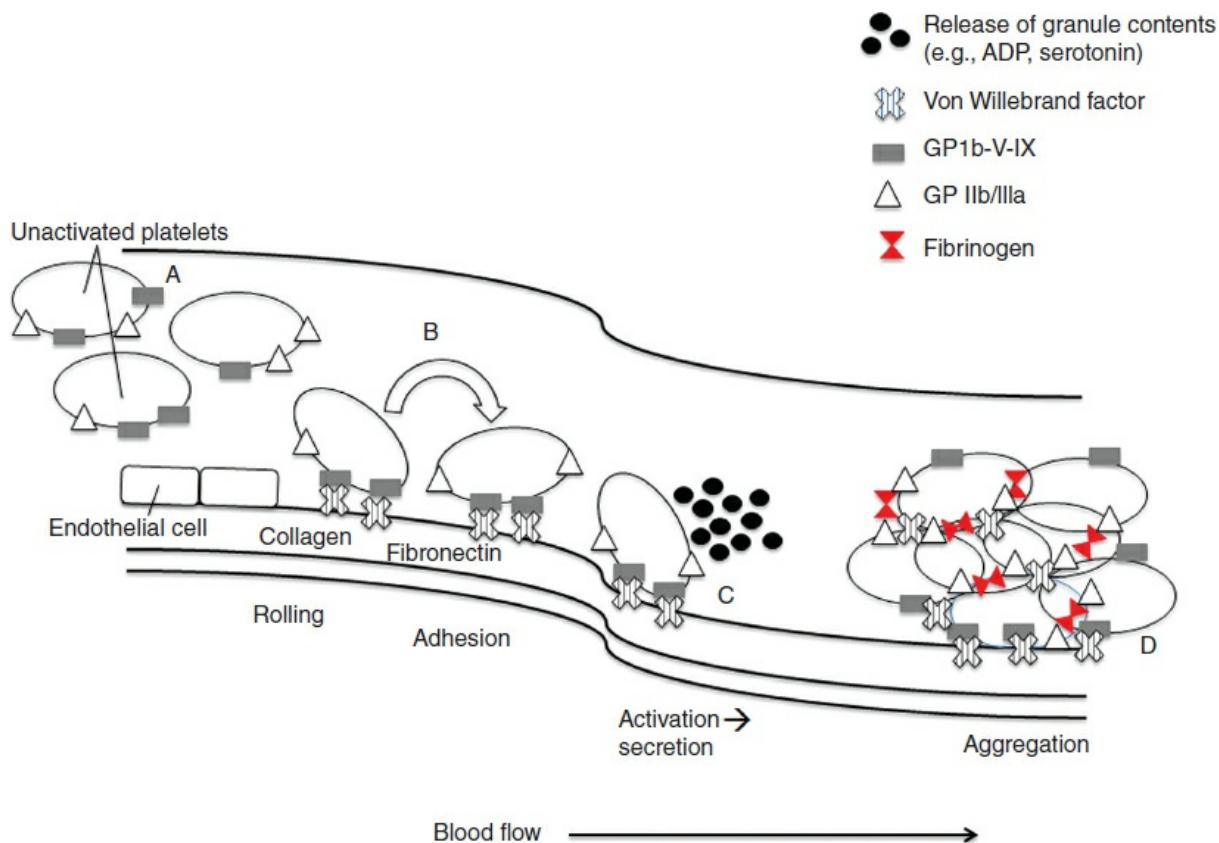


FIGURE 21.1 Primary hemostasis. **(A)** Normal conditions. Under physiologic conditions, platelets do not interact with the endothelium. **(B)** Adhesion. On disruption of the blood vessel wall, subendothelial collagen and fibronectin are exposed, leading to platelet adhesion. In the arterial/ arteriolar circulation, subendothelial VWF assists in the adherence of platelets to the site of injury via binding to the platelet GP Ib receptor. **(C)** Activation/ secretion. Tissue factor interacts with factor VIIa, present locally, to catalyze the formation of thrombin. Thrombin, collagen, and other molecules bind to receptors on the platelet membrane, leading to platelet activation. Activated platelets will release the contents of stored granules into plasma. These secretions (ADP, serotonin, platelet-activating factor, VWF, platelet factor 4, thromboxane A2) activate additional platelets. **(D)** Aggregation. Fibrinogen cross-links platelets via their GPIIb/ IIIa receptors, promoting the formation of an occlusive plug that prevents additional blood loss through the break in the vessel wall. VWF also bridges between platelets, via their GPIb and GPIIb/ IIIa receptors. ADP, adenosine diphosphate; GP, glycoprotein, VWF, von Willebrand factor.

Type 3 VWD (rare, 1 to 3 per million persons) is caused by a variety of mutations of

the VWF molecule, including larger deletions; patients may be homozygous for a given mutation or double heterozygotes. Severe bleeding manifests in childhood. FVIII is usually about 5%, and VWF levels usually are too low to detect.

Table 21.3 Laboratory Evaluation of VWD

Laboratory Parameter	Type					
	1	2A	2B	2M	2N	3
VWF: Ag	↓	↓	↓	↓	↓ or normal	↓↓↓
VWF: RCo	↓	↓↓	↓↓	↓	↓ or normal	↓↓↓
FVIII: C	↓ or normal	↓ or normal	↓ or normal	↓ or normal	↓↓	↓↓↓
VWF RCo: Ag ratio	Normal	<0.6	<0.6	<0.6	Normal	N/A
Multimers	Normal	High and intermediate molecular weight missing	High molecular weight missing	Normal	Normal	Absent
RIPA	Normal	↓	↑	↓	Normal	Absent

↓, Minimal decrease; ↓↓, moderate decrease; ↓↓↓, severe decrease; ↑, increase.

N/A, not available; VWF:Ag, von Willebrand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor activity; FVIII: C, factor VIII activity; RIPA, ristocetin-induced platelet aggregation.

Presentation

Bleeding symptoms usually involve mucous membranes and are similar to those seen with platelet disorders. Epistaxis, oral bleeding, menorrhagia, and gastrointestinal bleeding are common. Individuals with marked abnormalities of VWF usually present earlier in life with bleeding at the time of mucous membrane-related procedures (tooth extractions, tonsillectomy), or at menarche.

Diagnosis

The diagnosis of VWD is based on a typical history of bleeding (i.e., mucous membrane related) and confirmatory laboratory testing.¹⁴ The diagnosis can be difficult, given the large number of individuals whose VWF levels fall below the laboratory reference range, many of whom do not experience abnormal bleeding.

- The personal and family history of bleeding should be carefully documented.
- A complete blood count and routine coagulation studies should be performed, to exclude other diagnoses and assess for anemia.
- Initial testing for VWD (Table 21.3). A VWF antigen level (by enzyme-linked immunosorbent assay [ELISA]) and a VWF activity (by ristocetin cofactor assay) should be performed. The latter involves addition of ristocetin at 1.2 mg/ mL to a

mixture of patient plasma (the VWF source) and washed normal platelets. Ristocetin binds VWF, allowing it to bind GPIb on the platelet membrane, causing platelet aggregation. The factor VIII activity may be abnormal.

- | Secondary testing. A VWF multimer study detecting the distribution of multimers is performed once a diagnosis of VWD has been made, to assess for type 2 VWD ([Fig. 21.2](#)).
- | VWF levels < 30% are regarded by most clinicians as diagnostic for VWD. If borderline results are obtained, testing may need to be repeated up to three times to exclude the diagnosis. Menstruating females generally have the lowest VWF levels in the first 4 days of menstruation. Estrogen (oral contraceptive pills [OCPs], pregnancy), stress or acute illness increase VWF levels, and testing may need to be repeated. Hypothyroidism decreases VWF levels. Testing of family members also may aid in the diagnosis of patients with borderline results.
- | “Low VWF.” This classification was developed to encompass VWF levels in the 30% to 50% range, which are too high to be considered a definitive criterion for diagnosis, but may indicate a tendency to bleeding in selected patients. Hemostatic agents (see section Treatment in the following) may be used if bleeding (or a high risk of bleeding) is present.

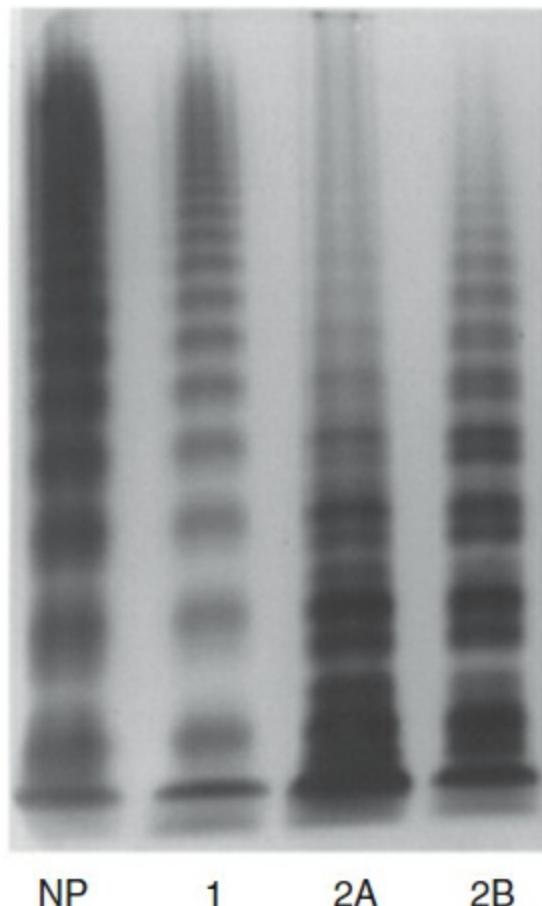


FIGURE 21.2 VWF multimer analysis. A VWF multimer assay

performed using normal NP demonstrates a normal distribution of VWF multimers. High-molecular-weight multimers are present at the top of the gel. A sample from type 1 VWD plasma (1) demonstrates a normal distribution, but the intensity of each band is reduced due to the quantitative deficiency. Type 2A VWD (2A) characteristically demonstrates an absence of large and intermediate multimers, as does type 2B VWD (2B). Platelet-type VWD shows a pattern that is similar to 2B VWD (i.e., an absence of high-molecular-weight multimers). Type 3 VWD, which is a severe quantitative deficiency of VWF, is characterized by few or no multimers (not shown).

NP, pooled plasma; VWD, von Willebrand disease; VWF, von Willebrand factor.

Adapted from Vogelsang G. Von Willebrand multimer patterns. ASH Image Bank (www.ashimagebank.org) 2012; image number 00010063, after Dent et al., ASH-SAP 2010, pp 263–289. © the American Society of Hematology. Used with Permission.

Treatment

The type of VWD, past response to bleeding challenges, current medications, and general medical condition should be considered (Table 21.4).¹⁴

- DDAVP indirectly causes release of VWF and factor VIII from storage sites (primarily the endothelium). After intravenous administration of 0.3 mcg/ kg IV, levels of both factors are increased two-to sevenfold for about 6 to 12 hours. Repeat testing at a later date should be considered in nonresponders due to improved response with age. More than two doses, given 12 to 24 hours apart, generally should be avoided in a 24- to 48-hour period, as tachyphylaxis and serious hyponatremia (due to fluid retention) can occur after repeated doses. Nonsteroidal anti-inflammatory agents (NSAIDs) may aggravate this latter effect. DDAVP is effective in almost all patients with mild or moderate type 1 disease¹⁵ and some patients with type 2 disease.
- VWF replacement products are used when bleeding is not controlled with DDAVP, or as prophylaxis prior to a major invasive procedure, or for clinically significant bleeding in patients who are less likely to be responsive to DDAVP (type 3 and some type 1 and 2 patients). Cryoprecipitate generally is not recommended because of its lack of viral inactivation.
- Antifibrinolytic agents (contraindicated in patients with hematuria or at increased risk of thrombosis) such as epsilon aminocaproic acid (Amicar) and topical agents (including topical thrombin, gel foam, and fibrin sealant) are used adjunctively, especially in cases of mucosal bleeding (e.g., dental).
- Avoidance of aspirin and other antiplatelet medications.

Table 21.4 Treatment of VWD-Related Bleeding

Type	Treatment ^a	Comment
1	DDAVP; VWF replacement product	DDAVP effective in most patients
1C	DDAVP; VWF replacement product	Response to DDAVP often short-lived
2A	DDAVP; VWF replacement product	Response to DDAVP may not be as marked as in type 1
2B	VWF replacement product	DDAVP may worsen thrombocytopenia; perform therapeutic trial with measurement of post-DDAVP platelet count
2N	VWF replacement product	Low baseline FVIII levels ^b typically require administration of FVIII replacement product in addition to VWF replacement product
2M	DDAVP; VWF replacement product	Response may not be as marked to DDAVP as in type 1
3	VWF replacement product ^c	Very low baseline FVIII levels ^b require administration of FVIII replacement product in addition to VWF replacement product

Desmopressin acetate (DDAVP) dose is 0.3 mcg/ kg IV in 50 mL saline more than 20 minutes, or nasal spray 150 mcg in each nostril (total, 300 mcg) for weight >50 kg or 150 mcg in one nostril for <50 kg, every 12 to 24 hours, maximum of 2 doses in a 48-hour period; restrict fluid and monitor for hyponatremia. A therapeutic trial is required to assess responsiveness before use in treatment of or prophylaxis of bleeding. VWF replacement products include Humate-P, Alphanate, and Wilate and are indicated for surgical prophylaxis, major bleeding or severe VWD; dose is 60 to 80 RCoF IU/ kg initially followed by 40 to 60 RCoF units/ kg q 12 hour. Give for 3 to 10 days for major bleeding or following surgery; longer treatment periods (e.g., up to 4 weeks) may be required for cases of intracranial hemorrhage. Recombinant VWF product (Vonvendi) dosing is 40 to 50 IU/ kg (minor bleeding) or 50 to 80 IU/ kg (major bleeding) initially; repeat every 8 to 24 hours as clinically indicated. Administration of a FVIII replacement product is advised when FVIII levels are below 40 IU/ dL or are unknown.

^aConsider administration of antifibrinolytic agents (such as epsilon aminocaproic acid, 50 mg/ kg 4× daily for 3–5 days; maximum 20 to 25 g/ day) in conjunction with other therapies in cases of refractory bleeding or bleeding at mucosal surfaces (e.g., dental bleeding or procedures, epistaxis).

^bDue to shortened FVIII half-life from deficient binding by abnormal or absent VWF.

^cPlatelet transfusions may be considered in addition to VWF replacement product if inadequate response is observed.

DDAVP, desmopressin acetate; VWF, von Willebrand factor.

Pregnancy and von Willebrand Disease

All pregnant women with VWD should be managed in consultation with a hematologist and should deliver at a specialized center for bleeding disorders. VWF levels increase two-to threefold during the last two trimesters of pregnancy; type 1 VWD patients whose VWF levels have reached the normal range during the third trimester may not require treatment during delivery. In more severely affected patients, VWF concentrates can be administered prophylactically, beginning usually after the onset of labor. Peripartum DDAVP use warrants caution due to the risk for hyponatremia and seizures.

The risk of postpartum hemorrhage may persist for up to a month after delivery.¹⁶

QUALITATIVE PLATELET DISORDERS

Introduction

Most disorders of platelet function are acquired. Heritable qualitative platelet disorders are individually rare (occurring in 0.01 to 1 per 100,000 population), but in aggregate may be commoner than once thought.¹⁷ Because of redundancy of biochemical and receptor pathways that mediate the function of platelets, certain defects may be detectable only on laboratory testing, whereas other qualitative defects characteristically produce clinically significant bleeding.

Review of Hemostasis and the Role of Platelet Biochemistry

Primary hemostasis describes the formation of a platelet plug at the site of vascular injury (Fig. 21.1). In a variety of reactions that are not entirely sequence-specific, individual circulating platelets must adhere to the denuded endothelial surface, undergo activation through receptor–ligand interactions, release the contents of their granules (i.e., platelet secretion), and aggregate to form a physical barrier to continued blood loss.¹⁸

- | Adhesion. Subendothelial molecules such as VWF, collagen, and fibronectin mediate adhesion of platelets to the exposed subendothelial matrix at sites of vessel wall compromise. In “high-shear” states such as arterioles, VWF is especially important, because it tethers the platelet to the endothelial surface via interaction with its receptor, platelet GPIb.
- | Activation. Subendothelial collagen activates platelets; thrombin, which has been generated locally in reactions following the interaction of factor VIIa and tissue factor (provided by the membranes of cells) also activates platelets by binding to receptors on the platelet surface and initiating a series of signal transduction events.
- | Secretion. Agonists such as collagen, thrombin, adenosine diphosphate (ADP), and epinephrine bind to their receptors on the platelet membrane and induce a series of biochemical events that cause platelets to release the contents of their granules (Table 21.5), which act to promote further activation and aggregation.
- | Aggregation. Binding of agonists also promotes a conformational change in the platelet GP IIb/ IIIa receptor, exposing its binding sites for fibrinogen and VWF; these molecules can then bridge between individual platelets at the site of vascular injury, promoting the formation and stability of the platelet plug.
- | Participation in coagulation reactions. The platelet membrane is rich in phospholipid, which is a required component for reactions involving clotting factor complexes.

Platelet Function Testing

- Platelet aggregation studies (platelet-rich plasma [PRP] system). According to the classical method, platelets in a suspension of PRP impede transmission of light. When any of a variety of agonists (collagen, thrombin, ADP, epinephrine) is added, aggregation occurs, consolidating the platelets and allowing the passage of light through the plasma (Fig. 21.3).
- The increase in light transmission as aggregation occurs is plotted as a function of time. Ideally, the waveform shows two physiologic processes: a primary wave represents initial aggregation as platelet receptors are activated and become available to bind proaggregatory molecules such as fibrinogen.
- A secondary wave indicates further aggregation that is stimulated by the release of platelet granule contents.
- Routinely, secretion of platelet granule contents (Table 21.5) is assessed in tandem with platelet aggregation; per one methodology, after stimulation of platelets with an agonist, release of adenosine triphosphate (ATP) into solution is measured through a chemiluminescence procedure, and plotted as a function of time.

Table 21.5 Characteristics of Platelet Granules

Parameter	Alpha Granules	Delta (Dense) Granules
Number per platelet	30–50	3–7
Visualization	Light microscopy (Wright's stain), electron microscopy	Electron microscopy
Contents	VWF, PDGF, PF4, TSP, FV, FXI, protein S, fibronectin; fibrinogen, IgG, P-selectin	ADP, ATP, serotonin, calcium

ADP, adenosine diphosphate; ATP, adenosine triphosphate; FV, factor V; FIX, factor IX; IgG, immunoglobulin G; PDGF, platelet-derived growth factor; PF4, platelet factor 4; VWF, von Willebrand factor.

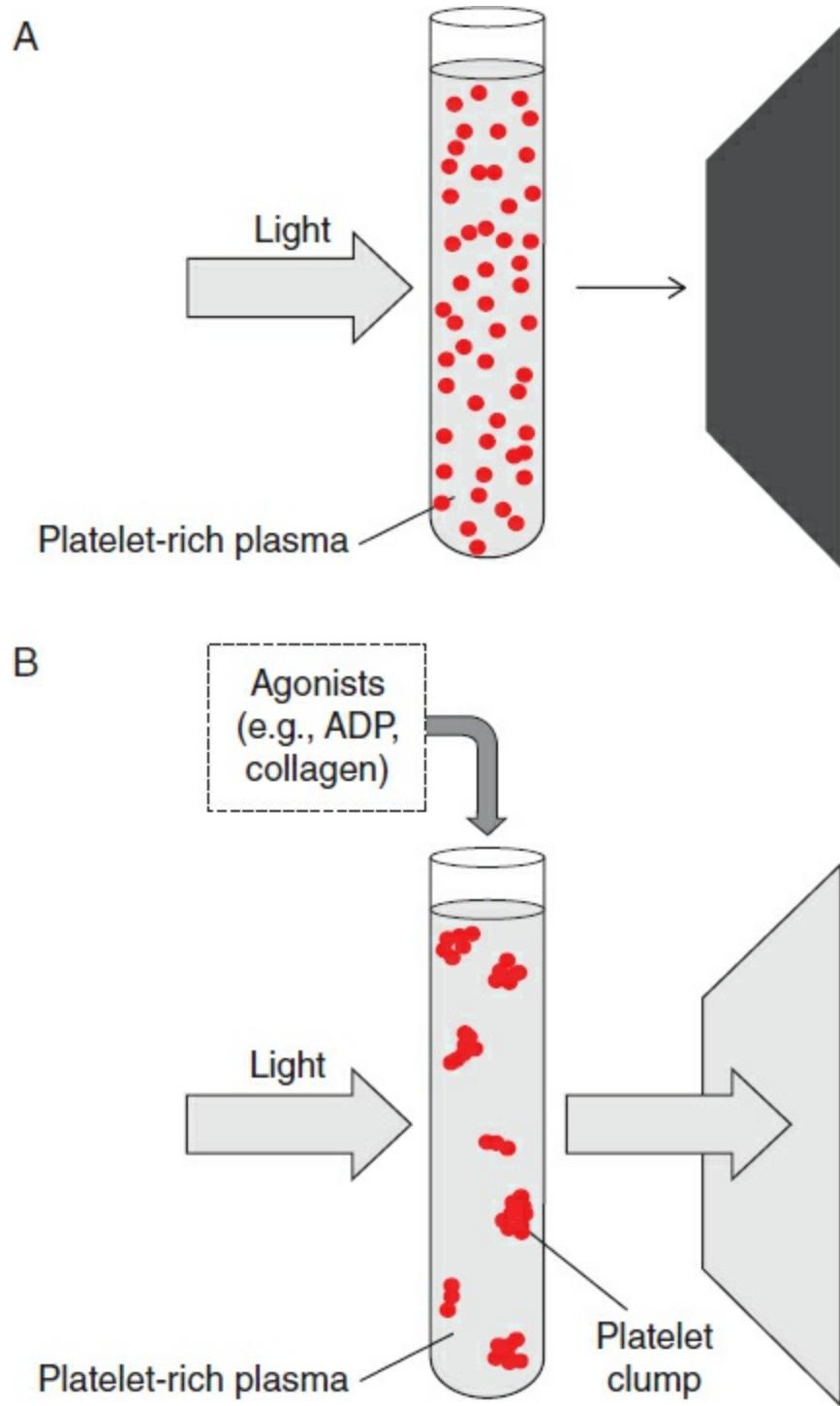


FIGURE 21.3 Platelet aggregation studies. **(A)** Gentle centrifugation of a blood sample yields PRP that is cloudy due to the presence of suspended platelets. Light transmission through the sample is limited. **(B)** Addition of an agonist (such as collagen, thrombin, ADP, arachidonic acid, or epinephrine) leads to platelet activation and aggregation. As the platelets aggregate (clump), the PRP becomes less cloudy, allowing more light to pass through (a fully aggregated sample of PRP will look like standard blood plasma). Because every

individual's plasma is slightly different, PPP is used to set the reference for baseline light transmission.

ADP, adenosine diphosphate; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

- | Platelet function analyzer (PFA-100TM, Dade Behring, Inc.). Citrated whole blood is aspirated through an aperture in a collagen-impregnated membrane, leading to platelet activation and aggregation; the time to occlusion of the aperture is measured and compared with a normal range. Though it may be helpful for assessing aspirin-associated platelet inhibition,¹⁹ the test lacks sufficient sensitivity and specificity to be used in screening for inherited platelet disorders.²⁰
- | Measurement of granule contents (rarely indicated). Centrifugation of PRP produces a platelet pellet; the platelet membranes are then disrupted, liberating intracellular/intragranular proteins into the lysate. The molecule of interest is then assessed (e.g., VWF, by ristocetin cofactor assay, for intragranular VWF).

Acquired Disorders

- | Drugs. The most common acquired qualitative platelet disorders are caused by the use of medications that directly or indirectly impair platelet function; of these, aspirin and the NSAIDs are most frequently responsible ([Table 21.6](#)). Thrombocytopenia has also been reported in patients using these agents and is thought to be immune mediated.²¹ Patients who present with bruising or platelet-type bleeding and whose platelet function testing shows abnormal aggregation or secretion should be questioned regarding current medications, especially recently initiated drugs and including over-the-counter, naturopathic, and herbal agents. Treatment of clinically significant bleeding due to drug-induced platelet dysfunction first involves discontinuation of the offending agent, and may require additional measures ([Table 21.6](#)).
- Aspirin irreversibly inhibits the platelet cyclooxygenase (COX) enzyme, which is responsible for the conversion of membrane-associated arachidonic acid to thromboxane A₂ (TxA₂); the inhibition is constant for the entire life span of the platelet (7 to 10 days). Platelet aggregation studies ([Fig. 21.4](#)) show decreased reactivity to most agonists, including low concentrations of thrombin and collagen, and normal aggregation with high concentrations of thrombin and collagen. Using the PFA-100 system, aspirin-induced platelet dysfunction is evident in an increased time to aperture occlusion with the epinephrine/ collagen reagent, while that of the ADP/collagen reagent is unaffected.¹⁹
- NSAIDs reversibly inhibit platelet COX; their inhibitory effect persists only as long as the drug is present in the circulation. Selective inhibitors of COX-2 do not bind or impair platelet COX-1.
- Platelet GP IIb/ IIIa inhibitors are used in the management of patients with acute

coronary syndromes or before or following percutaneous coronary intervention (PCI), frequently in conjunction with heparin. Eptifibatide is a small molecule that binds the GPIIb/ IIIa receptor, inhibiting the binding of its ligands, fibrinogen, VWF, and others, hindering platelet aggregation. Abciximab is a monoclonal antibody against GP IIb/ IIIa that also inhibits binding of these pro-aggregatory ligands. Abciximab-bound platelets can be cleared at an accelerated rate due to interaction between the Fc portion of the antibody and Fc receptors on reticuloendothelial macrophages in the liver and spleen, producing thrombocytopenia that in some cases (<1.0%) is severe.

- Ticlopidine, clopidogrel, and prasugrel irreversibly inhibit the binding of ADP to its receptor on the platelet membrane, impairing the ADP-dependent binding of fibrinogen to GPIIb/ IIa, decreasing platelet aggregation. Neutropenia and aplastic anemia have been reported with increased frequency in patients taking ticlopidine. Thrombotic thrombocytopenic purpura has been described with use of ticlopidine and less frequently with clopidogrel.²²
- Dipyridamole is used to prevent recurrent stroke or transient ischemic attack, usually in conjunction with aspirin. It inhibits ADP-and collagen-induced platelet aggregation via an effect on intracellular cyclic adenosine monophosphate (AMP).
- Other substances. Serotonin reuptake inhibitors (SSRIs) may impair the function of platelets by reducing the serotonin content of platelet-dense granules.²³ Omega-3 fatty acids may disrupt the phospholipid membrane of the platelet and interfere with reactions of coagulation that normally take place on the platelet surface.²⁴
- Myelodysplasia (MDS)/ myeloproliferation. The platelets that are produced in MDS and the myeloproliferative disorders (chronic myeloid leukemia, essential thrombocythemia, polycythemia vera, and idiopathic myelofibrosis) may show abnormal receptor-ligand interactions, ineffective signal transduction, or decreased secretion of platelet granule contents; in a minority of patients, these abnormalities lead to bleeding.²⁵
- Renal failure/uremia. Platelets from individuals with impaired kidney function frequently show abnormalities on aggregation testing. The degree of renal failure does not correlate with abnormal aggregation. Although plasma urea itself may not be causative, other factors, such as dysfunctional VWF and increased levels of nitric oxide and cyclic guanosine monophosphate (GMP) may lead to clinically important bleeding, especially gastrointestinal. DDAVP (standard doses), cryoprecipitate, and high-dose estrogens (Premarin, 50 mg single dose) have been suggested to be of benefit in uremia-related bleeding.²⁶ Because the presence of adequate numbers of intravascular red cells may facilitate interaction of platelets with the vessel wall, red cell transfusions or erythropoietin are recommended in patients with anemia related to renal failure who are bleeding, to keep the hematocrit above 30%.²⁶ Platelet transfusion may be beneficial temporarily if other measures fail and bleeding persists.

If a dialyzable substance in the uremic plasma is responsible for the defect in platelet function, hemodialysis also may be beneficial, albeit temporarily.²⁶

- | Cardiac bypass causes defects in both platelet number and function. As platelets pass through the extracorporeal oxygenating circuit, they contact the artificial surfaces of the system and are activated, they also are fragmented by distortional trauma. Both phenomena lead to their accelerated clearance. Additionally, complement activation, cytokine release, generation and thrombin and hypothermia may also contribute.²⁷ Following bypass, a decrease in the platelet count, abnormalities in platelet morphology on the blood smear, and impaired in vitro platelet aggregation are observed in most patients; but these effects typically persist for 24 to 48 hours following bypass. Platelet transfusion may be given for serious bleeding.
- | Acquired Glanzmann thrombasthenia is a rare phenomenon usually associated with antibodies (allo or auto) to platelet integrin $\alpha_{IIb}\beta_3$. It may occur in the setting of pregnancy, autoimmune disorders, or the use of integrin $\alpha_{IIb}\beta_3$ antagonists.

Table 21.6 Substances Associated With Platelet Dysfunction^a

Platelet-Directed Agents

Aspirin
NSAIDs (except COX-2 inhibitors)
Dipyridamole (Aggrenox)
Clopidigrel (Plavix)
Ticlopidine (Ticlid)
Prasugrel (Effient)
Abciximab (ReoPro)
Eptifibatide (Integrilin)
Tirofiban (Aggrastat)

Anaesthetics

Dibucaine
Procaine
Halothane

Antibiotics

Penicillins (penicillin G, ticarcillin, nafcillin, piperacillin, methicillin, ampicillin)
Cephalosporins (cefazolin, cefotaxime)
Nitrofurantoin

Chemotherapeutic Drugs

Carmustine (BCNU)
Daunorubicin
Mithramycin

Psychiatric Medications

Selective serotonin reuptake inhibitors (e.g., fluoxetine, paroxetine, sertraline)
Tricyclic antidepressants (e.g., imipramine, amitriptyline, nortriptyline)

Other Agents

Nitrates

Antihistamines (diphenhydramine, chlorpheniramine)

Ethanol

Omega-3 fatty acids (eicosapentaenoic acid)

“Wood ear” mushrooms

Radiographic contrast dye

Calcium channel blockers

Heparin

Alcohol

Dextran

^aMost of these agents have been reported to cause abnormalities in platelet aggregation or the bleeding time, rather than bleeding.

COX, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs.

Data from George JN, Shattil SJ. Acquired disorders of platelet function. In: Hoffman R, Benz EJ, Shattil SJ, et al. eds. Hematology: Basic Principles and Practice. 3rd ed. New York, NY: Churchill Livingstone; 2000:2174.

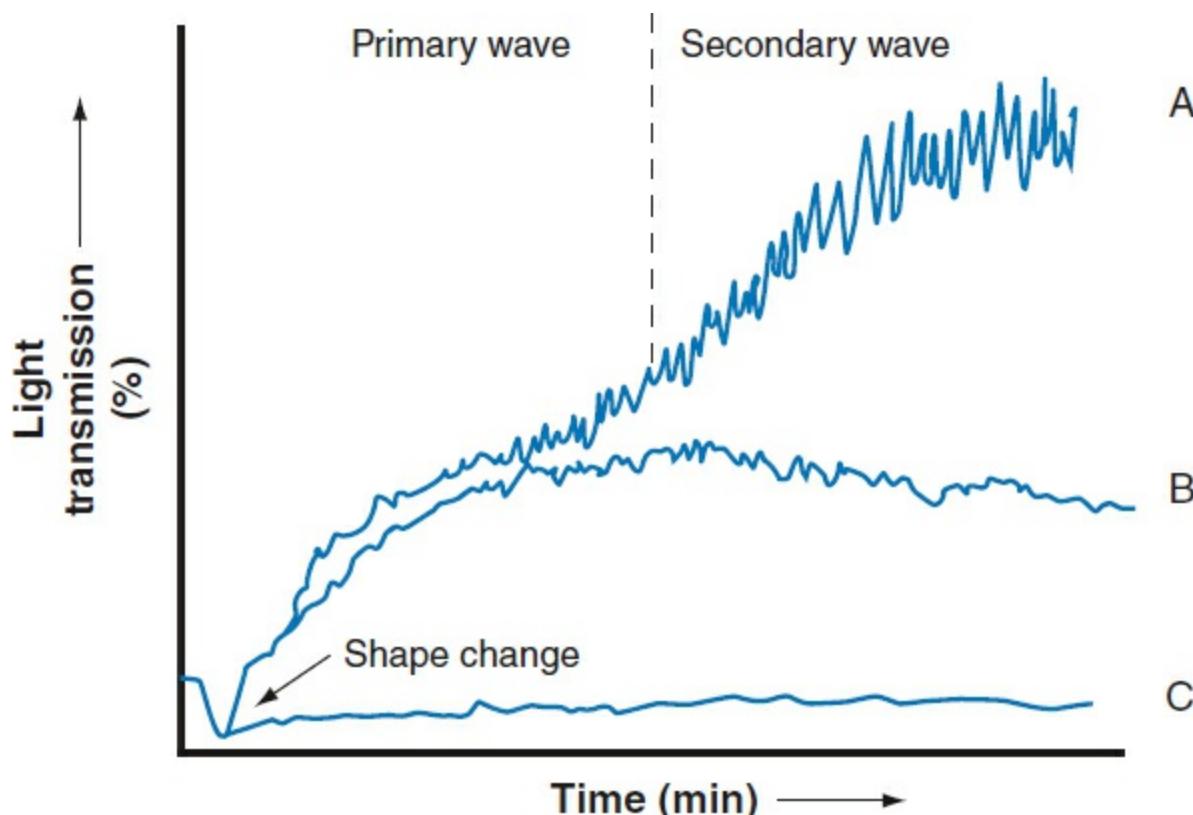


FIGURE 21.4 Platelet aggregation traces. (A) In the normal scenario, the binding of an agonist to its platelet receptor initiates a shape change that temporarily decreases light transmission; subsequently, a primary wave of platelet aggregation is recorded (as increased light transmission) as fibrinogen binds its receptor, GPIIb/ IIIa, and begins to cross-link platelets. Unlike the other agonists, collagen does not

induce a primary wave. A secondary wave occurs as signal transduction events (resulting from platelet activation) eventuate in augmented binding of GPIIb/ IIIa by fibrinogen and release of platelet granules, whose contents are able to induce further aggregation. (B) In SPD, platelet aggregation to ADP and other agonists typically shows an initial wave of aggregation, but the aggregates subsequently dissociate due to reduced or absent release of platelet granule contents. Because release of granules is largely dependent on thromboxane, the aspirin effect produces a similar platelet aggregation profile to that of SPD when ADP or epinephrine is used, but stronger agonists such as thrombin and collagen can circumvent the thromboxane pathway and produce a normal aggregation curve. (C) Due to lack of GP IIb/ IIIa expression on the platelet surface, platelets from patients with Glanzmann thrombasthenia show absent aggregation to all agonists except ristocetin.

ADP, adenosine diphosphate; GP, glycoprotein; SPD, storage pool disease.

Inherited Disorders

Inherited disorders of platelet function are rare and produce varying degrees of platelet-type bleeding, usually beginning within the first decade of life.¹⁷ Heritable disorders of platelet function may also remain clinically silent until unmasked by a significant hemostatic challenge. Prophylaxis before invasive procedures or treatment of significant hemorrhage typically involves transfusion of normal platelets. Early use of antifibrinolytic agents may decrease transfusion requirements. DDAVP may be effective in platelet disorders characterized by normal dense granules, whereas recombinant factor VIIa may be helpful as only an adjunctive measure to platelet transfusion when transfusions alone have been ineffective (Table 21.7).

Giant Platelet Disorders

- I Bernard–Soulier syndrome (BSS) comprises a triad of large platelets, moderate thrombocytopenia, and a prolonged bleeding time; individuals with this disorder have reduced or abnormal expression of platelet GPIb/ IX (the receptor for VWF) on the surface of their platelets. BSS is autosomal recessively inherited. Platelet aggregation studies are normal with all agonists except ristocetin. BSS is distinguished from VWD in that the reduced RIPA in BSS is corrected by the addition of normal platelets, whereas in VWD, it is corrected by the addition of normal plasma (which contains adequate VWF). The diagnosis can be confirmed by platelet flow cytometry.
-

Table 21.7 Management of Qualitative Platelet Disorders

Condition	Prophylaxis Before Invasive Procedures ^{a,b}	Treatment of Bleeding ^{a,b}
Acquired defects		
Drug-induced	Discontinue drug ≥7 days (ASA, clopidogrel, prasugrel), or ≥6–12 hrs (eptifibitide) or ≥24–48 hrs (abciximab) prior to procedure	Discontinue drug; platelets until drug has been cleared and/or hemostasis is achieved
MDS/ MPD	Platelets (only if significant thrombocytopenia or prior history of bleeding)	Platelets
Renal failure	Platelets; DDAVP ^c ; cryo; ESA; hemodialysis prior to procedure	Platelets, DDAVP ^c , cryo, high-dose estrogens; hemodialysis. Keep Hct > 30% ^d
Cardiac bypass related	(N/A)	Platelets, if clinically significant bleeding
Inherited defects		
Bernard–Soulier syndrome ^e	Platelets; rVIIa; DDAVP ^c	Platelets; rVIIa; DDAVP ^c
Glanzmann thrombasthenia ^e	Platelets; rVIIa ^f ; DDAVP ^c . Pregnancy: platelets at delivery and 3–14d postpartum	Platelets; rVIIa ^f ; DDAVP ^c
Storage pool disease	Platelets; DDAVP ^{c,g} ; rVIIa	Platelets; DDAVP ^{c,g} ; rVIIa
Disorders of signal transduction	Platelets; DDAVP ^c	Platelets; DDAVP ^c

^aGiven in order of preference.

^bAntifibrinolytics are to be considered adjunctive to other primary measures in almost all cases; see Table 21.3 for dosing.

^cSee Table 21.3 for dosing of DDAVP.

^dTypically through red cell transfusion, may use ESA.

^eIn addition, menses suppression in females to control menorrhagia is not uncommonly required.

^fSome clinicians use rVIIa to spare platelet transfusion and decrease the likelihood of formation of isoantibodies (see text).

^gDDAVP is less likely to be effective in dense granule deficiency than alpha granule deficiency.

Amicar, aminocaproic acid; ASA, aspirin; cryo, cryoprecipitate; DDAVP, desmopressin acetate; ESA, erythropoiesis-stimulating agent; Hct, hematocrit; MDS/ MPD, myelodysplasia/ myeloproliferative disorders; rhVIIa-recombinant human factor VIIa.

The May–Hegglin anomaly features mild to moderate thrombocytopenia, large platelets, and characteristic leukocyte azurophilic inclusions (Dohle bodies). Although the large size of the platelets implies a qualitative abnormality, patients generally do not bleed excessively and aggregation studies are normal. An autosomal dominantly inherited disorder, it is a manifestation of mutated non-muscle myosin heavy chain IIA,

which has been implicated in the related disorders Sebastian syndrome, Fechtner syndrome, and Epstein syndrome; these feature varying degrees of sensorineural hearing loss, nephritis, cataracts, and leukocyte inclusions. Eltrombopag has been used in some patients with severe thrombocytopenia with a resulting decrease in bleeding manifestations.²⁸

- | The gray platelet syndrome is a rare, inherited disorder characterized by abnormalities of platelet alpha granules, splenomegaly, elevated B12 levels, progressive thrombocytopenia, and fibrosis in the bone marrow; consanguinity is common. A lifelong history of mild to moderate mucocutaneous bleeding usually is present. Review of the blood smear typically shows agranular platelets that appear “gray” on Wright’s staining due to a lack of azurophilic granules. In contrast to dense granule deficiency, platelet aggregation to epinephrine, ADP, and arachidonic acid is often normal, while thrombin and collagen produce variable results. The diagnosis is confirmed with electron microscopy.
- | MYH9-related disease is associated with sensorineural deafness, cataracts, and glomerulonephritis. These disorders are caused by a mutation in the gene encoding the heavy chain of non-muscle myosin-9. Myosin precipitates may be seen on immunofluorescence analysis. Treatment with eltrombopag may be beneficial.²⁸
- | Filamin A disorders are associated with macrothrombocytopenia, coagulopathy, and hemorrhage. Abnormal Filamen A and a negative platelet population is seen on immunofluorescence analysis.²⁹

Other Disorders

Glanzmann thrombasthenia is a recessively inherited qualitative or quantitative abnormality in platelet integrin $\alpha_{IIb}\beta_3$ expression on the platelet surface.³⁰ Without adequate functional IIb/ IIIa to bind fibrinogen and VWF (both of which cross-link platelets), platelet aggregation is markedly impaired (Fig. 21.4). Patients may present with mucocutaneous bleeding in infancy. This may occur in combination with leukocyte dysfunction and should be suspected in infants with mucocutaneous bleeding, leukocytosis, delayed umbilical cord separation, or severe bacterial infections. In pregnant patients, the condition is associated with maternal and fetal hemorrhage, and a third of women experience primary postpartum hemorrhage (up to 20 days after delivery).³¹ Isoantibodies against the missing or defective platelet integrin may neutralize transfused platelets in some cases, suggesting a role for rVIIa as a platelet-sparing measure.³² The diagnosis can be confirmed by platelet flow cytometry. Severity of bleeding may decrease with age with the exception of menorrhagia and postpartum bleeding. Stem cell transplantation has been successfully used.³³

- | Storage pool disease (SPD) is characterized by abnormalities in number or content of platelet granules.³⁴ Defects in alpha-granules, dense granules, or both (Table 21.5)

may be present. Platelet aggregation to ADP (Fig. 21.4) typically shows an initial wave of aggregation, but the aggregates subsequently dissociate due to reduced or absent release of granule contents, which reinforce the aggregatory response. Most patients have a prolonged bleeding time. A variable bleeding diathesis results. More commonly, patients may have release defects wherein granules are present, but signaling necessary for release of granule contents is defective.

- Albinism-associated SPD occurs in the context of disorders characterized by oculocutaneous albinism, such as the Hermansky–Pudlak and Chediak–Higashi syndromes. Impaired biogenesis of dense granules, lysosomes, and melanosomes may be responsible for the reduced number of dense granules in these patients.
- Non-albinism-associated SPD occurs in a variety of other conditions (thrombocytopenia absent radii [TAR] syndrome), Ehlers–Danlos syndrome, Wiskott–Aldrich syndrome (immunodeficiency, dysfunctional platelets, and microthrombocytopenia), osteogenesis imperfecta. Defects in dense granules may relate more to granular content rather than number and may occur in conjunction with alpha-granule abnormalities (alpha-delta SPD).³⁵ Decreased or empty dense granules can be visualized on electron microscopy.
- Gray platelet syndrome (see earlier discussion).
- The Quebec platelet disorder³⁶ is an extremely rare disorder that is characterized by abnormal alpha-granule content and mild thrombocytopenia, leading to a moderate bleeding diathesis. Bleeding is unresponsive to platelet transfusion. Increased intraplatelet urokinase-type plasminogen activator results from a tandem duplication of the PLA2U gene.
- Scott syndrome³⁷ is an extremely rare disorder characterized by spontaneous bleeding and reduced thrombosis due to a defect in scramblase, which is necessary for adequate expression of phosphatidylserine on the outer leaflet of the platelet membrane and normal binding of coagulation factors. Patients may show a loss-of-function mutation in TMEM16F.³⁸
- Cytochrome c mutations have been described with resulting mild autosomal dominant thrombocytopenia and dysregulated platelet formation.³⁹
- Congenital disorders of signal transduction include defects in receptor–agonist interactions, G-protein activation, platelet enzymatic activity, and phosphorylation of signaling proteins.¹⁸
- Isolated laboratory-specific defects. Individuals with phenotypically normal hemostasis occasionally demonstrate reduced or (less frequently) absent aggregation to one or more agonists on platelet aggregation testing. These abnormalities, which may be genetically determined, probably reflect interindividual differences in the reactivity of platelets to certain ligands and do not necessarily indicate an increased risk for spontaneous or trauma-induced hemorrhage, unless a tendency to bleed has been previously demonstrated.

Note

1. Pseudo or platelet-type VWD is caused by a defect in the platelet GPIb molecule, allowing it to bind to the patient's normal VWF with increased avidity and leading to a type 2B clinical phenotype. Mixing studies using a modified RIPA (patient's platelets and control plasma) distinguish it from type 2B VWD.

References

1. Levi M, van der Poll T. A short contemporary history of disseminated intravascular coagulation. *Sem Thromb Hemost*. 2014;40(8):874–880.
2. Levi M, Schultz M, van der Poll T. Disseminated intravascular coagulation in infectious disease. *Semin Thromb Hemost*. 2010;36(4):367–377.
3. Taylor FB, Jr., Toh CH, Hoots WK, et al. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb Haemost*. 2001;86(5):1327–1330.
4. Franchini M, Manzato F, Salvagno GL, et al. Potential role of recombinant activated factor VII for the treatment of severe bleeding associated with disseminated intravascular coagulation: a systematic review. *Blood Coagulat Fibrinol*. 2007;18(7):589–593.
5. Thachil J, Toh CH, Levi M, et al. The withdrawal of activated protein C from the use in patients with severe sepsis and DIC [Amendment to the BCSH guideline on disseminated intravascular coagulation]. *Br J Haematol*. 2012;157(4):493–494.
6. Price VE, Ledingham DL, Krumpel A, et al. Diagnosis and management of neonatal purpura fulminans. *Semin Fetal Neonatal Med*. 2011;16(6):318–322.
7. Iba T, Saitoh D. Efficacy of antithrombin in preclinical and clinical applications for sepsis-associated disseminated intravascular coagulation. *J Intensive Care*. 2014;2(1):66.
8. Sibai BM, Ramadan MK, Usta I, et al. Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome). *Am J Obstetr Gynecol*. 1993;169(4):1000–1006.
9. Stenczer B, Molvarec A, Szabo G, et al. Circulating levels of thrombospondin-1 are decreased in HELLP syndrome. *Thromb Res*. 2012;129(4):470–473.
10. Chang H, Kuo MC, Shih LY, et al. Clinical bleeding events and laboratory coagulation profiles in acute promyelocytic leukemia. *Eur J Haematol*. 2012;88(4):321–328.
11. Schulman S, Zondag M, Linkins L, et al. Recurrent venous thromboembolism in anticoagulated patients with cancer: management and short-term prognosis. *J Thromb Haemost*. 2015;13(6):1010–1018.
12. Favaloro EJ. Diagnosis and classification of von Willebrand disease: a review of the differential utility of various functional von Willebrand factor assays. *Blood*

- Coagul Fibrinolysis. 2011;22(7):553–564.
13. Haberichter SL, Castaman G, Budde U, et al. Identification of type 1 von Willebrand disease patients with reduced von Willebrand factor survival by assay of the VWF propeptide in the European study: molecular and clinical markers for the diagnosis and management of type 1 VWD (MCMDM-1VWD). *Blood*. 2008;111(10):4979–4985.
 14. Laffan MA, Lester W, O'Donnell JS, et al. The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology. *Br J Haematol*. 2014;167(4):453–465.
 15. Castaman G, Tosetto A, Federici AB, et al. Bleeding tendency and efficacy of anti-haemorrhagic treatments in patients with type 1 von Willebrand disease and increased von Willebrand factor clearance. *Thromb Haemost*. 2011;105(4):647–654.
 16. Reynen E, James P. Von Willebrand disease and pregnancy: a review of evidence and expert opinion. *Semin Thromb Hemost*. 2016;42(7):717–723.
 17. Lambert MP. What to do when you suspect an inherited platelet disorder. *Hematol Am Soc Hematol Educ Program*. 2011;2011:377–383.
 18. Nurden AT, Nurden P. Congenital platelet disorders and understanding of platelet function. *Br J Haematol*. 2014;165(2):165–178.
 19. Crescente M, Di Castelnuovo A, Iacoviello L, et al. Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100. A systematic review. *Thromb Haemost*. 2008;99(1):14–26.
 20. Hayward CP, Harrison P, Cattaneo M, et al. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. *J Thromb Haemost*. 2006;4(2):312–319.
 21. Said SM, Hahn J, Schleyer E, et al. Glycoprotein IIb/ IIIa inhibitor-induced thrombocytopenia: diagnosis and treatment. *Clin Res Cardiol*. 2007;96(2):61–69.
 - 21a. George JN, Shattil SJ. Acquired disorders of platelet function. In: Hoffman R, Benz EJ, Shattil SJ, et al. eds. *Hematology: Basic Principles and Practice*. 3rd ed. New York, NY: Churchill Livingstone; 2000:2174
 22. Jacob S, Dunn BL, Qureshi ZP, et al. Ticlopidine-, clopidogrel-, and prasugrel-associated thrombotic thrombocytopenic purpura: a 20-year review from the Southern Network on Adverse Reactions (SONAR). *Semin Thromb Hemost*. 2012;38(8):845–853.
 23. Reikvam AG, Hustad S, Reikvam H, et al. The effects of selective serotonin reuptake inhibitors on platelet function in whole blood and platelet concentrates. *Platelets*. 2012;23(4):299–308.
 24. Cohen MG, Rossi JS, Garbarino J, et al. Insights into the inhibition of platelet activation by omega-3 polyunsaturated fatty acids: beyond aspirin and clopidogrel.

- Thromb Res. 2011;128(4):335–340.
- 25. Vladareanu AM, Vasilache V, Bumbea H, et al. Platelet dysfunction in acute leukemias and myelodysplastic syndromes. Rom J Intern Med. 2011;49(1):93–96.
 - 26. Hedges SJ, Dehoney SB, Hooper JS, et al. Evidence-based treatment recommendations for uremic bleeding. Nat Clin Pract Nephrol. 2007;3(3):138–153.
 - 27. Bonding Andreasen J, Hvas AM, et al. Marked changes in platelet count and function following pediatric congenital heart surgery. Paediatr Anaesth. 2014;24(4):386–392.
 - 28. Pecci A, Gresele P, Klersy C, et al. Eltrombopag for the treatment of the inherited thrombocytopenia deriving from MYH9 mutations. Blood. 2010;116(26):5832–5837.
 - 29. Nurden P, Debili N, Coupry I, et al. Thrombocytopenia resulting from mutations in filamin A can be expressed as an isolated syndrome. Blood. 2011;118(22):5928–5937.
 - 30. Nurden AT, Fiore M, Nurden P, et al. Glanzmann thrombasthenia: a review of ITGA2B and ITGB3 defects with emphasis on variants, phenotypic variability, and mouse models. Blood. 2011;118(23):5996–6005.
 - 31. Siddiq S, Clark A, Mumford A. A systematic review of the management and outcomes of pregnancy in Glanzmann thrombasthenia. Haemophilia. 2011;17(5):e858–869.
 - 32. Fiore M, Firah N, Pillois X, et al. Natural history of platelet antibody formation against alphaIIbbeta3 in a French cohort of Glanzmann thrombasthenia patients. Haemophilia. 2012;18(3):e201–209.
 - 33. Wiegering V, Sauer K, Winkler B, et al. Indication for allogeneic stem cell transplantation in Glanzmann's thrombasthenia. Hamostaseologie. 2013;33(4):305–312.
 - 34. Sandrock K, Zieger B. Current strategies in diagnosis of inherited storage pool defects. Transfus Med Hemother. 2010;37(5):248–258.
 - 35. White JG, Keel S, Reyes M, et al. Alpha-delta platelet storage pool deficiency in three generations. Platelets. 2007;18(1):1–10.
 - 36. Paterson AD, Rommens JM, Bharaj B, et al. Persons with Quebec platelet disorder have a tandem duplication of PLA2U, the urokinase plasminogen activator gene. Blood. 2010;115(6):1264–1266.
 - 37. Flores-Nascimento MC, Orsi FL, Yokoyama AP, et al. Diagnosis of Scott syndrome in patient with bleeding disorder of unknown cause. Blood Coagul Fibrinolysis. 2012;23(1):75–77.
 - 38. Lhermusier T, Chap H, Payrastre B. Platelet membrane phospholipid asymmetry: from the characterization of a scramblase activity to the identification of an essential protein mutated in Scott syndrome. J Thromb Haemost.

- 2011;9(10):1883–1891.
39. Morison IM, Cramer Borde EM, Cheesman EJ, et al. A mutation of human cytochrome c enhances the intrinsic apoptotic pathway but causes only thrombocytopenia. *Nature Genet*. 2008;40(4):387–379.

Venous Thromboembolism

Elisabet E. Manasanch and Jay N. Lozier

Venous thromboembolism (VTE) is a major health problem in the United States with more than 900,000 estimated cases annually.¹ The average yearly incidence is 117 cases per 100,000 population, with higher rates in women of childbearing age, males older than 45 years, and the elderly (where rates are up to fivefold higher). Pulmonary embolism (PE) occurs about 60% as often as deep venous thrombosis (DVT) and has a high mortality; the incidence may be higher because the diagnosis is often missed in hospitalized patients.^{2,3} Chronic VTE-induced pulmonary hypertension is a late complication of PE in 1% to 4% of cases after 4 years of follow-up, with all cases occurring before 2 years.^{4,5} Most cases of DVT occur in the lower extremities, but virtually any venous vascular bed can be involved. Upper extremity DVTs represent 1% to 5% of the total and are usually associated with long-term indwelling central venous access devices, thrombophilia, and/ or cancer.⁶ When upper extremity DVTs occur, the potential for subsequent pulmonary embolization is estimated to be as high as 36% in some trials,⁷ though fatal PE is less common than for DVT of the lower extremities.

DEEP VENOUS THROMBOSIS AND PULMONARY EMBOLISM

DVT of the extremity is often the precursor of PE, though both may become symptomatic at the same time, and it is possible that PE may occur without symptomatic DVT, due to complete embolism of nascent thrombus before complete occlusion of the vein occurs. One autopsy series indicates that a substantial number of patients with PE have no pathologic evidence for DVT.⁸ The signs and symptoms of DVT and PE are listed in Table 22.1.^{7,9}

The postthrombotic syndrome (PTS) is an important complication of DVT. It is caused by venous hypertension from outflow obstruction and valvular injury and varies from mild edema with little discomfort to incapacitating limb swelling with pain and

ulceration. The severity of PTS can be assessed using the Villalta scale,¹⁰ which is based on the cumulative rates of signs and symptoms characteristic of the syndrome (Table 22.2).

The reported incidence of PTS after an acute episode of DVT has been reported to range from 23% to 60%;¹¹ severe, disabling PTS with skin breakdown and ulceration is seen much less commonly, perhaps in <10% of cases, while mild symptoms probably are experienced in the majority of DVT cases. Surprisingly, contralateral extremities may also develop postthrombotic manifestations without prior evidence of overt DVT;¹¹ perhaps occult obstruction of the inferior vena cava (IVC) may be to blame. Sized-to-fit graded compression stockings (typically 20- to 40-mm pressure) should be applied shortly after diagnosis of DVT to prevent acute dilatation that may result in permanent damage to the valves. If there is poor circulation in the leg due to complete obstruction of venous outflow, compression stockings should be used cautiously or withheld if the increased compression threatens to stop blood flow altogether. After definitive therapy (thrombolysis or mechanical clot extraction) and venous flow are restored to some extent, compression stockings may be reconsidered to mitigate acute symptoms and eventual PTS. A sized-to-fit compression stocking can reduce the rate of PTS by about 50%.¹² Compression stockings do not substitute for adequate anticoagulation but are useful adjuncts to exercise-based rehabilitation programs. Stockings that are not fitted to provide 20 to 40 mmHg compression do not confer equivalent benefits as seen from fitted stockings. A poorly fitted stocking can actually be detrimental, for instance, when the upper parts of the stocking roll down and form a loose “tourniquet” and thereby impair return blood flow from the leg. Unfortunately, compression stockings or sleeves for upper extremity DVTs have not proven to be as beneficial, perhaps due to the lesser hydrostatic pressures involved with the upper extremity.

Table 22.1 Signs and Symptoms of Venous Thromboembolism

Sign/ Symptom	Occurrence (%)
Deep venous thrombosis	
Calf or leg swelling	88
Pain	56
Tenderness	55
Erythema	34
Homan sign	13
Palpable cord	6
Pulmonary embolism	
Dyspnea	77
Tachypnea	70

Tachycardia	43
Hypoxia/cyanosis	18
Hemoptysis	13
Syncope	10
Hypotension	10

Table 22.2 The Villalta Scale for Evaluation of Postthrombotic Syndrome

Symptoms
Pain
Cramps
Heaviness
Paresthesia
Pruritus
Clinical Signs
Edema
Venous dilatation/ ectasia
Hyperpigmentation
Erythema
Lipodermatosclerosis (skin induration)
Pain during calf compression

Characteristics of PTS. Scoring is based on the cumulative rating of the signs and symptoms, with each rated a 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). Total score: 0 to 4, no PTS; 5 to 14, mild-to-moderate PTS; ≥15 or presence of a venous ulcer, severe PTS.

Distal DVT is defined as thrombus found below the popliteal vein trifurcation and occurs most commonly (71% of the time) in the peroneal vein.¹³ The risk for PE is almost negligible, whether treated with anticoagulation, or not; propagation to other distal calf veins does occur in about half of all patients, and propagation to more proximal deep veins is seen in ~5% of patients.^{13,14} Lysis of isolated calf DVT typically occurs within 3 months.¹³ The risk of anticoagulation under these circumstances (0% to 6% bleeding risk) is approximately equal to the benefits realized (less propagation), so it remains controversial whether anticoagulation is necessary. A recent systematic review suggests that anticoagulation or serial imaging with noninvasive methods may be equally valid strategies.¹⁵ PTS occurs in ~5% of patients in the long term but is not characterized by severe changes such as skin ulceration.¹⁵

IMAGING OF DEEP VENOUS THROMBOSIS

Venography remains the standard for diagnostic imaging of DVT, but it is used less

commonly than ultrasonography because it is an invasive procedure that uses radiocontrast dye and requires a skilled operator to perform the injection. Further, there is the need to bring a patient to a fluoroscopy suite, which may not be feasible in the acutely ill patient in an intensive care unit with other comorbidities. In contrast, ultrasonography is noninvasive, portable, and does not use contrast to which the patient may be allergic. Doppler ultrasound instruments are typically portable and can be brought to the bedside even in the most acutely ill patients. Venography retains an advantage for diagnosis of small distal DVTs that are not well imaged by ultrasound, as well as thrombosis of the vena cava or iliac veins of the pelvis that are not accessible to ultrasound examination because they are obscured by bowel gas. Venography may also be useful in instances where ultrasound is not feasible or an unequivocal diagnosis of DVT must be made. Ultrasonography is considerably more sensitive for the detection of proximal DVT than for distal DVT.

Sensitivity of compression ultrasound with venous imaging ranges from 89% to 96% when DVT is diagnosed by a combination of direct visualization of an occlusive thrombus and noncompressibility of a vein. The specificity of this finding for DVT ranges from 94% to 99%, but, unfortunately, the sensitivity may be substantially diminished (47% to 62%) in patients with asymptomatic DVT.¹⁶ Serial ultrasound testing (which has little risk to the patient in contrast to venography) improves sensitivity, as a previously undiagnosed distal DVT may declare itself by proximal propagation. Furthermore, an ultrasound can accurately diagnose certain conditions that occasionally mimic DVT, such as Baker cyst. Impedance plethysmography may be useful in differentiating between a new or recurrent DVT, especially if the previous DVT has not resolved.

Magnetic resonance venography (MRV) and computed tomography venography (CTV) can diagnose DVT in a noninvasive manner. Prospective studies comparing CTV with venous ultrasound for diagnosis of DVT reported sensitivity rates of 100% and specificity of 96% to 100%.^{17,18} CTV can be easily combined with computed tomography angiography in patients suspected to have PE. However, it always requires the administration of IV contrast. One prospective blinded study reported the sensitivity to be >94% and the specificity to be >90% for the diagnosis of DVT using noncontrast MRV direct thrombus imaging.¹⁹ A major advantage for CTV and MRV is that deep abdominal, pelvic, and calf veins can be imaged. Furthermore, MRV can be successfully used avoiding contrast and its risks (such as gadolinium-associated systemic fibrosis in patients with chronic kidney disease).^{20,21} Major disadvantages include cost, availability, expert reading, and possible need for IV contrast use when compared to ultrasound techniques.

PULMONARY EMBOLISM DIAGNOSIS: ECHOCARDIOGRAPHY, ELECTROCARDIOGRAPHY, AND X-RAY

PE is thought to be the consequence of clot breaking free from a lower extremity DVT. Upper extremity DVT embolization is less common, but the increasing use of central venous access devices for cancer chemotherapy or other long-term parenteral treatment may increase its frequency. Less commonly, PE may originate in the IVC (particularly in association with renal cell carcinoma) or the right ventricle of the heart from mural thrombus. There is intriguing evidence from the study of acute trauma patients⁸ that pulmonary “embolism” can be seen without concurrent DVT, raising the possibility that some pulmonary emboli may actually be *in situ* pulmonary thrombosis. Regardless of the mechanism, imaging approaches to diagnose PE include pulmonary angiography (the standard), computed tomography (CT) angiography, ventilation/ perfusion scanning, and more recently MRI. Echocardiographic confirmation of right ventricular hypertension may assist in the decision regarding thrombolysis with right heart failure. Both echocardiography and spiral CT have a low sensitivity for PE located in peripheral pulmonary vessels. In severe cases, chest X-ray findings may include a Hampton hump (a wedge-shaped opacity with apex pointing to the hilum) or a focal paucity of blood vessel perfusion. Ancillary diagnostic chest radiography, electrocardiography (ECG), and echocardiographic findings suggestive of PE are listed in [Table 22.3](#).

Table 22.3 Ancillary Findings Diagnostic of Pulmonary Embolism

Chest Radiography

Hampton hump

Focal paucity of blood vessel perfusion (Westermark sign)

Dilated pulmonary artery proximal to the thrombus

Atelectasis

Pleural effusion

Elevated diaphragm

Electrocardiography

New right bundle branch block

S1Q3T3 pattern (sign of acute cor pulmonale)

Supraventricular arrhythmias

Echocardiography

Right ventricular dilatation, often with myocardial hypokinesis

Pulmonary artery dilatation

Right ventricular mural thrombi

Tricuspid regurgitation

LABORATORY DIAGNOSIS OF VENOUS THROMBOEMBOLISM

The D-dimer is a quantitative assay with good reproducibility that is often automated and can be used as an adjunct to imaging studies to exclude the presence of PE or DVT.^{22–24} When a thrombus is degraded by plasmin, D-dimers and other fibrin split products are formed from cross-linked chains of the fibrin clot—as such, they are markers for fibrin turnover. The use of D-dimer is preferable to the semiquantitative fibrin split products that are measured by latex agglutination methods at varying dilutions of patient plasma. If a patient has a low pretest probability of VTE and the sensitive enzyme-linked immunosorbent assay (ELISA) test for D-dimers is negative, as a practical matter VTE can be excluded. The low specificity for VTE requires that further diagnostic testing be conducted in the event of an elevated D-dimer to elucidate its etiology. Many other conditions can cause elevation of the D-dimer, including cancer, pregnancy, sepsis, sickle cell crisis, acute myocardial infarction, cardiopulmonary resuscitation, excessive bleeding, trauma, and recent surgery. Measurement of D-dimers may also guide the duration of anticoagulation for VTE, because the optimal course of therapy for such patients has not been established.²⁵

The PROLONG Study indicated that patients with positive D-dimers 1 month after the completion of at least 3 month anticoagulation with vitamin K antagonists (VKAs) for idiopathic VTE had a significantly higher risk for recurrent VTE, which was mitigated by prolonged anticoagulation. Patients with abnormal D-dimers who did not resume anticoagulation experienced a 15% incidence of rethrombosis over the 18-month observation period compared to 2.9% if anticoagulation was restarted.²⁶ The adjusted hazard comparing the rates of recurrence was 4.26 (95% confidence interval [CI] = 1.23 to 14.6, P = .02). A follow-up study, the PROLONG II, assessed the utility of repeated D-dimer testing in patients with a first unprovoked episode of VTE with normal D-dimer 1 month after stopping VKAs. D-dimer was tested at study initiation and every 2 months thereafter with a follow-up period of 13 months; 14% of patients with an initially negative D-dimer had a positive test at month 3 of evaluation. Furthermore, the D-dimer became abnormal at each subsequent time point in about 10% to 15% of patients up to 9 months of follow-up, when it decreased to 8% to 10%. An abnormal D-dimer at the first measurement or at day 30 usually remained abnormal over time in the majority of cases, and this pattern was associated with an increased risk of rethrombosis. The rates of recurrence for patients with an abnormal D-dimer at 3 months were 22.6% (95% CI = 10% to 41%) compared to 4.6% (95% CI = 2% to 9%) if D-dimer was normal (P = .003).²⁷ Repeat D-dimer testing may help identify the subgroup of patients at lower risk for recurrence, in whom anticoagulation could be

stopped. A multicenter prospective study (DULCIS, <http://clinicaltrials.gov>: NCT00954395) is currently underway to clarify this issue.

DEEP VENOUS THROMBOSIS IN SITES OTHER THAN THE DISTAL VEINS OF THE LOWER EXTREMITIES

Aside from typical presentations involving the lower extremities, DVT can occur in other sites such as veins of the upper extremities (particularly in conjunction with central venous catheters commonly used in cancer patients for chemotherapy), or in the veins of the chest or abdomen. Bilateral upper extremity DVTs are uncommon and should prompt a search for malignancy. The effects of DVT in these sites can be devastating, even in the absence of PE. Perioperative DVT of the splanchnic veins is common, occurring more frequently in laparoscopic procedures than with open ones. Unprovoked DVT of the splanchnic vessels should prompt a search for underlying abnormalities of hemostasis, such as deficiency of anticoagulant proteins, undiagnosed cancer, hematologic diseases such as myeloproliferative disorders (MPDs), or paroxysmal nocturnal hemoglobinuria (PNH).

Portal vein thrombosis can commonly occur as a complication of surgical procedures, especially splenectomy, or during pregnancy, or with peritonitis. In cases that are not associated with a precipitating factor, antiphospholipid antibodies (APAs), deficiency of protein C, protein S, or (less commonly) antithrombin III may be found; the factor V Leiden and prothrombin 20210 gene polymorphisms may also be seen in patients at rates greater than in the general population (as is true in any group of patients with pathologic thrombosis). Clonal V617F point mutation in the Janus 2 kinase (JAK2V617F) tyrosine kinase gene occurs in a large proportion of MPD (particularly polycythemia rubra vera) and is found in 45% of Budd–Chiari syndrome [BCS] and 34% of portal vein thrombosis. Table 22.4 indicates the prevalence of various prothrombotic states in patients with portal or hepatic vein thrombosis.^{28–30}

Table 22.4 Prevalence of Prothrombotic States in Portal or Hepatic Vein Thrombosis

Prothrombotic State	Portal Vein Thrombosis (%)	Hepatic Vein Thrombosis Budd–Chiari syndrome (BCS; %)
Antiphospholipid antibodies or lupus anticoagulant	11	5–19
Protein C deficiency	0–7	9–20
Protein S deficiency	2–30	0
Antithrombin	1–5	0
FV Leiden	3–13	22–26

Prothrombin 20210	3–35	5–6
Myeloproliferative disorder (PV, ET)	17–22	28–31
MTHFR C677T mutation	4–45	0

ET, essential thrombocythemia; PV, polycythemia vera.

Treatment options for splanchnic vein thrombosis include anticoagulation, thrombolysis, and in the most extreme cases, consideration should be given to orthotopic liver transplantation for those with liver failure. In addition, acute reduction of red blood cell mass and/ or platelet count may be beneficial if the splanchnic vein thrombosis is due to polycythemia vera (PV) or essential thrombocythemia.

ACQUIRED THROMBOPHILIC STATES

Heparin-Induced Thrombocytopenia and Heparin-Induced Thrombocytopenia With Thrombosis

Heparin-induced thrombocytopenia (HIT) is a prothrombotic state caused by a drug reaction to unfractionated heparin (UFH) and less commonly to low-molecular-weight heparin (LMWH), which can also exacerbate HIT due to cross-reactivity with UFH. Even small exposures to UFH, including flushing of IV lines, may precipitate HIT and even thrombosis, in patients with antibodies. Antibodies of the immunoglobulin G (IgG) class are formed that produce a strong activation of platelets through their Fc γ IIa receptors. They recognize large multimolecular complexes of platelet factor 4 bound to heparin (PF4/H), although only about 10% of all anti-PF4/H antibodies have platelet-activating properties.³¹ This activation promotes thrombosis in vivo in both venous and arterial sites. HIT is a “clinical-pathologic syndrome,” requiring both a compatible clinical picture and positive laboratory test results. In general, platelet counts begin to decrease 5 to 9 days after the initiation of heparin. Thrombocytopenia and thrombosis may occur earlier in patients primed by heparin administration in the prior 100 days. Clinical scoring systems have been implemented to evaluate the pretest probability of HIT: 4Ts³² and most recently, the HIT Expert Probability (HEP) score³³ (Tables 22.5 and 22.6).

Low scores usually indicate a <2% probability of having a positive platelet activation assay. Two types of assays are used to establish diagnosis: enzyme immunoassays, such as an ELISA that detects PF4-heparin antibodies, and platelet activation/ aggregation assays, which detect spontaneous aggregation of platelets induced by the addition of heparin to the patient’s platelet-rich plasma. Sensitivity of platelet aggregation assays can be increased when patient’s platelets are “loaded” with radioactive serotonin; serotonin release from platelets is detected as a marker of platelet activation in vitro, after the addition of heparin. The most sensitive test is the

serotonin-loaded platelet aggregation assay. False positive results are common with the ELISA test, and its specificity varies depending on the pretest clinical probability. A negative result usually excludes HIT.

Table 22.5 Pretest Prediction of Heparin-Induced Thrombocytopenia

4Ts Category	2 Points	1 Point	0 Point
Thrombocytopenia	PLT count fall > 50% from baseline AND PLT nadir $\geq 20 \times 10^9/\text{L}$	PLT count fall 30% to 50% from baseline OR PLT nadir $10\text{--}19 \times 10^9/\text{L}$	PLT fall < 30% from baseline OR PLT nadir $< 10 \times 10^9/\text{L}$
Time of PLT count fall	Clear onset 5–10d or PLT fall $\leq 1\text{d}$ with heparin exposure within 30 prior days	Fall in PLT between 5 and 10d but timing is not clear due to missing PLT counts or onset after day 10 of heparin exposure OR fall in PLT $\leq 1\text{d}$ with prior heparin exposure 30 and 100d ago	PLT count fall $< 4\text{d}$ without recent heparin exposure
Thrombosis	New thrombosis, skin necrosis, or acute systemic reaction after unfractionated heparin exposure	Thrombosis or unconfirmed but clinically suspected thrombosis	No thrombosis or thrombosis before heparin exposure
Other causes of thrombocytopenia	None apparent	Possible other causes	Probable other causes

Low score ≤ 3 ; intermediate score ≤ 5 ; high score ≤ 8 .

PLT, platelet.

Table 22.6 Heparin-Induced Thrombosis Expert Panel Probability Score

Clinical Features	Points
Magnitude of decrease in platelets	
<30%	-1
30%–50%	1
>50%	3
Timing of decrease after heparin exposure	
<4 d	-2
4 d	2
5–10 d	3
11–14 d	2
>14 d	-1

Nadir platelet count	
≤20,000/ µL	-2
>20,000/ µL	2
Thrombosis	
New venous thromboembolism (VTE)/ arterial thrombosis (ATE) > 4d after heparin	3
Progression of VTE/ ATE on heparin	2
Skin necrosis	3
Acute systemic reaction	2
Bleeding	-1
Other causes	
Chronic thrombocytopenia	-1
Medication	-2
Severe infection	-2
Severe disseminated intravascular coagulation (DIC)	-2
Indwelling arterial device	-2
Cardiopulmonary bypass within 96 h	-1
No other apparent cause	3

A score of 2 is 100% sensitive, but only 60% specific for heparin-induced thrombocytopenia (HIT). A score of 5 is 86% sensitive and 88% specific for HIT.

Two different classes of anticoagulants are used for the treatment of HIT in the presence or absence of thrombosis: direct thrombin inhibitors, lepirudin, bivalirudin, and argatroban, which are approved in the United States for the treatment of HIT and the factor Xa inhibitor fondaparinux, which is currently approved for DVT prophylaxis and treatment, but has growing evidence in literature to be an effective treatment for HIT.³⁴ Fondaparinux binds anti-PF4 antibodies, but does not activate platelets, unlike traditional LMWH preparations such as enoxaparin, dalteparin, and tinzaparin, which cross-react with the PF4-heparin antibodies and can activate platelets. Fondaparinux binds irreversibly to factor Xa and has a long active half-life (17 hours), which may prevent rebound hypercoagulability, but cannot be readily reversed if bleeding ensues. Dosing needs to be adjusted for impaired renal function. Lepirudin and argatroban require IV continuous infusions due to their short half-lives (<2 hours) and need PTT monitoring. Lupus anticoagulants (LAC) and/ or high factor VIII levels may result in prolonged or shortened clotting times, respectively, thereby complicating monitoring. Algorithms exist for infusions of argatroban without monitoring when LAC interfere with monitoring.³⁵ Levels of argatroban or other direct thrombin inhibitors can be monitored more directly by use of assays of the ecarin clotting time; ecarin is a snake venom that converts fibrinogen to fibrin but is not influenced by LAC or levels of factor VIII.³⁶ Lepirudin is antigenic, and antibody formation results in excessive

anticoagulation. Lepirudin is contraindicated in the presence of renal insufficiency. Bivalirudin (approved in HIT specifically for patients undergoing percutaneous coronary intervention) and argatroban are both hepatic-excreted, nonantigenic, and short-acting, and they should be used cautiously in patients with liver disease. Initiation of warfarin should be postponed at least until the platelet count is above 150,000/ μ L, because warfarin use during acute HIT is a major risk factor for venous limb gangrene, perhaps on the basis of rapid depletion of protein C, a vitamin-dependent anticoagulant protein with a short half-life (6 to 7 hours).

Nephrotic Syndrome

Patients with nephrotic syndrome are at higher risk for VTE, arterial thromboembolism (ATE), and renal vein thrombosis than in the general population (annual incidence, 9.85% [VTE] and 5.52% [ATE]).³⁷ Membranous glomerulonephritis confers the highest risk of VTE. Hypercoagulability appears due to alterations in plasma levels of proteins involved in coagulation and fibrinolysis. The anticoagulant proteins antithrombin III and protein S are lost in the urine, perhaps due to their relatively small size compared to procoagulant factors V, VIII, von Willebrand factor, and fibrinogen, which may be retained by the kidney.³⁸ Furthermore, fibrinogen, factor VIII, and von Willebrand factor are acute-phase reactants, and their levels may be increased by inflammation.³⁹ The routine use of prophylactic anticoagulation for patients with nephrotic syndrome has not been established by randomized controlled trials, but some experts advocate this when additional risk factors such as membranous glomerulonephritis or the antiphospholipid syndrome are present. LMWH must be used with extreme caution in patients with renal insufficiency in addition to nephrotic syndrome.

Antiphospholipid Antibody Syndrome

APAs may be associated with thrombosis on the basis of various mechanisms,⁴⁰ summarized in Table 22.7. Anticardiolipin antibodies are APA associated with infections such as syphilis, but these are transient and not usually associated with thrombosis.

Tests for APA include specific assays to quantify levels directly by ELISA or indirectly through the detection of anti β 2-glycoprotein I (GPI) antibodies. The APA that prolong phospholipid-dependent clotting assays (primarily the activated partial thromboplastin time [aPTT]) are designated as LAC. Other, less commonly used assays that are affected by LAC include the dilute Russell viper venom time (DRVVT), the dilute prothrombin, or tissue thromboplastin inhibition (TTI) test, and the kaolin clotting time (KCT). Confirmatory tests for LAC are performed by adding a source of phospholipids to a clotting reaction that is prolonged by the LAC to see if the abnormal

clotting time is corrected. Phospholipids may include platelets (in the platelet neutralization procedure, or PNP), or more recently phospholipids with defined physical properties, such as hexagonal-phase lipids that are used in some commercial tests. The hematologist consultant should be aware of the specific screening and confirmatory tests that are used in the local laboratory when diagnosing APA syndromes or LAC. Also important is that LAC tests may be positive at the time of an acute thrombotic event, but often are negative at subsequent time points only a few weeks later. In a study of 30 patients with lower extremity DVT that were treated with tissue plasminogen activator (tPA), 19 of the 30 were initially positive for LAC at the time of presentation, but 11 of these were documented to be negative 6 months later (or sooner), 3 remained positive at later time points, and there were 6 lost to follow-up and 1 with an indeterminate value at later time points (unpublished observations on patients described in Refs. 41 and 42).

Table 22.7 Mechanisms of Thrombosis Associated With Antiphospholipid Antibodies

Binding of APA to β_2 -glycoprotein I (β_2 -GPI)/ phospholipid complex exposed on the surface of injured or activated endothelial cells and monocytes
Overproduction of tissue factor by monocytes and endothelial cells
Activation of platelets that increases expression of glycoprotein IIb–IIIa and synthesis of thromboxane A2
Interaction with regulatory proteins such as annexin V, prothrombin, factor X, protein C, and plasmin

APA, antiphospholipid antibodies.

Two positive tests for APA (LAC, anticardiolipin antibody IgG or immunoglobulin M [IgM], and anti- β_2 -GPI antibody IgG or IgM) obtained at least 12 weeks apart are required to fulfill the laboratory criteria for the APA syndrome. Clinical criteria for the diagnosis of the APA syndrome include detection of venous and/ or arterial thrombotic events, autoimmune thrombocytopenic purpura, marantic endocarditis, multiple spontaneous abortions before the 10th week of gestation, or unexplained death of a morphologic normal fetus after the 10th week of gestation. Thrombosis can occur in virtually any vascular bed, and patients may present with a catastrophic syndrome with thrombosis in multiple vascular sites, including cerebrovascular accidents and DVT, with more than 50% mortality.

Treatment strategies should focus on modification or elimination of risk factors, such as smoking and oral estrogen contraceptives. For a thrombotic complication, systemic anticoagulation should be initiated. To prevent VTE recurrence, a randomized, double-blind, prospective study showed that dosing warfarin to an international normalized ratio (INR) of 2.0 to 3.0 was equally effective as higher-intensity warfarin regimens to achieve an INR of 2.5 to 3.5.⁴⁰ However, patients with recurrent VTE or arterial

thrombosis may need more aggressive treatment with an INR goal 3.0 to 4.0 or combined antithrombotic therapy (warfarin plus low-dose aspirin). This is based on the observation that the rate of recurrent thrombosis was low with an INR > 3.0, and is not endorsed by all experts.⁴³

Hypercoagulability of Malignancy

VTE frequently complicates malignancy and results in significant morbidity and mortality. The estimated prevalence of VTE in patients with cancer is 10% to 15% and can be as high as 28% to 30% in pancreatic cancer or malignant gliomas.⁴⁴ Certain sites have been associated with VTE associated with malignancy: intra-abdominal and bilateral lower extremity DVT ($P < .05$), which may justify cancer screening that otherwise might not be performed.⁴⁵ Malignancy promotes thromboses through a variety of mechanisms: release of tissue factor, activation of factor X by cancer procoagulants(s), endothelium–tumor cell interactions, and platelet activation. Hypercoagulability associated with malignancies is designated Trousseau syndrome and manifests as disseminated intravascular coagulation, nonbacterial thrombotic endocarditis, PE, DVT, and arterial thromboses. Occasionally, chemotherapy agents promote thrombosis, possibly through direct injury to the vascular endothelium. Of equal importance is the observation that adjuvant therapy with selective estrogen receptor modulators (SERMs), such as tamoxifen, or antiangiogenic medications, such as thalidomide and lenalidomide for the treatment of multiple myeloma, and bevacizumab for the treatment of breast, colon, brain, or lung malignancies, can all increase the potential thrombogenicity of the cancer type. Central venous indwelling catheters often complicate cancer care because of thrombus formation in the catheter itself and the vessel into which it has been inserted.⁴⁶

Treatment of cancer-associated thrombosis is challenging. Warfarin, the current mainstay of long-term anticoagulation, may be difficult to manage due to concomitant medications and thrombocytopenia from chemotherapy or radiation; further, rethrombosis is common despite correct warfarin dosing. LMWH is the agent of choice for anticoagulation in cancer patients. Dalteparin is approved for the extended treatment of symptomatic VTE in patients with cancer based on the large clinical CLOT trial that showed a reduction in objectively confirmed, symptomatic DVT and/ or PE during the 6-month study period when compared with oral anticoagulation (15.7% vs. 8%).⁴⁷ Dalteparin appeared to reduce the incidence of rethrombosis most significantly in the first month of treatment compared to warfarin with a statistically significant ($P = .03$) improvement in overall survival (a secondary end point in the study) in patients with nonmetastatic disease. A similar trial randomized 138 patients to receive enoxaparin 1.5 mg/ kg/ day followed by warfarin for 3 months or enoxaparin 1.5 mg/ kg/ day for 3

months. There was a trend toward a decrease in VTE recurrence or major bleeding with enoxaparin versus warfarin (21.1% vs. 10.5%; P = .09).⁴⁸ LMWH may exert an antineoplastic effect through interference with tumor cell adhesion, invasion, metastasis formation, and angiogenesis, all of which are needed for tumor progression. However, it is unclear whether this is true for all tumor types and further studies are needed.⁴⁹

Paroxysmal Nocturnal Hemoglobinuria

PNH causes intravascular hemolysis, bone marrow failure, and thrombotic events. Diagnosis can be made rapidly by detection of CD55-and CD59-deficient erythrocytes and neutrophils by flow cytometry. Patients with clones comprising greater than 50% of cells carry a high risk of thrombotic events (44% in 10 years), including unusual sites like the hepatic vein (BCS) being a frequent manifestation.⁵⁰ Thrombosis is the most common cause of death in PNH. The etiology of thrombosis may involve release of free hemoglobin (which activates the endothelium), complement-mediated damage of GPI-deficient erythrocytes, and/ or deficiency of GPI-anchored fibrinolytic factors such as urokinase/ plasminogen activator receptor.⁵¹ The humanized monoclonal antibody eculizumab targets the C5 terminal complement component and is Food and Drug Administration (FDA) approved for treatment of PNH. Eculizumab significantly reduced the rate of VTE in eculizumab-treated patients in a non-randomized trial when compared with the same patient's pretreatment (1.07/ 100 patient-years vs. 7.37/ 100 patient-years [P < .001]).⁵²

Surgery as a Risk for Acquired Thrombosis

Surgery is a major risk factor for thrombosis. Trauma to tissue results in endothelial injury, activation of the coagulation cascade (through the release of tissue factor), and platelet activation. The risk is modified by time of anesthesia, patient age, the presence of underlying heritable or acquired hypercoagulable states, and the nature of the surgical procedure. VTEs most frequently occur with hip or knee arthroplasty, hip fracture surgery, spinal cord injury, major trauma, and any surgery performed in the context of malignancy. Patients undergoing these procedures should receive thromboprophylaxis. Use of graduated pneumatic compression stockings plus LMWH, adjusted-dose heparin, fondaparinux, and oral anticoagulation with warfarin to achieve an INR goal of 2 to 3 are all reasonable options. DVT prophylaxis should be individualized depending on bleeding risk, history of previous thrombosis, history of HIT, presence of renal insufficiency, and type of surgery; published guidelines can assist in management.⁵³ Outpatient surgical procedures performed in patients younger than 40 years who can be made readily ambulatory do not require prophylactic anticoagulation. Prolonged prophylactic anticoagulation up to 30 days after surgery may be indicated for patients

undergoing total hip replacement (at least until the patient is mobile) and for those in whom a malignancy persists.

Laparoscopic surgery has become increasingly popular as a substitute for conventional open surgical procedures. Although there is less tissue damage, shorter procedure times, and quicker recovery, patients may be subjected to induction of pneumoperitoneum and prolonged use of the reverse Trendelenburg position to visualize and manipulate internal organs that may result in venous stasis and increased risk of thrombosis in some patients.⁵⁴ The American College of Chest Physicians' (ACCP) Clinical Practice Guidelines (8th edition) recommends against routine prophylaxis for those undergoing laparoscopic surgery without additional thromboembolic risk factors but recommends mechanical or pharmacologic prophylaxis in patients with any risk factors.⁵³ The Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) guidelines for DVT prophylaxis during laparoscopic surgery stratifies inpatients into low-, moderate-, and high-risk groups for thrombosis on the basis of a risk score imputed from the type of procedure and patient risk factors.^{55,56} Procedure-related risk factors include procedure lasting over 1 hour and pelvic procedures. Patient-related factors include age > 40 years, immobility, malignancy, thrombophilic states (protein C, protein S, or ATIII deficiency), obesity, peripartum state (or use of estrogens), heart failure, renal failure, varicose veins, inflammatory states, or infection. In the lowest risk group (procedure < 60 minutes in patients with no risk factors) elastic stockings and early ambulation suffice, and UFH or LMWH is optional. In the moderate-risk group (one patient risk factor in a procedure of less than 60 minutes, or any procedure >60 minutes with no patient risk factors) pneumatic compression devices or prophylactic heparin or LMWH are recommended. In the high-risk group (two or more risk factors in procedures >60 minutes), a combination of serial compression devices and prophylactic UFH or LMWH is recommended.^{55,56}

Myeloproliferative Disorders

MPDs are paradoxically associated with increased risks for both hemorrhage and thrombosis, but thrombosis is the most common cause of death in MPDs. The thrombotic risk of PV is often exacerbated by the hyperviscosity produced by a markedly increased red cell mass. Treatments for PV include serial phlebotomy to decrease red cell volume/ hyperviscosity and cytotoxic agents, such as hydroxyurea (HU), to reduce erythrocyte, leukocyte, and platelet production, with the ultimate goal to minimize the risk of thrombosis. ³²P or alkylators such as chlorambucil or busulfan are rarely used, except in the elderly where long-term leukemia risk is not as concerning as for other patients. The goal of PV therapy by phlebotomy is to keep the hematocrit below 45% in males and below 42% in females, so as to prevent stroke, heart attack, or DVT,

including the BCS.⁵⁷ PV-associated erythromelalgia is usually treated with low-dose aspirin 81 mg daily. Higher aspirin doses may be associated with thrombosis.

Therapy to prevent thrombosis is usually considered for patients with essential thrombocythemia who are older than 60 years of age or have a prior history of thrombosis. Guidance is derived from a randomized trial evaluating low-dose aspirin plus anagrelide versus HU. HU plus aspirin was associated with a lower risk of arterial thrombosis, serious hemorrhage, and transformation to myelofibrosis than anagrelide, a specific platelet-lowering noncytotoxic, and nonchemotherapy agent. VTE incidence was higher in the HU group; however, the totality of the data when analyzed for composite endpoints favored treatment with HU.⁵⁸ Blood counts should be followed closely during therapy, and HU is contraindicated in pregnant women or for women who desire to become pregnant.

Inherited Hypercoagulable States

Most inherited hypercoagulable states associated with an increased risk of VTE are deficiencies of one or more anticoagulant proteins or defects in coagulation factors that increase their level of expression or make them no longer subject to inhibition or regulation by anticoagulant proteins. There may also be metabolic problems such as homocysteinemia where toxic levels of homocysteine may be toxic to the endothelium and promote thrombosis. Inherited hypercoagulable states are listed in Table 22.8.

Persistent elevations of coagulation factors VIII, IX, or XI have also been implicated as inherited hypercoagulable states.^{59–63} Extremely rare inherited hypercoagulable states include dysfibrinogenemias or other deficiencies of the fibrinolytic system (plasminogen deficiency). Screening for a thrombophilic hypercoagulable state should be considered for a young patient with a positive family history of thrombosis, unprovoked thrombosis, or recurrent thrombosis. The timing of testing is critical. Elevations of factor VIII or fibrinogen can occur at the time of an acute thrombotic event, perhaps from inflammation, and only a persistently increased level is likely to be the cause for thrombosis. Anticoagulant medications can interfere with measurements of proteins C and S (warfarin) or antithrombin (heparin), and testing should be done when the patient is not taking these medications to ensure an accurate assessment of levels. Pregnancy is a time when levels of factor VIII rise, and free protein S decreases, so the baseline assessment of hypercoagulability is best done when a woman is not pregnant.

Activated Protein C Resistance (Factor V Leiden)

A polymorphism in the gene for coagulation factor V (Arg506Gln; factor V Leiden) results in a factor V protein that is not inactivated by activated protein C. The factor V Leiden polymorphism is seen in ~5% of the Caucasian population and is the most

common known inheritable risk factor for DVT or PE.⁶⁴ There are other rare factor V polymorphisms associated with resistance to activated protein C in Caucasians (factor V Cambridge, Arg306Thr)⁶⁵ and also in Chinese (factor V Hong Kong, Arg306Gly)⁶⁶ that will be missed by the DNA test for factor V Leiden. Most patients who carry the mutation do not develop thrombosis; however, additive risk factors, including estrogen use and the coexistence of the prothrombin mutation, greatly increase the risk of VTE. Laboratory testing includes direct detection of the characteristic mutation in the factor V gene, accomplished by the polymerase chain reaction (PCR) on peripheral blood leukocyte DNA. Indirect testing for inability of activated protein C to prolong the PTT (resistance to activated protein C) is done less commonly now, but may be useful if there is suspicion that a polymorphism other than the factor V Leiden may be present.

Table 22.8 Inherited Hypercoagulable States

Activated protein C resistance (factor V Leiden gene polymorphism, 506R → Q)

Prothrombin gene 20210 A → T polymorphism

Protein C deficiency

Protein S deficiency

Antithrombin deficiency

Hyperhomocysteinemia

Prothrombin Mutation (G20210A)

The second most common gene mutation responsible for congenital hypercoagulability in Caucasians is the prothrombin G20210A polymorphism. As with factor V Leiden, VTE is more commonly seen than arterial thrombosis. This mutation is associated with ~20% greater than normal prothrombin levels, seen almost exclusively in Caucasians, and was found to have a crude 2.8 odds ratio for the development of thrombosis.⁶⁷ The polymorphism apparently leads to more stable factor II messenger RNA (mRNA), and therefore greater prothrombin expression. G20210A can be detected directly by PCR analysis of the target site in the factor II gene; measurements of factor II are not particularly specific for the polymorphism, and should not be relied on to make the diagnosis.

Deficiencies of Proteins S and C and Antithrombin (Antithrombin III)

Proteins C and S and antithrombin (previously known as antithrombin III) are synthesized in the liver and serve to modulate reactions of blood coagulation. Protein C is a serine protease, and when activated by thrombin cleavage has the ability to catalytically cleave and inactivate factors V and VIII, thereby shutting down further synthesis of thrombin. It is a vitamin K-dependent protein, and its activity level goes

down in patients who are on warfarin. Protein S is a nonenzyme cofactor for activated protein C and is also a vitamin K-dependent protein that is inactivated in patients on warfarin. Protein S binds to (complement) C4b-binding protein,⁶⁸ which may mediate a decrease in protein S activity during acute inflammation. Antithrombin (previously known as antithrombin III) is a serine protease inhibitor that neutralizes thrombin and activated forms of other serine proteases such as factors Xa, XIa, IXa, and XIIa.⁶⁹ Antithrombin's activity as a serine protease inhibitor is accelerated by binding to heparin, a complex, sulfated polysaccharide that is a normal component of various tissues (lung, liver, intestine) and used in purified form as an anticoagulant medication. Deficiency of antithrombin is probably the most serious risk factor for thrombosis, especially VTE in association with surgery or invasive procedures.

Hyperhomocysteinemia

Homocysteine is an amino acid that does not appear in proteins but is an intermediate in the metabolism of methionine, a sulfur-containing, essential amino acid. Reactions of the metabolic pathways in which homocysteine participates require folic acid, cyanocobalamin (vitamin B₁₂), or pyridoxine (vitamin B₆) as cofactors. Deficiencies of these vitamins may cause accumulation of homocysteine to high levels in the bloodstream. Extremely high levels of homocysteine are seen in homocystinuria, and in methylene tetrahydrofolate reductase (MTHFR) gene defects. Homocysteine may be toxic to endothelial cells,⁷⁰ and accelerated atherosclerosis can be a feature of the disease homocystinuria.^{70,71} Accordingly, many investigators have sought evidence for an association between elevated homocysteine and thrombosis in people who are not suffering from the extreme manifestations of homocystinuria. Evidence that elevated homocysteine levels (>95th percentile) is associated with VTE was found in a study comparing patients with first time DVT with matched controls, where the odds ratio for elevated homocysteine was 2.5.⁷² It is not clear if lowering homocysteine levels by vitamin therapy can alter the risk for VTE, however. Despite lowering homocysteine levels in the treatment group of the 5,222 patient Heart Outcomes Prevention Evaluation-2 (HOPE-2) intervention trial, there was no difference in the incidence of VTE.⁷³ A meta-analysis of 31 published studies indicated that presence of the homozygous TT form of the MTHFR gene at amino acid 677 was only a very weak risk factor for thrombosis.⁷⁴ Therefore, it is our practice not to routinely seek expensive molecular studies of polymorphisms in the MTHFR gene as risk factors for VTE, but instead to measure homocysteine levels in serum.

TREATMENT

Prophylaxis

The American College of Physicians (ACP) recently published guidelines for VTE prophylaxis in medical patients and patients with an acute stroke.⁷⁵ Careful assessment of the risk for bleeding and thrombosis need to be performed prior to starting prophylaxis with heparin. The ACP guidelines recommend starting prophylaxis with heparin (either LMWH or UFH) in medical (including stroke) patients when the benefit outweighs the risk of bleeding. Pooled data from 18 trials showed that heparin prophylaxis was associated with a borderline statistically significant reduction in risk for mortality compared to no heparin prophylaxis (relative risk [RR] = 0.93, CI = 0.86 to 1.00), a statistically significant reduction in the risk of PE (RR = 0.70, CI = 0.56 to 0.87), at the expense of an increase in bleeding events, which was also statistically significant (RR = 1.28, CI = 1.05–1.56). Interestingly, no improvements in clinical outcomes were seen in three studies of mechanical prophylaxis in patients with stroke, but more patients had lower extremity skin damage (RR = 4.02, CI = 2.34 to 6.91) an increase of 39 events per 1,000 patients treated. As such, the ACP recommends against the use of mechanical prophylaxis with graduated compression stockings for the prevention of VTE.⁷⁵

The ninth edition guidelines of the ACCP address prophylactic treatment of VTE in nonorthopedic and orthopedic surgical patients.⁷⁶ Recommendations for or against anticoagulation or mechanical prophylaxis in general surgery patients are based on the type of surgery and individual thrombotic and bleeding risk assessment. Major orthopedic surgery has an estimated risk of symptomatic VTE of 4.3% in 35 days in patients receiving no prophylaxis.⁷⁶ Therefore, patients undergoing total hip arthroplasty, total knee arthroplasty, or hip fracture surgery who are not at increased risk of bleeding, should receive either thromboprophylaxis with LMWH (or alternative pharmacologic anticoagulation) or mechanical prophylaxis. Extended use of LMWH in the outpatient setting (up to 35 days post-surgery) is recommended based on data from three systematic reviews including seven controlled trials showing a decrease in symptomatic VTE of 9 per 1,000 patients without an appreciable increase in major bleeding.⁷⁶

Initial Treatment for Acute Venous Thromboembolism

Anticoagulation is the essential primary treatment and prophylaxis for VTE. Mechanical barrier devices (IVC filters) may be used in certain circumstances in lieu of anticoagulation, or as an adjunctive measure. Fibrinolytic therapy may also be beneficial in select patients as an adjunct to anticoagulation.

The ninth ACCP Guidelines for anticoagulation and treatment of VTE⁷⁷ suggest that a DVT or PE should initially be treated with LMWH, fondaparinux, or rivaroxaban, that LMWH and fondaparinux are preferable to UFH, thrombolytic therapy may be

advisable in the presence of hemodynamic compromise, and anticoagulation should be utilized for at least 3 months, in the setting of a provoked event, and longer for unprovoked events. The purpose of anticoagulation is to prevent additional clots from developing, to immediately stop further propagation of the existing clot, and to permit endogenous fibrinolysis to begin to dissolve the clot physiologically. If warfarin is used, it should be started after initiation of heparin, LMWH, or fondaparinux, typically at a 5-mg daily dose for most adults. A double-blind randomized study, however, showed that warfarin at 10 mg for 2 days followed by dose adjustment determined by a nomogram was also safe and effective.⁷⁸ Parenteral anticoagulation is continued until there are successive INR values of between 2 and 3 after adjustment of the warfarin dose. In the absence of PE, or with PE that is not complicated by cardiovascular compromise, or other reasons to admit the patient, this can typically be accomplished as an outpatient. Subcutaneous (SC) UFH versus LMWH was evaluated in the Fixed Dose (FIDO) study, a randomized trial of 708 patients, showing that fixed-dose SQ UFH is as effective and safe as LMWH in patients with acute VTE and is suitable for outpatient treatment.⁷⁹ LMWH and fondaparinux are less likely than UFH to cause HIT, however. LMWH requires monitoring of anti-Xa levels in patients at high or low extremes of weight, or who have renal failure, or are pregnant. LMWH and fondaparinux are relatively contraindicated in renal failure and should be used with extreme caution (using dose reduction and monitoring), as the kidneys excrete LMWH. UFH may be preferred in a patient at risk of bleeding, due to the ability to neutralize with protamine and its shorter duration of effect. See Chapter 23 for discussion of new oral anticoagulant medications.

Extended Treatment to Prevent Recurrent Venous Thromboembolism

Warfarin has been the only oral anticoagulant available for long-term anticoagulation until recently. Despite the introduction of new oral anticoagulants such as rivaroxaban, dabigatran, and apixaban that have indications to prevent DVT or stroke in atrial fibrillation, it remains a useful drug due to its lower cost and established risk–benefit profile. Dalteparin sodium is FDA approved for the extended treatment and subsequent prevention of recurrent symptomatic VTE in patients with cancer. Unique risks of long-term LMWH use include osteopenia and HIT (the latter risk is uncommon in this setting).

The ideal intensity of warfarin anticoagulation for long-term (indefinite duration) therapy to prevent recurrent VTE has been addressed in two well-designed trials yielding conflicting results. The PREVENT trial concluded that low-intensity anticoagulation with the target INR of 1.5 to 2.0 was successful in substantially reducing recurrent VTE risk.⁸⁰ In a randomized, two-arm study of standard warfarin

anticoagulation in Canada to achieve an INR between 2 and 3 versus a low-intensity INR arm with target INR between 1.5 and 2.0, the standard dosing regimen was more than 60% effective ($P = .03$) than low-intensity warfarin anticoagulation in reducing the cumulative probability of recurrent thromboembolism.⁸¹ There was no difference in bleeding complications between the two dosing intensities. The differences between the two studies may be related to trial design. For instance, the Canadian trial, in contrast to the PREVENT study, included cancer patients, who more likely would experience warfarin resistance.

At least 3 months of anticoagulation are recommended for idiopathic (unprovoked) DVT, and if there is no contraindication to anticoagulation, it should be continued indefinitely.⁷⁷ Similarly, life-threatening PE requires indefinite anticoagulation, unless the risk of life-threatening bleeding on anticoagulation exceeds the risk of a fatal PE; in this rare circumstance, an IVC filter may be utilized. The PREVENT trial corroborated that patients with idiopathic VTE have a high incidence of recurrent VTE and benefit from long-term anticoagulation.⁸⁰ As previously discussed, the APA syndrome requires prolonged anticoagulation. Individuals with cancer remain hypercoagulable as long as the malignancy is present. Patients with transient risk factors (i.e., trauma) usually require anticoagulation for 3 to 6 months. Patients with increased D-dimers, elevated FVIII activities, or evidence of significant residual DVT at 1 month after discontinuing anticoagulation are at increased risk of rethrombosis, and consideration should be given to resumption of long-term anticoagulation. The D-dimer can also be measured intermittently after discontinuation of therapy to assess for the risk of rethrombosis.²⁷

Inferior Vena Cava Filters in the Treatment of Deep Venous Thrombosis or Pulmonary Embolus

IVC filters are placed to prevent large clots in the lower extremities from embolizing to the pulmonary circulation. Major reasons for the placement of an IVC filter include strong contraindications to the use of anticoagulants, intolerance to or noncompliance with anticoagulants, and recurrent PE despite adequate systemic anticoagulation. All of these factors are relative indications for use of an IVC filter, and the decision to place one is not to be taken lightly. A randomized study revealed that, in the short term, an IVC filter decreases the incidence of pulmonary embolus from 4.8% to 1.1%, but by 2 years, the rate of recurrent DVT was 20.8% in the IVC filter group versus 11.6% in the nonfilter group, and overall mortality was not significantly different.⁸² Retrievable filters have the advantage that they may be removed after the risk of PE has passed, but they may be more prone to migration than permanent filters.

Fibrinolytic Therapy

Fibrinolytic therapy has typically been reserved for patients with massive PE associated with hemodynamic compromise.⁷⁷ Patients with systolic BP < 90 mm Hg, or a BP drop > 40 mm Hg for > 15 minutes, not caused by cardiac arrhythmias, sepsis, or hypovolemia, may benefit from thrombolytic therapy with improved survival.⁸³ Clinical studies do not show a survival advantage for thrombolytic agents in PE. Currently, tPA is the only fibrinolytic agent commonly available, and it has the advantage of a short half-life, and relative specificity for fibrin clot (as opposed to fibrinogen) when compared to urokinase or streptokinase. Fibrinolytic therapy is being investigated in the ongoing ATTRACT⁸⁴ and recently reported CAVENT⁸⁵ trials, in selected patients with massive iliac–femoral DVT. The CAVENT trial randomized 209 patients with iliofemoral DVT to anticoagulation with (101 patients) or without (108 patients) additional catheter-directed thrombolysis (CDT) with tPA. At 24 months, the patients who were given CDT had 41% incidence of PTS, versus 56% of the control group ($P = .047$). Patency of the iliofemoral system was seen in 66% of CDT patients versus 47% of the control group ($P = .012$). There was additional bleeding associated with CDT that included three major and five clinically significant bleeding episodes, consistent with typical estimates of bleeding risk from fibrinolytic therapy of ~8%.⁸⁶

At this time, catheter-directed fibrinolytic therapy with low doses of tPA may be considered for use in patients with DVT that is not responding to standard anticoagulation, and adjunctive measures such as stenting or balloon dilatation of venous segments with strictures may also be considered, but this is not yet standard practice.^{41,42,77}

References

1. The surgeon general's call to action to prevent deep venous thrombosis and pulmonary embolism, 2008. <https://www.ncbi.nlm.nih.gov/books/NBK44178/>. Accessed February 1, 2018.
2. Silverstein M, Heit J, Mohr D, et al. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Int Med. 1998;158:585–593.
3. Heit J. The epidemiology of venous thromboembolism in the community. Arterioscler Thromb Vasc Biol. 2008;28:370–372.
4. Pengo V, Lensing AW, Prins MH, et al. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. N Engl J Med. 2004;350:2226–2257.
5. Becattini C, Agnelli G, Pesavento R, et al. Incidence of chronic thromboembolic pulmonary hypertension after a first episode of pulmonary embolism. Chest. 2006;130:172–175.
6. Isma N, Svensson PJ, Gottsäter A, et al. Upper extremity deep venous thrombosis

- in the population-based Malmö thrombophilia study (MATS). Epidemiology, risk factors, recurrence risk, and mortality. *Thromb Res.* 2010;125(6):e335–338.
7. Prandoni P, Polistena P, Bernardi E, et al. Upper extremity deep vein thrombosis: risk factors, diagnosis, and complications. *Arch Intern Med.* 1997;157:57–62.
 8. Velmahos GC, Spaniolas K, Tabbara M, et al. Pulmonary embolism and deep venous thrombosis in trauma. Are they related? *Arch Surg.* 2009;144:928–932.
 9. Anderson FA, Wheeler HB, Goldberg RJ, et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. *Arch Intern Med.* 1991;151:933–938.
 10. Kahn SR. Measurement properties of the Villalta scale to define and classify the severity of the post-thrombotic syndrome. *J Thromb Haemost.* 2009;7:884–888.
 11. Ashrani A, Heit J. Incidence and cost burden of post-thrombotic syndrome. *J Thromb Thrombol.* 2009;28(4):465–476.
 12. Brandjes DP, Büller HR, Heijboer H, et al. Randomised trial of effect of compression stockings in patients with symptomatic proximal-vein thrombosis. *Lancet.* 1997;349(9054):759–762.
 13. Masuda EM, Kessler DM, Kistner RL, et al. The natural history of calf vein thrombosis: lysis of thrombi and development of reflux. *J Vasc Surg.* 1998;28:67–74.
 14. Lohr JM, James KV, Deshmukh RM, et al. Calf vein thrombi are not a benign finding. *Am J Surg.* 1995;170:86–90.
 15. Masuds EM, Kistner RL, Musikasinthorn C, et al. The controversy of managing calf vein thrombosis. *J Vasc Surg.* 2012;55:550–561.
 16. Segal J, Eng J, Tamariz L, et al. Review of the evidence on diagnosis of deep venous thrombosis and pulmonary embolism. Clinical practice guideline. American College of Physicians. *Ann Fam Med.* 2007;5:63–73.
 17. Loud PA, Katz DS, Klipstein DL, et al. Combined CT venography and pulmonary angiography in suspected thromboembolic disease: diagnostic accuracy for deep venous evaluation. *Am J Roentgenol.* 2000;174:61–65.
 18. Cham MD, Yankelvitz DF, Shaham D, et al. Deep venous thrombosis: detection by using indirect CT venography. The pulmonary angiography-indirect CT venography cooperative group. *Radiology.* 2000;216:744–751.
 19. Fraser DGW, Moody AR, Morgan PS, et al. Diagnosis of lower-limb deep venous thrombosis: a prospective blinded study of magnetic resonance direct thrombus imaging. *Ann Intern Med.* 2002;136:89–98.
 20. Ono A, Murase K, Taniguchi T, et al. Deep venous thrombosis: diagnostic value of non-contrast-enhanced MR venography using electrocardiography-triggered three-dimensional half-Fourier FSE. *Magen Reson Med.* 2010;64(1):88–97.
 21. Kay J, Czijak L. Gadolinium and systemic fibrosis: guilt by association. *Ann*

- Rheum Dis. 2010;69:1895–1897.
22. Aguilar C, Martinez A, Martinez A, et al. Diagnostic value of d-dimer in patients with a moderate pretest probability of deep venous thrombosis. *Br J Haematol*. 2002;118:275–277.
 23. Kulstad EB, Kulstad CD, Lovel EO. A rapid quantitative turbimetric D-dimer assay has high sensitivity for detection of pulmonary embolism in the ED. *Am J Emerg Med*. 2004;22:111–114.
 24. Rathbun SW, Whitsett TL, Raskob GE. Negative D-dimer result to exclude recurrent deep venous thrombosis: a management trial. *Ann Int Med*. 2004;141:839–846.
 25. Verhovsek M, Douketis JD, Yi Q, et al. Systematic review: D-dimer to predict recurrent disease after stopping anticoagulant therapy for unprovoked venous thromboembolism. *Ann Int Med*. 2008;149(7):481–490.
 26. Palareti G, Cosmi B, Legnani C, et al. D-dimer testing to determine the duration of anticoagulation therapy. *N Engl J Med*. 2006;355:2797.
 27. Cosmi B, Legnani C, Tosetto A, et al. Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study. *Blood*. 2010;115(3):481–488.
 28. Amitrano L, Brancaccio V, Guardascione MA, et al. Inherited coagulation disorders in cirrhotic patients with portal vein thrombosis. *Hepatology*. 2000;31:345–348.
 29. Denninger M-H, Chait Y, Casadevall N, et al. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. *Hepatology*. 2000;31:587–591.
 30. Jansen HLA, Meinardi JR, Vleggar FP, et al. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. *Blood*. 2000;96:2364–2368.
 31. Warkentin T. How I diagnose and manage HIT. *ASH Education Book. Hematology Am Soc Hematol Educ Program*. 2011;2011:143–149.
 32. Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol*. 2003;121:535–555.
 33. Cuker A, Arepally G, Crowther MA, et al. The HIT Expert Probability (HEP) Score: a novel pre-test probability model for heparin-induced thrombocytopenia based on broad expert opinion. *J Thromb Haemost*. 2010;8:2642–2650.
 34. Papadopoulos S, Flynn JD, Lewis DA. Fondaparinux as a treatment option for heparin-induced thrombocytopenia. *Pharmacotherapy*. 2007;27:921–926.
 35. Pendleton R, Wheeler MM, Rodgers GM. Argatroban dosing of patients with heparin-induced thrombocytopenia and an elevated aPTT due to antiphospholipid

- antibody syndrome. *Ann Pharmacother*. 2006;40:972–976.
36. Gosselin RC, King JH, Janatpour KA, et al. Comparing direct thrombin inhibitor using aPTT, ecarin clotting times, and thrombin inhibitor management testing. *Ann Pharmacother*. 2004;38:1383–1388.
37. Singhal R, Brimble KS. Thromboembolic complications in the nephrotic syndrome: pathophysiology and clinical management. *Thromb Res*. 2006;118:397–407.
38. Mahmoodi BK, ten Kate MK, Waanders F, et al. High absolute risks and predictors of venous and arterial thromboembolic events in patients with nephrotic syndrome: results from a large retrospective cohort study. *Circulation*. 2008;117:224–230.
39. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448–454.
40. Ruiz-Irastorza G, Crowther M, Branch W, et al. Antiphospholipid syndrome. *Lancet*. 2010;376(9751):1498–1509.
41. Chang R, Horne MK, Shawker TH, et al. Low-dose, once-daily, intraclot injections of alteplase for treatment of acute deep venous thrombosis. *J Vasc Interv Radiol*. 2011;22:1107–1116.
42. Lozier JN, Cullinane A, Nhgiem K, et al. Biochemical dynamics relevant to the safety of low-dose, intraclot alteplase for deep vein thrombosis. *Trans Res*. 2012;160:217–222.
43. Ruiz-Irastorza G, Cuadrado MJ, Ruiz-Arruza I, et al. Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients: report of a task force at the 13th International Congress on Antiphospholipid Antibodies. *Lupus*. 2011;20:206–218.
44. Saltzman HA, Alavi A, Greenspan RH, et al. Value of the ventilation/ perfusion in acute pulmonary embolism: results of the Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED). *JAMA*. 1990;263:2753–2759.
45. Tafur AJ, Kalsi H, Wysokinski WE, et al. The association of active cancer with venous thromboembolism location: a population-based study. *Mayo Clin Proc*. 2011;86:25–30.
46. Heit JA. The epidemiology of venous thromboembolism in the community: implications for prevention and management. *J Thromb Thrombolysis*. 2006;21:23–29.
47. Lee AYY, Levine MH, Baker RI, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *N Engl J Med*. 2003;349:146–153.
48. Meyer G, Marjanovic Z, Valcke J, et al. Comparison of low molecular weight heparin and warfarin for the secondary prevention of venous thromboembolism in patients with cancer: a randomized controlled study. *Arch Intern Med*.

- 2002;162(15):1729–1735.
49. Kuderer NM, Ortel TL, Francis CW. Impact of venous thromboembolism and anticoagulation on cancer and cancer survival. *J Clin Oncol*. 2009;27:4902–4911.
 50. Hall C, Richards S, Hillmen P. Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH). *Blood*. 2003;102:3587–3591.
 51. van Bijnen ST, van Heerde WL, Muus P. Mechanisms and clinical implications of thrombosis in paroxysmal nocturnal hemoglobinuria. *J Thromb Haemost*. 2012;10(1):1–10. doi:10.1111/j.1538-7836.2011.04562.x.
 52. Hillmen P, Muus P, Duhrsen U, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2007;110:4123–4128.
 53. Geerts WH, Bergqvist D, Pineo GF, et al. Prevention of venous thromboembolism. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest*. 2008;133:381S–453S.
 54. Ageno W, Squizzato A, Garcia D, et al. Epidemiology and risk factors of venous thromboembolism. *Semin Thromb Hemost*. 2006;32:651–658.
 55. VTE Prophylaxis for Laparoscopic Surgery Guidelines: An Update. <https://www.sages.org/publications/guidelines/guidelines-for-deep-venous-thrombosis-prophylaxis-during-laparoscopic-surgery/>. Accessed February 1, 2018.
 56. SAGES Guidelines Committee. Guidelines for deep venous thrombosis prophylaxis during laparoscopic surgery. *Surg Endosc*. 2007;21:1007–1009.
 57. Spivak JL. Polycythemia vera: myths, mechanisms, and management. *Blood*. 2002;117:47.
 58. Harrison CN, Campbell PJ, Buck G, et al. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *NEJM*. 2005;353:33–45.
 59. Kamphuisen PW, Eikenboom JCJ, Bertina RM. Elevated factor VIII levels and the risk of thrombosis. *Arterioscler Thromb Vasc Biol*. 2001;21:731–738.
 60. Rosendaal FR. High levels of factor VIII and venous thrombosis. *Thromb Haemost*. 2000;83:1–2.
 61. Lowe GDO, Woodward M, Vessey MP, et al. Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45–64 years: relationships to hormone replacement therapy. *Thromb Haemost*. 2000;83:530–535.
 62. Daly E, Vessey MP, Hawkins MM, et al. Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet*. 1996;348:977–980.
 63. Meijers JCM, Tekelenburg WLH, Bouma BN, et al. High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med*. 2000;342:696–701.
 64. Ridker PM, Miletich JP, Hennekens CH, et al. Ethnic distribution of factor V

- Leiden in 4047 men and women. Implications for venous thromboembolism screening. *JAMA*. 1997;277:1305–1307.
65. Williamson D, Brown K, Luddington R, et al. Factor V Cambridge: a new mutation (Arg306→Thr) associated with resistance to activated protein C. *Blood*. 1998;91:1140–1144.
 66. Chan WP, Lee CK, Kwong YL, et al. A novel mutation of Arg 306 of factor V gene in Hong Kong Chinese. *Blood*. 1998;91:1135–1139.
 67. Poort SR, Rosendaal FR, Reitsma PH, et al. A common genetic variation in the 3N-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88:3698–3703.
 68. Dahlback B, Stenflo J. High molecular weight complex in human plasma between vitamin K-dependent protein S and complement component C4b-binding protein. *Proc Natl Acad Sci U S A*. 1981;78:2512–2516.
 69. Zwicker J, Bauer KA. Thrombophilia. In: Kitchens CS, Alving BM, Kessler CM, eds. *Consultative Thrombosis and Hemostasis*. Philadelphia, PA: W.B. Saunders Company; 2002:181–196.
 70. Rees MM, Rodgers GM. Homocysteinemia: association of metabolic disorder with vascular disease and thrombosis. *Thromb Res*. 1993;71:337–359.
 71. Mudd SH, Skovby F, Levy HL. The natural history of homocystinuria due to cystathionine β -synthase deficiency. *Am J Hum Genet*. 1985;37:1–35.
 72. Den Heijer M, Koster T, Blom HK, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med*. 1996;334:759–762.
 73. Ray JG, Kearon C, Yi Qilong, et al. Homocysteine-lowering therapy and risk for venous thromboembolism: a randomized trial. *Ann Intern Med*. 2007;146:761–767.
 74. Ray JG, Shmorgun D, Chan WS. Common C677T polymorphism of the methylenetetrahydrofolate reductase gene and the risk of venous thromboembolism: meta-analysis of 31 studies. *Pathophysiol Haemost Thromb*. 2002;32:51–58.
 75. Qaseem A, Chou R, Humphrey LL, et al. Venous thromboembolism prophylaxis in hospitalized patients: a clinical practice guideline from the American College of Physicians. *Ann Intern Med*. 2011;155:625–632.
 76. Guyatt GH, Akl EA, Crowther M, et al. Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141:7S–47S.
 77. Kearon C, Akl EA, Comerata AJ, et al. Antithrombotic therapy for VTE disease antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e419S–e494S.

78. Kovacs MJ, Roger M, Anderson DR, et al. Comparison of 10-mg and 5-mg warfarin initiation nomograms together with low-molecular-weight-heparin for outpatient treatment of acute venous thromboembolism. A randomized, double-blind, controlled trial. *Ann Intern Med.* 2003;138:714–719.
79. Kearon C, Ginsberg JS, Julian JA. Comparison of fixed-dose weight-adjusted unfractionated heparin and low-molecular-weight heparin for acute treatment of venous thromboembolism. *JAMA.* 2006;296:935–942.
80. Ridker PM, Goldhaber SZ, Danielson E, et al. Long-term, low intensity warfarin for the prevention of recurrent venous thromboembolism. *N Engl J Med.* 2003;348:1425–1434.
81. Kearon C, Ginsberg JS, Kovacs MJ, et al. Comparison of low-intensity warfarin therapy with conventional-intensity warfarin therapy for long-term prevention of recurrent venous thromboembolism. *N Engl J Med.* 2003;349:631–639.
82. Decousus H, Leizorovicz A, Parent F, et al. A clinical trial of vena caval filters in the prevention of pulmonary embolism in patients with proximal deep-vein thrombosis. *N Engl J Med.* 1998;338:409–415.
83. European Society of Cardiology: Guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J.* 2000;21:1301–1336.
84. Comerota AJ. The ATTRACT Trial: rationale for early intervention for iliofemoral DVT. *Perspect Vasc Surg Endovasc Ther.* 2009;21:221–225.
85. Enden T, Haig Y, Klow N-E, et al. Long-term outcome after additional catheter-directed thrombolysis versus standard treatment for acute iliofemoral deep vein thrombosis (the CaVenT study): a randomised controlled trial. *Lancet.* 2012;379:31–38.
86. Vedantham S, Millward SF, Cardella JF, et al. Society of Interventional Radiology position statement: treatment of acute iliofemoral deep vein thrombosis with use of adjunctive catheter-directed intrathrombus thrombolysis. *J Vasc Interv Radiol.* 2006;17:613–616.

Consultations in Anticoagulation

Daisuke Araki and Jay N. Lozier

This chapter provides guidelines for the treatment of venous thromboembolism (VTE) in patients who require special consideration, such as those who have underlying cancer, malignancy, or who are pregnant. The chapter also discusses the use of inferior vena cava (IVC) filters, the prevention, diagnosis, and treatment of postthrombotic syndrome (PTS), and anticoagulant drugs with novel mechanisms that are now in development.

PROPHYLAXIS AND TREATMENT OF VENOUS THROMBOEMBOLISM IN THE PATIENT WITH CANCER IN SPECIFIC CLINICAL SETTINGS

Patients with malignancy have an increased risk for VTE due to multiple factors: Hypercoagulability resulting from increased production and release of microparticles containing procoagulants such as tissue factor; vessel wall damage, impaired blood flow (i.e., stasis) from extrinsic compression, prolonged immobility, anticancer therapy including cytotoxic chemotherapy, certain antiangiogenic agents, or hormonal therapy, and the increasing use of long-term indwelling devices, such as central venous catheters. Tumor angiogenesis, progression, growth, and the metastatic process are enhanced by, and depend on activation of blood coagulation. P-selectin, a cell adhesion molecule has also been identified as a risk factor for recurrent VTE and can be used as a predictive parameter for the development of VTE in cancer patients.¹ Similarly, elevated D-dimer and prothrombin fragment 1 + 2 levels independently predict development of VTE in cancer patients.² As long as the cancer is active, the increased risk for VTE is present; the cancers most commonly associated with VTE are carcinomas of the pancreas, stomach, kidney, lung, ovary, and bladder, certain hematologic malignancies, cancers of the testis and gliomas of the brain. Adenocarcinomas appear to be associated with a higher risk than squamous cell

cancers.

Studies performed during the current decade have demonstrated that low-molecular-weight heparin (LMWH) is more effective than oral anticoagulants in reducing the risk of recurrent VTE without increasing the risk of bleeding in patients with cancer and acute VTE. Products such as dalteparin, enoxaparin, nadroparin, and tinzaparin, as well as the synthetic factor Xa inhibitor, fondaparinux are approved by the U.S. Food and Drug Administration (FDA) for the prophylaxis and treatment of VTE. In general, unfractionated heparin (UFH), LMWH, fondaparinux and oral anticoagulants are the mainstay of therapy. Because LMWH undergoes renal excretion, patients with kidney impairment who receive LMWH should be monitored by measurement of the anti-factor Xa activity. The creatinine clearance should be estimated (or calculated) before initiation of LMWH in elderly patients, as they may have renal dysfunction despite having normal creatinine values. Specific dosing recommendations for enoxaparin in patients with severe renal insufficiency and/ or low body weight are presented in [Table 23.1](#). Monitoring of anti-Xa levels should also be used in severely obese patients (body mass index [BMI] ≥ 40) who receive therapeutic doses of LMWH and should be considered for those who are obese (BMI ≥ 30), especially if the patient has moderate to severe renal insufficiency (creatinine clearance less than 60 mL/ min).³ LMWH has close to 100% bioavailability but is primarily distributed in the plasma and highly vascular tissues with little distribution in fat tissue. As obese patients have a lower proportion of lean body mass compared with their total body weight, concern for potential overdosing of LMWH in obese patients has led some clinicians capping the dose at a maximum absolute dose, typically at the maximum prefilled syringe dose.⁴

Table 23.1 Enoxaparin and Fondaparinux Dosing in Patients With Renal Insufficiency per Manufacturer

Anticoagulant	CCr	Dose
Enoxaparin	≥ 30 mL/ min	40 mg sc daily for prophylaxis 1 mg/ kg sc BID or 1.5 mg/ kg sc daily for treatment
	<30 mL/ min	30 mg sc daily for prophylaxis 1 mg/ kg subcutaneous every 24 hours for treatment
Fondaparinux	≥ 30 mL/ min	2.5 mg sc daily for prophylaxis 7.5 mg sc daily for treatment
	<30 mL/ min	Contraindicated

In patients weighing <50 kg, the use of LMWHs with caution is recommended and the use of fondaparinux is not recommended because only limited or no data are available.

BID, twice daily; CCr, creatinine clearance rate; LMWH, low-molecular-weight heparin; sc, subcutaneous.

The target peak anti-factor Xa levels (measured four hours after injection) for patients who are treated with LMWH vary according to the product. The target

therapeutic range for enoxaparin is 0.6 to 1.2 U/ mL for twice-daily dosing and 1 to 2 U/ mL for once daily dosing; for dalteparin the target range is 0.5 to 1.5 U/ mL (see [Table 23.2](#)). Because fondaparinux is produced by complete chemical synthesis and its structure is completely defined, it is dosed on the basis of mass rather than anti-Xa activity. The assay for fondaparinux is often reported in terms of its concentration in mass/ volume (e.g., ng/ mL).

Primary Prophylaxis in Cancer Patients Undergoing Surgical Intervention

VTE is a common complication of cancer surgery and the most common cause of death at 30 days after surgery in the [@RISTOS](#) prospective observational study of cancer surgery patients.⁵ Prophylaxis should now be provided routinely to postoperative surgical patients, especially those with underlying cancer. Two randomized controlled trials have demonstrated that extending deep venous thrombosis (DVT) prophylaxis from 1 to 4 weeks reduces the incidence of VTE.^{6,7} Extended (up to 4 weeks) VTE prophylaxis is recommended for high-risk cancer surgery patients which include a previous episode of VTE, anesthesia times longer than 2 hours, advanced stage disease, perioperative bed rest \geq 4 days and patient age \geq 60 years.⁵

Laparoscopic surgery is rapidly becoming a common method for tumor resection. It is unclear how the recommendations developed for typical, open procedures should be applied to laparoscopic surgical procedures. Laparoscopic surgery offers the advantage of less tissue disruption, quicker recovery times, and shorter periods of postoperative immobilization. Intuitively, the reduction in tissue damage and the possibility of faster mobilization predicts lower risk of thromboembolic complications. Conversely, patients undergoing laparoscopic procedures are subjected to increased venous stasis as a result of the induction of pneumoperitoneum and prolonged use of the reverse Trendelenburg position to visualize and manipulate internal organs.⁸ The American College of Chest Physicians' clinical practice guidelines (eighth edition) recommend against routine prophylaxis (other than early and frequent ambulation) in patients undergoing laparoscopic surgery without thromboembolic risk factors, and recommends mechanical or pharmacologic prophylaxis in patients with any thromboembolic risk factors.⁹ The Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) guidelines for DVT prophylaxis during laparoscopic surgery stratifies inpatients into low, moderate, and high-risk groups for thrombosis on the basis of a risk score imputed from the type of procedure and patient risk factors.^{10,11} Procedure-related risk factors include procedure lasting over one hour, and pelvic procedures. Patient-related factors include age $>$ 40 years, immobility, malignancy, thrombophilic states (protein C, protein S, or ATIII deficiency), obesity, peripartum state (or use of estrogens), heart failure, renal failure, varicose veins, inflammatory states, or infection. In the lowest risk group

(procedure <60 minutes in patients with no risk factors) elastic stockings and early ambulation is all that is recommended, and UFH or LMWH is optional. In the moderate risk group (one patient risk factor in a procedure of <60 minutes, or any procedure >60 minutes with no patient risk factors) pneumatic compression devices or prophylactic heparin or LMWH are recommended. In the high risk group (two or more risk factors in procedures >60 minutes) a combination of serial compression devices and prophylactic UFH or LMWH are recommended.^{10,11}

Table 23.2 General Approaches to Therapeutic Anticoagulants Dosing and Monitoring

Agent	Dosing	Monitoring
LMWH		
Enoxaparin	1 mg/ kg sc every 12 hours or 1.5 mg/ kg daily	Anti-Xa level ^a 0.6–1.2 U/ mL (every 12 hours) Anti-Xa level 1–2 units/ mL (daily)
Dalteparin	200 units/ kg sc daily	Anti-Xa level 0.5–1.5 U/ mL
Tinzaparin	175 units/ kg sc daily	^b
Nadroparin	171 units/ kg sc daily	Anti-Xa level 1.2–1.8 U/ mL
Fondaparinux	5 mg (<50 kg); 7.5 mg (50–100 kg); 10 mg (>100 kg)	^c
UFH	80 units/ kg loading dose, then 18 units/ kg per hour infusion	aPTT of 2–2.5 × control or anti-Xa level 0.3–0.7 U/ mL
Warfarin	Initially, 5 mg daily, the titrate to target INR after heparinization	INR 2.0–3.0 with the exception of INR 2.5–3.5 for mechanical valve and INR 3.0–4.0 for antiphospholipid syndrome with arterial disease or recurrent events ⁶¹

^aAnti-Xa level for LMWH and fondaparinux is a peak level, 4 h after SC injection.

^bUnestablished, but may be similar to enoxaparin single dose daily: anti-Xa level 1–2 U/ mL.

^cAnti-Xa activity of fondaparinux has to be measured when fondaparinux is used as the calibrator. The activity of fondaparinux is expressed in milligrams of the fondaparinux and cannot be compared with activities of UFH or LMWH.

aPTT, activated partial thromboplastin time; INR, international normalized value; LMWH, low-molecular-weight heparin; sc, subcutaneous injection; UFH, unfractionated heparin.

Once daily LMWH appears to be as safe and effective as multiple daily injections of UFH and provides convenience as well as a better quality of life for the patient.^{5,6} In the Clinical Center at the National Institutes of Health, enoxaparin is the LMWH most commonly employed. However, fondaparinux or other LMWH, such as nadroparin, dalteparin, ardeparin, tinzaparin, and reviparin may be considered equivalent. The administration of warfarin at a low, fixed dose (e.g., 1 mg/ day) has not been shown to be of value for VTE prophylaxis, and is not recommended.

Primary Venous Thromboembolism Prophylaxis in Cancer Patients Receiving Chemotherapy, Hormonal, and/ or Anti-Angiogenic Treatment

Patients with cancer who are undergoing treatment should be considered for VTE prophylaxis if they have one or more of the following: A history of VTE, a large mass compressing a major vessel, or treatment which includes tamoxifen/ raloxifene, diethylstilbestrol or chemotherapy, especially use of bevacizumab, thalidomide-or lenalidomide-based combination regimens, particularly those given in combination with high-dose dexamethasone.¹² A recent clinical trial of patients with advanced cancer of lung, gastrointestinal, pancreatic, breast, ovarian, or head and neck undergoing chemotherapy (PROTECHT trial) showed a statistically significant ($P = .02$) decrease in thromboembolic events from 3.9% to 2.0% in the groups receiving prophylactic LMWH (i.e., nadroparin) or placebo, respectively.¹³ VTE prophylaxis in cancer patients undergoing treatment should be individualized; if prophylaxis is chosen, LMWH (i.e., enoxaparin 40 mg/ day) or UFH (low dose) should be considered (see Table 23.3). Aspirin prophylaxis (81 to 325 mg daily) is an option for patients receiving thalidomide or lenalidomide for multiple myeloma.¹² In chronic lymphocytic leukemia (CLL) patients treated with lenalidomide, it was shown that tumor necrosis factor α (TNF α), C-reactive protein, factor VIII, thrombomodulin, and soluble vascular cell adhesion molecule-1 (sVCAM1) were significantly increased from baseline after initiation of treatment ($P < .001$), and TNF α and sVCAM levels were more elevated in patients who subsequently had DVTs, suggesting inflammation, and endothelial cell dysfunction played an important role in VTE risk.¹⁴ Thus, anti-inflammatory effects of aspirin may contribute to prophylaxis against VTE in addition to antiplatelet effects otherwise not thought to be important for VTE prophylaxis.

Table 23.3 General Approaches to Prophylactic Anticoagulant Dosing

Agent	Dosing
Enoxaparin	40 mg sc daily
Dalteparin	5,000 units sc daily
Tinzaparin	4,500 units sc daily or 75 units/ kg sc daily
Nadroparin	2,850 units sc daily
Fondaparinux	2.5 mg sc daily
UFH	5,000 unit sc 3 times daily
Warfarin	Initial 5 mg daily after heparization, then dose adjusted to INR 2.0–3.0

INR, international normalized value; sc, subcutaneous injection; UFH, unfractionated heparin.

Primary Prophylaxis in Cancer Patients Who are Immobilized/

Hospitalized

VTE is a fairly common event in hospitalized cancer patients. A retrospective study of more than 66,000 hospitalized neutropenic adult cancer patients showed that 3% to 12% of these patients, depending on the type of malignancy, experienced VTE during their first hospitalization.¹⁵ Primary prophylaxis is effective in hospitalized medical patients, who undergo a threefold reduction in VTE when treated with enoxaparin at a daily dose of 40 mg, compared to controls receiving no treatment. This conclusion is derived from the MEDENOX (Prophylaxis in Medical Patients with Enoxaparin) trial,¹⁶ a double-blind randomized study of 1,102 patients with acute medical illnesses who received prophylaxis against VTE (14.9% of these patients had cancer or a history of cancer). Patients were randomized to one of three groups that would receive for 6 to 14 days the subcutaneous daily administration of 40 mg of enoxaparin, 20 mg of enoxaparin, or placebo. The primary outcome was VTE during the ensuing 3 months. The outcome favored prophylactic treatment with subcutaneous enoxaparin at a dose of 40 mg daily. Adverse events, which included hemorrhage, local reaction, thrombocytopenia, and death from any cause, were not different between the groups receiving enoxaparin and placebo. The weakness of this study was that a more appropriate control group should have been one receiving UFH. Therefore, it has been recommended that all hospitalized patients with cancer should receive anticoagulation therapy in the absence of contraindications. A subsequent randomized trial, the LIFENOX (study to evaluate the mortality reduction of enoxaparin in hospitalized acutely ill medical patients receiving enoxaparin) trial, indicates, however, that all-cause mortality is unchanged in medical patients undergoing thromboprophylaxis with LMWH.¹⁷ UFH is the agent of choice for thromboprophylaxis in hospitalized patients with a creatinine clearance of <30 mL/ min. A reduced enoxaparin dose of 30 mg daily can also be used in this situation and preferred if prolonged use is required. Occasional monitoring of anti-Xa levels may be appropriate in the setting of renal failure to prevent overdosage and bleeding.

Primary Prophylaxis in Patients With Brain Metastases and Primary Brain Tumors

The risk of VTE in patients with primary or metastatic brain tumors is increased for various reasons including expression of tissue factor¹⁸ and plasminogen activator inhibitor-1 (PAI-1)¹⁹ by gliomas, immobility due to paresis of limbs affected by the brain tumor or metastasis. In addition, the use of antiangiogenic agents such as Avastin (bevacizumab) may further increase the risk of arterial thrombosis and ironically increase the risk of bleeding.^{20,21} The challenge in using anticoagulation is balancing the risk of thrombosis with the risk of precipitating intracranial hemorrhage. Studies have shown both increased risk as well as benefit with the use of LMWH prophylaxis in the

nonsurgical setting.²²

For patients undergoing neurosurgery, the recommended prophylaxis is to initiate LMWH or low dose UFH 24 hours postoperatively, in combination with mechanical thromboprophylaxis, such as graduated compression stocking (GCS) and/ or intermittent pneumatic compression. This is associated with minimal risk for bleeding.²³ Initiation of prophylaxis before neurosurgery in patients with brain tumors may be associated with increased risk for intracranial hemorrhage, as shown in one such study terminated early due to increased bleeding.²⁴

Treatment of Venous Thromboembolism in Patients With Primary Brain Tumors or Brain Metastases

Patients with primary brain tumors or metastases who develop VTE can be treated with full doses of UFH or LMWH.²⁵ Here the use of UFH has the potential advantage over LMWH of a short half-life and the ability to administer protamine to neutralize it in the event of hemorrhage or overdosage. It may be advisable to forego the use of a bolus at the outset of treatment and simply start an infusion and increase the rate with frequent monitoring to prevent inadvertent overdosage. It is preferable to use anti-Xa activity monitoring (rather than the activated partial thromboplastin time [aPTT]) in such a setting due to the possibility that the aPTT is not as accurate in its ability to predict anticoagulant effects of heparin in the setting of lupus anticoagulants (LAs) or increased factor VIII levels that may result in spuriously long values or short values, respectively. A screening non-contrast head computed tomography (CT) may be considered to rule out recent intracranial bleeding before the initiation of anticoagulation,²⁵ especially in patients with certain types of brain metastases associated with high rates of spontaneous hemorrhage, such as thyroid cancer, melanoma, renal cell carcinoma, and choriocarcinoma. Evidence of recent spontaneous bleeding is generally considered a contraindication to anticoagulation. IVC filters have a role in this situation; however, they are often proposed in such settings on the mistaken assumption that the patient cannot be anticoagulated, and may be overutilized.

Treatment of Patients With Trousseau's Syndrome

Trousseau's syndrome is the constellation of venous and arterial thromboembolic disorders predating or associated with a malignancy.^{26,27} Patients with this syndrome, even if anticoagulated with warfarin with a therapeutic international normalized ratio (INR), may nevertheless have recurrent thrombi. Other clinical characteristics of Trousseau's syndrome include microangiopathy, chronic, low-grade disseminated intravascular coagulation (DIC), and non-bacteria thrombotic endocarditis. UFH, LMWH, and fondaparinux are more effective than warfarin in the treatment of

Trousseau's syndrome. The dosing of anticoagulation varies depending on the clinical setting. For example, a patient with an acute DVT requires enoxaparin at therapeutic doses, whereas DIC may be controlled with lower doses. Treatment is administered indefinitely (or as long as the tumor persists).

General Approach in Treating Venous Thromboembolism in Cancer Patients

In general, treatment of VTE in patients with cancer consists of acute therapy with LMWH or UFH for at least 5 to 7 days duration in patients without contraindications to anticoagulation followed by LMWH or warfarin for at least three months. The CLOT (randomized comparison of low-molecular-weight heparin versus oral anticoagulant therapy for the prevention of recurrent venous thromboembolism in patients with cancer) trial²⁸ showed an 8% absolute risk reduction without an increase in major bleeding when cancer-related VTE was treated with an LMWH (i.e., dalteparin) 6 months compared with warfarin. Chronic therapy with LMWH is associated with superior outcomes in cancer patients with VTE.

Cancer patients with a VTE should be treated for a minimum treatment time for at least 3 months while patients with PE should be treated for at least 6 months, ideally with LMWH. Anticoagulation for an indefinite duration may be considered in patients with active cancer or persistent risk factors who may be bedridden, critically ill, and/or malnourished. Extended anticoagulation therapy with a LMWH may require dosage reduction after an initial period. For example, in the CLOT study, the dalteparin dosing was lowered from 200 units/ kg every day to 150 units/ kg every day after 1 month.

In the event that warfarin will be used for chronic therapy (due to cost or patient's preference), there should be a transition phase of at least 5 days during which the acute parenteral anticoagulant (e.g., UFH, LMWH, or fondaparinux) is overlapped with warfarin until an INR of 2.0 or more is achieved. Clinicians should be aware that the warfarin modulation of anticoagulation intensity can be clinically challenging due to drug-drug interactions with commonly used chemotherapeutics, antimicrobials, and other new drugs such as those undergoing testing in phase 1 clinical trials.

Most institutions have nomograms for dosing and monitoring of UFH. Anti-Xa activity instead of the aPTT has been more frequently used to monitor UFH because of the observation of dissociation between the aPTT and heparin levels measured by anti-Xa activity, suggesting heparin resistance. Heparin resistance usually happens in patients with elevations in factor VIII or von Willebrand factor, anti-thrombin III (AT) deficiency, increased heparin clearance, elevations in heparin-binding proteins, and use of fibrinogen. Factor VIII, von Willebrand factor, and fibrinogen are acute phase proteins and elevated factor VIII levels shorten the aPTT.^{29–31} When UFH is used, the

target therapeutic anti-factor Xa level should be 0.3 to 0.7 U/ mL.

Anticoagulation Options in Cancer Patients

In patients who develop recurrent VTE despite adequate anticoagulation with warfarin (INR = 2.0 to 3.0) the etiology may be cancer-related hypercoagulability such as the Trouseau's syndrome, anatomic causes such as extrinsic vascular compression, and acquired or familial thrombophilia. Treatment can be switched to heparin (LMWH preferred) or fondaparinux. The use of heparin is preferred to the use of vitamin K antagonists in the setting of cancer.³² Switching to heparin therapy is an option following the failure of fondaparinux to prevent VTE recurrence and vice versa. Twice-daily dosing of enoxaparin is an option for patients exhibiting recurrent VTE while receiving once-daily therapy with a LMWH,³³ and escalating the dose of LMWH can be effective for treating cases that are resistant to standard, weight-adjusted doses of LMWH.³⁴ If thrombocytopenia occurs during anticoagulation, chemotherapy-induced thrombocytopenia, DIC, heparin-induced thrombocytopenia (HIT), antiphospholipid antibody syndrome (APS), thrombotic thrombocytopenic purpura, immune thrombocytopenic purpura, bone marrow failure, and folate or vitamin B₁₂ deficiency should all be part of the differential diagnosis. Thrombocytopenia does not protect against thrombosis. Anticoagulation therapy should not be withheld because of relative thrombocytopenia alone. The management of antithrombotic therapy in patients with thrombocytopenia requires individualized assessments of the risk of bleeding and the risk of thrombosis.³⁵ Low-dose enoxaparin (i.e., <1 mg/ kg/ day) may be considered safe at a platelet count in the range of 20 and $55 \times 10^9/ L$ in stem cell transplantation patients who weigh >55 kg.³⁶ On the other hand, thrombocytopenia in APS and HIT may indicate increased disease activity and increased thrombotic potential and thus, aggressive antithrombotic therapy may be warranted.³⁷ Clinical suspicion of HIT should be high when recurrent VTE is observed in a cancer patient receiving heparin-based therapy or in a patient who received such therapy in the recent past. In the typical presentation, platelet counts fall by more than 50% from baseline 5 to 8 days after exposure to heparin. The drop in platelet count can occur even sooner if the patient has been primed by treatment with heparin before the current exposure. A major difficulty in the diagnosis of HIT is that cancer patients often have multiple reasons for thrombocytopenia, including myelosuppressive drugs, radiation therapy, and infections. An algorithm for calculating pretest probability of HIT includes clinical elements such as thrombocytopenia, timing of drop in platelet count, other causes for thrombocytopenia, and thrombosis,³⁸ and has been modified since its introduction to improve the accuracy of pretest probability estimation.³⁹ Testing for antiplatelet factor 4 antibodies with a sensitive enzyme-linked immunosorbent assay (ELISA) method can

rule out HIT if negative. In cases in which the result is positive, the possibility of a false positive can be ruled out by the more specific test for release of ¹⁴C serotonin from labeled platelets in the patient serum and heparin. Once the diagnosis of HIT is established, all heparin must be discontinued (even apparently trivial catheter flushes), and an alternative anticoagulant must be started. Alternative anticoagulants that are useful in this setting include direct thrombin inhibitors such as argatroban, bivalirudin, or lepirudin. Long-term anticoagulation with warfarin may be initiated after the platelet count has recovered, and there must be at least five days of overlap with the alternative anticoagulant before its discontinuation. If warfarin is started while the patient is still in the acute phase of HIT, further acute thrombosis can be precipitated as protein C levels fall upon initiation of warfarin. The highest risk of HIT is seen with the use of UFH, and lower risk is seen with LMWH with shorter glycosaminoglycan chains such as enoxaparin. Fondaparinux is manufactured by a total chemical synthesis of the core pentasaccharide chain that is the minimal essential element required for anti-factor Xa anticoagulant activity, and has the least tendency to provoke antiplatelet factor 4 antibodies. Further, it can be used as an alternative to heparin in patients diagnosed with HIT.⁴⁰

Thrombosis and Venous Access Devices

Most cancer patients have, at one time or another, central venous catheters placed for various amounts of time to administer chemotherapy, antibiotics, drugs, or blood products. A frequent complication of long-term central venous catheters is thrombosis, that may involve the catheter tip, the entire length of the catheter, or the lumen of the vein in which the catheter resides.⁴¹ It would be desirable to prevent such thrombosis not only to preserve central venous access but also prevent morbidity from obstruction of the large veins of the arm or chest, and prevent further extension or embolization. Although many strategies for prophylaxis of catheter-related clots have been proposed, a recent Cochrane Database Systematic Review shows that there is no statistically significant effect of prophylactic heparin or low dose warfarin to prevent catheter-related DVT in patients with cancer.⁴² Therefore, for cancer patients with indwelling central venous catheters, neither prophylactic doses of LMWH nor “mini-dose” warfarin are recommended to prevent catheter-related thrombosis.

For those patients who have developed a catheter-related DVT, treatment can be initiated with LMWH for 5 to 7 days, followed by warfarin (INR 2 to 3) or enoxaparin at a dose of 1.5 mg/ kg/ day for the lifetime of the catheter for a total duration of therapy of at least 3 months (whichever is longer). If the catheter is required but DVT symptoms persist or the clot progresses despite anticoagulation, the catheter should be removed. Thrombolytic therapy with relatively small doses of tissue plasminogen activator (tPA;

e.g., one or two treatments of less than 10 mg) has been successfully employed to clear venous occlusions and maintain patency of the vessel without any observed bleeding.⁴³ Patients treated with tPA should have subsequent anticoagulation as above, in the absence of contraindication(s) to prevent repeat thrombosis. In some cases, dilatation of stenotic venous segments may be required when multiple catheters have been inserted and removed for therapy. Low doses of tPA (e.g., 2 mg) can be used to open catheters whose lumen is clotted.

INDICATIONS FOR INFERIOR VENA CAVA FILTERS

IVC filters should not be used as a routine method for the prevention of pulmonary embolism (PE) in patients with DVT. Rather, their use should be predicated on genuine contraindications to anticoagulation and/ or documented failure of adequate anticoagulation. Patients with baseline cardiac or pulmonary dysfunction severe enough to make any new or recurrent PE life-threatening, or documented multiple PE and chronic thromboembolic pulmonary hypertension, may also be candidates for an IVC filter. Patients with DVT who receive IVC filters are initially protected against PE. However, they are at an increased risk for future recurrent DVT, as well as future IVC thrombosis or postthrombotic changes in the lower extremities, presumably from increased resistance to venous outflow from the lower extremities. A randomized trial of the use of IVC filters compared to nonuse of IVC filters for DVT showed that the absolute risk of PE in the first 12 days (symptomatic or not) was reduced from 4.8% to 1.1%, but at 2 years the rate of recurrent DVT was 20.8% in the IVC filter group versus 11.6% in the nonfilter group; there was no difference in the mortality rates between the two groups.⁴⁴ There are retrievable as well as permanent IVC filters; however, retrievable filters are more prone to migration after placement than permanent filters and may rarely embolize. If the retrievable filter is not removed within the time frame indicated by the manufacturer (typically 180 days), it can become technically difficult to retrieve, and the vascular surgeon or interventional radiologist may recommend that it remain permanently in place. In cancer patients with a poor prognosis, the impetus for removing the IVC filter to prevent long-term problems is lessened. Empirically, the rate of postphlebitic syndrome in cancer patients who receive IVC filters does not appear to have increased.⁴⁵

Results from a recent systemic review of the use of retrievable IVC filters showed that the average retrieval rate was 34%; most filters, in fact, became permanent devices.⁴⁶ Serious complications, including strut fracture, with or without embolization or filter migration, vena cava perforation, or vena cava occlusion occur with an increasing incidence after prolonged implantation times (>30 days). In one retrospective series that evaluated 80 patients with retrievable filters placed between 2004 and 2009

strut fracture rate of 16% was observed, some with embolization of filter components to the heart.⁴⁷ At the present time, the objective comparison data of different filter designs do not support superiority of any particular design. In cancer patients IVC filters appear to be safe and effective,⁴⁸ but the outcome in patients treated with IVC filters and anticoagulation is no better than for the use of either treatment alone.⁴⁵

PREVENTION, DIAGNOSIS, AND TREATMENT OF VENOUS THROMBOEMBOLISM IN PREGNANCY

Although mortality is rare in pregnant women in developed countries, pregnancy-associated PE remains one of the most frequent causes of the rare deaths that do occur. Thrombosis during pregnancy and puerperium (the 6-week period following delivery) is attributable to venous stasis in the lower extremities caused by the gravid uterus, as well as alterations in hemostasis, such as the progressive increase in fibrin turnover, increased levels of coagulation factors, decreased fibrinolytic activity, and decreased free protein S levels. In addition, inherited thrombophilias and APS, as well as a previous history of thrombosis, may accentuate the risk for DVT during pregnancy and the postpartum period.⁴⁹ Although DVT incidence seems to be evenly distributed throughout the three trimesters, PE is disproportionately found in the puerperium. Poor obstetric outcomes (including preeclampsia, placental abruption, intrauterine growth delay, and fetal loss) may be associated with thrombophilia.⁵⁰

Diagnosis of Venous Thromboembolism During Pregnancy

In pregnant women, signs and symptoms such as lower extremity edema, back pain, and/or chest pain may be attributed to pregnancy rather than to a possible VTE. Because the levels of D-dimer increase during pregnancy, especially during the last trimester, the D-dimer assay may not be helpful in establishing a diagnosis of VTE in pregnant women. Radiologic studies have to be used judiciously, and considered with attention to potential risks to the fetus and mother, and are usually contraindicated. Compression ultrasonography of the whole leg is preferred as the initial test for suspected DVT or PE in symptomatic patients and does not pose significant risks to the fetus. If the ultrasound is positive for DVT in patients presenting with symptoms suggestive of PE, an indication for anticoagulation is established, the diagnosis of PE is inferred, and no further imaging is required to start treatment.⁴⁹ If pelvic DVT is a consideration, duplex ultrasonography rather than compression alone increase sensitivity. If results are equivocal or an iliac vein thrombosis is possible, then magnetic resonance venography should be considered, as it does not carry radiation risks and is reliable, though issues remain (e.g., the use of gadolinium and availability of the test). When using ventilation/perfusion (V/Q) lung scan, the perfusion scan should be completed first and if this is

normal, PE is excluded, and there is no need for additional radioisotope exposure from the ventilation assessment of the study. If abnormal, a ventilation scan should be performed to confirm mismatch. Those with an indeterminate V/ Q scan should have CT pulmonary angiography (CTPA). CTPA is also preferred with hemodynamic compromise. The fetal radiation exposure, even with current multidetector CT scanning instruments, varies according to trimester of pregnancy, with potentially greater exposure later in gestation if the scanner is programmed to increase the amount of scanner current, and radiation to compensate for greater tissue mass.⁵¹ It is best to discuss the diagnostic needs of the CT scan with the radiologist to minimize fetal radiation through physical measures (e.g., shielding), selection of imaging programs appropriate for the pregnant woman at her stage of gestation, and procedural measures (e.g., stopping the study once a diagnosis has been made). The risk to the fetus for developing a later malignancy after a CT imaging procedure has been estimated at one excess cancer per 1,000 abdominal/ pelvic CT procedures, which is a small, but not a negligible risk.⁴⁹ In addition to fetal risk, the female breast is radiosensitive and CTPA is associated with an increased lifetime risk of breast cancer of 13.6% with a historic background risk of 1/ 200.^{52,53} Reducing radiation to the maternal breast favors the use of perfusion scanning⁵⁴ and avoiding ventilation scanning, if possible.

Management of Pregnant Women at an Increased Risk of Venous Thromboembolism

Risk factors for thrombosis include a personal history of VTE, known inherited or acquired thrombophilic mutations/ polymorphisms, obesity, advanced maternal age, high parity, and prolonged bed rest. Based on safety data for the fetus, heparin compounds are preferred over warfarin for the prevention and treatment of VTE in pregnancy because UFH and LMWH do not cross the placenta. LMWH is preferred because it has better bioavailability, a longer plasma half-life, a more predictable dose response, and a better safety profile than UFH. The risks of HIT and osteoporosis appear lower with LMWH than with UFH.^{30,55} Warfarin is generally avoided in pregnant women, as it carries a risk of teratogenicity when administered between 6 and 12 weeks of gestation. After parturition, either warfarin or heparin anticoagulants can be safely used; because they do not appear in breast milk, they can be given to mothers who are nursing. The prophylactic anticoagulants should be continued for at least 6 weeks postpartum. The therapeutic anticoagulants should be for a minimum total duration of therapy of 6 months.

Tables 23.4 and 23.5 (adapted from Ref. 55) offer guidance on management in defined clinical settings. A complete description of thrombotic conditions, and appropriate treatments during pregnancy, as well as during the postpartum period, can

be found in that paper.

Table 23.4 Recommendations for Antepartum Management

Clinical Scenario	Management
Prior VTE receiving long-term anticoagulation	Full or 75% dose LMWH ^a
Prior VTE, not receiving long-term anticoagulation	Prophylactic or intermediate-dose LMWH ^b
<ul style="list-style-type: none"> ▪ Unprovoked VTE ▪ Estrogen-related VTE (i.e., OCP, pregnancy) 	
Prior VTE, not receiving long-term anticoagulation	Clinical vigilance
<ul style="list-style-type: none"> ▪ VTE associated with a transient major provoking risk factor 	
No prior VTE; homozygous factor V Leiden or prothrombin gene mutation (regardless of family history)	Prophylactic or intermediate-dose LMWH ^b
No prior VTE; other thrombophilia (regardless of family history)	Clinical vigilance

^aFor patients at risk for bleeding or osteoporosis, consideration can be given to decreasing dose to 75% of full treatment dose after at least 1 month of therapy.

^bAntepartum clinical vigilance is acceptable for patients accepting the risk of recurrence quoted above and for whom the burden of LMWH prophylaxis outweighs potential benefits. Intermediate-dose LMWH means dalteparin 5,000 U twice daily or enoxaparin 40 mg twice daily.

LMWH, low-molecular-weight heparin; OCP, oral contraceptive pills; VTE, venous thromboembolism.

Adapted with permission from Bates SM. Pregnancy-associated venous thromboembolism: prevention and treatment. Semin Hematol. 2011;48:271–284.

Table 23.5 Recommendations for Postpartum Management

Clinical Scenario	Management
Prior VTE receiving long-term anticoagulation	Resume long-term anticoagulation
Prior VTE, not receiving long-term anticoagulation	Prophylactic or intermediate-dose LMWH ^a or warfarin (INR 2.0–3.0) × 6 weeks
<ul style="list-style-type: none"> ▪ Unprovoked VTE ▪ Estrogen-related VTE (i.e., OCP, pregnancy) ▪ VTE associated with a transient major provoking risk factor 	
No prior VTE; homozygous factor V Leiden or prothrombin gene mutation (regardless of family history)	Prophylactic or intermediate-dose LMWH ^a or warfarin (INR 2.0–3.0) × 6 weeks
No prior VTE; other thrombophilia, + family history	Prophylactic or intermediate-dose LMWH or warfarin (INR 2.0–3.0) × 6 weeks
No prior VTE; other thrombophilia, no family history	Clinical vigilance

^aIntermediate-dose LMWH means dalteparin 5,000 U twice daily or enoxaparin 40 mg twice daily.

INR, international normalized ratio; LMWH, low-molecular-weight heparin; OCP, oral contraceptive pills; VTE, venous thromboembolism.

Adapted with permission from Bates SM. Pregnancy-associated venous thromboembolism: prevention

and treatment. Semin Hematol. 2011;48:271–284.

As parturition approaches, women who are receiving LMWH should plan to discontinue treatment 24 hours before delivery. This is particularly important for those in whom epidural or spinal anesthesia is to be used, due to the known additional risk for spinal hematoma that is conferred by the use of LMWH. The drug can be resumed 24 hours after delivery. UFH, which has a half-life of 90 minutes, may well be preferable for women who require anticoagulation but whose time of delivery is not predictable.

RECOGNITION OF POSTTHROMBOTIC (“POSTPHLEBITIC”) SYNDROME

PTS occurs within 1 to 2 years of an episode of DVT in as many as 20% to 50% of patients.⁵⁶ In some patients, it may take a several months for the initial pain and swelling associated with acute DVT to resolve, so a diagnosis of PTS should be deferred until after the acute phase has passed. PTS results from venous hypertension, which is due to obstruction and damage to the venous valves caused by DVT. The syndrome manifests as chronic pain, tingling, and edema in the affected leg, as well as hyperpigmentation of the skin, and painless ulcers on the medial malleolar surface in the worst cases. The diagnosis is primarily clinical; duplex scanning can be used if the symptoms increase in severity and if surgery is contemplated.

Long-term use of GCSs after symptomatic proximal DVT has been proved to reduce the risk of any PTS.⁵⁷ Application of GCS has been recommended to be initiated within 2 to 3 weeks after the first DVT and to be continued for at least 2 years. However, a recent trial reported that beyond an initial 6-month period use, there was no incremental benefit in prolonging compression therapy for an additional 18 months.⁵⁸ Although GCS are unlikely to cause harm, they are difficult to apply, uncomfortable, expensive, and require replacement every few months. Based on the current state of evidence on the use of GCS to prevent PTS, it should be applied to patients who have residual leg pain or swelling after proximal or distal DVT, and continue them for as long as the patient derives symptomatic benefit or is able to tolerate them.

RECOMMENDATIONS FOR MANAGEMENT OF POSTTHROMBOTIC SYNDROME

For minimally symptomatic PTS, graduated stockings with moderate compression (15 to 20 mmHg) or leg elevation at the end of the day may be palliative. An effective way for the patient to reverse or relieve the symptoms of venous hypertension is to elevate both legs above the level of the heart for 30 minutes three or four times daily. During the

night leg elevation can be achieved by elevating the foot of the bed (using blocks placed under the bed if necessary). In case of mild to moderate symptoms (edema, aching, and heavy legs) graduated stockings with firm compression, 20 to 30 mm Hg; extra firm is also available (30 to 40 mm Hg) with or without nighttime pneumatic device. Patients who have had ulcer formation should wear stockings daily throughout the entire day. Nonelastic stockings that are comprised of multiple layers attached by Velcro (CircAid) can also be used and may be easier to apply. Intermittent pneumatic compression in combination with sustained graduated compression has demonstrated improved outcome in patients with venous leg ulcers.⁵⁹

Pharmacologic Approach to Postthrombotic Syndrome

Horse chestnut seed extract (aescin) was found to be effective in short-term treatment of chronic venous insufficiency symptoms, such as leg pain and edema, when compared to placebo.⁶⁰ A short-term (i.e., up to 3 weeks) trial of twice-daily horse chestnut seed extract (available at natural product stores) may be suggested to patients whose postthrombotic symptoms are not adequately controlled by GCS. In patients with venous leg ulcers, oxyrutins have demonstrated efficacy in a small double-blind, randomized, controlled study,⁶¹ but larger and more rigorous trials are needed to confirm its long-term effectiveness and safety.

There is no evidence that diuretics are effective for the treatment of postthrombotic-related edema, or that nonsteroidal anti-inflammatory drugs improve symptoms of PTS beyond their analgesic effects.

Surgical Management of Postthrombotic Syndrome

Surgical correction of superficial venous reflux in addition to compression bandaging was not shown to improve ulcer healing but did reduce recurrent ulceration compared with compression therapy alone in a randomized trial.⁶² The available strategies to prevent and treat PTS is summarized in [Table 23.6](#) (adapted from Ref. 63).

ANTIPHOSPHOLIPID SYNDROME

APS is an acquired thrombophilic condition characterized by arterial or venous thrombosis or pregnancy morbidity in patients with persistent positive LA, anticardiolipin antibodies, or anti- β 2-glycoprotein I for at least 12 weeks apart. Antiphospholipid antibodies promote activation of endothelial cells, monocytes, and platelets; and overproduction of tissue factor and thromboxane A2. Complement activation might have a central pathogenic role. Of the different antiphospholipid antibodies, the LA is the strongest predictor of thrombotic outcomes related to antiphospholipid syndrome.⁶⁴

Prevention of thrombosis is a major goal in patients with antiphospholipid antibodies. The 13th International Congress on antiphospholipid antibodies recommended that all antiphospholipid antibodies carriers receive primary thromboprophylaxis with the usual doses of LMWH in high-risk situations, such as surgery, prolonged immobilization and puerperium, and indefinite anticoagulation at an INR of 2.0 to 3.0 for patients with antiphospholipid syndrome presenting with first venous events (secondary thromboprophylaxis). Antiphospholipid patients with the arterial disease or recurrent events, or both, may need a more aggressive treatment, such as warfarin with a target INR of more than 3.0 or combined antithrombotic therapy with aspirin or other antiplatelet medications. In cases of first venous event, patients with a single positive test for antiphospholipid antibodies (anticardiolipin or anti- β 2-glycoprotein 1), and a known transient precipitating factor, anticoagulation could be limited to 3 to 6 months. In patients with difficult management due to recurrent thrombosis, fluctuating INR levels, major bleeding or a high risk for major bleeding, alternative therapies could include long-term LMWH, hydroxychloroquine, or statins.^{64,65}

Table 23.6 Approaches to Prevent and Treat Postthrombotic Syndrome Prevention

Prevention

- Prevent the occurrence of DVT with the use of thromboprophylaxis in high-risk patients and settings, as recommended in evidence-based consensus guidelines.
- Prevent DVT recurrence by providing anticoagulation of appropriate intensity and duration for the initial DVT and by use of thromboprophylaxis in high-risk patients and settings if long-term anticoagulation is discontinued.
- GCS are not recommended for routine use to prevent PTS.
- The role of thrombolysis of acute DVT to prevent of PTS is not yet established. Catheter-directed thrombolytic techniques require further evaluation in properly designed trials before being endorsed as effective and safe to reduce the risk of PTS.

Management

- Use elastic compression stockings to reduce edema and improve PTS symptoms like leg heaviness.
- Consider the use of intermittent pneumatic compression units and/ or VenoWave device for severe symptomatic PTS.
- Consider the short-term use of venoactive agents such as aescin (horse chestnut extract) or rutosides, which appear to improve some PTS symptoms; large controlled trials addressing long-term effectiveness and safety are needed.
- Compression therapy, skin care, and topical dressings are used to treat venous ulcers.
- Providing patient support and ongoing follow-up is an important component of PTS management.

DVT, deep venous thrombosis; GCS, graduated compression stockings; PTS, postthrombotic syndrome.

Reprinted with permission from Kahn SR. The post-thrombotic syndrome. Am Soc Hematol Educ Program. 2010:216–220. Review.

NEW ANTICOAGULANTS

Warfarin has a narrow therapeutic window and its levels are affected by changes in diet and interactions with other drugs, so its use requires ongoing laboratory monitoring. Furthermore, the onset of action is delayed, and the full anticoagulant effect is not achieved for several days, necessitating concomitant use of parenteral anticoagulants with the rapid onset of action (e.g., LMWH), while the dose is titrated (“bridging therapy”). This has stimulated research on newer oral anticoagulants without these limitations. The new oral direct thrombin inhibitor (dabigatran) and oral factor Xa inhibitors (apixaban, rivaroxaban, and edoxaban) have good bioavailability, reliable anticoagulant activity, and rapid onset of action. In 2010, the FDA approved dabigatran (Pradaxa) 150 mg twice daily for the reduction of the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF). This approval was based on a multicenter, active-control trial, the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY), which demonstrated that dabigatran 150 or 110 mg twice daily was noninferior to warfarin with similar safety profiles, and the 150 mg regimen was significantly superior to warfarin and to the 110 mg dabigatran regimen.⁶⁶ Therefore, only the 150 mg daily dosing regimen was approved by the FDA.⁶⁷

Dabigatran was also approved by the FDA in 2014 for the treatment of VTE in patients who have been treated with a parenteral anticoagulant for 5 to 10 days, and reduce the risk of recurrent VTE in patients who have been previously treated, based on results from four phase III randomized trials. In the RE-COVER trials,^{68,69} a total of 5,153 patients with acute VTE who were initially given unfractionated or LMWH for a median of 9 days were randomly assigned to dabigatran 150 mg twice daily or warfarin (INR 2.0 to 3.0). These studies demonstrated that dabigatran had similar effects on the reduction of VTE recurrence and a lower risk of bleeding compared with warfarin; pooled analysis of these two studies gave hazard ratios (HRs) for recurrent VTE of 1.09 (95% CI = 0.76 to 1.57) and for any bleeding of 0.70 (95% CI = 0.61 to 0.79). The REMEDY trial,⁷⁰ which included a total of 2,866 patients with VTE who had completed at least 3 initial months of therapy, showed that dabigatran 150 mg twice daily was noninferior to warfarin in reducing recurrent VTE (HR = 1.44, P = .01 for non-inferiority) and was associated with lower rates of major or clinically relevant bleeding (HR = 0.54; 95% CI = 0.41 to 0.71). In the RE-SONATE trial,⁷⁰ a total of 1,353 patients were randomized between dabigatran and placebo. This study demonstrated that dabigatran significantly reduced the risk of recurrent VTE compared with placebo (HR = 0.08; 95% CI = 0.02 to 0.25; P < .001) and was associated with higher rates of major or clinically relevant bleeding (HR = 2.92; 95% CI = 1.52 to 5.60). Likewise, trials for prophylaxis of VTE in the setting of total hip^{71,72} or total knee replacement^{73,74} have shown noninferiority of dabigatran to warfarin for this indication, and dabigatran

was FDA approved for prophylaxis of VTE in the setting of hip surgery in November 2015.

In October 2015, the FDA granted accelerated approval to idarucizumab (Praxbind) for use in reversing dabigatran effect during life-threatening or uncontrolled bleeding, or emergency surgery. Approval was based on 3 phase I clinical trials^{75–77} involving healthy volunteers taking dabigatran and an interim analysis of the RE-VERSE AD trial involving 90 older adults who had clinically significant bleeding or the need for an urgent invasive procedure while on dabigatran for atrial fibrillation. In all studies, idarucizumab produced an immediate effect in either reducing the amount of dabigatran (measured as unbound dabigatran plasma concentrations), or normalization of clotting time, and/or surgical hemostasis. In RE-VERSE AD trial, both the thrombin time and dilute thrombin time rapidly returned to the normal range after idarucizumab administration; the thrombin time is extremely sensitive to dabigatran and a normal thrombin time suggests an absence of dabigatran effect; therefore, the thrombin time may be used to monitor reversal of dabigatran anticoagulation with idarucizumab.^{78,79}

In 2011, the FDA approved rivaroxaban (Xarelto) 10 mg once daily for 35 days following hip replacement and for 12 days following knee replacement for the prevention of DVT in patients undergoing joint replacement surgery. The approval is based on testing of rivaroxaban's safety and efficacy in the four RECORD (Regulation of Coagulation in Orthopedic Surgery to Prevent Deep Venous Thrombosis and Pulmonary Embolism) trials.^{80–83} The data from the RECORD trials showed significantly greater efficacy of rivaroxaban, both in head-to-head comparison with enoxaparin and when comparing extended-duration (5 weeks) of rivaroxaban with short-duration enoxaparin (2 weeks), followed by the placebo. In these trials, rivaroxaban and enoxaparin demonstrated similar safety profiles, including low rates of major bleeding. Moreover, in 2011, the FDA also approved rivaroxaban 20 mg once daily to reduce the risk of stroke in patients with NVAF, based on ROCKET-AF trial (Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation). In this study, rivaroxaban was non-inferior to warfarin for the prevention of stroke or systemic embolism. There was no significant between-group difference in the risk of major bleeding, although intracranial and fatal bleeding occurred less frequently in the rivaroxaban group.⁸⁴

Another oral factor Xa inhibitor, apixaban (Eliquis), was FDA approved in 2012 for the prevention of stroke in subjects with atrial fibrillation. In the Apixaban for reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial, apixaban at a dose of 5 mg twice daily was compared with warfarin (target INR = 2.0 to 3.0) in 18,201 patients with atrial fibrillation and at least one additional risk factor

for stroke. Apixaban was found to be superior to warfarin in preventing stroke or systemic embolism, caused less bleeding, and resulted in lower mortality in patients with atrial fibrillation.⁸⁵ Subsequently, the AMPLIFY and AMPLIFY-EXT studies⁸⁶ led to the FDA approval of apixaban for the treatment of VTE, as well as a reduction in the risk of recurrent VTE after the initial therapy. In the AMPLIFY trial, 5,395 patients with acute VTE were randomly assigned to apixaban (10 mg twice daily for first 7 days, followed by 5 mg twice daily for 6 months) or standard therapy (warfarin with INR goal 2.0 to 3.0, bridged with enoxaparin for at least first 5 days). The efficacy of apixaban was comparable to standard care with similar rates of recurrent symptomatic VTE or death related to VTE between these two arms (2.3% vs. 2.7%; 95% CI, 0.60 to 1.18), while apixaban was associated with lower risk of bleedings than standard care ($P < .001$). In the AMPLIFY-EXT trial, a total of 2,486 patients with VTE who had completed 6 to 12 months of standard anticoagulation or treatment as participants in AMPLIFY trial, had not had recurrent VTE during prior anticoagulant therapy, or there was clinical equipoise about the continuation or cessation of anticoagulant therapy, were randomized to apixaban 2.5 or 5 mg twice daily for 12 months versus placebo. The study demonstrated that the extended duration of anticoagulation with apixaban significantly reduced the rate of recurrent VTE without increasing the rate of major bleeding.

Edoxaban (Savaysa) 60 mg daily was recently FDA approved for the prevention of stroke and systemic embolism in subjects with NVAF and the treatment of DVT and PE following 5 to 10 days of initial therapy with parenteral anticoagulant, based on ENGAGE AF-TIMI 48 and HOKUSAI-VTE trials, respectively.^{87,88} The data from these studies demonstrated the similar efficacy of edoxaban to standard therapy with significantly lower rates of bleeding in edoxaban arm. Certain disadvantages remain for all of the new oral anticoagulant medications that are in advanced stages of clinical testing. Although Idarucizumab was FDA approved in 2015 as an effective antidote for dabigatran,^{75–77} there currently are no FDA-approved reversal agents for direct oral anticoagulants (DOACs) that inhibit factor Xa that, though a recent trial of the decoy peptide andexanet for reversal of various inhibitors of factor Xa is promising.⁸⁹ The previous trial using PER977 (Perosphere), which binds to various anticoagulants (i.e., UFH, LMWH, thrombin, and factor Xa inhibitors) through noncovalent hydrogen bonding and charge–charge interactions, as an antidote for edoxaban also showed a promising result, and phase 2 clinical studies are currently ongoing.⁹⁰ The rapid onset of anticoagulation, a benefit for most applications, is matched by an equally rapid decrease in the anticoagulant effect, in contrast to the gradual onset/ slow decrease of effect seen for warfarin. This may be problematic where a patient has poor compliance and frequently misses doses of a drug that disappear rapidly from the circulation,

particularly under conditions of high thrombotic risk. Although laboratory testing for anticoagulant effects can be accomplished by use of the ecarin clotting time, for instance, in the case of the direct antithrombin drugs, and anti-Xa activity measurement for inhibitors of factor Xa, these are not, as yet commonly available in contrast to the prothrombin time and INR. These drugs are all significantly more expensive than generic warfarin, and none are as yet given FDA approval for treatment of VTE (secondary prophylaxis), and a place for warfarin will likely remain even after FDA approval, in patients whose warfarin dose is stable and are not difficult to manage, or who cannot afford the more expensive novel agents.

These concerns aside, a new era of easier-to-use oral anticoagulants for the prevention and treatment of VTE is now beginning, which is a welcome development.

References

1. Ay C, Simanek R, Vormittag R, et al. High plasma levels of soluble P-selectin are predictive of venous thromboembolism in cancer patients: results from the Vienna Cancer and Thrombosis Study (CATS). *Blood*. 2008;112:2703–2708.
2. Ay C, Vormittag R, Dunkler D, et al. D-dimer and prothrombin fragment 1 + 2 predict venous thromboembolism in patients with cancer: results from the Vienna Cancer and Thrombosis Study. *J Clin Oncol*. 2009 Sep 1;27:4124–4129.
3. O’Shea SI, Ortel TL. Issues in the utilization of low molecular weight heparins. *Semin Hematol*. 2002;39:172–178.
4. Lim W. Using low molecular weight heparin in special patient populations. *J Thromb Thrombolysis*. 2010;29:233–240.
5. Agnelli G, Bolis G, Capussotti L, et al. A clinical outcome-based prospective study on venous thromboembolism after cancer surgery: the @RISTOS project. *Ann Surg*. 2006;243:89–95.
6. Bergqvist, D, Agnelli, G, Cohen, AT, et al. Duration of prophylaxis against venous thromboembolism with enoxaparin after surgery for cancer. *N Engl J Med*. 2002;346:975–980.
7. Rasmussen MS, Jorgensen LN, Wille-Jorgensen P, et al. Prolonged prophylaxis with dalteparin to prevent late thromboembolic complications in patients undergoing major abdominal surgery: a multicenter randomized open-label study. *J Thromb Haemost*. 2006;11:2384–2390.
8. Ageno W, Squizzato A, Garcia D, et al. Epidemiology and risk factors of venous thromboembolism. *Semin Thromb Hemost*. 2006;32:651–658.
9. Geerts WH, Bergqvist D Pineo GF, et al. Prevention of venous thromboembolism. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th ed.) *Chest*. 2008;133:381S–453S.
10. VTE Prophylaxis for Laparoscopic Surgery Guidelines: An Update.

<https://www.sages.org/publications/guidelines/guidelines-for-deep-venous-thrombosis-prophylaxis-during-laparoscopic-surgery/>. Accessed February 1, 2018.

11. Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) Guidelines Committee. Guidelines for deep venous thrombosis prophylaxis during laparoscopic surgery. *Surg Endosc*. 2007;21(6):1007–1009.
12. Palumbo A, Rajkumar S V, Dimopoulos MA, et al., on behalf of the International Myeloma Working Group. Prevention of thalidomide-and lenalidomide-associated thrombosis in myeloma. *Leukemia*. 2008;22:414–423.
13. Agnelli G, Gussoni G, Bianchini C, et al., on behalf of the PROTECHT Investigators. Nadroparin for the prevention of thromboembolic events in ambulatory patients with metastatic or locally advanced solid cancer receiving chemotherapy: a randomised, placebo-controlled, double-blind study. *Lancet Oncol*. 2009;10:943–949.
14. Aue G, Lozier JN, Tian X, et al. Inflammation, TNF α and endothelial dysfunction link lenalidomide to venous thrombosis in chronic lymphocytic leukemia. *Am J Hematol*. 2011;86:835–840.
15. Khorana AA, Francis CF, Culakova E, et al. Thromboembolism in hospitalized neutropenic cancer patients. *J Clin Oncol*. 2006;24:484–490.
16. Samama MM, Cohen AT, Darmon JY, et al. A comparison of enoxaparin with placebo for the prevention of venous thromboembolism in acutely ill medical patients. Prophylaxis in medical patients with enoxaparin study group. *N Engl J Med*. 1999;341:793–800.
17. Kakkar AK, Cimminiello C, Goldhaber SZ, et al, for the LIFENOX investigators. Low molecular weight heparin and mortality in acutely ill medical patients. *N Engl J Med*. 2011;365:2463–2472.
18. Hamada K, Kuratsu J, Saitoh Y, et al. Expression of tissue factor correlates with grade of malignancy in human glioma. *Cancer*. 1996;77:1877–1883.
19. Sawaya R, Ligon L. Thromboembolic complications associated with brain tumors. *J Neurooncol*. 1994;22:173–181.
20. Bevacizumab package insert. December 21, 2011. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125085s0169lbl.pdf. Accessed February 1, 2018.
21. Norden AD, Young GS, Setayash K, et al. Bevacizumab for recurring malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology*. 2008;70:779–787.
22. Agnelli G, Piovella F, Buoncristiani P, et al. Enoxaparin plus compression stockings compared with compression stockings alone in the prevention of venous thromboembolism after elective neurosurgery. *N Engl J Med*. 1998;339:80–85.
23. Goldhaber SZ, Dunn K, Gerhard-Herman M, et al. Low rate of venous

- thromboembolism after craniotomy for brain tumor using multimodality prophylaxis. *Chest*. 2002;122:1933–1937.
24. Dickinson LD, Miller LD, Patel CP, et al. Enoxaparin increases the incidence of postoperative intracranial hemorrhage when initiated preoperatively for deep venous thrombosis prophylaxis in patients with brain tumors. *Neurosurgery*. 1998;43:1074–1081.
 25. Gerber DE, Grossman SA, Streiff MB. Management of venous thromboembolism in patients with primary and metastatic brain tumors. *J Clin Oncol*. 2006;24(8):1310–1318. Review.
 26. Levine MN, Lee AYY, Kakkar AK. From Trouseau to targeted therapy: new insights and innovations in thrombosis and cancer. *J Thromb Haemost*. 2003;1:1456–1463.
 27. Walsh-McMonagle D, Green D. Low-molecular-weight heparin in the management of Troussseau's syndrome. *Cancer*. 1997;80:649–655.
 28. Lee AYY, Levine MH, Baker RI, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *N Engl J Med*. 2003;349:146–153.
 29. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448–454.
 30. Hirsh J, Rascke R. Heparin and low-molecular weight heparin. The seventh ACCP conference on antithrombotic and thrombolytic therapy. *Chest*. 2004;126:188S–203S.
 31. Levine MN, Hirsh J, Gent M, et al. A randomized trial comparing activated thromboplastin time with heparin assay in patients with acute venous thromboembolism requiring large daily doses of heparin. *Arch Intern Med*. 1994;154:49–56.
 32. Akl EA, Barba M, Rohilla S, et al. Low-molecular-weight heparins are superior to vitamin K antagonists for the long term treatment of venous thromboembolism in patients with cancer: a Cochrane Systemic Review. *J Exp Clin Cancer Res*. 2008;27:21–31.
 33. Merli G, Spiro TE, Olsson C-G, et al. Subcutaneous enoxaparin once or twice daily compared with intravenous unfractionated heparin for treatment of venous thromboembolic disease. *Ann Int Med*. 2001;134:191–202.
 34. Carrier M, Le Gal G, Cho R, et al. Dose escalation of low molecular weight heparin to manage recurrent venous thromboembolic events despite systemic anticoagulation in cancer patients. *J Thromb Haemost*. 2009;7:760–765.
 35. Arnold D, Lim W. A rational approach to the diagnosis and management of thrombocytopenia in the hospitalized patient. *Semin Hematol*. 2011;48:251–258.
 36. Ibrahim RB, Peres E, Dansey R, et al. Safety of low-dose low-molecular-weight heparins in thrombocytopenic stem cell transplantation patients: a case series and

- review of the literature. *Bone Marrow Transplant.* 2005;35:1071–1077.
- 37. Lim W. Antiphospholipid antibody syndrome. *Hematology Am Soc Hematol Educ Program.* 2009:233–239. Review.
 - 38. Warkentin TE. Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol.* 2003;121:535–555.
 - 39. Cuker A, Arepally G, Crowther MA, et al. The HIT Expert Probability (HEP) Score: a novel pre-test probability model for heparin-induced thrombocytopenia based on broad expert opinion. *J Thromb Haemost.* 2010;8:2642–2650.
 - 40. Papadopoulos S, Flynn JD, Lewis DA. Fondaparinux as a treatment option for heparin-induced thrombocytopenia. *Pharmacotherapy.* 2007;27:921–926.
 - 41. Horne MK III, Chang R. Thrombosis related to venous access devices. In Kitchens C, Alving B, Kessler C, eds. *Consultative Hemostasis and Thrombosis.* 2nd ed. Philadelphia, PA: W. B. Saunders; 2007:553–559.
 - 42. Akl EA, Kamath G, Yosuico V, et al. Thromboprophylaxis for patients with cancer and central venous catheters (Cochrane Database Systematic Review). *Cancer.* 2011;112:2483–2492.
 - 43. Chang R, Horne MK, Shawker TH, et al. Low-Dose, once-daily, intraclot injections of alteplase for treatment of acute deep venous thrombosis. *J Vasc Interv Radiol.* 2011;22:1107–1116.
 - 44. Decousus H, Leizorovicz A, Parent F, et al. A clinical trial of vena caval filters in the prevention of pulmonary embolism in patients with proximal deep-vein thrombosis. *N Engl J Med.* 1998;338:409–415.
 - 45. Raman, R, Leming PD, Bhandari M, et al. Inferior vena cava filters in the management of cancer-associated venous thromboembolism: a systematic review. *Oncol Rev.* 2010;4:147–157.
 - 46. Angel LF, Tapson V, Galgon R, et al. Systematic review of the use of retrievable inferior vena cava filters. *J Vasc Intervent Rad.* 2011;22:1522–1530.
 - 47. Nicholson W, Nicholson WJ, Tolerico P, et al. Prevalence of fracture and fragment embolization of bard retrievable vena cava filters and clinical implications including cardiac perforation and tamponade. *Arch Int Med.* 2010;170:1827–1831.
 - 48. Wallace MJ, Jean JL, Gupta S, et al. Use of inferior vena caval filters and survival in patients with malignancy. *Cancer.* 2004;101:1902–1907.
 - 49. Marik PE, Plante LA. Venous thromboembolic disease and pregnancy. *N Engl J Med.* 2008;359:2025–2033.
 - 50. Battinelli EM, Bauer KA. Hematologic disorders in pregnancy. *Hematol Oncol Clin North Am.* 2011;25:323–333.
 - 51. Gilet AG, Dunkin JM, Fernandez TJ, et al. Fetal radiation dose during gestation estimated on an anthropomorphic phantom for three generations of CT scanners. *Am J Radiol.* 2011;196:1133–1137.

52. Kalra MK, Maher MM, Toth TL, et al. Strategies for CT radiation dose optimization. *Radiology*. 2004;230:619–628.
53. Remy-Jardin M, Remy J. Spiral CT angiography of the pulmonary circulation. *Radiology*. 1999;212:615–636.
54. Shahir K, Goodman LR, Tali A, et al. Pulmonary embolism in pregnancy: CT pulmonary angiography versus perfusion scanning. *Am J Roentgenol*. 2010;195:W213–W220.
55. Bates SM. Pregnancy-associated venous thromboembolism: prevention and treatment. *Semin Hematol*. 2011;48:271–284.
56. Kahn SR, Ginsberg J. Relationship between deep venous thrombosis and the post-thrombotic syndrome. *Arch Intern Med*. 2004;164:17–26.
57. Kolbach D, Sandbrink M, Hamulyak K, et al. Non-pharmaceutical measures for prevention of post-thrombotic syndrome. *Cochrane Database Syst Rev*. 2004;(1):CD004174.
58. Aschwanden M, Jeanneret C, Koller MT, et al. Effect of prolonged treatment with compression stockings to prevent post-thrombotic sequelae: a randomized controlled trial. *J Vasc Surg*. 2008;47:1015–1021.
59. Comerota AJ. Intermittent pneumatic compression: physiologic and clinical basis to improve management of venous leg ulcers. *J Vasc Surg*. 2011;53:1121–1129.
60. Pittler MH, Ernst E. Horse chestnut seed extract for chronic venous insufficiency. *Cochrane Database Syst Rev*. 2006 Jan 25;(1):CD003230.
61. Petruzzellis V, Troccoli T, Candiani C, et al. Oxerutins (Venoruton): efficacy in chronic venous insufficiency—a double-blind, randomized, controlled study. *Angiology*. 2002;53:257–263.
62. Gohel MS, Barwell JR, Taylor M, et al. Long term results of compression therapy alone versus compression plus surgery in chronic venous ulceration (ESCHAR): randomised controlled trial. *Br Med J*. 2007;335:83–88.
63. Kahn SR. The post-thrombotic syndrome. *Am Soc Hematol Educ Program*. 2010:216–220. Review.
64. Ruiz-Irastorza G, Crowther M, Branch W, et al, Antiphospholipid syndrome. *Lancet*. 2010;376:1498–1509.
65. Ruiz-Irastorza G, Cuadrado MJ, Ruiz-Arruza I, et al. Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients: report of a Task Force at the 13th International Congress on Antiphospholipid Antibodies. *Lupus*. 2011;20:206–218.
66. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139–1151.
67. Beasley BN, Unger EF, Temple R. Anticoagulant options— why the FDA approved a higher but not a lower dose of Dabigatran. *N Engl J Med*. 2011;364:1788–1790.

68. Schulman S, Kearon C, Kakkar AK, et al. RE-COVER Study Group. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. *N Engl J Med.* 2009;361:2342–2352.
69. Schulman S, Kakkar AK, Goldhaber SZ. Treatment of acute venous thromboembolism with dabigatran or warfarin and pooled analysis. *Circulation.* 2014;129:764–772.
70. Schulman S, Kearon C, Kakkar AK, et al. Extended use of dabigatran, warfarin, or placebo in venous thromboembolism. *N Engl J Med.* 2013;368:709–718.
71. Eriksson BI, Dahl OE, Rosencher N, et al. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomized, double-blind, non-inferiority trial. *Lancet.* 2007;370:949–956.
72. Eriksson BI, Dahl OE, Huo MH, et al. Oral dabigatran versus enoxaparin for thromboprophylaxis after primary total hip arthroplasty (RE-NOVATE II*). A randomised, double-blind, non-inferiority trial. *Thromb Haemost.* 2011;105:721–729.
73. Eriksson BI, Dahl OE, Rosencher N, et al. Oral dabigatran etexilate vs. subcutaneous enoxaparin for the prevention of venous thromboembolism after total knee replacement: the RE-MODEL randomized trial. *J Thromb Haemost.* 2007;5:2178–2185.
74. Ginsberg JS, Davidson BL, Comp PC, et al. Oral thrombin inhibitor dabigatran etexilate vs. North American enoxaparin regimen for prevention of venous thromboembolism after knee arthroplasty surgery. *J Arthroplasty.* 2009;24:1–9.
75. Pollack CV, Reilly PA, Eikelboom J, et al. Idarucizumab for dabigatran reversal. *N Engl J Med.* 2015;373:511–520.
76. Glund S, Stangier J, Schmohl M, et al. Safety, tolerability, and efficacy of idarucizumab for the reversal of the anticoagulant effect of dabigatran in healthy male volunteers: a randomised, placebo-controlled, double-blind phase 1 trial. *Lancet.* 2015;386:680–690.
77. Glund S, Moschetti V, Norris S, et al. A randomised study in healthy volunteers to investigate the safety, tolerability and pharmacokinetics of idarucizumab, a specific antidote to dabigatran. *Thromb Haemost.* 2015;113:943–951.
78. Lessire S, Douxfils J, Baudar J, et al. Is thrombin time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study. *Thromb Res.* 2015;136(3):693–696.
79. Sié P. Spotlight on idarucizumab and its potential for the reversal of anticoagulant effects of dabigatran. *Drug Des Devel Ther.* 2016;10:1683–1689.
80. Eriksson BI, Borris LC, Friedman RJ, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med.* 2008;358:2765–2775.
81. Lassen MR, Ageno W, Borris LC, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med.* 2008;358(26):2776–

82. Kakkar AK, Brenner B, Dahl OE, et al. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomized controlled trial. *Lancet*. 2008;372:31–39.
83. Turpie AG, Lassen MR, Davidson BL, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. *Lancet*. 2009;373:1673–1680.
84. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med*. 2011;365:883–891.
85. Granger CB, Alexander JH, McMurray JJV, et al. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2011;365:981–992.
86. Agnelli G, Buller HR, Cohen A, et al. Apixaban for extended treatment of venous thromboembolism. *N Engl J Med*. 2013;368:699–708.
87. Giugliano RP, Ruff CT, Braunwald E, et al. Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2013;369:2093–2104.
88. Hokusai-VTE Investigators, Büller HR, Décousus H, et al. Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. *N Engl J Med*. 2013;369:1406–1415.
89. Connolly SJ, Milling TJ, Eikelboom JW, et al. Andexanet alfa for acute major bleeding associated with factor Xa inhibitors. *N Engl J Med*. 2016;375:1131–1141.
90. Ansell JE, Bakhru SH, Laulicht BE, et al. Use of PER977 to reverse the anticoagulant effect of edoxaban. *N Engl J Med*. 2014;371:2141–2142.

Blood Transfusion

Harvey G. Klein

BLOOD CELL ANTIGENS

Red blood cell (RBC) antigens are classified according to their biochemical, phenotypic, and immunologic characteristics. Based on these characteristics, they have been separated into different blood group systems. Currently, 36 major blood group systems are recognized encompassing 41 genes and more than 1,500 individual alleles, the most clinically relevant being ABO, Rh, Kell, Kidd, and Duffy. The clinically important alloantibodies (specific for antigens not found on the individual's red cells) can cause destruction of transfused red cells or are implicated in hemolytic disease of the newborn (HDN).

The antiglobulin or Coombs test (see the following discussion) is used to detect antibodies to red cell antigens and in crossmatching compatible blood units for transfusion. When a clinically important alloantibody is present in a recipient's serum, antigen-negative blood must be selected. If the alloantibody is against a very high frequency antigen (present in greater than 90% of individuals) or when multiple alloantibodies are present, procurement of compatible blood may be difficult or impossible. Occasionally, the red cell autoantibodies in the recipient makes all units appear incompatible. Further investigations are necessary in these cases to rule out an underlying alloantibody.

“Naturally occurring” antibodies such as anti-A and/ or anti-B are present in the absence of prior sensitizing stimulus, whereas the development of most other alloantibodies require prior sensitization by exposure to the corresponding red cell antigen in a previous transfusion or pregnancy.

- | Blood group A individuals have naturally occurring anti-B
- | Blood group B individuals have naturally occurring anti-A
- | Blood group O individuals have naturally occurring anti-A and anti-B

- | Blood group AB individuals have neither anti-A nor anti-B.

LABORATORY DETERMINATION OF MAJOR BLOOD GROUPS

An individual's blood group is determined by performing a forward and reverse grouping (ABO typing):

- | In **forward** grouping, reagents of known antibody specificity (anti-A or anti-B) are added to the patient's RBCs of unknown phenotype (A, B, AB, or O) and the mixtures are examined for visible agglutination; the absence of agglutination on combining the patient's cells with anti-A or anti-B reagent indicates that the patient's cells do not have the corresponding antigen. For example, red cells of group O will not agglutinate in the presence of anti-A and/ or anti-B.
- | In **reverse** grouping, the patient's serum is added to reagent cells of known phenotype (A, B, or O) and the mixtures are examined for visible agglutination; the agglutination on combining the patient's serum with reagent cells of A or B phenotype indicates that the patient's serum contains the corresponding antibody. For example, serum-containing anti-A and anti-B (blood group O) agglutinate in the presence of both group A and group B red cells.

The forward and reverse groups must be consistent. A specific blood group may not be assigned with certainty until an ABO discrepancy is resolved.

Some common causes of apparent ABO discrepancies are¹ presence of A or B subgroups,² missing/ weakly reacting antibodies (can occur with newborns, the elderly, or in hypogammaglobulinemic states) or weak expression/ absence of expected antigens (can occur with lymphoproliferative disorders and post-hematopoietic stem cell transplantation [HSCT]),³ unexpected or nonspecific antibodies such as cold reactive alloantibodies or autoantibodies,⁴ interfering substances such as Wharton's jelly from the umbilical cord of a newborn, or⁵ hyperproteinemic states causing Rouleaux formation.

ANTIGLOBULIN TEST

The antiglobulin test uses antibodies with specificity for immunoglobulins (Igs) or complement to detect the presence of an antibody (or complement) on the RBC surface or in a patient's serum.

The direct antiglobulin test (DAT), or the direct Coombs test, detects the antibody or complement coating RBC, and may be positive in various settings, including those listed as follows:

- | Hemolytic transfusion reactions
- | HDN (usually strongly positive)

- | Autoimmune hemolytic anemias (AIHA)
- | With some pharmacologic agents (penicillins, cephalosporins)
- | After administration of intravenous immunoglobulin (IVIG) or plasma (passively acquired)
- | Post–marrow or organ transplant (passenger lymphocyte syndrome)
- | Autoimmune diseases
- | Some normal individuals

A positive DAT does not necessarily indicate in vivo hemolysis or shortened RBC survival. False-positive results may occur when Rouleaux formation is mistaken for agglutination.

The indirect antiglobulin test (IAT), or indirect Coombs test, is used to screen for antibodies when looking for compatible blood for transfusion (“type and screen”) and in the serologic crossmatch (patient serum and donor/ reagent red cells). The IAT detects antibody present in the serum, but not bound to the RBC. When the IAT is positive, the antibody must be identified and the corresponding antigen always avoided in transfusions. A negative IAT does not necessarily indicate absence of alloantibodies: The titer of antibody may be below the level of detection or the antibody might be directed against a low-incidence antigen (present in <1% of individuals) not present on reagent testing cells. A negative IAT does not guarantee that blood is compatible, nor does a weak IAT indicate that hemolysis is likely to be mild. A positive IAT always requires further investigation.

In AIHA, wherein the antibody may be present in the serum and on the RBC of the patient, both the DAT and IAT may be positive.

Red cell antigen prediction through genotyping has proven advantageous in several clinical scenarios including recently transfused patients, patients with antibodies to high- or low-incidence antigens for whom serology has limited value (especially “historical” antibodies no longer detectable), and patients receiving monoclonal therapies such as daratumumab that obscure traditional typing assays.²

BLOOD COMPATIBILITY

In general, blood components that contain more than 2 mL of RBC must be compatible with the patient’s plasma. Particular attention is paid to the Rh type because less than 1 mL of RBC, a volume that is found in most platelet concentrates, is sufficient to sensitize an Rh-negative patient.¹ Plasma-containing components, including platelet preparations, should be ABO compatible with the patient’s RBC when possible to prevent passive immune hemolysis from antibodies in the plasma ([Table 24.1](#)).

Table 24.1 Compatibility of Recipient Blood With Donor Blood Components

Patient ABO Group	Whole Blood	Red Blood Cells	Platelets	Plasma	Cryoprecipitate
O	O	O	Any (O preferred)	O, A, B, AB	N/ A
A	A	A or O	Any (A preferred)	A or AB	N/ A
B	B	B or O	Any (B preferred)	B or AB	N/ A
AB	AB	AB, A, B, or O	Any (AB preferred)	AB	N/ A

The most basic practical application of blood group serology involves the selection of compatible blood. The absence or presence of blood cell antigens can have important biological and clinical implications. Compatible blood takes time to prepare.

- | In an emergency, group O Rh-negative RBC may be released uncrossmatched with the consent of the requesting physician; testing is completed after release. Group-specific red cells and an abbreviated crossmatch can be prepared in 15 minutes.
- | Fully tested red cells can be prepared in 45 to 60 minutes; cryopreserved RBC and fresh-frozen plasma (FFP) may take longer. In the setting of transfusion or pregnancy in the previous 3 months a pretransfusion sample cannot be more than 3 days old.⁶

ABO INCOMPATIBILITY IN TRANSPLANT SETTINGS

Optimal results in HSCT rely on human leukocyte antigen (HLA) compatibility, so that selection of the recipient–donor pair is determined by similarity of HLA potentially at the expense of ABO compatibility. Because HLA and ABO genes are inherited independently, some (20% to 40%) of these transplants will be ABO incompatible.¹

Whereas ABO incompatibility does not appear to impact graft failure, potential complications of mismatches include acute or delayed hemolysis and delayed RBC engraftment.^{2–5}

Minor Incompatibility

- | Donor's serum contains antibodies against RBC antigens of the recipient (e.g., recipient blood group A, B, or AB, and donor blood group O).
- | Prior to infusion of the stem cell preparation, plasma containing anti-A and anti-B can be removed to prevent immediate postinfusion hemolysis of the recipient's RBC (plasma reduction).
- | Of minor ABO-incompatible transplant recipients, 10% to 15% may experience abrupt onset of hemolysis 5 to 15 days posttransplant when immune-competent B lymphocytes in the graft mount a response against the recipient RBC antigens (passenger lymphocyte syndrome).
- | Hemolysis may be severe or even fatal unless recognized promptly.

Major Incompatibility

- Recipient's serum contains antibodies against the RBC antigens of the donor (e.g., recipient group O and donor group A, B, or AB; recipient group A or B and donor group AB).
- Hemolysis of RBC in the stem cell preparation on infusion may occur if the graft is not processed to remove RBC prior to infusion (red cell reduction).
- Post-transplant, the recipient may produce antibodies against donor red cell antigens for months, especially with nonmyeloablative regimens.
- RBC engraftment and erythropoiesis may be delayed, resulting in red cell aplasia.^{5,6}

Minor and major (bidirectional) incompatibility between the donor and recipient occurs when each has antibodies against the ABO blood group antigens of the other (combination of group A donor and B recipient, or vice versa).

Table 24.2 Transfusion in Minor and Major ABO-Incompatible Transplants

Recipient	Donor	Phase I All Components	Phase II RBC	Phase II Platelets	Phase III FFP	Phase III All Components
Minor Mismatch						
A	O	Recipient group	O	A; AB; B; O	A; AB	Donor group
B	O	Recipient group	O	B; AB; A; O	B; AB	Donor group
AB	O	Recipient group	O	AB; A; B; O	AB	Donor group
AB	A	Recipient group	A	AB; A; B; O	AB	Donor group
AB	B	Recipient group	B	AB; B; A; O	AB	Donor group
Major Mismatch						
O	A	Recipient group	O	A; AB; B; O	A; AB	Donor group
O	B	Recipient group	O	B; AB; A; O	B; AB	Donor group
O	AB	Recipient group	O	AB; A; B; O	AB	Donor group
A	AB	Recipient group	A	AB; A; B; O	AB	Donor group
B	AB	Recipient group	B	AB; B; A; O	AB	Donor group
Minor and Major and Mismatch						
A	B	Recipient group	O	AB; A; B; O	AB	Donor group
B	A	Recipient group	O	AB; B; A; O	AB	Donor group

Phase I: from the time patient is prepared for hematopoietic progenitor cell transplant.

Phase II: from initiation of myeloablative therapy from the time DAT is negative and isoantibodies against donor are no longer detectable (for RBC) or when the recipient's erythrocytes are no longer detectable.

Phase III: after the forward and reverse type of the patient are consistent with donor ABO group. Italicized blood groups indicate next best choice in order of preference.

All cellular components should be irradiated.

DAT, direct antiglobulin test; FFP, fresh-frozen plasma; RBC, red blood cells.

Adapted from Brecher ME, ed. Technical Manual. 15th ed. Bethesda, MD: AABB Press; 2005:600; Friedberg RC, Andrzejewski C. Transfusion therapy in hematopoietic stem cell transplantation. In: Mintz PD, ed. Transfusion Therapy: Clinical Principles and Practice, 2nd ed. Bethesda, MD: AABB Press; 2005.

Table 24.2 describes appropriate transfusion management during transplantation. All transfusion components must be irradiated.^{7,8}

Rh Incompatibility

Rh incompatibility occurs in 10% to 15% of stem cell transplants. Transfusion practice is analogous to that of major and minor ABO incompatibility, but the consequences are less severe.

For Rh-negative recipients of Rh-positive hematopoietic stem cell preparations

- | Transplant product should be red cell reduced to decrease risk of alloimmunization similar to major ABO incompatibility.

For Rh-positive recipient from Rh-negative donor previously alloimmunized to the Rh antigen

- | Monitor the patient for signs of delayed hemolysis (as in minor incompatibility).

BLOOD COMPONENTS AND DERIVATIVES

Blood Components and Transfusion Therapy

Blood components can be separated from the whole blood by centrifugation or by apheresis. Approximately 29 million blood components (RBCs, platelets, plasma, and cryoprecipitate) are transfused annually in the United States.

Storage and infusion of blood products

- | Blood components should be infused through standard 170- to 260- μm infusion filters to remove any clots that form during storage.⁶
- | An approved infusion pump may be used for strict control of transfusion rate. Nonapproved pumps may damage or hemolyze cells.
- | Bedside leukoreduction filters may be used when leukoreduction is indicated for the whole blood, packed RBCs, and platelets that have not been leukocyte reduced prior to storage.
- | Hypotensive reactions have been associated with bedside leukoreduction, especially in patients receiving angiotensin-converting enzyme (ACE) inhibitors.
- | Allow blood to filter by gravity.
- | Granulocyte concentrates must never be infused through leukoreduction filters.
- | Whole blood and other cellular blood components may be infused with isotonic solutions: USP 0.9% NaCl (normal saline) and certain U.S. Food and Drug

- Administration (FDA)-approved electrolyte solutions.
- Cellular blood products must never be infused with hypertonic or hypotonic solutions, for example, solutions containing glucose or calcium, such as D5W (5% dextrose in water) or lactated Ringer solution as hemolysis, clotting, or agglutination of RBC may result.
- Medications should never be added to blood components.
- Never store blood components in unmonitored refrigerators in nursing units or surgical suites; the risk for administration of blood components to the wrong patient increases in these cases.
- Return blood to storage (or blood bank) if the transfusion is not started within 30 minutes of issue. Further warming damages the red cells.
- Warming devices with internal monitors are available for blood products to avoid transfusion of large volumes of cold fluid.
- Blood components should never be warmed in uncertified devices (e.g., microwave ovens or water baths) as it can result in hemolysis and can be lethal.

Most adverse transfusion reactions occur in the first 15 minutes:

- Administration of blood products should start slowly and under close observation.
- The time of transfusion should not exceed 4 hours as the risk of bacterial growth and cell damage increases with time at room temperature.
- If transfusion is anticipated to take longer, the transfusion service can divide the unit into smaller aliquots.

See [Table 24.3](#) for blood component administration. See [Table 24.4](#) for indications for additional modifications to blood components.

Storage conditions for different blood components vary and are designed to maximize preservation and effectiveness:

- Red cells are refrigerated (at 1°C to 6°C) for up to 42 days.
- Platelets are stored at room temperature and expire in 5 days or 7 days if retested.
- Plasma components are stored frozen for a year (at -18°C) or more (at -65°C), but must be thawed before use, and therefore, are not immediately available.

As with any medical treatment, blood transfusion requires informed consent:

- Patients must be advised of the indications and common adverse events, as well as any potential alternatives to allogeneic transfusion.

Whole Blood

A unit of whole blood typically has a volume of 450 to 500 mL and a hematocrit of 35% to 45%. Whole blood is rarely available and infrequently used except for trauma.

Indications: Acute hypovolemia with red cell loss, massive transfusion, and

exchange transfusion

Not indicated: Chronic anemia (in which blood volume is often increased)

Whole blood is not a reliable source of functional platelets or granulocytes, which deteriorate in less than 72 and 24 hours, respectively at refrigerator temperatures.

Table 24.3 Blood Component Administration per NIH Practice^a

	Whole Blood	Packed Red Blood Cell	Granulocytes	Platelets	Plasma
Adults					
First 15 min	2 mL/ min	2 mL/ min	2 mL/ min	2–5 mL/ min	2–5 mL/ min
Subsequently	100–230 mL/ hr	100–230 mL/ hr	75–100 mL/ hr	200–300 mL/ hr	200–300 mL/ hr
Pediatric					
First 5 min	N/ A	N/ A	N/ A	5% of total volume ordered for transfusion	5% of total volume ^a ordered for transfusion
First 15 min	5% of total volume ^b ordered for transfusion	5% of total volume ^b ordered for transfusion	5% of total volume ordered for transfusion	N/ A	N/ A
Subsequently	Variable (as tolerated)	2–5 mL/ kg/ hr	Over 2–3 hr (for a 200-mL product)	As tolerated	1–2 mL/ min

^aFlow rates are guidelines to be adjusted to patient tolerance.

^bVolume ordered for pediatric transfusion should be based on the child's weight (10–15 mL blood product/kg); excludes granulocyte transfusion.

NIH, National Institutes of Health.

Red Blood Cells

RBC (packed red cells) are separated from whole blood by centrifugation. A unit of RBC contains approximately 200 mL and a hematocrit of 60% to 80%. In general, 1 unit of packed RBC will increase the hemoglobin (Hb) by 1 g/ dL in an average-sized adult. In the average pediatric patient, transfusion of 8 to 10 mL/ kg of RBC is expected to increase Hb by 3 g/ dL. The decision to transfuse should be based on an assessment of symptoms, coexisting or underlying medical conditions, and the cause of anemia, and patients should not be transfused based solely on their Hb level. Numerous observational studies and meta-analyses indicated that patients with cardiovascular disease are more sensitive to anemia and do better at a higher Hb level.^{9,10}

Indications: Treatment of symptomatic anemia.

Although it is generally accepted that patients with Hb < 6 g/ dL should be transfused

and that transfusion is rarely required when it exceeds 10 g/ dL, the interval between these values is an area of controversy. Practice guidelines support an Hb level of less than 7 g/ dL as generally acceptable for the initiation of RBC transfusion in asymptomatic patients.¹¹ Patients at particular risk for bleeding (thrombocytopenia, recent hemorrhage) or with cardiac and pulmonary compromise should be maintained at a higher Hb.

Not indicated: RBC should not be transfused for volume expansion or nutritional purposes

Transfusion is rarely indicated in otherwise treatable anemia, including anemia associated with vitamin B₁₂, iron, or folate deficiency; if symptoms are severe, these patients may benefit from a single-unit transfusion as the underlying cause is corrected.

Platelets

Platelets may be separated from the whole blood shortly after collection (“random donor” or “whole blood-derived” platelet concentrates) or collected by apheresis (“single donor” or “apheresis platelets”). Platelets for prophylactic administration are always stored at room temperature. Cold-stored platelets may be more effective for treatment of bleeding, but circulate for only a matter of hours; cold-stored platelets are not widely available.

Table 24.4 Indications for Additional Modifications of Cellular Blood Components

	Leukoreduction	Irradiation (Red Blood Cells, Platelets, Granulocytes)	Washing (Plasma Removal)	Volume Reduction	Freezing Deglycer (Red Blood Cells)
Description	Filtration of component after collection, at bedside, or removal of WBC during automated collection for a 3 log (99.9%) WBC reduction Final WBC content <5 × 10 ⁶	Gamma irradiation (cesium, cobalt, X-ray) of cellular component with 2,500 cGy to inactivate viable lymphocytes within component	Component washed with sterile normal saline to remove >98% of plasma proteins, electrolytes, and antibodies WBC content 5 × 10 ⁸	Removal of plasma from cellular components (mainly platelets); RBC concentrates have very little plasma	Addition of glycerol and freezing general 6d of collection (depending on the additive solution and the time of collection) glycerol freezing used)
Purpose	Reduction of FNHTR Reduction of CMV transmission (CMV-safe) Reduction of HLA	Prevention of transfusion-associated GVHD	Prevention of allergic reactions Decrease risk of hyperkalemia	Reduction of circulatory overload Removal of antibodies	Long-term storage of autologous allogeneic blood phenotypic

	alloimmunization				
Indications	Patients who have experienced an episode of FNHTR Alternative to CMV-seronegative components (from donor tested negative for CMV) for at-risk patients such as neonates and transplant patients	Recipients of bone marrow/hematopoietic stem cell transplants Recipients of transfusion from blood relatives Patients on immunosuppressive regimens, particularly with purine analogues Patients with congenital immunodeficiencies and certain malignancies Patients with B-cell malignancies Hodgkin lymphoma	Patients who experience recurrent severe allergic reactions (not responsive to premedication with antihistamines) IgA-deficient patients when IgA-deficient component is not available Recipients at risk from hyperkalemia such as newborns and fetuses receiving intrauterine transfusions	Patients with expanded plasma volume such as those with normovolemic chronic anemia, thalassemia major, sickle cell disease, congestive heart failure Pediatric, elderly, and other patients susceptible to volume overload	Patients with blood phenotypic multiple alloantibodies
Comments	Equivalent to CMV-seronegative components Not effective and not indicated for prevention of transfusion-associated graft-versus-host disease	Not indicated for prevention of FNHTR and unnecessary for patients with aplastic anemia (despite ATG therapy) or patients with HIV in the absence of other indications for irradiation (above), or platelets inactivated with psoralen and UV irradiation	Washing results in a 15%–20% loss of red cells or platelets. RBCs must be used within 24 hrs and platelets must be used within 4 hrs of washing because of increased risk of contamination	Platelets must be used within 4 hr of volume reduction because of decrease in amount of plasma/volume remaining for optimal platelet metabolism Not equivalent to washing for	May not be feasible RBCs with certain abnormalities such as hereditary spherocytosis and paroxysmal nocturnal hemoglobinuria (PNH) Depending on method glycerol freezing

	associated with opening of a closed system	prevention of allergic reactions	open or system deglyce shelf life 24 hrs c respect
	Not equivalent to "leukoreduced"	Not equiv "leukore (may re >95% o	

ATG, antithymocyte globulin; CMV, cytomegalovirus; FNHTR, febrile nonhemolytic transfusion reactions; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; RBC, red blood cells; WBC, white blood cells.

A therapeutic dose of platelets for an adult is 1 unit of platelets (5.5×10^{10} platelets)/ 10 kg of body weight, which should increase the platelet count in an average-sized adult by approximately 5,000/ μL . Each apheresis (single-donor) platelet product is expected to contain at least 3×10^{11} platelets, roughly equivalent to 4 to 6 units of random donor platelets. The indications for use are the same for both preparations. Alloimmunized refractory patients may require single-donor HLA-matched platelets. Single-donor platelets also offer the additional advantage of decreased donor exposure and a lower risk of bacterial sepsis.

Indications: Prevention and treatment of hemorrhage in patients with thrombocytopenia or platelet function defects.

Not indicated: Bleeding unassociated with thrombocytopenia (in the absence of a clinically significant platelet function defect), other defects in hemostasis (such as factor deficiencies).

Platelets are traditionally contraindicated in thrombotic thrombocytopenic purpura (TTP),¹² because they could potentially precipitate thrombosis; this belief has been recently challenged.¹³ Patients with TTP who develop life-threatening hemorrhage may benefit from a cautious trial of transfused platelets.

The threshold for prophylactic platelet transfusion varies based on the patient's underlying condition and likelihood of hemorrhage:

- | A threshold of 10,000/ μL is effective in preventing morbidity and mortality from bleeding in stable oncology patients with no other hemostatic defects undergoing chemotherapy.
- | A platelet count greater than 50,000/ μL is desirable prior to most invasive procedures and in the immediate postprocedure period. Lower counts are appropriate for line placement and bone marrow aspirate/ biopsy.
- | Platelet counts closer to 100,000/ μL may be prudent for patients at high risk for intracranial hemorrhage, such as those with cerebral leukostasis, or when undergoing

neurosurgical or ocular procedures.

- Stable chronically thrombocytopenic patients, such as those with aplastic anemia or myelodysplasia, may tolerate platelet counts as low as 5,000/ μ L in the absence of complicating factors such as fever, infection, and additional defects in hemostasis.¹

More aggressive support is indicated for patients who are unstable— febrile, infected, receiving multiple medications— especially if the platelet counts are decreasing.^{1,14}

Platelet transfusions should be monitored by a 1- to 24-hour posttransfusion platelet or complete blood count (CBC) to assess response and guide subsequent transfusion therapy. A corrected count increment (CCI) may be used to determine the increase in platelet count in an individual post-platelet transfusion:

$$\text{CCI} = \frac{(\text{Posttransfusion platelet count}^* - \text{pretransfusion platelet count}) \times \text{Body surface area}^{**}}{\text{Number of platelets transfused}^{***}}$$

*Posttransfusion platelet count, expressed per microliter, is best obtained 15 minutes to 1 hour posttransfusion.

**Body surface area = the square root of [(height in cm \times weight in kg)/ 3,600], expressed in meters squared.

***Expressed as multiples of 1×10^{11} .

An absolute posttransfusion increment of 10,000/ μ L or greater (approximately 2,000/ μ L/ unit of random donor platelets) in an average-sized adult corresponds to a CCI of 5,000.

Platelet Refractoriness

Patients who respond poorly to repeated platelet infusions are termed refractory. Posttransfusion platelet counts (performed at 1 and 24 hours) are useful tests to determine refractoriness. The CCI should also be calculated and failure to achieve a CCI of 5,000 or greater is a cause to suspect platelet refractoriness. Refractoriness may be immune or non-immune mediated. Immune-mediated refractoriness indicates alloimmunization to HLA or human platelet antigens (HPA).

- Non-immune-mediated causes of platelet refractoriness: Fever, infection, splenomegaly, disseminated intravascular coagulation (DIC), massive bleeding, and medications that enhance platelet destruction; more likely to affect the 24-hour posttransfusion count.
- Immune-mediated platelet refractoriness caused by alloimmunization to HLA and HPAs usually associated with multiparity or exposure to nonleukocyte-reduced platelet transfusions; more likely to affect the 1-hour posttransfusion count.

In practice, the distinction between immune- and non-immune-mediated platelet refractoriness becomes less clear as alloimmunized patients often have multiple medical issues predisposing to nonimmune refractoriness. When immune-mediated platelet refractoriness is suspected and CCI is less than 5,000 after each of two platelet transfusions the following steps should be taken:

- | ABO-compatible fresh (<72 hours in storage) platelets should be used for two subsequent transfusions.
- | If CCI still does not exceed 5,000, HLA antibody screen to detect alloantibodies, or commercial platelet compatibility tests should be performed.
- | When alloantibodies with broad specificity are found (for HLA A and B loci) platelets from HLA-matched donors are indicated.
- | Crossmatch compatible platelets may be beneficial when HLA antibody status of the recipient cannot be determined, HLA-matched platelets cannot be obtained, or when the patient is refractory to HLA-matched platelets (up to 40% to 50% of cases).
- | Corticosteroids, washed platelets, or IVIG have not proved useful in the treatment of refractoriness.

Granulocytes

Granulocytes are collected by apheresis for specific patients, from donors who are mobilized before collection with corticosteroids and/ or granulocyte-colony-stimulating factor (G-CSF).

- | Granulocytes can be stored at room temperature up to 24 hours post-collection but optimally should be administered within 6 hours of collection.
- | Granulocyte collections have a volume of 250 mL and contain plasma, approximately 30-mL RBC, and variable amounts of mononuclear leukocytes and platelets.
- | Granulocyte concentrates should be ABO, Rh, and RBC crossmatch compatible.
- | Products should be irradiated because of the viable lymphocytes in the collection.
- | The minimal therapeutic dose is 1×10^{10} granulocytes/ unit; however, increments are unlikely to be seen unless greater than 3 to 4×10^{10} granulocytes/ unit are infused.¹

Indications: Patients with absolute neutrophil counts of less than 0.5×10^9 / L and documented bacterial or fungal infection refractory to antimicrobials. Recipients must have a reasonable expectation of achieving hematopoietic recovery or engraftment (endogenous granulocyte production). Infants with bacterial sepsis, whose granulocyte counts are less than 3×10^9 / L with postmitotic neutrophils comprising less than 10% of their nucleated marrow cells, may benefit from granulocyte transfusions.¹ Granulocyte transfusions act as a “bridge” to neutrophil recovery.

A 1- to 6-hour posttransfusion CBC with differential for determination of absolute neutrophil count (ANC) may help assess efficacy. A 6-hour posttransfusion increment

may be higher than a 1-hour posttransfusion ANC because granulocytes are sequestered in the lungs before equilibrating in peripheral blood. If the patient's ANC fails to reach expected levels or if a clinical reaction occurs, an HLA antibody screen and tests for antibodies to human neutrophil antigens (HNA) are indicated to look for an immunologic cause.

Not indicated: Patients whose bone marrow function is not likely to recover. Contraindicated in patients with prior severe pulmonary reactions to HLA or HNA antibodies or HLA or HNA alloimmunization.

- | Alloimmunized patients may develop chills, fever, rigors, shortness of breath, wheezing, pulmonary infiltrates, cyanosis, and hypotension^{6,15}; rigors and fever may respond to intravenous (IV) meperidine.
- | Pulmonary toxicity may be exacerbated when granulocytes and amphotericin B are administered in close temporal proximity.¹⁶ At National Institutes of Health (NIH), amphotericin B administration and granulocyte transfusions are separated by at least 4 hours.
- | Granulocyte transfusion therapy should be evaluated after an initial course of four infusions and then periodically.
- | Granulocyte concentrates may contain leukocyte-associated pathogens such as cytomegalovirus (CMV), which may be a particular concern for immunosuppressed stem cell transplant recipients, solid organ transplant recipients, neonates undergoing extracorporeal membrane oxygenation, and low-birth-weight and premature infants.
- | While granulocyte transfusions decrease the length of bacterial infection, proof that granulocyte transfusions decrease mortality has been elusive.^{17,18}

Fresh-Frozen Plasma

Plasma separated from the whole blood or collected by apheresis and frozen within 8 hours is labeled FFP. FFP contains plasma proteins at the time of thaw in about the same concentrations as at the time of collection. The volume of a unit of plasma is approximately 200 mL.

- | By convention, 1 mL of FFP is expected to provide 1 unit of activity of all factors (except labile factors V and VIII). In practice, individual units may vary in content.
- | The usual dose is 10 to 20 mL/kg in adults (equivalent to approximately 4 to 6 units of FFP) to increase coagulation factor levels by 20%.

Indications: Correction of multiple clotting factor deficiencies in patients who are bleeding or prior to an invasive procedure, replacement of factors in consumptive coagulopathy, coagulation factor deficiencies caused by liver disease, dilutional coagulopathy after massive transfusion, replacement fluid for plasma exchange in the treatment of TTP, replacement of single congenital factor deficiencies when no virus-

safe fractionated product is available (mostly applies to factor V and XI deficiency).

- | A PT greater than 1.5 normal or aPTT ratio greater than 2.0 in the microvascular bleeding is a guide to consider treatment.¹¹
- | Note, in the setting of life-threatening bleeding associated with warfarin (Coumadin) therapy where rapid reversal is necessary, or in over-anticoagulated patients with volume overload a more appropriate therapy is prothrombin complex concentrate (PCC). See section blood derivatives.

Not indicated: Volume expansion, protein replacement in nutritional deficiencies.

Cryoprecipitate

Cryoprecipitate (cryo) is the cold-insoluble portion of plasma, which contains factor VIII, fibrinogen, von Willebrand factor, factor XIII, and fibronectin. Ordinarily stored frozen, cryo can be kept at room temperature for up to 6 hours; on pooling it must be transfused within 4 hours.

- | Compatibility testing is unnecessary.
- | A unit of cryo is usually less than 15 mL of plasma and contains more than 80 international units (IU) of factor VIII and more than 150 mg of fibrinogen.
- | One unit of cryo can increase fibrinogen in an average adult by 5 to 10 mg/ dL.
- | A therapeutic dose for an adult is 80 to 150 mL of cryo (8 to 10 units pooled).

Indications: Treatment of acquired fibrinogen deficiency, dysfibrinogenemia, factor XIII deficiency, DIC (if fibrinogen < 1.0 g/ L).

- | Note: Pathogen-inactivated fibrinogen concentrate (see blood derivatives) is preferred for the treatment of congenital fibrinogen deficiency.

Cryo has also been used to correct the platelet defect of uremic bleeding, although with variable success.

The dosage of cryo depends on the underlying deficiency and the plasma volume of the patient. To determine the number of bags of cryo to replace fibrinogen

$$\frac{(\text{Desired fibrinogen level mg / dL} - \text{Initial fibrinogen level mg / dL}) \times \text{Patient's plasma volume dL}^*}{250 \text{ mg(fibrinogen per cryo bag)}}$$

*The plasma volume for an average adult = $(1\% \text{ hematocrit} / 100) \times \text{patient weight in kg} \times 70 \text{ mL/kg}$. For infants and children weighing less than 40 kg, the plasma volume = $(1\% \text{ hematocrit} / 100) \times \text{patient weight in kg} \times 80 \text{ to } 85 \text{ mL/kg}$.

Not indicated: Factor VIII deficiency and von Willebrand disease, for which more specific and safer products now exist.

Hematopoietic Stem and Progenitor Cells

Optimal outcomes in HSCT depend on the successful procurement of cells from patients (autografts) or donors (allografts). Advances in this field continue to improve clinical outcomes for an increasing range of patients with malignant and nonmalignant disorders.

Stem cell sources now include related and unrelated bone marrow, peripheral blood, and umbilical cord blood. The vast majority of HSCT now use mobilized peripheral blood, although guidelines for the management of aplastic anemia still recommend using a bone marrow source, if possible.¹⁹

Historically, mobilization of stem cells into peripheral blood for autografting was done using myelosuppressive chemotherapy such as cyclophosphamide as increased number of cells entered the circulation during the recovery phase of the bone marrow. As cytokines such as G-CSF became available, they were used either alone or in combination with chemotherapy as mobilizing agents. G-CSF is now the standard for this indication.

A significant proportion of patients do not mobilize well with G-CSF with or without chemotherapeutic agents. The factors that have been shown to predict poor mobilization include advanced age, increasing the number of cycles of prior chemotherapy, prior radiation therapy, and marrow metastasis.^{20–22} Lenalidomide, purine analogs, and alkylating agents are particularly marrow suppressive and have been associated with poor mobilization. There is a correlation between the number of circulating CD34⁺ cells and the probability of obtaining an adequate collection for transplant.²³

In 2008, plerixafor (AMD3100) was approved for mobilization in conjunction with G-CSF. In contrast to G-CSF that needs multiple injections over several days a single dose of plerixafor mobilizes stem cells into the periphery beginning at 1 hour and peaking at 10 hours.²⁴ In addition, the quality of the stem cell graft mobilized by plerixafor may be superior. Compared to G-CSF mobilized cells, the plerixafor-mobilized cells were more primitive, and therefore more quiescent and produced superior engraftment in both murine and human recipients.²⁵ The combination of G-CSF and plerixafor has also been studied and produces greater increases in CD34⁺ cells than with either agent alone.²⁶ Plerixafor has emerged as a primary agent in an expanding list of diagnoses and various clinical settings.²⁷

Standard collections of allogeneic peripheral blood stem cell (PBSC) involve 3 to 4 hours per apheresis procedure, during which approximately 10 L of blood are processed. At least two collections are usual, but large volumes of 25 to 30 L are more efficient and used increasingly to allow complete collections with a single procedure.²⁸ Donor demographic and laboratory predictors such as platelet count and CD34⁺ mononuclear cell count can be used to customize the length of the collection

procedure.²⁴

As G-CSF is still the standard agent used, complications seen with stem cell mobilization are often related to this cytokine. Bone pain, headache, fatigue, insomnia, and gastrointestinal disturbances are usually mild and respond to the administration of acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs). Spleen size increases in almost all individuals on G-CSF and this has been associated with splenic rupture. Donors should be advised to refrain from contact sports for a few weeks after the last mobilization.²⁹ Vascular complications and citrate toxicity are not unlike those experienced in other long apheresis procedures (see later).

PBSC grafts are infused as fresh collections or stored frozen with the cryoprotectant dimethyl sulfoxide (DMSO) in liquid nitrogen. Thawed cells infused with DMSO may cause nausea, vomiting, fever, dyspnea, hypotension, and anaphylaxis. Reactions are dose dependent and may be lessened by prophylactic antihistamines. PBSCs carry the risk of transfusion-transmitted infectious agents and are tested in the same manner as are other blood components. However, given their highly specialized use and their lifesaving potential, exceptions are made to donor selection criteria normally used for allogeneic blood collections, with the concurrence of the treating physician and the recipient.

Adequate cell dose for engraftment depends on whether the procedure is an autograft, a related, or an unrelated allograft. Cell dose, cell source, and patient characteristics are all important variables. The dose of stem cells for unrelated donors (National Marrow Donor Program) is 2 to 4×10^8 nucleated cells/ kg of recipient weight, with 2 to 4×10^6 CD34⁺ cells/ kg of recipient weight deemed as an adequate dose for transplantation and doses of greater than 5×10^6 CD34⁺ cells/ kg of recipient weight associated with more rapid engraftment.³⁰ Lower doses may be adequate in related donor settings, but engraftment of both leukocytes and platelets correlates with CD34⁺ cell content.

Cord blood is being used increasingly as a stem cell source. Less acute and chronic graft-versus-host disease (GVHD) is reported as grafts have less alloreactivity even when mismatched as compared with PBSC or bone marrow grafts. In addition, because mismatching is better tolerated, chances improve of finding a suitable donor. However, because of lower numbers of progenitor cells, hematopoietic reconstitution is delayed and as a result recipients of umbilical cord transplants have a higher risk of developing fatal infections. Cells are obtained from the placenta during the third stage of delivery or postdelivery, with the consent of the mother, and stored in liquid nitrogen. The component volume is usually 50 to 100 mL and may be further reduced by removing red cells and plasma. The small volumes and yields of CD34⁺ progenitor cells currently make cord blood most suitable for children and smaller adults. Although simultaneous

infusion of two and even three cord blood units have resulted in successful engraftment for larger adults, in pediatric patients they are not associated with improved outcomes relative to single cord transplants.³¹ PBSCs stored in liquid nitrogen likely remain stable for many years, but maximum safe storage periods have not been determined. Recipients of minor mismatched cord blood seem less likely to make red cell alloantibodies than do recipients of mismatched marrow or PBSC.

Blood Derivatives

Derivatives or blood products are produced commercially by fractionation of plasma and include colloids such as albumin and plasma protein fraction, immune globulins, coagulation factor concentrates, and a variety of orphan proteins such as α -1-antitrypsin and antithrombin.

Prothrombin Complex Concentrate

PCCs are derived from pooled human plasma. They were initially developed for the prophylaxis and treatment of patients with factor IX deficiency. The three products available in the United States (Profilnine SD and Bebulin VH) are termed 3 factor-PCCs as they contain low levels of factor VII whereas Kcentra contains 4 factors.

- | All PCCs contain the vitamin K-dependent factors II, IX, and X while 4 factor-PCCs contain higher levels of factor VII.
- | Thromboembolism is a potential adverse effect and may occur less in 3-factor than 4-factor products.³²
- | As compared to FFP, PCC for warfarin reversal is associated with lower mortality, more rapid international normalized ratio (INR) reduction, and less volume overload without an increased risk of thromboembolic events.³³

Indications: Emergency treatment of bleeding due to overanticoagulation with warfarin. Should always be used in combination with vitamin K therapy.

See [Table 24.51](#) for more blood derivatives and [Table 24.6](#) for coagulation factor preparations.

Rh Immune Globulin

Rh immune globulin (RhIg) is available in intramuscular (IM) form and IV form.

Indications: Prevention of alloimmunization of Rh-negative recipients exposed to Rh-positive RBC, immune thrombocytopenic purpura (ITP).

- | Prevention of Rh immunization in Rh-negative women with Rh-positive fetuses and subsequent HDN or after blood transfusion with Rh-positive blood.
- | Greater than 99% success in preventing Rh alloimmunization in pregnancy³⁴; failure is usually because of missed or insufficient injections.

Treatment of ITP in Rh-positive patients only (IV RhIg).

Table 24.5 Selected Blood Derivatives

Derivative	Indications	Cautions	Derivation	Content
Albumin 5% solution	Hypovolemia Volume expansion Acute liver failure Osmotic pressure binding excess bilirubin Cardiopulmonary bypass surgery Hemodilution	Patients at risk of hypervolemia	Pooled human plasma concentrated by fractionation and treated to nearly eliminate risk of virus transmission osmotically and oncotically equivalent to plasma	At least 95% albumin and the remainder globulins and other proteins 145 mEq/ L sodium
Albumin 25% solution	Maintenance of plasma colloid osmotic pressure Can be given 24 hr after extensive burns treated initially with crystalloids Binding excess free bilirubin Can decrease risk of kernicterus when given prior to exchange transfusion for HDN	Patients at risk of hypervolemia Hyperoncotic	Pooled human plasma concentrated by fractionation and treated to nearly eliminate risk of virus transmission Hyperoncotic	At least 95% albumin and the remainder globulins and other proteins 145 mEq/ L sodium
PPF ^a (available only as 5% solution)	Similar to 5% albumin	Hypotension administration at greater than 10 mL/ min, especially in patients taking ACE inhibitors Contraindication Intra-arterial administration in cardiopulmonary bypass setting	Similar to 5% albumin	At least 83% albumin and the remainder globulins and other proteins 145 mEq/ L sodium
IVIG ^b	Prophylaxis Passive immunity and passive antibody Replacement Primary immunodeficiencies Immunomodulation Some autoimmune disorders (e.g.,	Attenuated vaccines should not be administered in close temporal association with attenuated vaccines (3 mo) Rapid administration	Pooled human plasma concentrated by fractionation and treated to nearly eliminate risk of virus transmission	90% IgG, trace IgM, trace IgA Half-life in preparation: 18–32 d

	refractory ITP) Treatment of Certain Infectious Disorders Pediatric HIV infection, CMV interstitial pneumonitis, posthematopoietic transplantation Thrombocytopenia related to HIV, neurologic disorders Guillain–Barre syndrome and chronic inflammatory demyelinating polyneuropathy Relative indications PTP Neonatal alloimmune thrombocytopenia Refractory warm type autoimmune hemolytic anemia	IgA deficiency IgA-deficient patients must receive Ig from IgA deficient donors only Route of administration Intramuscular Ig must never be administered intravenously IVIG should not be administered intramuscularly	A subcutaneous formulation is available
Fibrinogen	Treatment of acute bleeding episodes in patients with congenital fibrinogen deficiency, including afibrinogenemia and hypofibrinogenemia	Thrombotic events have been reported	Pooled human plasma concentrated by fractionation and treated to nearly eliminate risk of virus transmission 900–1,300 mg lyophilized fibrinogen concentrate powder for reconstitution printed on label

Other plasma derivatives include antithrombin complex, protein C concentrate, C1-esterase inhibitor, a-1-proteinase inhibitor, fibrinogen, and factor XIII, which are indicated for the corresponding specific deficiencies.

^aPlasma protein fraction.

^bIntravenous immune globulin.

ACE, angiotensin-converting enzyme; CMV, cytomegalovirus; HDN, hemolytic disease of the newborn; Ig, immunoglobulin; ITP, immune thrombocytopenic purpura; IV, intravenous; PPF, PTP, posttransfusion purpura.

Adapted from Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine. 12th ed. Oxford, UK: Wiley Blackwell; 2014.

Table 24.6 Selected Coagulation Factor Preparations

Coagulation Factor	Content	Indications	Risks and Cautions
Recombinant factor VIIa ^a (rVIIa)	Activated coagulation factor VII	Licensed use Refractory hemophilia A or B High-factor VIII or IX inhibitor levels in hemophilia A or B	Increased risk of thrombosis, particularly in patients with DIC and

		bleeding episodes in patients with factor VII deficiency Used successfully Severe bleeding refractory to other therapy Glanzmann thrombasthenia	atherosclerotic cardiovascular disease Allergic reactions Hypertension
Factor VIII concentrate	Factor VIII Humate P (CSL Behring, King of Prussia, PA) has vWF	Hemophilia A factor VIII deficiency (other than hemophilia A) von Willebrand disease	Development of factor VIII inhibitor (10% of severely hemophilic patients) Viral transmission potential (small) Hemolysis (passive anti-AB antibodies)
Recombinant factor VIII	Factor VIII ReFacto (Wyeth, Madison, NJ) (b domain deleted preparation) does not contain human albumin	Hemophilia A	Albumin-containing preparations have small risk of viral transmission Allergic reactions
Factor IX complex (prothrombin complex)	Specified amount of concentrated factor IX, and variable amounts of activated factors II, VII, and X, and protein C	Hemophilia B Factor X deficiency (rare) Factor VII deficiency (rare)	Increased risk of thrombosis in liver disease Viral transmission (small) Hemolysis (passive anti-AB antibodies)
Coagulation factor IX	Purified factor IX and nontherapeutic amounts of factors II, VII, and X	Hemophilia B	Less risk of thrombosis than with factor IX concentrate Viral transmission potential (small) Hemolysis (passive anti-AB antibodies)
Recombinant factor IX	Factor IX	Hemophilia B	Less risk of thrombosis than with factor IX concentrate Allergic reactions

^aHemostatic agent.

DIC, disseminated intravascular coagulation; vWF, von Willebrand factor.

Intramuscular RhIg is used in Rh-negative females of childbearing age after exposure to small-volume Rh-positive RBC (with platelet transfusions or the accidental transfusion of an Rh-positive unit of RBC). RhIg IV is used for large volume exposures.

Use of RhIg in Rh-negative males and females without childbearing potential is controversial but may protect from complications of future transfusions.

In a pregnancy where the mother is Rh negative and the father Rh positive, the fetus

may be Rh positive, and therefore there is a risk of Rh immunization of the mother. In these cases a prophylactic dose of 300 µg of anti-D is given at 28 weeks gestation.

A full dose containing 300 µg of anti-D to cover an exposure of 15 mL of Rh-positive RBC is also given:

- | To Rh-negative women at risk of Rh immunization within 72 hours of delivery.
- | After amniocentesis and chorionic villus sampling, with manipulations such as external cephalic version, ectopic pregnancy, abortion, and abdominal trauma after 20 weeks gestation.

A minidose containing 50 µg of anti-D to cover an exposure of 2.5 mL of Rh-positive RBC can be given if these events occur before 12 weeks, although 300 µg is often given as it is more readily available.

Erroneous transfusions:

- | RhIg dose should be calculated based on the RBC volume transfused.

The half-life of RhIg is 21 days. Additional RhIg should be administered in the following situations:

- | Expected ongoing risk of fetomaternal hemorrhage.
- | Nonobstetric cases with additional transfusion of products containing Rh-positive RBC 21 days or more after the last dose of RhIg.

RhIg should be given within 72 hours but if not feasible it should still be administered as soon as the need is recognized for up to 14 days. Some authorities maintain that it may be useful up to 28 days.³⁵

Not indicated: Rh-positive individuals, previously immunized Rh-negative individuals, Rh-negative females with known Rh-negative fetuses or newborns, or in Rh-negative patients with ITP.

Derivative and Recombinant Coagulation Factors

Recombinant and plasma-derived coagulation factors provide a concentrated source of the desired factor for prevention and treatment of bleeding episodes in patients with factor deficiencies. Recombinant factors contain no other human-derived products and no risk of viral disease transmission ([Table 24.6](#)).

TRANSFUSION REACTIONS AND ADVERSE SEQUELAE

Any adverse response to blood component transfusion is considered a transfusion reaction. Most reactions occur at the beginning or during transfusion and are termed acute. Others, including development of alloantibodies, iron overload, and some parasitic and viral infections, do not become apparent for weeks, months, or years and

are termed chronic.

- Because most transfusion reactions occur within 15 minutes, close monitoring of vital signs and status at the beginning of the transfusion may prevent more severe reactions.
- If a reaction is suspected, the infusion should be halted, the transfusion service notified, appropriate samples collected, and the patient monitored.

Transfusion reactions can be classified as hemolytic versus nonhemolytic and acute versus delayed. Hemolytic reactions may be immune-or non-immune-mediated ([Table 24.7](#)).

Table 24.7 Transfusion Reactions

Mechanism	Acute/ Severe	Delayed/ Potentially Severe	Other
Immunologic	Acute hemolytic transfusion reaction	DHTR (red cell antigen alloimmunization)	Mild allergic/ urticarial
	Sickle cell hyperhemolysis transfusion syndrome	Human leukocyte antigen alloimmunization	Posttransfusion purpura
	Anaphylaxis	Transfusion-associated GVHD	Febrile nonhemolytic transfusion reaction
	Transfusion-related acute lung injury	Post-transfusion purpura	
Nonimmunologic	Bacterial sepsis	Viral transmission	Angiotensin-converting enzyme inhibitor-related hypotension
	Circulatory overload	Parasitic transmission	
	Air embolism	Prion transmission Hemosiderosis	

DHTR, delayed hemolytic transfusion reaction; GVHD, graft-versus-host disease.

Acute Hemolytic Transfusion Reaction

Acute hemolytic transfusion reactions may be severe and fatal and may occur with as little as 10 mL of incompatible blood. Most result from ABO blood group incompatibility between patient plasma and donor RBC, and are usually the result of the incorrect transfusion of a unit (or several units) of blood intended for another patient.

Presentation: Fever, chills, flank/ back pain, dyspnea, chest pain, anxiety that can progress to hypotension, renal failure, shock, and death, if severe.

Mechanism: ABO incompatibility resulting from the destruction of transfused RBC by preformed, naturally occurring IgM anti-A and/ or anti-B isohemagglutinins in the recipient's plasma.

- Intravascular hemolysis is caused by complement fixation by IgM antibodies.

- | Hemoglobinemia occurs followed by hemoglobinuria.
- | Complement activation promotes histamine and serotonin release that causes wheezing, chest pain or tightness, and gastrointestinal symptoms.
- | Acute hemolysis results in anemia.
- | Cytokine release contributes to renal failure, hypotension, shock, and DIC.

Evaluation:

- | Clerical check of component bag and compare with patient identification.
 - | Submit to the blood bank:
 - | The infusion set, the implicated unit, and any units transfused within 4 hours of the reaction.
 - | Blood specimens from the patient for repeat ABO group and Rh type determination, crossmatch, and DAT (usually positive); assessment of other parameters such as hematocrit (decreased), lactate dehydrogenase (increased), haptoglobin (decreased), and bilirubin levels (increased usually by 6 hours)¹ as indices of hemolysis.
 - | First posttransfusion-voided urine to examine for hemoglobinuria.
 - | Transfusion reaction report detailing the events, signs/ symptoms, and documenting the patient's pretransfusion and posttransfusion vital signs.

Management: Mainly supportive, transfusion must be stopped and disconnected at the hub of the needle and IV access maintained with physiologic saline.

- | Support of blood pressure and renal blood flow with fluids and pressors if necessary, and induction of diuresis to maintain urine output at greater than 1 mL/ kg/ hr.⁶
- | Withhold further transfusion until the cause of the reaction is determined.
- | Coagulation status should be monitored and DIC treated, if present.

Prevention: Meticulous clerical check of the blood unit and patient identification. In case of error in patient identification, immediate steps must be taken to insure that a second patient does not receive the wrong unit (companion error).

Hemolysis Associated With Passive Antibody Infusion

- | Severe acute hemolysis can occur if large volumes of ABO-incompatible plasma (usually group O FFP or apheresis platelets into group A patient) are infused.
- | Recognition and management of acute anemia is ordinarily sufficient.

Sickle Cell Hemolytic Transfusion Syndrome

Patients with sickle cell anemia are at an increased risk from hemolytic transfusion reactions. A fall in Hb after transfusion of red cells is suggestive of the hyperhemolytic syndrome. This entity is poorly understood but occurs when both the patient's and the

transfused red cells are destroyed.^{1,36} Transfusion-associated hemolysis may mimic a severe posttransfusion pain crisis which is mediated by factors such as decreasing Hb, complement activation, and increased oxygen consumption with fever. Stopping transfusion is imperative as additional transfusion may result in exacerbation of the syndrome. In the United States, inherent differences in RBC antigen phenotypes between patients with sickle cell anemia (almost exclusively of African descent) and the majority of blood donors (primarily non-African) place patients with sickle cell anemia at increased risk of alloantibody formation and immune hemolysis. In addition, patients with sickle cell disease are often chronically and heavily transfused. Extended red cell phenotyping of patients with sickle cell anemia in the early stages of transfusion therapy reduces the risk of alloimmunization.^{37,38}

Delayed Hemolytic Transfusion Reaction

Delayed hemolytic transfusion reaction (DHTR) occurs within days, weeks, or even months after transfusion in patients who have been previously immunized by transfusion or by pregnancy (primary immunization). These reactions rarely, if ever, occur as a result of primary immunization and are usually a result of second transfusions. Because its manifestation may be mild and symptoms delayed in onset, DHTR may not be immediately recognized and this complication is likely underreported.³⁹ Serious sequelae are rare. The importance of recognizing these reactions is to document antibody formation and prevent hemolytic reactions with future transfusions.

Presentation: Fever with or without chills, and symptoms of anemia. Jaundice may be present.

- | Subtle decrease in Hb may be the only clinical manifestation.
- | DAT is usually positive.
- | Hemoglobinuria is rare because hemolysis is extravascular.

Mechanism: Repeat stimulation and accelerated (anamnestic) appearance of the antibody in a previously alloimmunized patient on reexposure to the offending antigen.

Management: Close monitoring of the patient's Hb for evidence of continuing hemolysis and supportive therapy. Eculizumab has been used in some patients with sickle cell disease and life-threatening hyperhemolysis to interdict complement-mediated cell lysis.

Prevention: Future transfusions must be antigen-negative for the corresponding implicated antibody even if the antibody is no longer detectable.

- | Notification of the patient to prevent future reactions (as by antibody card or bracelet).

Other Causes of Hemolysis

The other causes of hemolysis temporally associated with transfusion that may mimic hemolytic transfusion reactions include drug-induced hemolysis, mechanical and thermal hemolysis, and hemolysis related to bacterial contamination of the RBC unit.

- | Drug-induced hemolysis that may be either intra-or extravascular may present with anemia, positive DAT, and elevated LDH and bilirubin.
- | Hemolysis may result from administration of blood with hypotonic solutions (D5W, hypotonic saline, and distilled water) or medications.
- | Mechanical hemolysis may result from prosthetic heart valves and other intravascular devices and transfusion through small-bore catheters or with the use of roller pumps.
- | Thermal hemolysis results from exposure of red cells to cold (ice, temperatures below 1°C to 6°C, or use of unmonitored refrigerators) or to temperatures above 42°C (malfunction of blood warmers, or unmonitored unconventional blood warming methods); the DAT should be negative in these cases; some of these reactions have been fatal.

Anaphylactic Transfusion Reactions

Anaphylactic transfusion reactions usually occur at the start of transfusion when small amounts of blood containing plasma are transfused. Although rare, anaphylactic reactions may be rapidly fatal.

Presentation: Sudden onset of urticaria, flushing, chills, vomiting, diarrhea, initial hypertension followed by hypotension, angioedema, coughing, stridor, laryngeal edema, and progression to respiratory distress and shock. Fever is not a feature of anaphylaxis.

Mechanism: IgE-mediated response to transfused proteins.

Rare reactions may occur in patients with medication, peanut, or latex allergy when exposed to plasma containing these allergens.

IgA deficiency is common. Only IgA-deficient patients with antibodies to IgA are susceptible to anaphylaxis on receiving plasma containing IgA. These reactions as well as reactions to other plasma proteins such as haptoglobin, are rare.

Management: Discontinue transfusion and employ standard measures for anaphylaxis: Epinephrine, corticosteroids, and circulatory support.

Anti-IgA or antibody to a subspecies must be demonstrated to confirm whether the patient is IgA deficient.

Prevention: Usually not predictable.

Transfusion-Related Acute Lung Injury

Transfusion-related acute lung injury (TRALI) is characterized by noncardiogenic pulmonary edema and is associated with transfusion of plasma-containing blood components. Previously believed to be underreported,³⁹ it has an estimated frequency of

between 1:1,300 and 1:5,000 transfusions and is the leading cause of transfusion-associated deaths reported to the FDA with a mortality rate of 6% to 10%.^{1,6}

Presentation: Acute respiratory insufficiency, tachycardia, dyspnea, hypotension, oxygen desaturation (O₂ saturation < 90% on room air), chills, rigors, fever with 1°C to 2°C temperature increase, and a bilateral pulmonary infiltration by chest X-ray in the absence of heart failure or elevated central venous pressure, with no other causes of acute lung injury evident.^{39,40}

- | Occurs during or within 6 hours (most commonly after 2 hours) of transfusion.
- | Hypoxemia may require intubation (70% to 75% of cases).³⁹
- | Symptoms subside rapidly; chest X-ray normal within 96 hours; clinical recovery in 48 to 96 hours in 80% of the patients.⁶

Mechanism: Reaction of antineutrophil antibodies and/ or anti-HLA class I and II antibodies, usually donor derived, to corresponding recipient antigens; destruction occurs in the pulmonary vasculature and results in endothelial damage.

- | Multiparous females are more likely to be HLA/ HNA alloimmunized, as are donors who have themselves been transfused.
- | Antibodies from the recipient against cells in the donor plasma implicated in approximately 5% of cases.
- | In 10% of cases no donor or recipient antibodies are identified.

A second possible pathophysiologic mechanism of TRALI is the “two-hit” theory, which suggests an interaction between primed pulmonary neutrophils in patients with underlying illness (in proinflammatory states) and biologically active response modifiers (lipids, cytokines) introduced by transfusion.⁴¹

Management: Discontinue transfusion and provide respiratory and circulatory supportive care; steroids and diuretics are not useful.

Prevention: A patient who has experienced TRALI is not necessarily at increased risk for development of TRALI with future transfusions, unless receiving blood from the same donor. Implicated donors are ordinarily deferred indefinitely. Blood centers now draw plasma preferentially from male donors to reduce the risk from multiparous female donors and apheresis platelets are routinely screened for the HLA-antibodies.⁴²

Transfusion-Associated Circulatory Overload

Transfusion-associated circulatory overload (TACO) can present in a similar fashion to TRALI but is much more commonly seen. Unlike TRALI, circulatory overload is associated with central venous pressure elevation and cardiac failure. Pulmonary edema in TACO is cardiogenic in origin and may result in the development or exacerbation of congestive heart failure.³ Children, the elderly, those with compromised cardiac, renal,

or pulmonary function, and patients in states of plasma volume expansion (normovolemic chronic anemia, thalassemia major, and sickle cell disease) are at particular risk.^{6,39}

Presentation: Cough, dyspnea, cyanosis, orthopnea, chest discomfort, rales, headache, distension of jugular veins, and tachycardia.

Management: Discontinue transfusion and administer supportive care (oxygen, diuresis, and phlebotomy), if necessary.

Prevention: Patients at risk should receive smaller aliquots of blood infused at slower rates (1 to 4 mL/ kg/ hr) in as concentrated a form as possible.

Transfusion-Associated Graft-Versus-Host Disease

Transfusion-associated GVHD (TA-GVHD) is a rare but severe adverse outcome of transfusion. Those at risk include the immunocompromised, patients with certain malignancies such as lymphoma, neuroblastoma, and sarcoma, patients receiving directed donations (from family members or relations), and premature infants. Patients with HIV and aplastic anemia do not need irradiated blood products^{1,6,39} (see Table 24.4).

Presentation: Rash, diarrhea, hepatitis, mucositis, and pancytopenia; almost universally fatal with rare reports of survival.¹

Mechanism: Immunocompetent lymphocytes from the donor engraft recognize the patient's antigens as foreign and initiate an immune response.

Management: Supportive; no specific measures have proved effective.

Prevention: Irradiation of blood components.

- | Irradiation dose of 2,500 cGy.
- | Leukoreduction does not prevent TA-GVHD.

Bacterial Contamination and Sepsis

The initial symptoms of bacterial contamination occur shortly after the start of transfusion and usually within 2 hours. These include chills and fevers. Temperature increase can be less marked in patients premedicated with antipyretics or receiving corticosteroids. In addition, mild septic reactions may be initially obscured by underlying conditions that predispose the patient to fever or manifest similar signs and symptom.⁶

Presentation: Rigors and shaking chills, fever, nausea, vomiting, abdominal cramp, bloody diarrhea, severe hypotension, and rapid progression to circulatory compromise, renal failure, shock, and DIC.

- | Not all contaminated blood products result in reactions, clinically detectable sepsis, and fewer yet are fatal.

Mechanism: Common sources of bacterial contamination are subclinical bacteremia in the donor or skin contaminants at the phlebotomy site.

- | Commonly implicated in RBC contamination are bacteria that survive the cold storage conditions and thrive on iron, such as Yersinia and Pseudomonas.
- | Platelets stored at room temperature are the component most susceptible to bacterial growth.
- | Contamination occurs in approximately 1:5,000 collections, sepsis in 1:50,000 transfusions, and death in 1:1,000,000 transfusions.⁶³
- | More than half of bacterial contaminations of platelets are caused by skin flora, Staphylococci, Streptococci, Propionibacterium; many species are not implicated in serious transfusion reactions.^{6,43}
- | Currently, the residual risk of septic transfusion reactions from platelets is estimated to be more than twice as high for pooled whole blood derived platelets, not tested by culture method, than for culture-negative apheresis platelets, 1:33,000 versus 1:75,000.⁶³
- | The highest mortality is usually associated with blood components contaminated with endotoxin-producing Gram-negative bacteria.

Evaluation and management: Transfusion must be stopped and broad spectrum antibiotics commenced.

- | All transfusion units and blood component bags transfused within 4 hours should be returned to the blood bank for culture.
- | Blood samples from both the blood component unit(s) and the patient should be sent for culture.

Prevention: Strict hygienic must be practiced from collection to processing, storage, and administration of the component and transfusion of blood components within the allotted 4 hours. All blood platelets are now tested by culture and/ or rapid assay prior to release and a method of pathogen inactivation has been licensed for platelets and plasma.

Mild Allergic/ Urticarial Transfusion Reactions

Allergic transfusion reactions are relatively common, do not generally progress to anaphylaxis, are rarely lethal, and do not necessarily recur with subsequent transfusions.

Presentation: Localized erythema, pruritus, flushing, and urticaria, usually near the IV site.

- | Severe urticaria and pruritus may be the initial signs of anaphylaxis.

Mechanism: Release of histamine and other anaphylotoxins.

Evaluation and management: Mild allergic reactions generally resolve when

transfusion is temporarily stopped, and symptoms improve with the administration of oral or parenteral antihistamines.

- | Mild allergic reactions (hives only): Transfusion with the same unit may be resumed, at a slower rate and with close monitoring of the patient.
- | Transfusion reaction evaluation generally not necessary.

Prevention: Mild allergic reactions are considered atopic reactions and generally unpredictable.

- | No method to prescreen for all possible offending antigens.
- | Antihistamines are effective in treating, but not in preventing allergic reactions.

Posttransfusion Purpura

Posttransfusion purpura (PTP) is a profound thrombocytopenia that may occur after transfusion with any blood component but is usually associated with the transfusion of red cells or whole blood.

Presentation: Abrupt decline in the platelet count within days to 2 to 3 weeks posttransfusion (mean 9 days).⁶

- | Usually self-limiting: resolves within 2 to 3 weeks without treatment.

Mechanism: Not well understood; antibodies against human platelet (platelet-specific) antigens to which the patient may have become sensitized as a result of pregnancy or prior transfusion destroy both transfused and autologous platelets (innocent bystander effect).

- | Most commonly anti-HPA 1a (HPA-1a).

Management: IVIG is the current treatment of choice.

Although PTP almost never recurs, antigen-negative components should be transfused in patients with previously documented PTP.

- | Family members provide a good source of donors if antigen-negative blood is otherwise not available.
- | Severe PTP that does not resolve spontaneously and if refractory to high-dose IVIG may respond to plasmapheresis.^{1,39}

Hypotension Associated With Transfusion

Mechanism: Transient isolated hypotension that resolves after discontinuation of transfusion may be caused by activation of bradykinin and is associated with use of bedside leukoreduction filters and apheresis procedures (especially with albumin replacement).³⁹

- Patients receiving ACE inhibitors are at particular risk as ACE inhibitors block normal metabolism of bradykinin.

Evaluation and Management: Severe transfusion reactions such as anaphylaxis, acute hemolytic transfusion reaction, TRALI, and bacterial contamination (sepsis) must be excluded.

Prevention: Avoid bedside leukoreduction (especially for patients taking ACE inhibitors).

Febrile Nonhemolytic Transfusion Reaction

Febrile nonhemolytic transfusion reactions (FNHTR) are defined by a greater than 1°C increase in temperature for which no other cause is identifiable. FNHTR is a diagnosis of exclusion, made after consideration of acute hemolytic transfusion reaction, TRALI, and sepsis and determination that the symptoms are not related to the patient's underlying medical condition or medications. The incidence of FNHTR varies among patient populations, and depends on the age and type of blood component and a variety of donor and recipient factors. Platelets are more likely to be implicated than are RBC or FFP, and older blood components more than fresh, leukocyte-reduced components (as cytokines accumulate during storage). The incidence is higher in patients who have received multiple transfusions.³⁹

Presentation: Chills, fever, rigors that may be preceded by headache, and nausea; the patient may also experience tachycardia, tachypnea, and general discomfort.

- Patients who sustain significant fever with transfusion are likely to have repeated reactions.

Mechanism: Interaction of antibodies in the recipient against donor leukocytes or platelets and subsequent cytokine release; accumulated cytokines in blood component bag during storage of cellular components.

Evaluation and management: Same as with a hemolytic reaction.

- Antipyretics may be administered.

Prevention: Prestorage leukoreduction, removal of plasma in extreme situations, and premedication with antipyretics.

Hemosiderosis

Hemosiderosis or iron overload occurs in chronically transfused patients (usually a cumulative dose of 50 to 100 red cell transfusions).

Presentation: Classical symptoms include “bronzing” of skin, hepatomegaly, hepatic fibrosis and malfunction, diabetes and other endocrine gland dysfunction, and

cardiac failure.

Mechanism: Accumulation of iron in the skin, liver, heart, and endocrine organs.

Evaluation and management: Iron studies and iron chelation or phlebotomy when appropriate.

Prevention: Consider chelation for more than 50 units red cell burden; red cell exchange using apheresis helps delay iron accumulation in patients with sickle cell disease.⁶

Table 24.8 Estimated Risk of Transfusion Transmission of Viral Diseases in the United States with Current Testing

Virus	Risk per transfusion
HIV 1 and 2	1:1,467,000
Hepatitis C	1:1,149,000
Hepatitis B	1:280,000
West Nile Virus	Seasonal and regional variability
Zika Virus	Seasonal and regional variability

Adapted from Stramer SL. Current risks of transfusion-transmitted agents: a review. Arch Pathol Lab Med. 2007;131:702–707; Dodd RY, Notari EP IV, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion. 2002;42:975–979.

Transfusion-Transmitted Infections

Current testing of donor blood prior to release of blood components includes the following:

- Antibodies to HIV types 1 and 2 (anti-HIV-1/ 2); hepatitis C virus (HCV; anti-HCV); hepatitis B core antigen (anti-HBc); human lymphotrophic virus types I and II (antihuman T-cell leukemia virus [HTLV] I/ II); Trypanosoma cruzi; Treponema pallidum.
- Surface antigen: Hepatitis B surface antigen (HBsAg).
- Nucleic acid testing (NAT) for HIV; HCV; West Nile virus (WNV), Zika virus (ZikV).

With current mandated testing, the estimated risk of transmission of viral infections per transfusion is reported in Table 24.8.^{43–46} Blood is also tested by enzyme-linked immunoassay for the detection *T. cruzi* parasite, the causative agent for Chagas disease.

Other infectious agents and diseases transmissible by transfusion^{47,48} for which the blood supply is not currently routinely tested include the following:

- Hepatitis A virus, parvovirus B19, chikungunya, and dengue viruses
- Parasitic diseases, not as common in the United States, including malaria, babesiosis, and leishmaniasis

- The protozoal disease toxoplasmosis which affects mainly immunocompromised patients
- Prions (protein particles) responsible for the transmission of variant Creutzfeldt–Jakob disease (vCJD).⁴⁹

Alternatives to Allogeneic Blood Transfusion

Alternatives to the use of blood component therapy are available and may be particularly useful for bleeding patients who refuse allogeneic blood component transfusions (usually for religious beliefs) or bleeding patients unresponsive to appropriate transfusion therapy.

Patients with religious concerns about blood transfusion must be informed of any human-derived content in products that may be administered, allowing them to make an informed decision.

Examples of alternatives to allogeneic blood transfusion^{6,50} are listed in Table 24.9.

Indications for use of some pharmaceutical hemostatic agents^{1,51} are summarized in Table 24.10.^{1,52}

Massive Transfusion

Massive transfusion is the administration of blood components over a 24-hour period in amounts that equal or exceed the total blood volume of the patient (10 or more units of whole blood or 20 units of packed RBC in an adult). After transfusion of one or more blood volumes, an abbreviated RBC crossmatch (see general concepts) is performed to provide RBC more rapidly. Group O, Rh-negative, or Rh-positive blood (acceptable in male patients or postmenopausal females) may be released initially and once a specimen is received by the transfusion service ABO-compatible blood can be provided.

- Adequate intravascular blood volume and blood pressure may be maintained initially with colloids (albumin, plasma protein fraction) or crystalloids (lactated Ringer solution or normal saline).
- Transfusion of packed RBC may become necessary after a loss of more than 25% to 30% of blood volume, depending on the rate of blood loss, tissue perfusion, and oxygenation status of the patient.⁵³
- Transfusion of blood components based on fixed ratios or algorithms should be avoided, with the possible exception of 1:1:1 trauma packets early in trauma resuscitation.⁵³

Table 24.9 Examples of Alternatives to Allogeneic Blood Transfusion

Preoperative	Intraoperative	Postoperative
--------------	----------------	---------------

Autologous blood collection	Blood salvaged from a sterile surgical field	Blood recovered from drainage
Donor's Hb should meet or exceed 11 g/ dL	Use in oncologic procedures is controversial	Used primarily with cardiac and orthopedic surgery ⁶
Donor must be at no increased risk of bacterial infection	Gross contamination of surgical field with malignant cells constitutes a relative contraindication	
Donations up to every 5d with last collection no later than 72 hr before procedure	Blood salvaged/ processed by devices that collect, centrifuge, wash, and concentrate red blood cells	
Autologous blood subject to same shelf-life limitations as allogeneic blood components	May be stored at room temperature for 4 hr from the end of collection and at 1°C–6°C for up to 24 hr if refrigeration began within 4 hr from completion of processing ⁵⁰	
Unit(s) may be frozen until used	Acute normovolemic hemodilution	
Prevents	Whole blood is collected from patient and replaced with crystalloid or colloid and reinfused after cessation of major blood loss, or sooner if indicated	Transfusion should start within 6 hr of initiation of collection ^{6,50}
Transmission of viral infections; red blood cell antigen alloimmunization; some transfusion reactions	May be stored at room temperature up to 8 hr or refrigerated at 1°C–6°C up to 24 hr after collection ⁵⁰	
Risk of bacterial contamination and of clerical error leading to transfusion of ABO-incompatible units not decreased significantly ⁶	Other blood components	
	Examples include platelet-rich and platelet-poor plasma and cryoprecipitate intended for transfusion or for topical use	
	Must be stored at room temperature and administered before the patient leaves the operating room ⁵⁰	

Hb, hemoglobin.

Adverse Sequelae of Massive Transfusion

- | Dilution and/ or consumption of hemostatic constituents of blood. Platelet count, prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen levels should be determined frequently.
- | Replacement therapy is warranted if platelet count is less than 50, PT/ PTT greater than 1.5, and fibrinogen concentration less than 100 mg/ dL.
- | Hypothermia, acidosis, hypocalcemia, and other biochemical disturbances may occur

- and electrolytes, particularly potassium and calcium, should be monitored.^{1,53}
- Hypocalcemia secondary to citrate accumulation may occur when large volumes of blood are administered at rapid rates (more than 100 mL/ min), especially in the liver and renal dysfunction.¹
 - Trauma-associated coagulopathy (TAC) is unpredictable and highly lethal and is best managed by early and aggressive component therapy.⁵³

Table 24.10 Selected Pharmacologic Hemostatic Agents

Agent	Indications	Nonindications and Adverse Effects	Administration and Preparations
rFVIIa	Licensed Use Refractory hemophilia A or B High-factor VIII or IX inhibitor levels in hemophilia A or B Off-label use Severe bleeding refractory to other therapy Glanzmann thrombasthenia Bleeding with need for rapid reversal of warfarin anticoagulants	Potential for thromboembolic events in patients predisposed to thrombosis No suitable assay for monitoring drug efficacy; laboratory values do not correlate with hemostatic effectiveness	Usual dose 90 µg/ kg repeated every 2 hr
Vitamin K	Vitamin K deficiency resulting in coagulopathy (factors II, VII, IX, X) Reversal of warfarin anticoagulation when prolonged effectiveness is desired and simple discontinuation of warfarin is not feasible	Not effective for urgent reversal of warfarin or correction of vitamin K-dependent factors IV administration associated with anaphylaxis (rare), which may be fatal	IV faster effect than subcutaneous (SC) or oral (PO) Solution and tablet forms: 0.5–20 mg
Protamine	Neutralization of anticoagulation from unfractionated heparin (displaces antithrombin III and complexes heparin) After cardiac bypass surgery in patients who have received unfractionated heparin	Possible heparin rebound because of shorter half-life of protamine compared with heparin Requires close monitoring of coagulation parameters Hypotension Increased pulmonary artery pressure Allergic reactions	Immediate onset of action 1 mg of protamine neutralizes 80–100 USP heparin Half-life: 2 hr Activated clotting time used to monitor effectiveness and determine dosing Dosing should not exceed 100 mg in 2 hr
Conjugated Estrogens	Coagulopathy related to uremia, GI bleeding associated with	Not useful when immediate	Onset of effect within 6 hr and duration of

	angiodyplasia (Osler–Weber–Rendu syndrome), end-stage renal disease, von Willebrand disease	hemostasis is required May be associated with gynecomastia, weight gain, and dyspepsia	up to 2 weeks Maximum effectiveness between 5–7 d Usual dose IV: 0.6 mg/ kg Patch: 50–100 µg/ 24 hr PO: 50 mg Peak response IV within 1 hr and duration of up to 12 hr Usual dose IV: 0.3 µL/ kg in 50 mL normal saline (in 10 mL normal saline for children weighing <10 kg) over 30 min SC: 0.3 µg/ kg Intranasal: 300 µg (adults)
DDAVP ^a	Bleeding associated with Hemophilia A; von Willebrand disease; Bernard–Soulier disease; aspirin ingestion; platelet hemostasis; defects where other treatment options are not effective	Not useful in type 2B von Willebrand disease because of thrombocytopenia and increased affinity of vWF for platelets May be associated with hyponatremia when administered with hypotonic fluids Electrolytes and volume should be closely monitored Hypertension, facial flushing, nausea, increased risk of myocardial infarction in cardiac surgery patients Tachyphylaxis with repeated administration	
AMCA ^b / ACA ^c	Excessive fibrinolysis: Congenital α2-antiplasmin deficiency GI and uterine bleeding where antifibrinolytic action is needed Some acquired causes of fibrinolysis (e.g., cardiac bypass surgery) May be useful as irrigation in intractable bleeding from the bladder A megakaryocytic and peripheral immune-mediated thrombocytopenia AMCA: Used in patients with hemophilia A and B during dental procedures along with fibrin sealant and DDAVP	Contraindicated in thrombotic DIC or active intravascular clotting Reduce dose in renal insufficiency	AMCA is 10-fold more potent than EACA Usual dose EACA: 2–4 g/ 3–4 hr Total of 10–24 g/ 24 hr AMCA: 1 g/ 6–8 hr Total of 3–4 g/ 24 hr Half-life for both: 2–10 hr

^a1-deamino-8-d-arginine vasopressin (desmopressin).

^bTranexamic acid.

^cε-Aminocaproic acid.

ACA; AMCA; EACA; DDAVP; DIC, disseminated intravascular coagulation; GI, gastrointestinal; IV, intravenous; vCJD, variant Creutzfeldt–Jakob disease; vWF, von Willebrand factor.

Adapted from Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine. 14th ed. Oxford, UK: Blackwell; 2014; Bolan CD, Klein HG. Blood component and pharmacologic therapy of hemostatic disorders. In: Kitchens C, Kessler C, Alving BM, eds. Consultative Hemostasis and Thrombosis. 22nd ed. Philadelphia, PA: Elsevier; 2007:461–490.

Disseminated Intravascular Coagulation

DIC probably complicates massive transfusion less often than suspected, but DIC is associated with shock, independent of blood loss or transfusion. TAC is a particularly lethal variant.

- | Laboratory coagulation test results are consistent with a consumptive coagulopathy.
- | Treatment is correction of the underlying disorder while transfusion therapy is supportive.
- | Administer cryoprecipitate or fibrinogen concentrate when fibrinogen falls less than 100 mg/ dL.
- | Other components, such as platelets may be necessary, especially if bleeding is severe.
- | If multiple factors are consumed, plasma factor levels of above 30% can be achieved with an FFP dose of 10 to 20 mL/ kg.¹

IMMUNOHEMATOLOGIC DISORDERS

Hemolytic Disease of the Newborn

HDN is the destruction of fetal erythrocytes by maternal IgG antibodies that cross the placenta and react with a paternally derived antigen present on the fetal RBC. Although traditionally associated with Rh antibodies (anti-Rh D), other antibodies including anti-A, anti-B, and anti-K:1 have been implicated and may cause significant HDN.

- | Mild cases: The newborn is asymptomatic and laboratory findings of a positive DAT and mild hyperbilirubinemia are the only abnormalities.
- | Severe cases: May result in intrauterine death (hydrops fetalis, erythroblastosis fetalis). There is a high risk of kernicterus caused by high unconjugated bilirubin.

Treatment: Intrauterine RBC transfusion (in severe cases) using antigen compatible (with the mother's antibody), irradiated, CMV-negative, sickle-negative RBC suspended in 5% albumin or compatible FFP.

- | Usually a two-blood-volume red cell exchange removes approximately 25% of excess bilirubin, provides albumin to which excess bilirubin can bind, and removes antibody and approximately 70% of RBC coated with antibody.
- | Additional exchange transfusions may be necessary if level of bilirubin continues to

rise.

Neonatal Alloimmune Thrombocytopenia and Maternal Immune Thrombocytopenic Purpura

Neonatal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT) is the destruction of platelets that carry paternally derived antigens by maternal antibodies that cross the placenta. As with HDN, NAIT may vary in severity from very mild and asymptomatic thrombocytopenia to life-threatening bleeding, and it may occur in utero or in the neonatal period. The vast majority of NAIT is associated with antibody (IgG) against the common platelet antigen HPA-1a (PLA1), especially in the HLA DRw52a phenotype.¹

NAIT is usually self-limiting and resolves within 2 to 3 weeks. If NAIT is suspected, often as a result of a previously affected pregnancy, cordocentesis to determine platelet counts may be performed in conjunction with the administration of compatible platelets (maternal platelets or platelets known to be negative for the implicated antigen).

- | In utero NAIT: IVIG is given with or without weekly corticosteroid administration to the mother (1 g/ kg) until delivery.
- | If there is high risk of intracranial hemorrhage, platelet transfusion is performed immediately before delivery.
- | When compatible platelets are unavailable, high-dose IVIG has been administered to the neonate with variable effectiveness.
- | An increase in platelet counts within 24 to 48 hours may be seen in patients who respond to IVIG.⁶

Maternal Immune Thrombocytopenic Purpura

In maternal ITP, antibodies against maternal platelets can cross the placenta and cause thrombocytopenia in the fetus.

- | Degree of thrombocytopenia is milder than that associated with NAIT with a lower risk of fetal or neonatal intracranial hemorrhage.
- | Maternal platelets and random donor platelets are generally not required but may be needed in about 44% of newborns.⁵⁴
- | IVIG may also be beneficial.
- | Maternal ITP usually resolves in days to weeks (on clearance of maternal antibodies from the neonate's circulation).

Autoimmune Hemolytic Anemias

AIHA are characterized by the antibodies against the individual's own RBC antigens (autoantibodies), resulting in accelerated destruction of RBC. AIHA may be associated

with autoimmune disorders, infections, medications, or malignancies, or it may be primary. The laboratory hallmark is the positive DAT, indicating the antibody or complement-coated red cells. Antibody may also be present in the serum such that positive DAT and IAT may coexist, making identification of underlying alloantibodies and compatibility testing difficult.

Warm Autoimmune Hemolytic Anemia

The majority of AIHA are caused by warm reacting antibodies. The implicated antibody is usually IgG and reacts with all cells, although occasionally a warm autoantibody appears to have specificity against Rh antigens and several others. Patients with compensated warm AIHA require no specific treatment but should be investigated for an underlying condition such as systemic lupus erythematosus or a lymphoproliferative disorder. In children, viral illness may be accompanied by transient AIHA. Medications, particularly purine nucleoside analogues, are commonly associated with warm AIHA. Warm-reacting autoantibodies may be present only as a laboratory finding, or they may cause severe, even life-threatening hemolysis; these antibodies react optimally at 37°C in vitro. Patients are often totally asymptomatic, but may present with fatigue, jaundice, or mild anemia. Moderate splenomegaly occurs in about one-third to one-half of the cases, and hepatomegaly in one-third of the patients. Hemolysis is usually not severe and is mainly extravascular.⁵⁵

Laboratory findings include a positive DAT, spherocytes on the blood smear, elevated unconjugated bilirubin and LDH as indices of red cell turnover, and a high reticulocyte count. Rarely, reticulocytopenia may be seen, either because of inadequate bone marrow response or because the autoantibody reacts with red cell precursors, as well as with mature cells.

Red cell alloantibodies as a result of previous transfusions or pregnancies, found in approximately one-third of patients with AIHA, are capable of causing severe hemolytic transfusion reactions. Broadly reactive autoantibody may mask underlying alloantibodies and make procurement of compatible blood difficult.

Occasionally hemolysis may result in severe symptomatic anemia. Transfusion should not be delayed even when compatible blood cannot be obtained. The term “least incompatible” has not been adequately defined, does not correlate with clinical events, and would be best abolished.⁵⁶

Oral glucocorticoids (prednisone at 1 mg/ kg/ day) are the current standard of care and about 50% of patients will respond. Splenectomy is effective in approximately half of those who are refractory to steroids. Immunosuppressive agents and IVIG may benefit selected patients.⁵⁶ Refractory AIHA, especially when associated with lymphoproliferative disease, may respond to the monoclonal antibody rituximab (see

later).

Cold Agglutinin Syndrome

Cold-reacting antibodies are common and usually of no significance, but some cold agglutinins, especially those with very high titer at 4°C but broad thermal amplitude (reactivity up to 30°C) may result in cold agglutinin syndrome (cold hemagglutinin disease). IgM is the immune globulin classically implicated and the DAT is almost always positive for C3d alone. Cold agglutinin syndrome may be primary (idiopathic) or secondary, often to a viral infection or lymphoproliferative disorder. Acute cold agglutinin syndrome may be associated with *Mycoplasma pneumoniae* and Epstein–Barr virus, is seen mostly in children and young adults, and tends to be transient and self-limited. Chronic cold agglutinin syndrome is most often seen in the elderly and may be associated with lymphoma, chronic lymphocytic leukemia, and Waldenstrom macroglobulinemia. Patients may present with acrocyanosis and hematuria precipitated by cold, and/or severe pain in the nose, ears, and distal extremities on cold exposure. Severe anemia is rare in the chronic form.

Transfusion is rarely necessary, but, when performed, the typing specimen must be kept at body temperature from the time of phlebotomy through the testing procedure. Up to 50% of transfused cells may be destroyed by autoantibodies of the patient even when blood warmers are used.

Treatment with corticosteroids and splenectomy is not effective, and most patients do well simply by avoiding exposure to the cold. Rituximab, the anti-CD20 monoclonal antibody, has proved effective when administered as four weekly infusions.⁵⁶

Paroxysmal Cold Hemoglobinuria

Paroxysmal cold hemoglobinuria (PCH) is a rare form of AIHA that results from a biphasic IgG antibody (Donath–Landsteiner antibody). Originally associated with untreated syphilis, it is now found most often with viral infections in children. The Donath-Landsteiner antibody binds to the RBC at cold temperatures and causes intravascular hemolysis as complement is fixed at warmer temperatures, accounting for the paroxysms of hemoglobinuria. Anemia associated with PCH is usually transient and self-limiting over 2 to 3 weeks. If transfusion support becomes necessary, crossmatch-compatible blood may be found if the antibody is not reactive at temperatures above 4°C. Unavailability of compatible RBC should not preclude transfusion in life-threatening anemia associated with hemolysis, despite shortened survival of the transfused RBC.^{1,6,55}

Therapeutic Apheresis in the Management of Immunohematologic Disorders

Apheresis is the process by which selected components or substances in blood are removed from the circulation and the remainder of the blood returned to the patient. Apheresis is used to collect routine blood components (platelets, plasma, and stem cells) from donors for transfusion. It also has therapeutic utility in many disorders where a pathologic substance is found in the blood. It is also used to remove excess of cells, normal or abnormal, in the blood, known as cytapheresis. See [Table 24.11](#) for commonly accepted indications for therapeutic apheresis for hematologic disorders endorsed by the American Society for Apheresis (ASFA).^{57,58}

- | The kinetics of most intravascular substances indicate that the exchange of 1 to 1.5 plasma volumes results in the highest efficiency removal with progressively decreased efficiency and added toxicity with each additional consecutive exchange.
- | The volume of blood processed to attain the desired apheresis effect depends on the nature of the specific component, including its intravascular distribution and concentration in the particular patient.
- | The patient's total blood volume determines the safe extracorporeal blood volume (which should not exceed 15% of blood volume).
- | Small patients may require that the instrument be primed with saline or blood.

Apheresis is generally safe, especially for normal component donors. Complications mainly relate to vascular access, hemodynamic changes (especially for patients with cardiovascular disease), and a variable loss of blood components. The risks associated with apheresis are usually associated with a patient's underlying disease.

- | Plasma exchange may result in a 30% or more decrease in platelet counts.⁵⁹
- | Platelet transfusion may be required for patients with low platelet counts and hemostatic problems.
- | Cellular blood component counts return to normal after a few days and proteins and electrolytes re-equilibrate within hours, although fibrinogen may remain below baseline levels after 72 hours.⁶⁰
- | Hypotension may occur as a result of volume shifts and bradykinin activation from blood contact with plastic⁶¹; withhold ACE inhibitors, which potentiate this effect, from patients for 24 to 48 hours before an apheresis procedure.
- | Plasma exchange may reduce blood levels of certain medications, especially those bound to plasma proteins or those with a long plasma half-life.⁶¹
- | Citrate is used to prevent coagulation of blood in the circuit and may result in citrate toxicity: Binding of calcium, decreased levels of ionized calcium, and potentially symptomatic hypocalcemia.
- | Hypocalcemia may present with mild perioral tingling and discomfort, chest tightness, and tetany in severe cases; if symptoms do not subside with adjustment of citrate and whole blood flow rates, administration of oral calcium (as chewable tablets) or IV

calcium gluconate replace the bound calcium and prevent the accompanying syndromes.⁶²

Table 24.11 Recommendation for Therapeutic Apheresis in Hematologic Disorders

Category I: Accepted as Standard First-line or Primary Therapy

- Babesiosis—severe (red cell exchange)
- Cryoglobulinemia (plasma exchange)
- Cutaneous T-cell lymphoma (photopheresis)
- Hereditary hemochromatosis (red cell exchange)
- Hyperviscosity in monoclonal gammopathies (plasma exchange)
- Hyperleukocytosis (cytapheresis)
- Sickle cell disease with acute stroke, prophylaxis (red cell exchange)
- TTP (plasma exchange)
- Polyneuropathy with IgM, with or without Waldenstrom macroglobulinemia (plasma exchange)
- HUS— atypical, complement-mediated (plasma exchange)

Category II: Generally Accepted as Adjunctive or Supportive Therapy

- ABO-incompatible hematopoietic progenitor cell/ marrow transplantation (plasma exchange recipient)
- GVHD— skin (photopheresis)
- Malaria— severe (red cell exchange)
- Myeloma with acute renal failure (plasma exchange)
- Red cell alloimmunization in pregnancy^a (plasma exchange)
- Sickle cell disease— primary/ secondary prophylaxis/ iron overload prevention (red cell exchange)
- Thrombocytosis— symptomatic (cytapheresis)
- Pure red blood cell aplasia (plasma exchange)

Category III: Optimal Role of Apheresis Therapy Not Established

- Aplastic anemia (plasma exchange)
- Erythrocytosis or polycythemia vera (phlebotomy/ cytapheresis)
- Warm autoimmune hemolytic anemia (plasma exchange)
- GVHD— nonskin (photopheresis)
- Multiple myeloma with polyneuropathy (plasma exchange)
- Thrombocytosis— prophylactic (cytapheresis)
- PTP (plasma exchange)
- Catastrophic antiphospholipid syndrome

^aIf fetus <20-week gestation and previous severely affected pregnancy.

GVHD, graft-versus-host disease; HUS, hemolytic uremic syndrome; PTP, Posttransfusion purpura; TTP, thrombotic thrombocytopenic purpura.

Adapted from Szczepiorkowski ZM, Winters JL, Bandarenko N, et al. Guidelines on the use of therapeutic apheresis in clinical practice—evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. *J Clin Apher*. 2010;25(3):83–177.

Thrombocytapheresis

Increased platelet counts, particularly in myeloproliferative disorders in which platelets are also qualitatively abnormal, may be associated with bleeding or thrombosis.

Patients who are bleeding (e.g., with chronic myelogenous leukemia [CML]) may obtain immediate benefit from therapeutic cytapheresis. Generally plateletpheresis is a

first-line therapy for thrombocytosis (platelet counts greater than 500,000/ μL) in symptomatic patients.

- | Each procedure will lower the count 30% to 50%.
- | Cytoreductive chemotherapy should be initiated simultaneously, as plateletpheresis is not effective long term.^{57,61}

Leukocytapheresis (Leukapheresis)

Malignant leukocytosis or hyperleukocytosis (immature white blood cell counts of greater than 100,000/ μL), in association with some leukemias, can result in leukostasis in the central nervous system, kidneys, and lungs. Symptoms may occur with rapidly rising blast counts less than 100,000/ μL , especially in AML and CML.

- | Changes in mentation, dizziness, blurred vision, hypoxia, or respiratory symptoms constitute a medical emergency where rapid cytoreduction is imperative.
- | Therapeutic leukapheresis can reduce the leukocyte count by 30% to 50% in hours.
- | Symptoms may abate promptly.
- | Reduction of the white cell count permits cytoreductive chemotherapy and reduces the risk of developing tumor lysis syndrome.
- | Chemotherapy with hydroxyurea (if myeloid malignancy) or a similar agent should be initiated concurrently as repeated leukocytapheresis may not control hyperleukocytosis.

Photopheresis (Extracorporeal Photochemotherapy)

Photopheresis is the separation of the patient's leukocytes by apheresis for extracorporeal treatment with the chemotherapeutic agent 8-methoxy-psoralen (8-MOP) and photoactivation by ultraviolet light.

A (ultraviolet [UVA]) light for subsequent reinfusion to the patient.

- | It has some efficacy in the treatment of refractory cutaneous T-cell lymphoma, allograft rejection, refractory acute and chronic GVHD, scleroderma, and other autoimmune diseases.
- | Mechanism of action is not fully understood; possibly related to apoptosis of pathogenic T lymphocytes and antigen-presenting cells or anti-idiotype cytotoxic T-cell response.^{1,61}
- | Use of 8-MOP is contraindicated in patients with light-sensitive disorders such as xeroderma pigmentosa, albinism, and certain porphyrias.⁶¹

Erythrocytapheresis/ Red Cell Exchange

Red cell exchange involves the removal of abnormal red cells.

- | Patient's RBC are replaced with normal donor RBC in patients with sickle cell

disease.

- | Erythrocytapheresis may be used to reduce red cell mass acutely in symptomatic (visual disturbances, confusion, lethargy, hemorrhage, threatened stroke, or thrombosis of abdominal vasculature) patients with excessive polycythemia.^{57,61}
- | In polycythemia rubra vera (PRV) saline or colloid volume replacement is administered to maintain isovolemia.

Red Cell Exchange and Sickle Cell Anemia

Red cell exchange may be used acutely to treat some complications of sickle cell disease,⁵⁷ including acute chest syndrome, stroke, retinal infarction, early priapism, and hepatic crisis, or as protracted or chronic treatment for the prevention of complications such as stroke and recurrent severe painful crises, and for reduction of iron overload secondary to transfusion.⁶¹

- | In the perioperative setting, simple transfusion or a single red cell exchange prevents morbidity associated with sickle cell disease.
- | The goal is to achieve HbS of less than 30%.
- | Transfusion and exchange have been used to treat sickle complications during pregnancy but routine use is not warranted. Exchange transfusion can raise HbA to levels difficult to achieve with simple transfusion and may benefit patients in the third trimester for preeclampsia, sepsis, and preoperative management.⁶¹

Red Cell Exchange and Parasitemia

Red cell exchange has been used as antiparasitic treatment in malaria to decrease the circulating parasite load when it exceeds 5%.⁷

Plasmapheresis

Plasmapheresis may be used to collect plasma for transfusion or manufacturing of plasma derivatives, or to remove undesirable substances from the circulation. Colloids or saline (plasma with TTP) are administered to maintain isovolemia. See Tables 24.11 and 24.12 for common indications for therapeutic plasmapheresis.^{56,57}

Table 24.12 Categories I and II Recommendations for Therapeutic Plasma Exchange

Category I	Category II
ANCA-associated rapidly progressive glomerulonephritis (granulomatosis with polyangiitis and microscopic polyangiitis)	ABO-incompatible renal allograft (plasma exchange recipient) ABO-incompatible hematopoietic progenitor cell/ marrow transplantation (plasma exchange recipient) ^a
Acute inflammatory demyelinating	Demyelinating disease

polyradiculoneuropathy (Guillain–Barré syndrome)	
Antiglomerular basement membrane antibody disease	Coagulation factor inhibitors
Chronic inflammatory demyelinating polyradiculoneuropathy	Cryoglobulinemia with polyneuropathy
Cryoglobulinemia	
Demyelinating polyneuropathy with IgG and IgA	Familial hypercholesterolemia
Focal segmental glomerulosclerosis	
Homozygous familial hypercholesterolemia (selective adsorption)	Lambert–Eaton myasthenia syndrome
Hyperviscosity with monoclonal gammopathy	
Myasthenia gravis	
Sydenham's chorea	Myeloma with acute renal failure
Polyneuropathy with IgM (with or without Waldenstrom macroglobulinemia)	
Progressive multifocal leukoencephalopathy associated with natalizumab	
Renal transplantation— antibody-mediated rejection	Pediatric autoimmune neuropsychiatric disorders (PANDAS)
TTP	Phytanic acid storage disease rheumatoid arthritis— refractory (immunoabsorption)

^aRemoval of RBC from the hematopoietic progenitor cell/ marrow.

Ig, immune globulin; TTP, thrombotic thrombocytopenic purpura.

Adapted from Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice—evidence-based approach from the Writing Committee of the American Society for Apheresis. The Sixth Special Issue, J Clin Apher. 2013;28:145–284.

Thrombotic Microangiopathies

TTP and hemolytic uremic syndrome (HUS) belong to a spectrum of thrombotic microangiopathies: TTP may be associated with prominent neurologic symptoms; HUS presents with a more prominent renal component. Characteristic findings of TTP include fever, renal impairment, neurologic symptoms such as change in mental status, seizures, or coma, thrombocytopenia (platelet counts usually less than 30,000/ μ L), and microangiopathic hemolytic anemia with schistocytes.

TTP results from the accumulation of ultra-large von Willebrand factor multimers caused by congenital absence of or inhibitory antibodies to the vWF-cleaving metalloprotease ADAMTS13.^{1,61} When TTP and HUS-like syndromes are associated with immunosuppressive agents (typically vinca alkaloids, mitomycin, bleomycin, BL22, cisplatin, tacrolimus, and cyclosporin A), post-bone marrow transplant or cancer they do not respond well to therapeutic plasma exchange (TPE).

- TPE is first-line therapy for the treatment of TTP and generally not effective for typical HUS.
- TPE should be performed as soon as TTP is suspected; plasma infusion may be used at the onset of TTP before TPE can be performed.
- The effectiveness of TPE in TTP depends on the removal of ultra-large vWF multimers and reduction of the IgG antibodies against vWF-cleaving protease (ADAMTS13).
- Plasma (FFP) is the fluid replacement of choice in TPE for TTP and also replaces the vWF-cleaving protease.
- TPE is often done daily, then tapered until platelet counts stabilize at more than 100,000/ μL for 2 consecutive days.
- Response should be monitored by clinical assessment and laboratory measurements (platelet count, LDH, and extent of schistocytosis).

Platelet transfusion is generally discouraged as this is thought to potentially precipitate thrombosis. This dogma has recently been challenged.¹³ Platelet transfusion may be necessary in the event of life-threatening hemorrhage.⁶

Dysproteinemias

Complications of paraproteinemias of multiple myeloma, Waldenstrom macroglobulinemia, and cryoglobulinemia respond to TPE.

- Hyperviscosity syndrome with mental status changes, mucosal and gastrointestinal bleeding, retinopathy, and hypervolemia constitutes a medical emergency.
- Hyperviscosity responds to even small volume exchanges, but procedures need to be repeated until the paraprotein is controlled with chemotherapy.⁶¹

References

1. Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine. 12th ed. Oxford, UK: Blackwell; 2014.
2. Mielcarek M, Leisenring W, Torok-Storb B, et al. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence for graft-versus-plasma cell effect. *Blood*. 2000;96:1150–1156.
3. Worel N, Greinix HT, Keil F, et al. Severe immune hemolysis after minor ABO-mismatched allogeneic peripheral blood progenitor cell transplantation occurs more frequently after nonmyeloablative than myeloablative conditioning. *Transfusion*. 2002;42:1293–1301.
4. Griffith LM, McCoy JP, Bolan CD, et al. Persistence of recipient plasma cells and anti-donor isohaemagglutinins in patients with delayed donor erythropoiesis after major ABO incompatible non-myeloablative haematopoietic cell transplantation.

- Br J Haematol. 2005;128:668–675.
5. Bolan CD, Leitman SF, Griffith LM, et al. Delayed donor red cell chimerism and pure red cell aplasia following major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation. *Blood*. 2001;98:1687–1694.
 6. Fung M, ed. Technical Manual. 18th ed. Bethesda, MD: AABB Press; 2014.
 7. Brecher ME, ed. Technical Manual. 15th ed. Bethesda, MD: AABB Press; 2005:600.
 8. Friedberg RC, Andrzejewski C. Transfusion therapy in hematopoietic stem cell transplantation. In: Mintz PD, ed. *Transfusion Therapy: Clinical Principles and Practice*, 2nd ed. Bethesda, MD: AABB Press; 2005.
 9. Cortés-Puchl, Wiley BM, Klein HG, et al. Restrictive red blood cell transfusion strategies in patients with cardiovascular disease: a meta-analysis. *Ann Int Med* (in press).
 10. Docherty AB, O'Donnell R, Brunskill S, et al. Effect of restrictive versus liberal transfusion strategies on outcomes in patients with cardiovascular disease in a noncardiac surgery setting: systematic review and meta-analysis. *BMJ*. (Online) 2016;352.
 11. Carson JL, Guyatt G, Heddle NM, et al. Clinical practice guidelines from the AABB: Red Blood Cell Transfusion Thresholds and Storage. *JAMA*. 2016;316:2025.
 12. Fontana S, Kremer Hovinga JA, Lämmle B, et al. Treatment of TTP. *Vox Sang*. 2006;90:245–254.
 13. Swisher KK, Terrell DR, Vesely SK, et al. Clinical outcomes after platelet transfusions with TTP. *Transfusion*. 2009;49:873–887.
 14. Kaufman RM, Djulbegovic B, Gernsheimer T, et al. Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med*. 2015; 162:205–213.
 15. Stroncek DF, Leonard K, Eiber G, et al. Alloimmunization after granulocyte transfusions. *Transfusion*. 1996;36:1009–1015.
 16. Wright DG, Robichaud KJ, Pizzo PA, et al. Lethal pulmonary reactions associated with the combined use of amphotericin B and leukocyte transfusions. *N Engl J Med*. 1981;304:1185–1189.
 17. Price TH, Boeckh M, Harrison RW et al. Efficacy of transfusion with granulocytes from G-CSF/ dexamethasone-treated donors in neutropenic patients with infection. *Blood*. 2015;29(126):2153.
 18. Strauss RG. Neutrophil/ granulocyte transfusions collected from G-CSF + dexamethasone-stimulated donors. *Curr Opin Hematol*. 2015;22:565–567.
 19. Georges GE, Storb R. Hematopoietic stem cell transplantation for acquired aplastic anemia. *Curr Opin Hematol*. 2016;23:495–500.
 20. Carral A, de la Rubia J, Martin G, et al. Factors influencing the collection of peripheral blood stem cell in patients with acute myeloblastic leukemia and non-

- myeloid malignancies. *Leuk Res.* 2003;27:5–12.
- 21. Vasu S, Leitman SF, Tisdale JF, et al. Donor demographic and laboratory predictors of allogeneic peripheral blood stem cell mobilization in an ethnically diverse population. *Blood.* 2008;112(5):209–210.
 - 22. Panch SR, Yau YY, Kang EM, et al. Mobilization characteristics and strategies to improve hematopoietic progenitor cell mobilization and collection in patients with chronic granulomatous disease and severe combined immunodeficiency. *Transfusion.* 2015;55(2):265–274.
 - 23. Fruehauf S, Schmitt K, Veldwijk MR, et al. Peripheral blood progenitor cell (PBPC) counts during steady-state haemopoiesis enable the estimation of the yield of mobilized PBPC after granulocyte colony-stimulating factor supported cytotoxic chemotherapy: an update on 100 patients. *Br J Haematol.* 1999;105:786–794.
 - 24. Devine SM, Flomenberg N, Vesole DH, et al. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol.* 2004;22:1095–1102.
 - 25. Hess D, Wirthlin L, Craft T, et al. Human CD34+ cells mobilized by AMD3100 demonstrate enhanced NOD/ SCID repopulating function compared to CD34+ cells mobilized by granulocyte colony stimulating factor. *Blood.* 2005;106:1962.
 - 26. Yuan S, Wang S. How do we mobilize and collect autologous peripheral blood stem cells? *Transfusion.* 2016;57(1):13–23. doi: 10.1111/trf.13868.
 - 27. Pantin J, Purev E, Tian X, et al. Effect of high-dose plerixafor on CD34+ cells mobilization in healthy stem-cell donors: results of a randomized crossover trial. *Haematologica.* 2016;102(3):600–609. doi: 10.3324/haematol.2016.147132.
 - 28. Bolan CD, Carter CS, Wesley RA, et al. Prospective evaluation of cell kinetics, yields and donor experiences during a single large-volume apheresis versus two smaller volume consecutive day collections of allogeneic peripheral blood stem cells. *Br J Haematol.* 2003;120:801–807.
 - 29. Stroncek D, Shawker T, Follmann D, et al. G-CSF-induced spleen size changes in peripheral blood progenitor cell donors. *Transfusion.* 2003;43:609–613.
 - 30. Snyder EL, Haley NR, eds. *Cellular Therapy: A Physician's Handbook.* 1st ed. Bethesda, MD: AABB Press; 2004:11–23.
 - 31. Wagner JE Jr. Eapen M, Kurtzberg J. One-unit versus two-unit cord-blood transplantation. *N Engl J Med.* 2015; 372:288–293.
 - 32. Chai-Adisaksopha C, Hillis C, Siegal DM, et al. Prothrombin complex concentrates versus fresh frozen plasma for warfarin reversal. A systematic review and meta-analysis. *Thromb Haemost.* 2016;116:879–890.
 - 33. Ageno W, Gallus AS, Wittkowsky A, et al. Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest.*

- 2012;141(2 Suppl):e44S–88S.
34. Sandler SG, Gottschall JL. Postpartum Rh immunoprophylaxis. *Obstet Gynecol*. 2012;120(6):1428–1438.
 35. ACOG Practice Bulletin. Prevention of RhD alloimmunization. Number 4, May 1999 (replaces educational bulletin Number 147, October 1990). Clinical management guidelines for obstetrician-gynecologists. American College of Obstetrics and Gynecology. *Int J Gynecol Obstet*. 1999;66:63–70.
 36. Petz LD, Calhoun L, Shulman IA, et al. The sickle cell hemolytic transfusion reaction syndrome. *Transfusion*. 1997;37:382–392.
 37. Chou ST, Fasano RM. Management of patients with sickle cell disease using transfusion therapy: guidelines and complications. *Hematol Oncol Clin North Am*. 2016 Jun;30(3):591–608.
 38. Vichinsky EP, Luban NL, Wright E, et al. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. *Transfusion*. 2001 Sep;41(9):1086–1092.
 39. Vlaar AP, Juffermans NP. Transfusion-related acute lung injury: a clinical review. *Lancet*. 2013;382(9896):984–994.
 40. Toy P, Popovsky MA, Abraham E, et al. Transfusion-related acute lung injury: definition and review. *Crit Care Med*. 2005;33:721–726.
 41. West FB, Silliman CC. Transfusion-related acute lung injury: advances in understanding the role of proinflammatory mediators in its genesis. *Expert Rev Hematol*. 2013;265–276.
 42. Eder AF, Herron R, Strupp A, et al. Transfusion-related acute lung injury surveillance (2003–2005) and the potential impact of the selective use of plasma from male donors in the American Red Cross. *Transfusion*. 2007;47:599–607.
 43. Zhou S, Dorsay KA, Notari EP, et al. Prevalence, incidence, and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blood donors since the introduction of nucleic acid testing. *Transfusion*. 2010;50:1495–1504.
 44. Zou S, Stramer SL, Notari EP, et al. Current incidence and residual risk of Hepatitis B infection among blood donors in the United States. *Transfusion*. 2009;49(suppl 2):1S–235S.
 45. Stramer SL. Current risks of transfusion-transmitted agents: a review. *Arch Pathol Lab Med*. 2007;131:702–707.
 46. Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion*. 2002;42:975–979.
 47. Dodd RY. Transmission of parasites by blood transfusion. *Vox Sang*. 1998;74(Suppl 2):161–163.
 48. Stramer SL. Emerging infectious diseases. *ISBT Sci Ser*. 2014;9(1):30–36.

49. Health Protection Agency Press Statement. Fourth case of transfusion-associated vCJD infection in the United Kingdom. *Euro Surveill*. January 18, 2007.
50. Santrach P, ed. Standards for Perioperative Autologous Blood Collection and Administration. 4th ed. Bethesda, MD: AABB Press; 2009.
51. Simmons JW, Powell MF. Acute traumatic coagulopathy: pathophysiology and resuscitation. *Br J Anaesth*. 2016 Dec;117(Suppl 3):iii31–iii43.
52. Bolan CD, Klein HG. Blood component and pharmacologic therapy of hemostatic disorders. In: Kitchens C, Kessler C, Alving BM, eds. Consultative Hemostasis and Thrombosis. 22nd ed. Philadelphia, PA: Elsevier; 2007:461–490.
53. Webert KE, Mittal R, Sigouin C, et al. A retrospective 11-year analysis of obstetric patients with idiopathic thrombocytopenic purpura. *Blood*. 2003;102:4306–4311.
54. Petz LD, Garratty G, eds. Immune Hemolytic Anemias. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2004.
55. Schöllkopf C, Kjeldsen L, Bjerrum OW, et al. Rituximab in chronic cold agglutinin disease: a prospective study of 20 patients. *Leuk Lymphoma*. 2006;47(2):253.
56. Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: The Sixth Special Issue. *J Clin Apher*. 2013;28:145–284.
57. Rogers RL, Johnson H, Ludwig R, et al. Efficacy and safety of plateletpheresis by donors with low-normal platelet counts. *J Clin Apher*. 1995;10:194–197.
58. Szczepiorkowski ZM, Winters JL, Bandarenko N, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. *J Clin Apher*. 2010;25(3):83–177.
59. Flbaum MA, Cuneo RA, Appelbaum FR, et al. The hemostatic imbalance of plasma-exchange transfusion. *Blood*. 1979;54:694–702.
60. McLeod BC, ed. Apheresis: Principles and Practice. 3rd ed. Bethesda, MD: AABB Press; 2010.
61. Bolan CD, Cecco SA, Wesley RA, et al. Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation. *Transfusion*. 2002;42:935–946.
62. Griffith KS, Lewis LS, Mali S, et al. Treatment of malaria in the United States: a systematic review. *JAMA*. 2007;297(20):2264–2277.
63. Eder AF, Kennedy JM, Dy BA, et al. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). *Transfusion*. 2007;47:1134–1142.

Suggested Reading

1. Klein HG, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine. 12th ed. Oxford, UK: Blackwell; 2014.
2. McLeod BC. Therapeutic Apheresis: A Physician's Handbook. 3rd ed. Bethesda, MD: AABB Press; 2010.
3. Sweeney J, Lozano M, eds. Platelet Transfusion Therapy. Bethesda, MD: AABB Press; 2013.
4. Wong ECC, Roseff SD. Pediatric Transfusion: A Physician's Handbook, 4th ed . Bethesda, MD: AABB Press; 2014.
5. Babic AM, Hendrickson JE, Eapen K et al. Core Principles in Cellular Therapy, 3rd ed. Bethesda, MD: AABB Press; 2014.
6. Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis. *J Clin Apher*. 2013;28:145–284.
7. Zou S, Dorsay KA, Notari EP, et al. Prevalence, incidence, and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blooddonors since the introduction of nucleic acid testing. *Transfusion*. 2010;50:1495–1504.

Hemochromatosis

Sandhya R. Panch, Charles D. Bolan, and Susan F. Leitman

EPIDEMIOLOGY

Classic hereditary hemochromatosis (HH), is an autosomal recessive disorder caused by inappropriate dietary absorption of iron and abnormal iron cycling. It is characterized by progressive accumulation of iron in tissues, particularly the liver, pancreas, heart, endocrine organs, and skin, which may lead to end-stage organ damage, usually during or after middle age.^{1–3} It is one of the most common single-gene disorders in Caucasians of northern European descent, with an incidence of 1 in 200 and a carrier rate of 1 in 10 persons. However, the clinical penetrance of the disorder is highly variable, and only a minority of affected persons develops severe or life-threatening organ dysfunction.^{4,5}

Genetic Basis for Classic Hemochromatosis: HFE Mutations

- Mutations in HFE, a major histocompatibility complex (MHC) class-I-like gene on chromosome 6, are found in nearly 90% of persons with the clinical phenotype and 100% of affected persons with a strong family history of the disorder.^{6,7}
- Substitution of tyrosine for cysteine at amino acid 282 of the HFE gene product (C282Y) is considered the founder mutation; and by linkage disequilibrium analysis, the mutation originated recently, within the past 2,000 years. C282Y occurs with the highest frequency in northwestern European populations, reaching 14% in areas of Great Britain (Table 25.1). Allele frequency decreases in a north-to-south and west-to-east direction across Europe, and the ancestral haplotype may have been of Viking or Celtic origin; it is extremely rare in African and Asian populations. Homozygosity for C282Y is seen in 64% to 96% of persons with clinical hemochromatosis.
- A second HFE mutation, replacement of histidine by aspartate at residue 63 of the

HFE protein (H63D) is frequently found on the non-C282Y-containing chromosome of individuals with clinical hemochromatosis who are heterozygous for C282Y.⁶ H63D is an older mutation with a wider population distribution, having an allele frequency of 5% to 14% throughout Europe and Asia. It appears to be a genetic polymorphism without much clinical impact in the absence of another genetic or environmental factor. Compound heterozygosity for C282Y/ H63D is seen in 4% to 7% of persons with a hemochromatosis phenotype.

Seventeen additional polymorphisms in HFE have been described. Of these, only the S65C mutation appears to have clinical impact and may cause mild iron overload when compound heterozygous with C282Y or H63D.

Table 25.1 Frequency of HFE Genotypes in the US Caucasian Population

Genotype	Frequency (%)
C282Y/ C282Y	1 in 200 (0.5%)
C282Y/ wt	1 in 7–12 (8%–14%)
H63D/ H63D	1 in 40 (2.5%)
H63D/ wt	1 in 4 (25%)
S65C/ wt	1 in 25 (4%)

wt, wild type.

PATHOPHYSIOLOGY

Since iron excretion in the gut is fixed at 1 mg/ day, normal iron balance must be maintained by meticulous control of iron absorption in the intestine and iron release from macrophages. These are modulated in response to body iron stores and the erythropoietic demand for iron.

Hepcidin: Key Regulator of Iron Homeostasis

Hepcidin, a liver-derived peptide hormone, is a key negative regulator of iron release into the plasma by intestinal enterocytes, macrophages, hepatocytes, and placental cells.⁸ It binds to and causes internalization and degradation of the cell surface iron exporter, ferroportin⁹ as shown in Figure 25.1. Hepcidin excess decreases intestinal iron absorption and macrophage iron release, and causes anemia. Hepcidin deficiency promotes intestinal iron absorption and leads to tissue iron overload. Hepcidin gene expression is enhanced by iron overload and inflammation, and suppressed by anemia and hypoxia. Recently, erythroid hormones, erythroferrone (ERFE), and additional candidates (TWSG1 and GDF15) were identified as potential mediators of hepcidin suppression during ineffective erythropoiesis. Although hepcidin is ordinarily induced by dietary iron loading, its expression is inappropriately reduced in all forms of

inherited hemochromatosis.^{10–12}

Iron Overload Disorders and Hepcidin Deficiency

Hepcidin deficiency plays a central role in the pathogenesis of the inherited hemochromatosis disorders, including those due to mutations in the HFE gene, the hemojuvelin gene (HJV), the transferrin receptor 2 gene (TfR2), and hepcidin itself (HAMP; Table 25.2). Hemojuvelin acts as a coreceptor in the bone morphogenetic protein (BMP) pathway, interacting with BMP ligands and BMP type I and II receptors to generate an active signaling complex.¹⁰ This complex activates a SMAD receptor signaling cascade and translocation of a SMAD complex to the nucleus, where it increases HFE transcription. HJV and HAMP mutations are critical components in the same common pathway; their negative effect on hepcidin expression is associated with severe iron loading in childhood or juvenile hemochromatosis.

HFE Localization and Function

HFE is highly expressed in Kupffer cells of the liver and in tissue macrophages. Binding to β2 microglobulin (β2m) allows expression of HFE/ β2m on the cell surface,¹³ where it forms a stable complex with transferrin receptor 1 (TfR1). The C282Y mutation prevents the formation of a disulfide bond in HFE, disabling β2m binding, and preventing cell surface expression. Disruption of the HFE/ β2m/ TfR1 complex and mutations in TfR2 are associated with adult-onset iron overload. HFE and TfR2 may regulate hepcidin expression by enhanced iron transport into the cell (endocytosis of diferric transferrin), by upstream regulation of hepcidin, or as weak coreceptors for BMP–SMAD signaling. According to this model, it might be possible to treat hemochromatosis by hepcidin replacement.

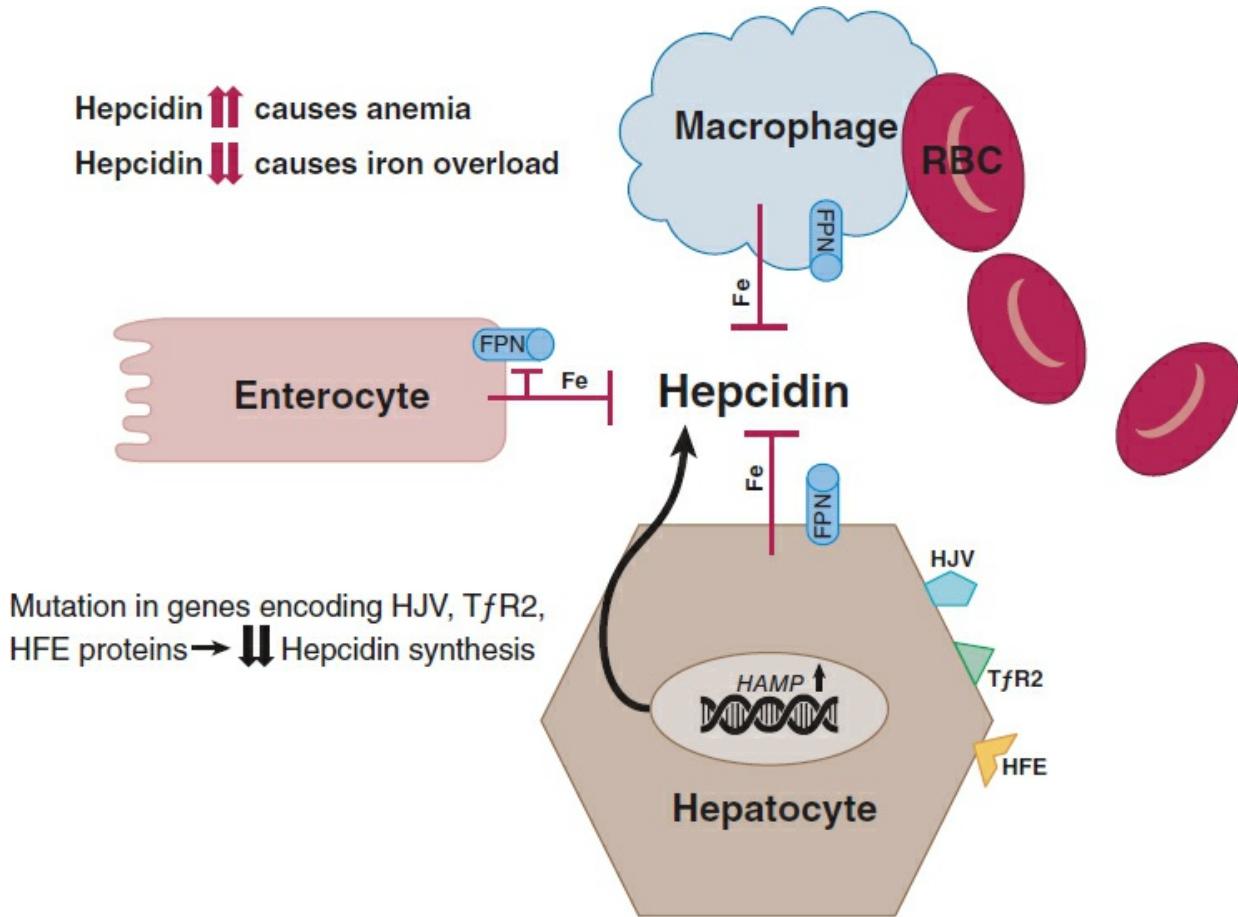


FIGURE 25.1 Regulation of iron homeostasis. Iron homeostasis: Hepcidin “applies the brakes” on transmembrane receptor, ferroportin (FPN) to prevent iron release into plasma. FPN is present on intestinal enterocytes, hepatocytes, and macrophages engulfing senescent red blood cells (RBC). Most inherited hemochromatosis disorders result from hepcidin deficiency, due to mutations in homojuvelin (HJV), transferrin receptor 2 (TfR2), HFE, hepcidin (HAMP) genes encoding the respective proteins

Table 25.2 Classification of Iron Overload Disorders

Primary (Genetic) Hemochromatosis	Secondary Hemochromatosis
Type 1: Classical/ hereditary hemochromatosis (HFE gene)	1. Transfusional siderosis
Type 2: Juvenile hemochromatosis (severe phenotype)	2. Congenital anemia with ineffective erythropoiesis (thalassemia, red cell enzyme deficiencies)
2a. Hemojuvelin mutations (HJV gene, 1q-linked)	
2b. Hepcidin mutations (HAMP gene)	
Type 3: Transferrin receptor 2 deficiency (TfR2 gene)	3. Acquired sideroblastic and dyserythroblastic anemias

Type 4. Ferroportin deficiency

(SLC11A3 gene)

Type 5. African iron overload

The most common form of secondary hemochromatosis is transfusional iron overload: 1 mL of red cells contains about 1 mg of iron. Inappropriate absorption of iron in the gut may also occur in association with ineffective erythropoiesis. In this case, the erythropoietic stimulus to decrease hepcidin levels overrides the effect of iron overload on increasing hepcidin expression.

Table 25.3 Body Iron Distribution

Body Iron	Men (g)	Women (g)
Hemoglobin (red cells)	3.0	2.4
Storage iron (liver)	1.0	0.4
Myoglobin and respiratory enzymes (muscle)	0.3	0.2
Total non-hemochromatosis adult	4.4	3.1
Total hemochromatosis adult	5–20	4–10

Iron Homeostasis

The distribution of body iron is shown in Table 25.3, with a comparison of iron stores in the normal state and in subjects with hemochromatosis.

- Excess iron and tissue injury. When the capacity for iron storage is exceeded, excess tissue iron causes cellular damage by catalyzing the formation of oxyradicals.¹⁴ Oxidative damage to lipids, proteins, carbohydrates, and DNA may lead to widespread impairment in cell function and integrity. In particular, lipid peroxidation may result in impaired membrane-dependent mitochondrial and lysosomal function. Oxidative injury to DNA, particularly in hepatocytes, may predispose to mutagenesis and cancer.
- Non-transferrin bound iron (NTBI). It represents “free iron in serum.” NTBI enters cells freely, independent of receptor-mediated uptake. NTBI levels are low or undetectable at transferrin saturation (TS) below 40% and increase linearly with TS levels above 40% to 50%. NTBI and its intracellular labile iron counterpart may be the direct mediators of oxidant stress.¹⁴

CLINICAL FEATURES AND DIAGNOSIS OF HFE-HEMOCHROMATOSIS

Before the availability of biochemical and genetic screening tests, HFE-

hemochromatosis was identified by damage to the liver, pancreas, heart, and joints, and diagnosed by demonstrating increased iron stores on liver biopsy. The “classic triad” of cirrhosis, diabetes, and skin pigmentation appeared in many publications and textbooks.¹ Patients typically presented with:

- Severe liver disease due to hepatic fibrosis or cirrhosis
- Cardiac failure and refractory arrhythmias
- Polyendocrine failure: Insulin-dependent diabetes and hypogonadotropic hypogonadism
- Debilitating symmetric polyarthritis
- Greyish skin pigmentation

It is now recognized that this severe clinical phenotype is relatively rare and only develops in 1% to 4% of untreated C282Y homozygotes over their lifetime.⁴ Between 40% and 60% of C282Y homozygote males and 60% to 80% of homozygote females remain asymptomatic or have minimal clinical manifestations throughout their lives; of the 40% to 50% who do develop symptoms that affect quality of life, arthritis, fatigue, and sexual dysfunction are the most common complaints (Table 25.4).^{15,16}

New Diagnostic Definition

In the current era of molecular testing, recognizing that clinical penetrance can be highly variable, the diagnosis of hemochromatosis is established by the detection of two mutated HFE alleles. This definition does not require active symptoms or signs of illness or the presence of iron overload. The four stages of the disorder are recognized as¹⁷:

- . Genetic predisposition with no other abnormality (age 0 to 20 years, 0- to 5-g tissue iron storage)
- . Iron overload without symptoms (age > 20 years, >5-g iron storage)
- . Iron overload with early symptoms (age >30 years, >8-g iron storage)
- . Iron overload with organ damage (age >40, 10- to 20-g iron storage).

Table 25.4 Clinical Features of Hereditary Hemochromatosis: Historical Versus Current

Historical Description	Current Common Presentation
Liver disease	Fatigue
Skin “bronzing”	Arthropathy
Diabetes	Impotence (men)

Common Clinical Presentation

The most common clinical presentation of HH is with nonspecific symptoms, and therefore practitioners should have a low threshold for ordering serum TS and ferritin studies in patients with unexplained chronic fatigue, arthralgias or arthritis, sexual dysfunction, hepatomegaly, or elevated liver function studies (alanine aminotransferase [ALT]). As such symptoms are easily overlooked, the single most common event currently leading to a diagnosis of hemochromatosis is the incidental detection of an abnormal laboratory test result, either an elevated TS, serum ferritin, or ALT. In hemochromatosis subjects diagnosed with fatigue on presentation, screening laboratory tests to evaluate possible concomitant thyroid disease should be obtained.

Typical findings related to the most common clinical signs and symptoms of hemochromatosis are shown in [Table 25.5](#). It is difficult to assign a frequency to these symptoms since there is a continuum of increasing frequency with increasing age, and with male versus female sex.¹⁷ Arthritis is the clinical feature with the greatest impact on the quality of life.¹⁸ In contrast to significant cardiac abnormalities described in hemochromatosis patients who presented with very high iron before the advent of more frequent screening and the availability of a genetic test, heart disease now is generally absent or clinically insignificant in newly diagnosed, asymptomatic subjects.¹⁹

The considerable variability in clinical penetrance of C282Y homozygosity, both in the rate of accumulation of iron stores and appearance of organ dysfunction, may be due to environmental, lifestyle, and genetic factors ([Table 25.6](#)).

LABORATORY TESTING

Once the clinical suspicion of hemochromatosis is raised, the diagnosis should be confirmed by laboratory testing, including:

- . Confirmatory lab tests
 - Serum iron (SI), transferrin, and TS: Transferrin is the major iron transport protein in plasma. Several assay methods for TS exist: Most accurate among them is direct colorimetric analysis of SI combined with nephelometric assay of transferrin, wherein $TS = \frac{molar\ concentration\ of\ iron}{twice\ the\ molar\ concentration\ of\ transferrin} \times 100\%.$ Less expensive but also less robust methods include chemical analyses of total SI binding capacity (TIBC) and unbound iron capacity (UIBC). Saturation of SI binding capacity is measured by dividing the SI by either TIBC (SI/TIBC) or by the sum of iron and UIBC ([SI]/[SI + UIBC]). Normal range for TS is 15% to 45%.
 - Serum ferritin: Major intracellular iron storage protein; measured immunologically. Estimates degree of iron overload and size of mobilizable iron stores (1 $\mu\text{g}/\text{L}$)

ferritin = 7 to 8 mg stored iron; e.g., 1,000 µg/ L ferritin = 7,000 to 8,000 mg stored iron). Used to determine the pace of initial phlebotomy therapy. Normal levels < 350 µg/ L in men, < 120 µg/ L in women.

- **HFE genotype:** Definitive diagnostic test, assesses predisposition to serious illness, useful for family counseling.
- . Ancillary lab tests
 - ALT: To assess degree of liver injury
 - Complete blood count (CBC): Obtain baseline hemoglobin and red cell mean corpuscular volume (MCV), which can be monitored during therapy (decrease in MCV is an indicator of iron-limited erythropoiesis).
 - Blood glucose and electrolytes
 - Total and free testosterone: As indicated by symptoms
 - Thyroid function tests: As indicated by symptoms
 - Alpha-fetoprotein: As baseline for subsequent monitoring for liver cancer
 - Serologic tests for exposure to hepatitis B and C (hepatitis B virus surface antigen [HBsAg] and anti-hepatitis C virus [HCV]): Active viral hepatitis worsens liver injury; useful to guide vaccine administration.

Table 25.5 Clinical and Laboratory Features of Hereditary Hemochromatosis (C282Y Homozygotes)

Sign/ Symptom	Frequency (%)	Features
Fatigue	30–50	Maybe related to liver disease, endocrine dysfunction
Arthritis	30–60	Major quality of life feature; more likely in subjects with higher iron burden at presentation. Symmetric, degenerative noninflammatory osteoarthritis; radiographic features include sclerosis, joint space narrowing, subchondral cysts, osteophytes, osteopenia. Chondrocalcinosis (pseudogout) and gout more common than in non-HH population. Disproportionate involvement of hands and feet, with MCP and MTP joints commonly affected. Hip replacement more common than in age-adjusted non-HH population.
Sexual dysfunction	30–50	Excess iron deposited in anterior pituitary and testes. Reduced shaving, loss of libido, erectile dysfunction, gynecomastia in men. Low free testosterone levels, inappropriately low LH and FSH. Testosterone replacement therapy may restore libido and potency.
Skin changes	10–20	Greyish or gray–brown hue; bronzing is rare
Hepatomegaly	10–20	Portal circulation leads directly from GI tract to liver; liver is first site of

Hypothyroidism	10–15	iron deposition; hepatic iron loading precedes other organs. 70% of all HH-related deaths are due to liver disease. Primary hypothyroidism; thyroid gland fibrotic; elevated TSH, associated with anti-thyroid antibodies
Elevated TS	>80	TS > 50% in 94% of men and 82% of women over age 40 y
Elevated ferritin	>60	Ferritin > normal in 90% of men and 60% of women over age 40 y
Elevated ALT	10–25	Influenced by other factors: alcohol, drugs, obesity

ALT, alanine aminotransferase; FSH, follicle-stimulating hormone; HH, hereditary hemochromatosis; LH, luteinizing hormone; MCP, metacarpophalangeal; MTP, metatarsophalangeal; TS, transferrin saturation; TSH, thyroid-stimulating hormone.

Table 25.6 Factors Influencing Clinical Penetrance

Factors That Accelerate Iron Overload	Factors That Lessen Iron Overload and Organ Damage
Environmental / lifestyle	<ul style="list-style-type: none"> ■ Alcohol use ■ Oral iron supplementation ■ Dietary habits (meat-rich diet) ■ Exogenous estrogen, vitamin C <ul style="list-style-type: none"> ■ Blood donation ■ Multiparity/ menorrhagia (women) ■ Dietary habits (vegetarian diet, tea) ■ Medications (proton pump inhibitors)
Genetic / acquired disorders	<ul style="list-style-type: none"> ■ Hepatitis B or C infection (HBV, HCV) ■ Nonalcoholic steatohepatitis (NASH) ■ Porphyria cutanea tarda (PCT) ■ Alpha-1 antitrypsin deficiency (AAT) ■ Mutations in hepcidin, ferroportin, transferrin receptor, other genes

Role of Liver Biopsy

Liver biopsy is generally not required for diagnosis. Although it previously served as the “gold standard” for both diagnosis and prognosis, the diagnosis is now more safely and reliably made with use of the HFE genotype.¹⁷

Indications for Biopsy

- . For prognostic purposes, to confirm high clinical suspicion of cirrhosis:
 - a. Ferritin > 3,000 µg/ L
 - b. Hepatomegaly and/ or signs of portal hypertension (large spleen, low platelets)
 - c. ALT does not normalize with phlebotomy.
- . If concomitant hepatitis B virus (HBV) or HCV infection present
- . In diagnostic workup of elevated ferritin and ALT, with normal HFE genotype and absence of other genetic causes listed earlier.

Histologic Findings

- Marked increase in hepatocellular iron, with relative sparing of Kupffer cells. Iron is distributed in a decreasing gradient from the periportal to the centrilobular areas.
- With progressive damage, may see portal fibrous expansion, bridging fibrosis with piecemeal necrosis, and macro or micronodular cirrhosis.
- Hepatic iron index (hepatic iron concentration/ $56 \times \text{age}$) > 1.9 (in absence of transfusional siderosis) strongly suggests iron overload is due to hemochromatosis rather than other causes.

Radiographic and Other Tests

- Skeletal films: Performed to evaluate symptomatic joints.
- Liver ultrasound: Useful in workup of non-HH causes of elevated ferritin; may show steatosis. Important in surveillance for liver cancer.
- Computed tomography (CT) and/ or magnetic resonance imaging (MRI) of liver: Not indicated diagnostically. Useful for suspected liver cancer.
- Superconducting quantum interference device (SQUID) assessment: Provides most sensitive noninvasive quantitative assessment of iron stores; has limited availability.

POPULATION SCREENING

The clinical course of HH meets the definition of a disorder for which population screening should be performed^{2,3}:

- High prevalence in selected populations
- Burden of disease (clinical penetrance) high enough to warrant medical and public attention
- Prolonged presymptomatic phase, during which detection and treatment lead to reductions in morbidity and mortality (early detection prevents complications and improves outcomes)
- Availability of reliable, accurate, easily available, and inexpensive screening tests
- Treatment is effective, safe, inexpensive, and easily accessible.

Thus, the costs of widespread testing and preventive treatment are considered favorable (more effective and less expensive) than delaying until development of late symptoms—particularly as the early, presenting symptoms are nonspecific, are often not recognized as being caused by hemochromatosis, and are associated with a 5- to 10-year delay until accurate diagnosis.

Laboratory Screening

- TS: The single best screening test is the TS: It is inexpensive, widely available, highly sensitive, and specific for the presence of the C282Y HFE allele.²⁰ The decision threshold at which confirmatory testing should be initiated ranges from TS values of 45% to 62%, depending on whether sensitivity or specificity is preferred (Table 25.7). Because TS is affected by dietary and diurnal variation, an elevated value should be confirmed by a second TS after an overnight fast, in the absence of oral iron supplements. Phenotype screening with TS is not advised until the age of 20 to 30 years, as iron burdens are generally low below this age.¹⁷ An algorithm for workup of persons detected through screening programs is shown in Figure 25.2.
- Ferritin screening: Ferritin is an acute phase reactant; levels rise with inflammation, infection, and non-HH liver disease. Lack of sensitivity and specificity make it a less reliable screening test.
- Genotype screening: A 1998 consensus conference decided against widespread population screening using genetic tests. The high cost of genetic tests and variable clinical penetrance of HH, coupled with concerns over stigmatization, discrimination, and insurability, led to rejection of this approach at the time.²¹

Screening of Family Members of C282Y Homozygotes

- Screening of children: Most cost-effective test is HFE genotype; biochemical screening is also acceptable; testing should be delayed until the age of 20 to 30 years. If more than two children are involved, the best approach may be genotyping of the other parent.²²
- Screening of siblings: All siblings should be counseled to undergo either genetic or phenotypic screening. The most cost-effective test is HFE genotype, but phenotypic screening with combination of TS and ferritin is also acceptable.²²

TREATMENT

Phlebotomy Therapy

- Phlebotomy: Periodic removal of 1 unit (500 mL) of whole blood. Safe, inexpensive, standard of care since the past 50 years.²³ One unit of whole blood removes 200 to 250 mg of iron. Double red cell collection by apheresis removes 360 mL of packed red cells (400 to 420 mg of iron) and may be particularly useful in blood center setting.

Table 25.7 Diagnostic Yield of Transferrin Saturation Screening

Gender	TS Decision Threshold (%)	Sensitivity (Detection Rate in %)	Specificity (False Positive in %)
--------	---------------------------	-----------------------------------	-----------------------------------

Male	≥ 50	94	7
	≥ 60	86	1.5
Female	≥ 50	82	5
	≥ 60	67	0.6

Further evaluation is recommended if TS > 55%–62% in men and > 45%–50% in women.

TS, transferrin saturation.

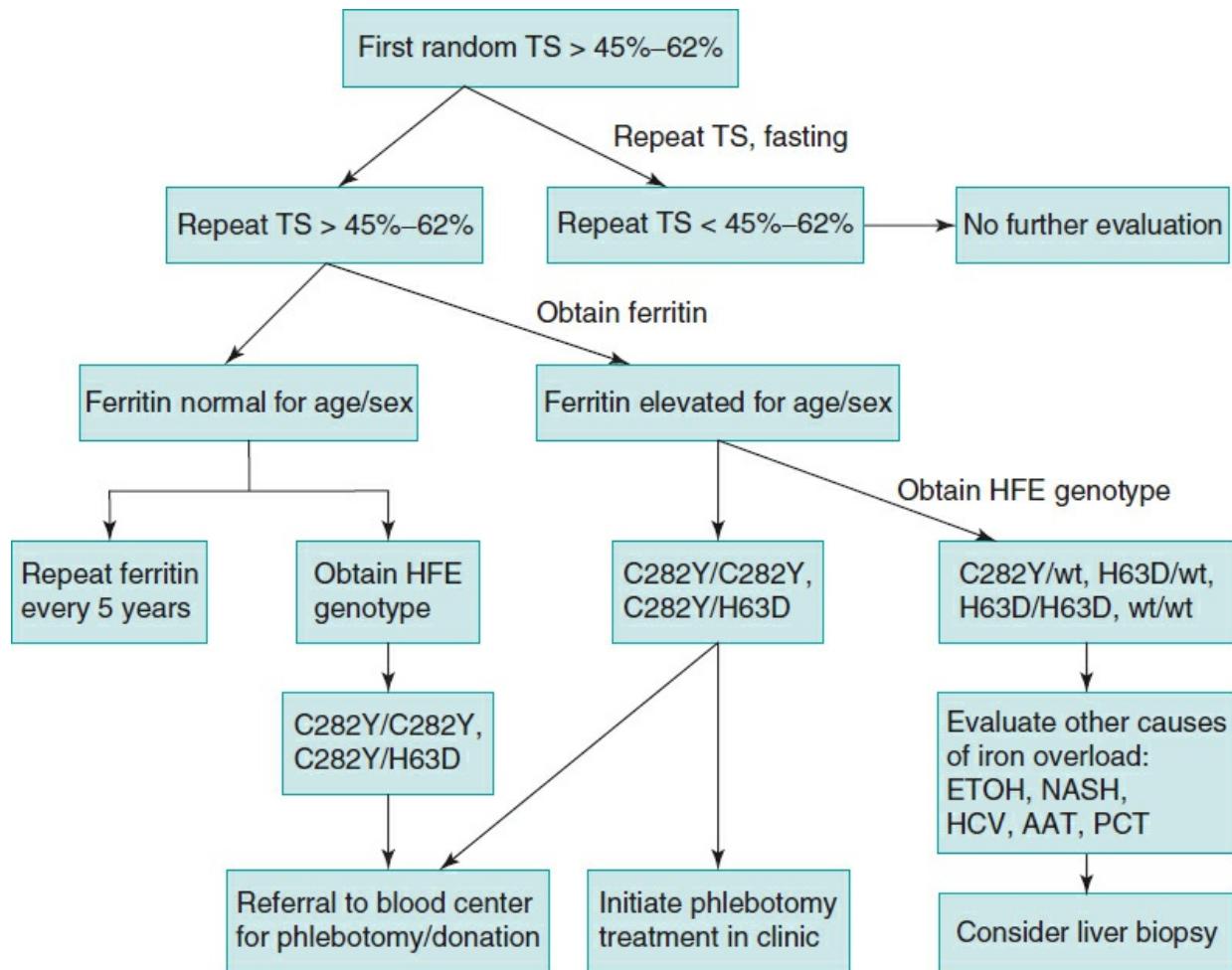


FIGURE 25.2 Decision tree for hemochromatosis population screening.

AAT, alpha-1 antitrypsin deficiency; ETOH, ethyl alcohol; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PCT, porphyria cutanea tarda; TS, transferrin saturation; wt, wild type.

Controversy regarding treatment indications for subjects with modest iron burdens.

- Patients generally desire treatment; are eager and willing to be blood donors
- Therapy safe, accessible, and prevents late organ damage
- Referral to blood center shifts argument in favor of treatment (double benefit, to subject and to community; efficient care; no charge for procedure).

Guidelines for Phlebotomy Therapy

Phase 1: Iron Depletion

- | Pace: Initiate phlebotomy at 1- to 4-week intervals, depending on ferritin, hemoglobin, ALT, gender, and weight. As iron depletion approaches, decrease pace to monthly.
- | Target of “de-ironing” therapy: Several assays may be used
 - Ferritin < 30 µg/ L
 - TS < 30%
 - Decrease in red cell MCV to 3% to 5% below pre-phlebotomy level²⁴
- | Monitoring parameters

Pre-phlebotomy fingerstick hemoglobin or hematocrit (\pm venous CBC) at each visit to avoid anemia

Ferritin every 4 to 8 weeks initially, then ferritin \pm TS every 1 to 2 treatments once ferritin <100 µg/ L.
- | Safety guide: Threshold hemoglobin for therapeutic bleed \geq 12.5 g/ dL (hematocrit \geq 38%). In general, do not bleed below this level; defer therapy for 1 to 4 weeks until hemoglobin recovers. Iron deficiency is not necessary during treatment; anemia should be avoided.
- | General guide: For initial ferritin of 500 to 1,500, patients generally require 15 to 30 bleeds to achieve iron depletion. If initial ferritin >2,000 µg/ dL, may require >40 to 50 bleeds.

Phase II: Preventing Reaccumulation (Maintenance)

- | Pace: Five hundred milliliter removed every 8 to 26 weeks (mean: 10 to 12 weeks), depending on gender, weight, age, and dietary habits. This is usually a lifelong requirement, although some subjects reaccumulate iron very slowly.
- | Goals of maintenance therapy

Ferritin 30 to 50 µg/ L

TS < 50%

Hemoglobin > 12.5 g/ dL
- | Monitoring parameters

Pre-phlebotomy fingerstick hemoglobin or hematocrit (\pm venous CBC) at each visit, and ferritin and/ or TS every 1 to 2 treatments.

Evaluation of Anemia During Phlebotomy Therapy

Development of a hemoglobin <12.5 g/ dL despite elevated ferritin levels may be due to occult bleeding, medications such as proton pump inhibitors,²⁵ or endocrine causes such as hypothyroidism (men and women), or decreased testosterone levels (men). If

concomitant disorder of erythroid production is present (thalassemia, or renal insufficiency) and urgent need for phlebotomy exists, weekly erythropoietin may be helpful. Anemia may also be due to development of liver cancer.

Use of Iron Chelators

Phlebotomy therapy remains the safest, and the most efficacious treatment for HH. Iron chelators may only be considered transiently in individuals who cannot tolerate regular phlebotomies (e.g., in refractory heart failure with unstable hemodynamics). Deferrioxamine and/ or deferiprone have been used in these instances with improvement in left ventricular end-diastolic volume and ejection fraction. Combined therapy with intravenous (IV)/ subcutaneous deferoxamine and oral deferiprone has successfully reversed iron-related cardiac disease, preventing early cardiac deaths in patients with transfusion-dependent thalassemia. NTBI iron is the major component chelated with Desferrioxamine and liver iron is removed first. With prolonged and continuous high IV or subcutaneous doses, cardiac iron is depleted. Deferiprone, being more lipophilic than Desferrioxamine is more efficient at removing intracellular iron and directly chelates cardiac iron. With the decrease in cardiac siderosis and an improvement in ejection fraction, phlebotomy therapy may be resumed in HH patients.²⁶

Arthritis, Endocrine Replacement, Vaccinations, and Cancer Surveillance

- | Arthritis: Responds moderately well to nonsteroidal anti-inflammatory agents
Joint aspiration to exclude gout or pseudogout in acutely inflamed joints
Orthopedic evaluation for joint replacement for severe chronic hip, knee, or ankle pain
Cumulative incidence of major joint replacement in C282Y + / + HH subjects is 30% by the age of 70 years.
- | Testosterone replacement: Consider in males with symptomatic sexual dysfunction and low testosterone levels
- | HAV and HBV vaccination: Should be given as prophylaxis against future hepatic injury in unexposed patients
- | Alpha fetoprotein and liver ultrasound: Surveillance for hepatocellular cancer. Repeat every 6 months if cirrhosis documented by biopsy.

Dietary and Lifestyle Counseling

- | Avoid oral iron supplements
- | Limit alcohol intake to protect the liver
- | Red meat in moderation, but major change in dietary habits not required. Iron stores are most efficiently controlled by adjusting frequency of bleeds rather than reducing the intake of iron-rich foods.

- | Avoid raw shellfish until iron depletion achieved (avoid *vibrio vulnificus*)
- | If ALT is elevated:
 - Discontinue alcohol intake until iron depletion completed and ALT normal
 - Consider discontinuation of medications with potential hepatic toxicity.

PROGNOSIS AND RESPONSE TO THERAPY

If cirrhosis is not present, long-term survival is unchanged from non-HH population.²⁵ If cirrhosis is present, risk of hepatic cancer is increased and persists for life: 18.5% of subjects with cirrhosis develop liver cancer, which may not be detected until 5 to 10 years after iron depletion. The overall incidence of hepatic cancer is 100-fold greater in HH than non-HH subjects and accounts for 10% to 30% of HH-related deaths. Progression of cirrhosis due to HH is slower than in other types of cirrhosis (alcoholic, viral); however, HH subjects undergoing liver transplant for end-stage liver disease or liver cancer have a higher than average peritransplant mortality. The response to phlebotomy varies by tissue site ([Table 25.8](#)).

HEMOCHROMATOSIS SUBJECTS AS BLOOD DONORS

- | Regulatory issues. US Food and Drug Administration (FDA) allows blood centers to obtain a “variance” from federal code to permit blood from HH subjects to be made available for transfusion into others, even if collected more frequently than 56-day interval
- | FDA requirements. Phlebotomy must be performed:
 - Under a physician’s direction
 - Without charge regardless of whether subjects qualify as donors
 - With periodic laboratory monitoring
- | Logistics and safety²⁷
 - Seventy-five percent of all HH subjects meet allogeneic donor eligibility criteria
 - Fifty-five percent of HH subjects were blood donors before the knowledge of their diagnosis
 - Potential HH-donor contribution estimated at 1 to 2 million red cell units per year in the United States
 - Recent rapid increase in the number of US blood centers with FDA-approved variances to allow HH subjects to be blood donors (81 centers, May 2007)
 - HH subjects documented to be safe, reliable donors
- | Advantages of phlebotomy care in the blood center
 - Treatment is free, consistent, accessible, and convenient
 - Increased patient satisfaction: Avoid frustration of knowing blood will be discarded

- Alleviate national blood shortages.

Table 25.8 Response to Phlebotomy Therapy in Hemochromatosis

Complication	Prevents	Reverses or Improves
Arthropathy	Unknown	Partly, if initiated early in course
Fatigue	Yes	Yes, to a variable degree
Skin greying	Yes	Yes
Liver fibrosis	Yes	Partly, if initiated early in course
Cirrhosis	Yes	No; but portal hypertension may improve
Cardiomyopathy	Yes	Partly, if initiated early in course
Diabetes	Yes	No
Hypogonadism	Yes	No
Hypothyroidism	Yes	No

NON-HFE IRON OVERLOAD

Ferroportin disease, the most common hereditary, non-HFE cause of iron overload,²⁸ is an autosomal-dominant disorder caused by mutations in the gene coding for ferroportin that is the main iron export protein in mammals.²⁹ This condition is not restricted to Caucasians and is characterized by elevated ferritin levels despite low normal TS, iron accumulation in organs and reticuloendothelial macrophages, and marginal anemia with poor tolerance of phlebotomy therapy. Nonalcoholic fatty liver disease is a common acquired cause of iron overload, often associated with features of the metabolic syndrome such as obesity, hypertension, hypercholesterolemia, and elevated fasting glucose levels or type II diabetes. Subjects present with elevated liver transaminases and ferritin levels without the degree of elevation in TS that accompanies classic HFE-associated hemochromatosis. Liver ultrasound may demonstrate findings consistent with fatty infiltration, diagnostic histopathologic features of this disorder are present on liver biopsy. Phlebotomy therapy may decrease serum ferritin levels, however, a recent prospective randomized study showed no improvement in hepatic steatosis or occurrence of liver injury.³⁰ Emphasis of patient management should be directed toward the patient's underlying medical conditions. Phlebotomy therapy has also been applied in patients with transfusional siderosis, following cure of primary disease and hematologic count recovery. Etiology of hyperferritinemia at baseline is varied in these subjects and may include graft-versus-host disease, hepatic dysfunction, residual infections, and iron overload. Phlebotomy parameters are typically extrapolated from those used in HH patients. While hyperferritinemia, TS, and liver function tests improve over time, the specific role of phlebotomy therapy is debatable in the absence of

randomized controlled data. Management of infectious and immunologic disease sequelae may supersede therapeutic phlebotomies. Table 25.9 summarizes specific clinical indications, areas of controversy, and contraindications for the use of phlebotomy therapy.³¹

FUTURE CHALLENGES

The process of molecular discovery is rapidly leading to a more comprehensive understanding of the role of HFE protein in iron homeostasis. At the same time, the availability of a genetic test has focused increased public and medical attention on hemochromatosis. Robust population screening studies are currently in progress to more accurately determine clinical penetrance, both for early as well as late complications. It is hoped that the increased emphasis on educational campaigns to foster prompt recognition of early symptoms by primary care providers complement or perhaps even alleviate the need for targeted screening programs. Better appreciation of the advantages of referral to the blood center may improve the quality and accessibility of care and also confer a benefit to the general public health.

Table 25.9 Indications for Therapeutic Phlebotomy

Indication	Pathophysiology	Diagnosis	Phlebotomy Target ^a	Clinical Outcomes	Comments
Primary iron overload (IO)					
Hereditary hemochromatosis	Homozygous HFE (p.C282Y) mutation ^b → decreased hepcidin synthesis → excess iron absorption → end-organ damage	SF > 300 in M; >200 in F; TS > 45%; HFE gene studies	Weekly bleeds until SF < 50, then maintenance bleeds every 3–4 months to keep SF < 50–100	Prevention of liver, heart, endocrine injury; reversal of liver damage; survival benefit related to prevention of advanced liver disease	Linear decrease in serum ferritin over time
Secondary iron overload					
Hematologic malignancies (leukemia, MDS)	Parenteral iron loading due to transfusions	SF > 1,000 ^c ; LIC by biopsy, T2-MRI, SQUID	SF < 200–300 (in the absence of GVHD/infection)	Improved transaminitis; survival benefit unclear	Indicates transitory independence after therapy
Sickle cell disease	Parenteral iron loading due to transfusions	SF > 1,000	SF < 200–300 (bleed only if Hb > 7 g/dL)	Resolution of IO; improved liver histology	Indicates transitory independence

			and transfusion independent)		after
Thalassemia	Ineffective erythropoiesis → enteral iron loading + transfusional siderosis	SF > 1,000 ^c ; LIC by biopsy, T2-MRI, SQUID	SF < 200–300 (in the absence of GVHD/infection)	Reversal of liver damage ^d , improved cardiac function	Indicates trans. independence after
Dyserythropoietic anemias (HSA, CDA)	Ineffective erythropoiesis → enteral iron loading ± transfusional siderosis	Hyperferritinemia; molecular studies	SF in normal range, normalization of MCV	Paradoxical improvement of anemia	Initial Hb decrements increase
Iron toxicity ± overload					
Porphyria cutanea tarda	Enzyme defect in heme synthetic pathway → photoactive prophyrin accumulation, mild IO; worsened by HCV, HH, or excess alcohol	Skin lesions; elevated porphyrins in urine, feces; SF normal or elevated	SF < 50; typically 4–5 bleeds total	Improved skin lesions; normalized porphyrin levels	
Chronic HCV infection	Hepcidin suppression by HCV-associated ROS	HCV PCR; SF > 200	Phlebotomy not indicated	Treatment by newer highly effective antiviral agents	
African IO	Diet, genetic	SF > 300	SF < 300	Theoretical benefit	No clinical benefit
Nonalcoholic fatty liver disease (NAFLD)	Hepatic inflammation ↓ hepcidin suppression	Clinical diagnosis of metabolic syndrome; SF > 300; < ALT ± steatosis on ultrasound or liver biopsy	Phlebotomy not indicated	Treatment directed at diet, exercise, weight loss	Elevated gene expression biopsy
Peripheral arterial disease (PAD)	Elevated iron stores may lead to ROS-mediated cardiovascular insult	Advanced PAD and SF > 400	Investigational use only; SF = 25–60, per study	May decrease risk of stroke, MI in subjects ≤ 60	General performance outcomes clinic
Increased RBC mass					
Polycythemia rubra	Neoplastic clone	Erythrocytosis,	HCT < 45%;	Decreased risk	Concomitant

vera	of red cell progenitors	suppressed EPO levels, JAK2/ other mutations	maintenance bleeds may be needed	of CVA, DVT, improved survival	aspirin hydr. mos.
Secondary erythrocytosis	Excess EPO production (gene mutations in O ₂ sensing pathway, EPO-secreting tumors, COPD, high altitude)	Erythrocytosis, increased EPO, clinical history of associated conditions	HCT < 55% and/ or symptom improvement	Decreased thrombotic events, symptomatic relief	Cytoreductive therapy cont.

^aUnless otherwise specified, weekly or bimonthly therapeutic phlebotomy of 5–10 mL/kg until target reached.

^bOther rare causes of genetic hemochromatosis are not considered.

^cLittle evidence for cut-off but used in most clinical studies to define transfusional iron overload.

^dOnly documented in thalassemia with extreme iron loading; hepatic damage reversible if not progressed to cirrhosis.

ALT, alanine aminotransferase; CDA, congenital dyserythropoietic anemia; COPD, chronic obstructive pulmonary disease; EPO, erythropoietin; F, female; GVHD, graft-versus-host disease; Hb, hemoglobin; HCV, hepatitis C virus; HCT, hematocrit (%); HSCT, hematopoietic stem cell transplant; HSA, hereditary sideroblastic anemia; LIC, liver iron concentration; M, male; MCV, mean corpuscular volume; MDS, myelodysplastic syndrome; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; ROS, reactive oxygen species; SF, serum ferritin (mcg/L); SQUID, superconducting quantum interference device; TS, transferrin saturation (%).

References

- Bothwell TH, MacPhail AP. Hereditary hemochromatosis: etiologic, pathologic, and clinical aspects. *Semin Hematol*. 1998;35:55–71.
- Bomford A. Genetics of hemochromatosis. *Lancet*. 2002;360:1673–1681.
- Tavill AS. Diagnosis and management of hemochromatosis: AASLD practice guidelines. *Hepatology*. 2001;33:1321–1328.
- Beutler E. Penetrance in hereditary hemochromatosis. The HFE Cys282Tyr mutation as a necessary but not sufficient cause of clinical hereditary hemochromatosis. *Blood*. 2003;101:3347–3350.
- Ajioka R, Kushner JP. Clinical consequences of iron overload in hemochromatosis patients. *Blood*. 2003;101:3351–3354.
- Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996;13:399–408.
- Jazwinska EC, Cullen LM, Busfield F, et al. Haemochromatosis and HLA-H. *Nature Genetics*. 1996;14:249–251.
- Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood*. 2003;102:783–788.

9. Nemeth E, Tuttle M, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;306:2090–2093.
10. Babitt JL, Huang FW, Wrighting DM, et al. Bone morphogenetic signaling by hemojuvelin regulates hepcidin expression. *Nat Gen*. 2006;38:531–539.
11. Bridle KR, Frazer DM, Wilkins SJ, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *Lancet*. 2003;361:669–673.
12. Kim A, Nemeth E. New insights into iron regulation and erythropoiesis. *Curr Opin Hematol*. 2015 May;22(3):199–205.
13. Feder JN, Penny DM, Irrinki A, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc Natl Acad Sci USE*. 1998;95:1472–1477.
14. Brissot P, Loreal O. Role of non-transferrin bound iron in the pathogenesis of iron overload and toxicity. In: Hershko C, ed. Iron Chelation Therapy. New York, NY: Kluwer Academic/ Plenum Publishers; 2002:45–53.
15. Olynyk JK, Cullen DJ, Aquilia S, et al. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med*. 1999;341:718–724.
16. Bulaj ZJ, Ajioka RS, Phillips JD, et al. Disease-related conditions in relatives of patients with hemochromatosis. *N Engl J Med*. 2000;343:1529–1535.
17. Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis— Part II. Expert document. *J Hepatol*. 2000;33:487–496.
18. Adams P, Speechley M. The effect of arthritis on the quality of life in hereditary hemochromatosis. *J Rheumatol*. 1996;23:707–710.
19. Shizukuda Y, Bolan C, Tripodi D, et al. Left ventricular systolic function during stress echocardiography in subjects with asymptomatic hereditary hemochromatosis. *Am J Cardiol*. 2006;98:694–698.
20. Bradley LA, Haddow JE, Palomaki GE. Population screening for hemochromatosis: expectations based on a study of relatives of symptomatic probands. *J Med Screen*. 1996; 3:171–177.
21. Burke W, Thomson E, Khouri MJ, et al. Hereditary hemochromatosis. Gene discovery and its implications for population-based screening. *J Am Med Assoc*. 1998;280:172–178.
22. El-Serag HB, Inadomi JM, Kowdley KV. Screening for hereditary hemochromatosis in siblings and children of affected patients. *Ann Int Med*. 2000;132:261–269.
23. Barton JC, McDonnell SM, Adams PC, et al. Management of hemochromatosis. *Ann Intern Med*. 1998;129:932–939.
24. Adams PC, Barton JC. How I treat hemochromatosis. *Blood*. 2010;116:317–325.
25. Leitman SF, Browning JN, Yau YY, et al. Hemochromatosis subjects as allogeneic

- blood donors: a prospective study. *Transfusion*. 2003;43:1538–1544.
- 26. Fabio G, Minonzio F, Delbini P, et al. Reversal of cardiac complications by deferiprone and deferoxamine combination therapy in a patient affected by a severe type of juvenile hemochromatosis (JH). *Blood*. 2007;109(1):362–364.
 - 27. Niederau C, Fischer R, Purschel A, et al. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology*. 1996;110:1107–1119.
 - 28. Pietrangelo A, Caleffi, Corradini E. Non-HFE hepatic iron overload. *Semin Liv Dis*. 2011;31:302–318.
 - 29. Pietrangelo A. The ferroportin disease. *Blood Cells Mol Dis*. 2004;32:131–138.
 - 30. Adams LA, Crawford DH, Stuart K, et al. The impact of phlebotomy in nonalcoholic fatty liver disease: a prospective, randomized, controlled trial. *Hepatology*. 2015;61(5):1555–1564.
 - 31. Panch SR, Yau YY, West K, et al. Initial serum ferritin predicts number of therapeutic phlebotomies to iron depletion in secondary iron overload. *Transfusion*. 2015;55(3):611–622.

Consultative Hematology

Pierre Noel

HEMATOLOGIC COMPLICATIONS OF PREGNANCY

Anemia in Pregnancy

During normal pregnancies, plasma volume increases by 40% to 60% and red cell mass by 20% to 40%. The hematocrit typically decreases 30% to 32%, and the lower limit of normal for hemoglobin declines to 11 g/ dL in the first trimester and 10 g/ dL in the second and third trimesters. The most common forms of anemia of pregnancy in North America are due to iron and folate deficiencies.

One thousand milligrams of additional iron are required during pregnancy. The normal 500-mg iron storage pool is insufficient, and iron-deficiency anemia develops in the absence of iron supplementation throughout pregnancy. The risk of iron deficiency and anemia is increased in women with multiple gestations. The recommended daily allowance for iron during pregnancy is 27 mg of elemental iron. The US Centers for Disease Control and Prevention recommends routine low-dose iron supplementation (30 mg of elemental iron daily) for all pregnant women, beginning at the first prenatal visit. Calculations of dosage for iron preparations should be based on the amount of elemental iron in each preparation: Ferrous sulfate contains 20% elemental iron, ferrous gluconate 12%, and ferrous fumarate 33%. Low values of serum iron and ferritin are reliable indicators of iron deficiency in pregnancy. The consequences of maternal iron deficiency on the neonate are controversial. Mild-to-moderate maternal iron-deficiency anemia is not associated with significant anemia in the fetus.

Folate needs are increased during pregnancy. Folate deficiency is associated with anemia, neural tube defects, and cleft palate. Neural tube closure occurs during the fourth week of pregnancy; therefore, folate supplementation is necessary before conception to prevent neural tube defects. Most prenatal vitamins contain both folate and iron.

Sickle Cell Disease in Pregnancy

Women with sickle cell anemia are in a high-risk pregnancy group. With modern obstetric and perinatal care, maternal mortality is less than 1%, and perinatal mortality is less than 15%.

Hydroxyurea should be discontinued 3 months before pregnancy. Iron-chelating therapy should be discontinued during pregnancy. Both deferoxamine and deferasirox are classified as category C drugs.

Prophylactic red cell transfusions are associated with a reduction in maternal mortality, vaso-occlusive pain episodes, pulmonary complications, pulmonary embolism, pyelonephritis, perinatal mortality, neonatal death, and preterm birth. The goal is to maintain the Hb level at 10 to 11 g/ dL, and the hemoglobin S (HbS) level \leq 30%, via transfusions given every 3 to 4 weeks.

The adverse events associated with prophylactic exchange transfusion include alloimmunization and delayed hemolytic transfusion reactions.

Thrombocytopenia in Pregnancy

Platelet count decreases by approximately 10% during pregnancy, mostly in the third trimester.

The most common cause of thrombocytopenia is incidental thrombocytopenia of pregnancy (75%), followed by thrombocytopenia complicating hypertensive disorders of pregnancy (20%) and finally immunologic disorders of pregnancy (5%).

Thrombocytopenia of less than 100,000/ μ L in the first trimester of pregnancy is most typical for immune thrombocytopenic purpura (ITP). Thrombocytopenia of more than 70,000/ μ L occurring late during the second trimester or during the third trimester, in the absence of hypertension or proteinuria, usually represents incidental thrombocytopenia of pregnancy. Platelet-associated immunoglobulin G (IgG) is elevated in both incidental thrombocytopenia of pregnancy and ITP.

It is important in any patient with thrombocytopenia to consider HIV infection, systemic lupus erythematosus, and thrombocytopenia associated with antiphospholipid antibodies in the differential diagnosis.

Incidental Thrombocytopenia of Pregnancy

The platelet count in incidental thrombocytopenia generally remains more than 100,000/ μ L. Incidental thrombocytopenia usually develops in the third trimester and is not associated with neonatal thrombocytopenia. The likelihood of a more serious cause of thrombocytopenia increases when the platelet count drops less than 70,000/ μ L. The pathogenesis of incidental thrombocytopenia is not clearly defined but may involve a combination of hemodilution and decreased platelet half-life.

Incidental thrombocytopenia remains a diagnosis of exclusion. The diagnosis is made by the lack of other physical or laboratory abnormalities in patients who do not have an antecedent history of ITP. Women with incidental thrombocytopenia should receive standard obstetrical care. A platelet count greater than 80,000/ μ L is felt to be sufficient for epidural anesthesia.

Immune Thrombocytopenic Purpura

ITP is the most common cause of severe thrombocytopenia in the first trimester of pregnancy. An antecedent history of ITP or autoimmune disorder makes the diagnosis more likely. The nadir platelet count in ITP usually occurs in the third trimester.

Patients with platelet counts greater than 20,000 to 30,000/ μ L and no evidence of bruising or mucosal bleeding generally do not require treatment in the first two trimesters of pregnancy. A platelet count greater than 50,000/ μ L is considered safe for normal vaginal delivery or cesarean section. Although there is no consensus, a platelet count greater than 80,000/ μ L is sufficient for epidural anesthesia. The bleeding time is not an accurate predictor of risk of bleeding in these situations.

Optimal first-line therapy for ITP in pregnant patients is controversial. Corticosteroids are the least expensive option but are associated with pregnancy-induced hypertension, gestational diabetes, osteoporosis, excessive weight gain, and premature rupture of fetal membranes. The placenta metabolizes 90% of the administered dose of prednisone, and thus serious fetal side effects are unlikely. Prednisone is initiated at a dose of 10 mg/ day and subsequently adjusted to maintain a platelet count $>30 \times 10^9/ L$. The dose of prednisone required is seldom in excess of 30 mg/ day. Intravenous immunoglobulin (IVIg) should be considered if the maintenance dose of prednisone is in excess of 10 mg/ day. IVIg given at a dose of 1 g/ kg (single dose or divided in two doses, based on prepregnancy weight) is associated with a response in more than 60% of patients, and response duration averages from 3 weeks to 1 month.

Splenectomy should be considered in patients refractory to corticosteroids and IVIg. Splenectomy is best performed in the second trimester of pregnancy. Splenectomy in the first trimester may induce labor, and splenectomy in the third trimester may be technically difficult. Splenectomy has been successfully performed laparoscopically during pregnancy.

The use of anti-D is limited in pregnancy because of the risk of acute hemolysis and anemia. Little data is available to determine the safety and efficacy of chimeric anti-CD20 monoclonal antibody rituximab in pregnancy. Rituximab crosses the placenta.

Very little data are available regarding the safety and efficacy during pregnancy of thrombopoietin receptor mimetic agonists (romiplostim and eltrombopag). Experience

with immunosuppressive and cytotoxic agents during pregnancy also is limited. Danazol and vinca alkaloids are best avoided. Interventions that raise maternal platelet count are not effective in augmenting the platelet count of the fetus.

The use of nonsteroidal anti-inflammatory drugs should be avoided postpartum in patients with platelet counts less than 100,000/ μ L. Thromboprophylaxis should be considered in all women with a platelet count greater than 50,000/ μ L; if they have undergone surgical delivery, are immobilized for a prolonged amount of time, or have acquired or congenital thrombophilia.

Neonatal mortality is less than 1% in ITP. Five percent of neonates have a platelet count of less than 20,000/ μ L; most hemorrhagic events in neonates occur 24 to 48 hours following delivery, at the nadir of the platelet count. There is no evidence that cesarean section is safer for the neonate than is vaginal delivery. The mode of delivery should be decided on the basis of routine obstetric indications.

Maternal platelet count, maternal platelet antibody levels, or a history of maternal splenectomy for ITP are not accurate predictors of neonatal platelet counts. The most accurate predictor of fetal thrombocytopenia is a history of thrombocytopenia at delivery in a prior sibling. Fetal scalp blood sampling and cordocentesis have been generally abandoned.

A cord platelet count should be determined following delivery in every neonate. Thrombocytopenic neonates should be followed closely following delivery, the platelet count nadir may not occur before 2 to 5 days. Neonates presenting with clinical bleeding or a platelet count less than 20,000 μ L should be managed using IVIg at a dose of 1 g/ kg. Life-threatening bleeding can be treated with a combination of IVIg and platelet transfusions. Neonates with platelet counts less than 50,000/ μ L should undergo transcranial ultrasound to exclude intracranial hemorrhage.

Preeclampsia and Hemolysis, Elevated Liver Enzymes, and Low Platelets Syndrome

Preeclampsia is defined as hypertension (systolic pressure greater than 140 mm of mercury or diastolic pressure greater than 90 mm of mercury) and proteinuria (greater than 300 mg of protein/ 24 hours) occurring after 20 weeks of gestation. Preeclampsia occurs in 3% of all pregnancies and accounts for 18% of maternal deaths in the United States. Predisposing factors include age younger than 20 years or older than 30 years, increased body mass index, chronic hypertension, and insulin resistance. Thrombocytopenia develops in 50% of patients with preeclampsia. Endothelial damage and activation of the coagulation system with thrombin generation may explain the thrombocytopenia. D-dimers and thrombin–anti-thrombin complexes are increased in patients with thrombocytopenia.

The criteria for HELLP syndrome (**hemolysis**, elevated liver enzymes, and low platelets) are as follows:

- | Microangiopathic hemolytic anemia
- | Increased transaminases
- | Lactic dehydrogenase greater than 600 units/ mL
- | Thrombocytopenia (less than 100,000/ μ L).

HELLP occurs in up to 10% of women with severe preeclampsia. Proteinuria is present in 75% of patients with HELLP syndrome, but only 50% to 60% have hypertension. The syndrome usually occurs in white, multiparous women elder than 25 years. Maternal mortality is 3% to 4% and fetal mortality is 10% to 25%. The severity of thrombocytopenia is correlated with maternal morbidity and perinatal mortality. Fetal mortality is attributed to placental ischemia, abruption of the placenta, immaturity, and intrauterine asphyxia. Neonatal thrombocytopenia can occur in both preeclampsia and HELLP. The mechanism of neonatal thrombocytopenia remains unclear. There is a 3% risk of recurrence of HELLP in subsequent pregnancies.

The definitive treatment for eclampsia and HELLP is delivery of the fetus. Management focuses on stabilization of the patient and maturation of the fetal lung. The presence of multiorgan dysfunction, fetal distress, or a gestational age greater than 34 weeks warrants immediate delivery. Plasma exchange appears to be beneficial in patients with platelet counts $<50 \times 10^9/ \text{L}$ or in patients with evidence of clinical deterioration. Coagulopathy resulting from preeclampsia-associated diffuse intravascular coagulation (DIC) occurs in 20% of patients. The clinical manifestations of preeclampsia and HELLP resolve within a few days of delivery. Rarely, HELLP syndrome can present postpartum, up to 72 hours after delivery. If the manifestations worsen or persist after 1 or 2 days, plasma exchange is indicated.

Acute fatty liver of pregnancy usually affects primigravid women; it occurs in the third trimester and is associated with hypertension and proteinuria in 50% of patients. Presentation tends to be nonspecific and consist of headache, fatigue, nausea, vomiting and right-upper quadrant or epigastric pain. There may be progression to liver failure and encephalopathy. Microangiopathic hemolytic anemia and thrombocytopenia are not prominent in this syndrome.

Early delivery is recommended and recovery occurs over a period of 1 to 4 weeks postpartum. Plasma exchange appears beneficial, particularly if started early following diagnosis.

Approximately one in five women who develop acute fatty liver disease of pregnancy may carry a fetus that is deficient of the enzyme long-chain 3-hydroxyacyl-CoA dehydrogenase.

Thrombotic Thrombocytopenic Purpura and Atypical Hemolytic Uremic Syndrome

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) occur in only 0.004% of pregnancies. The following are the classic pentad of symptoms of TTP:

- | Microangiopathic hemolytic anemia
- | Thrombocytopenia
- | Neurologic abnormalities
- | Fever
- | Renal dysfunction.

However, the classic pentad is present in only 40% of patients. Pregnancy is a precipitating factor for TTP. The mean time of onset of TTP is at 23.5 weeks of pregnancy. Plasma exchange therapy is recommended for the management of the pregnant TTP patient, and delivery is indicated only for patients who do not respond to plasma exchange. Pregnancy termination is not considered therapeutic in TTP or HUS.

Ultralarge von Willebrand factor (VWF) multimers are found in TTP, thought to be secondary to the deficiency of a specific VWF-cleaving protease, identified as ADAMTS-13. ADAMTS-13 levels in TTP are typically less than 10%. ADAMTS-13 deficiency can be congenital or acquired. Two-thirds of women presenting with acute TTP for the first time in pregnancy have late-onset congenital disease. Acquired deficiency is associated with autoantibodies directed against ADAMTS-13. Reduced ADAMTS-13 levels are not specific for TTP; reduced levels are seen in the third trimester of pregnancy, in uremia, acute inflammation, malignancy, and in DIC. Most patients with TTP respond to plasma exchange. Steroids may be used until an anti-ADAMTS13 antibody is excluded. Rituximab is reserved for patients with refractory immune-mediated TTP, where the mother's life is in danger. Patients who develop pregnancy-associated TTP are at high risk of recurrence with subsequent pregnancies.

Atypical HUS (aHUS) is a disease of microvascular endothelial activation associated with complement deregulation, leading to an increase in activity in the alternative complement pathway. Mutations in complement genes controlling the alternative complement pathway or complement-activating genes account for more than 50% of the cases. Most cases occur postpartum with a mean time of onset of 26 days following delivery. Patients with aHUS present with microangiopathic hemolytic anemia and acute renal failure. VWF levels are usually elevated while multimer analysis may or may not show ultralarge multimers. Deficiency of VWF-cleaving protease is usually not associated with this syndrome.

Several women with a familial history of pregnancy-associated HUS have developed their first episode of HUS during pregnancy, and HUS has occurred in such patients

with the use of oral contraceptives. Postpartum HUS is associated with a poor prognosis; two-third of cases develop end-stage renal failure within 1 month. Plasma exchange can be initiated while ADAMTS13 levels are being processed in the laboratory. Once TTP is ruled out, eculizumab (humanized monoclonal antibody inhibitor of complement C5) becomes the treatment of choice. Dialysis and other supportive-care measures may also need to be initiated (Table 26.1).

Diffuse Intravascular Coagulation

Placental abruption is the most common cause of DIC (Table 26.2). There is an increased incidence of placental abruption in cocaine addicts. The incidence of DIC complicating placental abruption and dead fetus syndrome has decreased with advances in ultrasonography and prenatal care.

Table 26.1 Pregnancy-Associated Microangiopathies

Diagnosis	Preeclampsia	HELLP	PP-HUS	TTP
Time of onset	>20 weeks	>34 weeks	Postpartum (90%)	<24 weeks
MAHA	No	Yes	Yes	Yes
Thrombocytopenia	Yes	Yes	Yes	Yes
Coagulopathy	No	20%	No	No
Renal failure	Rare	Rare	Yes	Possible
Liver disease	No	Yes	No	No
Hypertension	Yes	Possible	Possible	Possible
Effect of delivery on disease	Yes	Yes	None	None

HELLP, hemolysis, elevated liver enzymes, and low platelets; MAHA, microangiopathic hemolytic anemia; PP-HUS, postpartum hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura.

Table 26.2 Causes of Obstetrical Disseminated Intravascular Coagulation

Placental abruption
Fetal death syndrome
Amniotic fluid embolism
HELLP syndrome
Clostridial sepsis
Sepsis
Major obstetrical hemorrhage

HELLP, hemolysis, elevated liver enzymes, and low platelets.

Fetal death syndrome is recognized by ultrasonography. Delivery of the dead fetus removes the source of tissue thromboplastin release. Blood component support and the

use of antithrombin-3 have been useful in the management of the coagulopathy.

Placental abruption is managed with blood component support followed by delivery. Antithrombin-3 and activated protein C have been used with success in this disorder.

Transient DIC occurs in patients undergoing hypertonic saline abortions, but the DIC usually resolves once the fetus is delivered. Clostridial sepsis following abortions is associated with DIC and poor clinical outcome.

Venous Thromboembolism in Pregnancy

The per-day risk of venous thromboembolism (VTE) is increased 7- to 10-fold for antepartum VTE and 15- to 35-fold for postpartum VTE. The risk of VTE diminishes rapidly following delivery, returning to the antepartum risk level by 3 weeks postpartum and to the nonpregnant level after 6 weeks. Venous thrombi occur predominantly in the left leg, partly because of the compression of the left iliac vein by the right iliac artery as they cross. Most deep venous thrombosis (DVTs) affect the proximal veins, with >60% restricted to ileofemoral veins.

Hemodynamic changes causing venous stasis and hypercoagulability play a role in the increased risk of VTE during pregnancy. Hypercoagulability is thought to be secondary to an increase in fibrinogen, factor VIII, and VWF. Furthermore, a decrease in protein S, the development of acquired protein C resistance, and reduced fibrinolytic activity from increased plasminogen activator inhibitor type 1 and 2 activity and decreased tissue plasminogen activator activity may contribute to the risk of VTE during pregnancy.

History of prior VTE, body mass index greater than 25, prolonged immobilization, inherited thrombophilias, antiphospholipid antibodies, and a family history of thrombosis all increase the risk of VTE during pregnancy.

Diagnosis of Venous Thromboembolism in Pregnancy

The diagnosis of VTE during pregnancy is complicated by the potential for fetal oncogenicity and teratogenicity associated with the use diagnostic ionizing radiation.

Compression ultrasonography (CU) of the entire proximal venous system to the trifurcation should be performed as the initial test for suspected deep vein thrombosis (DVT) in pregnancy. A normal CU does not exclude a calf DVT. The CU needs to be repeated at day 2 and day 7 to exclude an extending calf-vein thrombosis. A limited venogram with fetal shielding can be used in equivocal cases. When iliac DVT is suspected, pulsed Doppler ultrasound should be used; if the results are negative or equivocal, magnetic resonance venography (MRV) or venography should be considered.

In patients with suspected pulmonary emboli during pregnancy, bilateral compression lower extremity ultrasounds should be performed. If the ultrasound is

negative, a ventilation/ perfusion lung scan (V/ Q) should be the next procedure. If the results of the V/ Q scan are equivocal, computed tomography pulmonary angiography (CTPA) should be performed. However, should an isolated sub-segmental defect be suggested by CTPA, additional testing is suggested because of the high false-positive rate.

D-dimer levels increase throughout pregnancy. The D-dimer test has a high sensitivity, relatively low specificity, and very high negative predictive value.

Treatment of Venous Thromboembolic Disease in Pregnancy

Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) do not cross the placenta; therefore, there is no risk of fetal bleeding or teratogenicity. Heparin-induced thrombocytopenia, bleeding, and heparin-induced osteoporosis are more common with UFH than with LMWH.

There are case reports describing the use of fondaparinux, argatroban, and lepirudin in pregnant women with heparin-induced thrombocytopenia. The three drugs are classified under US Food and Drug Administration (FDA) Class B, indicating that animal studies have not shown harm in pregnancy, but there are no data from humans. Fondaparinux appears to cross the placenta in very low concentrations, but lepirudin and argatroban do not appear to cross the placenta. Coumarin derivatives do transit the placenta and have been associated with fetal bleeding and teratogenicity. Central nervous system abnormalities have occurred after the use of coumarin derivatives in every trimester of pregnancy: nasal hypoplasia and/ or stippled epiphyses have been associated with these drugs employed between the sixth and twelfth week of pregnancy. Due to the paucity of human data, direct oral anticoagulants (DOACs) should not be used in pregnant or lactating patients.

The activated partial thromboplastin time response to UFH is blunted in pregnancy because of increased factor VIII levels and increased heparin-binding proteins. This blunted response may lead to heparin overdosing. Measuring anti-activated factor (FXa) levels may obviate the problem. LMWHs have less nonspecific binding to heparin-binding proteins; hence they have a more predictable dose-response than UFH.

The quantity of UFH and LMWH detected in breast milk is clinically not significant. Argatroban and bivalirudin are not detected in breast milk, but fondaparinux is. Clinical evidence suggests that warfarin sodium is not excreted in breast milk and that breastfeeding is safe when mothers are treated with warfarin sodium.

The initial dose of LMWH is based on patient weight. Because of the variation of weight and glomerular filtration rate during pregnancy, it is recommended to monitor anticoagulation by monthly anti-FXa levels. Dose reduction to three-fourth dose after 3 to 4 weeks of full treatment appears safe and may obviate the need for continued anti-

FXa monitoring. LMWH should be discontinued 24 hours prior to elective induction of labor and neuroaxial anesthesia. Intravenous UFH can be initiated in patients at high risk for thrombosis and discontinued 6 hours before the time of expected delivery. LMWH can usually be restarted within 12 hours of delivery.

UFH or LMWH should be continued for at least 4 days after initiation of warfarin, until the international normalized ratio has been therapeutic, 2.0 or more, for 2 consecutive days.

The placement of an inferior vena cava filter should be considered, if the VTE is diagnosed after 37 weeks of pregnancy. Discontinuation of anticoagulation for delivery without a filter in place is associated with high rates of morbidity and mortality.

Prophylactic Anticoagulation in Patients With a Previous History of Venous Thromboembolism

Prophylactic antepartum anticoagulation is indicated in patients with a history of unprovoked VTE. Patients with a previous provoked VTE secondary to a temporary risk factor, who do not have an identifiable thrombophilia, are at low risk of recurrence at the time of a subsequent pregnancy and do not require antepartum prophylactic anticoagulation. All women with prior VTE should receive prophylactic postpartum anticoagulation for 6 weeks.

Patients with no prior VTE who are heterozygous for factor V Leiden or prothrombin gene mutation have a low risk of antepartum VTE without prophylaxis. Antepartum anticoagulation is warranted in patients with antithrombin III deficiency and in patients who are double heterozygotes for factor V Leiden and prothrombin gene mutation ([Table 26.3](#)).

Patients with idiopathic VTE who are pregnant or plan to become pregnant should undergo screening for thrombophilias. Patients with history of fetal loss, abruption, preeclampsia, and intrauterine fetal growth retardation should also be screened for thrombophilias.

Cesarean section is not a risk factor for VTE. Pharmacologic or mechanical thromboprophylaxis is recommended in patients with one VTE risk factor. Combined pharmacologic and mechanical thromboprophylaxis are recommended in patients with multiple VTE risk factors. In high-risk patients, 6 weeks of thromboprophylaxis is recommended.

Thrombophilias and Recurrent Miscarriage

Recurrent miscarriage is defined as three consecutive, spontaneous abortions of an intrauterine pregnancy, each occurring at less than 20 weeks gestation. Anticardiolipin antibodies have been linked to recurrent miscarriage. There are insufficient data to include inherited thrombophilias in the evaluation of women with recurrent miscarriage.

Prednisone, low-dose aspirin, UFH, LMWH, and IVIg have been used in the management of this problem. Prednisone was found to be equally effective as low-dose subcutaneous UFH in preventing pregnancy loss, but aspirin was associated with increased toxicities. UFH and aspirin have been shown to be superior to aspirin alone in preventing pregnancy loss. LMWH can be employed instead of UFH. The optimal dosages of UFH and LMWH remain to be defined.

Table 26.3 Most Common Thrombophilias

Inherited

Factor V Leiden
Prothrombin G20210A mutation
4G/ 4G mutation of the plasminogen activator inhibitor gene (PAI-I)
Thermolabile variant of methylenetetrahydrofolate reductase, the most common cause of homocystinemia
Antithrombin III deficiency
Protein C deficiency
Protein S deficiency

Acquired

Antiphospholipid antibody

HEMATOLOGICAL MANIFESTATIONS OF TROPICAL DISEASE

Malaria

Anemia is a serious complication of malaria, especially *Plasmodium falciparum* infection. The prevalence and degree of anemia depend on the nutritional and immune status of the patient. The degree of anemia cannot be explained entirely by intravascular rupture of parasitized red cells. Several mechanisms are involved in the anemia of malaria (Table 26.4). *Plasmodium vivax* and *Plasmodium ovale* invade only reticulocytes, *Plasmodium malariae* invades only mature red cells, and *P. falciparum* invades red cells of all ages. The proportion of cells parasitized in *P. vivax* malaria rarely exceeds 1%, whereas as many as 50% of red cells may be parasitized in *P. falciparum* infections.

P. vivax uses the Duffy antigen as a receptor for junction formation during invasion. *P. falciparum* does not use the Duffy antigen as a receptor for invasion but rather sialic acid residues of glycophorin A and B. Certain inherited defects confer resistance to parasitization by malarial organisms (Table 26.5).

There are two major clinical patterns in malaria: (1) acute malaria in the nonimmune and (2) recurrent malaria. Acute malaria is associated with a rapid drop in hemoglobin. Recurrent malaria causes splenomegaly and less severe anemia, and there are only scanty asexual forms and some gametocytes on the peripheral blood smear (Table 26.6).

In tropical areas, anemia tends to be more prevalent and most severe in children from 1 to 5 years and during pregnancy. Pregnant women who are not immune to *P. falciparum* develop severe malaria during pregnancy, and they have high rates of abortion, premature delivery, and perinatal and maternal mortality. In women who are immune, extravascular hemolysis and secondary folic acid deficiency play a major role in the pathogenesis of anemia. The extravascular hemolysis in immune women peaks during the second trimester and is accompanied by progressive splenomegaly.

Hyperreactive malarial splenomegaly (HMS) is characterized by splenomegaly, hypersplenism, a polyclonal B-lymphocyte proliferation, high immunoglobulin M (IgM) levels, and raised titers of antibodies against the predominant species of malaria. Sickle cell trait is protective against HMS. Patients with HMS have a persistence of malaria-induced IgM lymphocytotoxic antibodies, which reduce the numbers of T-suppressor lymphocytes and permit the proliferation of B-lymphocytes. HMS has been associated with the development of splenic lymphoma with villous lymphocytes. Significant lymphocytosis develops in 15% of patients with HMS, and may be mistaken for chronic lymphocytic leukemia.

Visceral Leishmaniasis

Visceral leishmaniasis (VL, Kala-Azar) is caused by one of the three species of *Leishmania donovani* complex. *L. donovani* is transmitted by phlebotomine sand flies. VL can also be transmitted through sexual contact, blood transfusions, and vertically.

L. donovani infects macrophages throughout the reticuloendothelial system. Patients develop irregular patterns of fever, weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia. The pancytopenia is secondary to hypersplenism and is worsened by folic acid deficiency. Monocytosis and lymphocytosis are typically present.

Chronic VL infection can cause marrow hypoplasia, gelatinous transformation, dyserythropoiesis, and myelofibrosis.

Table 26.4 Causes of Anemia in Malaria

Intravascular rupture of parasitized red cells	hypersplenism
Autoimmune hemolysis (50% of patients have a positive direct Coombs)	
Reticulocytopenia (anemia of chronic disease),	dyserythropoiesis (cytokine mediated)
Secondary bacterial, fungal, or viral infections	
Nutritional anemias	

Table 26.5 Protective Genetic Alterations

Southeast Asian ovalocytosis (autosomal dominant, 27 base pair deletion in the band 3 gene).

Heterozygotes for β -thalassemias (protection against *Plasmodium falciparum*)
HbE, HbS
Hereditary persistence of fetal hemoglobin
Glucose-6-phosphate dehydrogenase deficiency
Duffy-null phenotype (the Duffy antigen receptor for chemokines serves as a receptor for red cell invasion by *Plasmodium vivax*. Individuals who are Duffy-null are resistant to vivax malaria.)
Glycophorin A-deficient phenotypes [En(a-), M^k] (glycophorins are important ligands for the attachment and invasion of *P. falciparum* merozoites).
Glycophorin B-deficient phenotypes [S-s-U-]
CD35 (Knops antigen) variants (CD35 is involved in the resetting of *P. falciparum*-infected red cells with uninfected cells)

HbE, hemoglobin E; HbS, hemoglobin S.

Table 26.6 Hematologic Manifestations of Malaria

Parameters	Acute Malaria (Nonimmune)	Recurrent Malaria
Drop in hemoglobin	Drop in Ht within 24–48 hrs of onset of symptoms	Chronic
Severity of anemia	Hemoglobin can drop down to 2 g/ dL	2 g/ dL lower than noninfected controls
Neutrophils	Neutrophilia in the first 2 d, followed by neutropenia for 1–2 weeks, followed by neutrophilia	May be decreased because of hypersplenism
Monocytes	Monocytosis	Variable
Lymphocytes	Lymphocytosis	Variable
Platelets	Thrombocytopenia	Maybe decreased because of hypersplenism
Hypersplenism	No	Yes

African Trypanosomiasis (Sleeping Sickness)

African trypanosomiasis (AT or sleeping sickness) is endemic in sub-Saharan Africa. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are the etiologic agents. The tsetse fly is the vector. The infection is associated by the proliferation of macrophages and lymphocytes. Patients typically develop splenomegaly, pancytopenia secondary to hypersplenism, polyclonal hypergammaglobulinemia, monocytosis, and lymphocytosis.

Helminth Infections

Eosinophilia is present during the invasive migrating phase of hookworms, *Strongyloides*, and *Ascaris*. Hookworm is second in frequency only to malaria as an infectious cause of anemia. The daily loss of blood in the gut is 0.03 to 0.05 mL for each *Necator americanus* worm and 0.15 to 0.23 mL for each *Ancylostoma duodenale* worm.

The development of iron deficiency is related to the dietary intake of iron, the size of the iron stores, and the hookworm load. Iron depletion is more common in women, during pregnancy, and in children. Less frequent causes of iron deficiency are outlined in [Table 26.7](#).

Table 26.7 Iron Deficiency Associated With Helminth Infections

Helminth	Site of Blood Loss
Trichuriasis (whipworm)	Intestinal bleeding
Urinary schistosomiasis	Bladder
Intestinal schistosomiasis	Colon

Table 26.8 End-Organ Damage Associated With Hypereosinophilia

End Organ	Eosinophil Granule Proteins	Clinicopathologic Manifestations
Heart	Peroxidases, eosinophil major basic protein, eosinophil cationic protein	Constrictive pericarditis, fibroblastic endocarditis, endomyocardial fibrosis, myocarditis, intramural thrombus formation, mitral and tricuspid regurgitation, coronary arterial thrombi
Nervous system	Eosinophil-derived neurotoxin	Mononeuritis multiplex, paraparesis, central nervous system dysfunction, cerebellar involvement, recurrent subacute encephalopathy, cerebral infarction, seizures, eosinophilic meningitis
Lungs		Infiltrates, fibrosis, pleural effusions, pulmonary nodules
Skin		Angioedema, urticaria, papulonodular lesions, mucosal ulcerations (buccal and genital)
Eyes		Retinal vasculitis, microthrombi
Gastrointestinal/hepatic		Ascites, diarrhea, gastritis, colitis, pancreatitis, hepatitis, hepatic nodules
Muscle/ joints		Destructive arthritis, effusions, arthralgia, myositis

CLONAL EOSINOPHILIC DISORDERS

Blood eosinophilia is defined as an eosinophil count superior to 450/ μL . Eosinophils are much more abundant in tissues than in the peripheral blood. Sustained eosinophilia is associated with end-organ damage in a minority of patients ([Table 26.8](#)).

IL-5, IL-3, and granulocyte-colony-stimulating factor (G-CSF) all stimulate eosinophil production and inhibit eosinophil apoptosis. Eotaxin-1, eotaxin-2, and RANTES (regulated on activation T-cell expressed and secreted) are chemotactic cytokines, causing eosinophils to migrate into tissues. Eosinophils are the source of

multiple cytokines (IL-2, IL-3, IL-4, IL-5, IL-7, IL-13, IL-16, tumor necrosis factor α [TNF α], transforming growth factor β [TGF β], and RANTES). Eosinophils are also the source of cationic proteins such as eosinophil cationic protein, eosinophil peroxidase, major basic protein, eosinophil-derived neurotoxin, and Charcot–Leyden crystal lysophospholipase.

Helminthic infections are the most common cause of eosinophilia worldwide, and atopic disorders are the most common cause in industrialized countries. Clonal eosinophilic disorders account for only a small proportion of all eosinophilias ([Table 26.9](#)).

Sustained hypereosinophilia, whether reactive or clonal, can cause end-organ damage. The risk factors for end-organ damage are undefined.

The evaluation of a patient with eosinophilia is influenced by the patient's geographical origin and travel history. Serial stool examinations for ova and parasites may need to be supplemented by endemically relevant serologies and occasionally tissue biopsies.

Table 26.9 Diseases Commonly Associated With Eosinophilia

Infectious (helminth, protozoa, fungi, HIV, HTLV-1)

Allergic diseases (asthma, atopic dermatitis, allergic rhinitis, urticarias, allergic drug reactions)

Respiratory tract disorders (hypersensitivity pneumonitis, Loeffler's syndrome, allergic bronchopulmonary aspergillosis, tropical pulmonary eosinophilia)

Endocrinologic disorders (Addison's disease)

Gastrointestinal disorders (inflammatory bowel disease, eosinophilic gastroenteritis)

Cutaneous and subcutaneous disorders (atopic dermatitis, eosinophilic cellulitis, scabies, episodic angioedema with eosinophilia, chronic idiopathic urticaria, recurrent granulomatous dermatitis, eosinophilic fasciitis)

Immunodeficiency syndromes

Connective tissue disease (Churg-Strauss and cutaneous necrotizing eosinophilic vasculitis)

Neoplastic (lymphomas, T-ALL, T-cell lymphoproliferative disorders, solid tumors)

Myeloid leukemias and myeloproliferative disorders (acute eosinophilic leukemia, myelomonocytic leukemia with eosinophilia, chronic myelomonocytic leukemia with eosinophilia, chronic myeloid leukemia)

Idiopathic hypereosinophilic syndrome

Cytokines (IL-2, GM-CSF)

L-tryptophan and toxic oil syndrome

GM-CSF, granulocyte macrophage colony-stimulating factor; HTLV-1, human T-cell leukemia virus; IL-2, interleukin 2; T-ALL, T-cell acute lymphoblastic leukemia.

A clonal eosinophilic disorder needs to be investigated in patients without evidence of infectious or reactive causes of eosinophilia. Clonal eosinophilic disorders can be

subdivided into (1) clonal T-cell disorders, (2) clonal myeloid disorders, and (3) suspected clonality that cannot be proven (idiopathic hypereosinophilic syndrome [IHES]). Several patients classified as having IHES is decreasing as our diagnostic tools are improving ([Table 26.10](#)).

The clonality of eosinophils can be demonstrated by the expression of a single alloenzyme of glucose-6-phosphate dehydrogenase in purified eosinophils from female heterozygotes. Polymerase chain reaction amplification of the human androgen receptor gene locus (HUMARA) can also document clonality of eosinophils in female patients.

T-Cell Clonal Disorders

Interleukin-5 (IL-5) overproduction by TH2 lymphocytes has been demonstrated in both clonal and reactive hypereosinophilic disorders. Aberrant clones of T-lymphocytes are present in 25% of patients with clonal hypereosinophilic disorders. The finding of isolated T-cell clonality by polymerase chain reaction (PCR) without T-cell immunophenotypic abnormalities or demonstration of Th2 cytokine production is insufficient to establish a diagnosis of lymphocyte-variant hypereosinophilia. The aberrant phenotypes are heterogeneous ([CD3⁺, CD4⁺, CD8⁻], [CD3⁺, CD4⁻, CD8⁺], [CD3⁺, CD4⁻, CD8⁻], [CD3⁻, CD4⁺]). In most cases, an activated T-cell phenotype is present with expression of CD25 and HLA-DR. In 50% of patients, a clonal rearrangement of the T-cell receptor gene (β) or (γ) is found. T-cell lymphomas develop in a proportion of these patients.

Patients with aberrant CD4⁺, CD3⁻ T cells producing high levels of IL-5, IL-4, and IL-13 typically present with skin manifestations, lack of severe end-organ involvement, and they have elevated IgE levels and polyclonal hypergammaglobulinemia.

The optimal treatment of patients with aberrant T-cell clones remains unclear. Corticosteroids are effective and currently represent first-line therapy. Most patients require steroid-sparing immunosuppressive agents. Elevated serum IgE and thymus and activation-regulated chemokine (TARC) levels predict response to corticosteroids. Hydroxyurea and imatinib are less likely to be effective. Interferon- α and cyclosporine have been used in this setting. The role of alemtuzumab in the management of CD52⁺ clonal T-cell disorders is under evaluation.

Table 26.10 Clonal Hypereosinophilic Disorders

Clonal T-Cell Disorders

- T-ALL
- T-cell lymphomas
- Aberrant T-cell clones ([CD3⁺, CD4⁺, CD8⁻], [CD3⁺, CD4⁻, CD8⁺], [CD3⁺, CD4⁻, CD8⁻], [CD3⁻, CD4⁺])

Clonal Myeloid Disorders

- Acute leukemias (M2 AML with eosinophilia, M4-Eo AML with inv(16) (p13;q22), t(16;16) (p13;q22))
- Chronic myelomonocytic leukemias with eosinophilia
- Myeloproliferative disorders with eosinophilia (polycythemia vera, chronic myelogenous leukemia, essential thrombocythosis, agnogenic myeloid metaplasia)
- Systemic mast cell disease with eosinophilia
- FIP1L1-PDGFR- α hypereosinophilic disorders

Clonal Hypereosinophilic Disorders

The evaluation of patients with suspected clonal hypereosinophilic disorders should include:

- CBC-differential and peripheral blood smear
- Chemistry group
- Serum IgE
- B12
- Serum tryptase (Increased in mast cell disease with eosinophilia and the myeloproliferative variant of FIP1L1-PDGFR- α hypereosinophilic disorders.)
- Peripheral blood flow cytometry (Used to identify an aberrant population of T-lymphocytes.)
- T-cell receptor gene (β) or (γ) rearrangement studies
- HIV serology
- CT scans of the chest, abdomen, and pelvis
- Bone marrow aspirate and biopsy (with reticulin and tryptase staining of the biopsy)
- Bone marrow cytogenetics
- PCR for FIP1L1-PDGFR- α fusion gene and/ or CHIC-2 fluorescent in situ hybridization

AML, acute myeloid leukemia; CBC, complete blood count; CHIC-2, cysteine-rich hydrophobic domain 2; CT, computed tomography; IgE, immunoglobulin E; PCR, polymerase chain reaction; PDGFRA- α , platelet-derived growth factor receptor- α ; T-ALL, T-cell acute lymphoblastic leukemia.

Acute Leukemias

Acute eosinophilic leukemia is rare. Cyanide-resistant peroxidase can be used to identify eosinophilic blasts. Myelomonocytic leukemia (M4-Eo) with eosinophilia is associated with inv (16) (p13;q22) and t(16;16) (p13;q22). The core binding factor- β is a transcription factor located at 16q22, and the smooth muscle myosin heavy chain is located at 16p13. The eosinophils in M4-Eo frequently have a dysplastic appearance.

Chronic Myelomonocytic Leukemia With Eosinophilia

The two predominant subtypes of chronic myelomonocytic leukemia with eosinophilia involve, respectively, platelet-derived growth factor receptor β (PDGFR- β) and fibroblast growth factor receptor 1 (FGFR1). In both subtypes, fusion oncoproteins are constitutively activated and are able to activate downstream stimulatory and antiapoptotic pathways.

Chronic Myelomonocytic Leukemia With Eosinophilia Subtypes

PDGFR- β subtype:

- Age 50 to 60 years
- Male predominance (>90%)
- Monocytosis, eosinophilia, splenomegaly

- Most common chromosome abnormality is t(5;12)(p12;q31-32) [ETV6-PDGFR]
- Imatinib responsive

Fibroblast growth factor receptor 1 subtype (FGFR1):

- Median age: 32 years
- Male to female ratio (1.5:1)
- Associated with lymphoblastic lymphoma transformation (B and T)
- Associate with rearrangement of FGFR1 at the 8p11-12 locus
- Not responsive to imatinib.

FIP1L1-PDGFR- α Hypereosinophilic Disorders

FIP1L1-PDGFR- α is a constitutively activated tyrosine kinase that was first described in a patient with hypereosinophilic syndrome, who genetically had an occult 800-kb interstitial deletion of chromosome 4q12. The FIP1L1-PDGFR- α cannot be detected by conventional karyotyping; fluorescence in situ hybridization (CHIC-2) or reverse transcription polymerase chain reaction is required. An imatinib dose of 100 mg daily may be sufficient to achieve a molecular remission in some patients, others may require doses in the range of 300 to 400 mg daily. Most patients ($\geq 95\%$) with the FIP1L1-PDGFR- α treated with imatinib obtain a molecular remission within 3 months of initiating therapy. The optimal maintenance dose and duration of treatment remain to be defined. Imatinib suppresses but does not eliminate FIP1L1-PDGFR, discontinuation of imatinib leads to relapse in most patients. Molecular remissions can be reestablished by reinitiating imatinib therapy. Resistance to imatinib is associated with a T674I mutation in PDGFR- α ; this mutation occurs in the adenosine triphosphate (ATP)-binding region of PDGFR- α at the same position as the T315I mutation in BCR-ABL.

Imatinib can elicit durable remissions in patients with rearrangements of PDGFR- β or PDGFR- α variants.

A serum troponin and cardiac echocardiogram should be obtained before initiating imatinib. An increased level of serum cardiac troponin correlates with cardiomyopathy. Prophylactic use of corticosteroids during the first 7 to 10 days of treatment with imatinib is recommended in patients with evidence of eosinophil-mediated cardiomyopathy and in patients with other cardiac comorbidities.

Idiopathic Hypereosinophilic Syndrome

Idiopathic hypereosinophilic syndrome is arbitrarily defined as eosinophilia in excess of 1,500/ μ L for more than 6 months with evidence of end-organ damage, without an evident primary or secondary cause of eosinophilia. Idiopathic hypereosinophilia is the term favored when end-organ damage is absent. Corticosteroids represent the first-line therapy for idiopathic hypereosinophilic syndrome. Hydroxyurea, interferon- α , and

monoclonal antibodies directed against IL-5 have been used in steroid-resistant patients. The role of imatinib as frontline therapy in patients without FIP1L1-PDGFR- α fusion proteins remains to be established.

NEUTROPENIA

Neutropenia is defined as a decrease in neutrophils less than 1,500/ μL . Severe neutropenia is defined as a decrease in neutrophils less than 500/ μL . In patients of African heritage and some Middle-Eastern groups, the neutrophil count may normally be as low as 800 to 1,000/ μL .

Neutropenias are classified as either intrinsic disorders of the hematopoietic system or secondary forms. The secondary forms are caused by extrinsic factors such as hypersplenism, infections, drugs, and immune destruction ([Table 26.11](#)).

Intrinsic Disorders

Congenital Neutropenias

Congenital neutropenias include Kostmann syndrome, cyclic neutropenia, congenital immunodeficiency syndromes, as well as several other rare syndromes that are not discussed in this chapter.

Table 26.11 Neutropenia Classification

Intrinsic Disorders
Congenital
Acquired
Extrinsic Disorders
Immune neutropenias
Neutropenia associated with autoimmune disorders
Neutropenia associated with large granular lymphocytes
Hypersplenism
Neutropenia associated with infectious diseases
Drug-related neutropenias
Nutritional deficiencies (B12, folate, copper)

Kostmann syndrome is an autosomal-dominant disorder presenting in the newborn. Characteristic findings include neutrophils less than 200/ μL , monocytosis, anemia, thrombocytosis, splenomegaly, and maturation arrest in the marrow at the promyelocyte level. The accelerated apoptosis of neutrophilic precursors is secondary to a mutation of neutrophil elastase. Ninety percent of children with Kostmann syndrome respond to granulocyte colony-stimulating factor (G-CSF). Evolution to myelodysplasia and acute leukemia occurs in some patients. It is unclear if G-CSF increases this risk.

Cyclic neutropenias can be congenital (autosomal-dominant congenital disorder) or acquired with clonal large granular lymphocyte (LGL) syndrome. Congenital cyclic neutropenia is due to mutations at the enzyme active site of the neutrophil elastase gene, which leads to accelerated apoptosis of neutrophils. Clinically, patients present with cycles of neutropenia every 21 to 56 days. The neutropenia can be severe and last 3 to 6 days. Fever, mucosal ulcers, and lymphadenopathy can occur during the nadir of the cycles. G-CSF is useful in the management of cyclic neutropenia.

Congenital immunodeficiency syndromes frequently associated with neutropenia include X-linked agammaglobulinemia, X-linked hyper-immunoglobulin M syndrome, and reticular dysgenesis.

Acquired Neutropenias

Acquired intrinsic disorders include leukemias, myelodysplastic syndromes, lymphoproliferative disorders, aplastic anemia, neutropenia of prematurity, and chronic idiopathic neutropenia.

Chronic idiopathic neutropenia occurs in both children and adults. The neutropenia in some patients can be severe. Patients have negative antineutrophil antibodies, normal marrow cytogenetics, and either normocellular marrows or marrows showing decreased postmitotic cells. The prognosis is excellent; patients do not progress to myelodysplasia or leukemia. A proportion of these patients may have autoimmune neutropenia (AIN), with undetectable antineutrophil antibodies. G-CSF is effective in increasing the neutrophil count.

Extrinsic Disorders

Immune Neutropenias

Five neutrophil-specific antigens carried on two different glycoproteins have been described. The neutrophil-specific antigens (NA) (NA1, NA2, and SH) are expressed on Fc γ RIIIb (CD16), which is a low-affinity receptor for IgG1 and IgG3. The NB antigen is expressed on glycoprotein CD177. There are data suggesting that ANCA can be implicated in the pathogenesis of secondary AIN. The granulocyte immunofluorescence test (GIFT), the granulocyte agglutination test (GAT), and the monoclonal antibody immobilization of granulocyte antigens assay (MAIGA) can be used to detect antineutrophil antibodies. A combination of GIFT and GAT is recommended as the best approach.

Alloimmune neonatal neutropenia occurs when maternal antibodies cross the placenta and react with the infant's neutrophils. In isoimmune neutropenia, the mother produces an antibody to the paternal CD16 isotype that is different from her own.

Primary AIN is diagnosed in patients with isolated neutropenia who have detectable

antineutrophil antibodies. NA1 antibodies are detected in 35% to 40% of patients. The clinical course is usually benign, and spontaneous remissions are common.

Neutropenia Associated With Autoimmune Disorders

AIN is associated with common variable immunodeficiency. The condition should be excluded in patients with recurrent immune cytopenias and granulomatous disease. A high incidence of antineutrophil antibodies is found in patients with X-linked autoimmune lymphoproliferative syndrome (ALPS).

In systemic lupus erythematosus, Fas-mediated apoptosis of mature neutrophils and CD34⁺ hematopoietic progenitor cells play an important role in the pathogenesis of neutropenia. Sjogren's syndrome, systemic sclerosis, and primary biliary cirrhosis and Grave's disease have all been associated with AIN.

Felty's syndrome patients typically have deforming rheumatoid arthritis, splenomegaly, and elevated rheumatoid factor titers. The neutropenia in Felty's is thought to be antibody mediated. In a proportion of patients with Felty's syndrome, the neutropenia is secondary to clonal LGLs.

Neutropenia Associated With Large Granular Lymphocyte Syndrome

LGL syndrome is caused by an expansion of either T-lymphocytes or natural killer (NK) cells. The NK-cell subtype is more aggressive and accounts for 15% of cases. Forty percent of LGL is associated with other diseases such as rheumatoid arthritis.

The T-cells in clonal LGL express the CD3–TCR complex and have rearranged T-cell receptor genes. These cells are thought to represent *in vivo* activated cytotoxic T cells. Clonal LGLs express high levels of Fas ligand. Normal neutrophil survival is regulated by the Fas–Fas ligand apoptotic system. The neutropenia in clonal LGL syndrome appears to be mediated by increased peripheral destruction of neutrophils secondary to immune complexes and bone marrow suppression of granulopoiesis by Fas ligand secretion.

Neutropenia Associated With Infectious Diseases

The most common cause of acquired neutropenia is infection. Gram-negative septicemia, *Staphylococcus aureus*, typhoid fever, paratyphoid fever, tularemia, and brucellosis can cause neutropenia. Infectious hepatitis, influenza, measles, Colorado tick fever, mononucleosis, cytomegalovirus, Kawasaki disease, HIV, and parvovirus B12 are included in the differential diagnosis of neutropenia associated with infectious diseases.

Parvovirus B19 is frequently associated with transient neutropenia and may cause protracted leucopenia in immunosuppressed patients. Neutropenia is seen in more than 70% of patients with acquired immunodeficiency syndrome and can be associated with

hypersplenism and antineutrophil antibodies.

Drug-Induced Neutropenia

The second most common cause of neutropenia is medication exposure: approximately 70% of agranulocytosis cases in the United States are attributed to medications. Almost all classes of medications have been implicated in causing drug-induced neutropenia. Anti-infectives, psychotropic agents, and antithyroid medications are most commonly implicated. Three pathogenetic mechanisms for isolated neutropenia include dose-dependent inhibition of granulopoiesis, immune-mediated destruction of neutrophils and their precursors, and direct toxic effect on marrow granulocytic precursors ([Table 26.12](#)).

The onset of neutropenia is rapid (1 to 2 days) in immune-mediated destruction of neutrophils and more variable with agents causing either direct toxic effect or dose-dependent inhibition. Immune-mediated destruction of neutrophils and their precursors occurs by two mechanisms. With hapten mediation, the agent acts as a hapten to induce antibody formation and needs to be present for neutropenia to occur. In the immune complex mechanism, once the complex is formed, continued drug presence is not required for neutrophil destruction.

Table 26.12 Mechanisms of Drug-Induced Isolated Neutropenia

Dose-Dependent Inhibition of Granulopoiesis

- β-Lactam antibiotics, carbamazepine, valproic acid

Immune-Mediated Destruction of Neutrophils and Neutrophil Precursors

- Agent acts as hapten to induce antibody formation, complement fixation, and neutrophil destruction: penicillin, gold, cephalosporins, antithyroid drugs
- Immune complex related: quinidine

Direct Toxic Effect on Marrow Granulocytic Precursors

- Sulfasalazine, captopril, phenothiazine, clozapine
- Chemotherapy drugs seldom cause isolated neutropenia

Ipilimumab, fludarabine, and rituximab have been associated with AIN. Rituximab-associated neutropenia is delayed. Rituximab late-onset neutropenia appears after a median of 38 to 175 days from the last rituximab dose, and its median duration is 5 to 77 days. The pathogenesis is not completely understood. The role of G-CSF remains controversial, and the decision regarding its use should be made on an individual basis.

Suggested Readings

1. Abdalla SH, Pasvol G. Approach to the patient in the tropics with anemia. In: Guerrant RL, Walker DH, Weller PF, eds. Tropical Infectious Diseases:

- Principles, Pathogens and Practice. 3rd ed. Philadelphia, PA: Elsevier; 2011.
2. Bartels M, Murphy K, Bruin M. Understanding chronic neutropenia: life is short. *Br J Haematol*. 2016; 172:157–169.
 3. Bennet A, Chunilal S. Diagnosis and management of deep vein thrombosis and pulmonary embolism in pregnancy. *Sem Thromb Hemost*. 2016; 42:760–773
 4. Bergmann F, Rath W. The differential diagnosis of thrombocytopenia in pregnancy. *Dtsz Arxtebl Int*. 2015; 112:795–802.
 5. Boga C, Ozdogu H. Pregnancy and sickle cell disease: a review of the current literature. *Crit Rev Oncol Hematol*. 2016;98:364–374.
 6. Dale DC, Bolyard AA. An update on the diagnosis and treatment of chronic idiopathic neutropenia. *Curr Opin Hematol*. 2017; 24(1):46-53.
 7. Checkley AM, Chiodini PL, Dockrell DH, et al. Eosinophilia in returning travellers and migrants from the tropics: UK recommendations for investigation and initial management. *J Infect*. 2010;60:1–10.
 8. Gotlib J. World Health Organization-defined eosinophilic disorders: 2015 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2015; 90:1078–1089.
 9. Newberger PE, Dale DC. Evaluation and management of patients with isolated neutropenia. *Semin Hematol*. 2013; 50:198–206.
 10. Piatek CI, El-Hemaidi I, Feinstein DI, et al. Management of immune-mediated cytopenias in pregnancy. *Autoim Rev*. 2015;14:806–811.
 11. Pick AM, Nystrom KK. Nonchemotherapy drug-induced neutropenia and agranulocytosis: Could medications be the culprit? *J Pharm Pract*. 2014; 27:447–452.
 12. Pierangeli SS, Leader B, Barilaro G, et al. Acquired and inherited thrombophilia disorders in pregnancy. *Obstet Gynecol Clin North Am*. 2011;38:271–295.
 13. Simcox LE, Ormesher L, Tower C, et al. Thrombophilia and pregnancy complications. *Int J Mol Sci*. 2015; 16:28418–28428.
 14. Thomas MR, Robinson S, Scully MA. How we manage thrombotic microangiopathies in pregnancy. *Br J Haematol*. 2016; 173:821–830.

Interpretation of Standard Hematologic Tests

Vid Leko, Katherine R. Calvo, and Geraldine P. Schechter

CELLULAR ANALYSIS OF THE PERIPHERAL BLOOD AND BONE MARROW

Rapid and accurate analysis of the cellular elements of blood and bone marrow is essential for the practice of clinical hematology. Standard automated complete blood counters typically provide valuable information within minutes, while the clinical information from microscopic evaluation of peripheral blood or bone marrow can be derived in less than an hour.^{1,2}

Complete Blood Count

Automated Complete Blood Counters

By combining different detection modalities—electrical impedance, flow cytometry, and spectrophotometry—automated blood counters analyze complete blood count (CBC), leukocyte differential, and hemoglobin concentration. Newer analyzers also provide information about reticulocyte counts, presence of immature granulocytes (IGs), nucleated red blood cells (nucleated RBCs), and immature platelet fractions (IPFs). Some models also feature an integrated slide maker/stainer unit, which automatically prepares peripheral blood films from samples that are flagged as abnormal by the analyzer.

RBCs and platelets are analyzed using the direct current (DC) detection method, which exploits a physics phenomenon known as the Coulter principle. A portion of aspirated blood is diluted and allowed to flow in electrical current through an aperture between two parts of an electrical circuit. As each cell passes through the orifice, the

conduction through the circuit briefly decreases, leading to generation of a “pulse.” These signals, whose amplitude is proportional to the cell size, are then converted into graphical displays called histograms. To create an RBC histogram (Fig. 27.1A), which provides information about RBC number and size distribution, the detection threshold is adjusted so that particles smaller than the red cells (platelets) are not counted. This still allows detection of white blood cells (WBCs), but as their concentration in the intact blood is 10^3 times lower than the RBC count, the latter is not significantly affected. To obtain a platelet histogram, on the other hand, red cells are lysed and the threshold adjusted to include only the smaller-size particles, thus excluding WBCs (Fig. 27.1B). Both RBCs and platelets can be also measured by using flow cytometry.

WBC populations, reticulocytes, nucleated RBCs, and occasionally platelets are analyzed using flow cytometry. A diluted portion of a blood sample is treated with surfactant, which lyses the RBCs and permeabilizes the WBCs and platelets and mixed with fluorescence markers that bind to intracellular nucleic acids. Cells are then passed through a flow channel so that each cell is exposed to a laser beam, causing a scatter of light and emission of side fluorescence. The magnitude of forward-directed scatter (FSC) depends on the cell size and therefore measures the cell volume, while the side scatter (SSC) depends on granularity and measures internal cell complexity. Side fluorescent light emission (SFL), which depends on the amount of cellular DNA/ RNA, serves as a measure of nucleic acid content. Several combinations of these three signals are then used to create scattergrams, graphical displays that enable enumeration and characterization of different cell populations (Fig. 27.1C and 27.1D).

The hemoglobin concentration is measured using spectrophotometry: RBCs are first lysed with sodium lauryl sulfate (SLS), which then binds to oxidized heme moieties of hemoglobin molecules and forms complexes that absorb monochromatic light of a certain wavelength. The absorbance, measured by a photosensor, is proportional to the hemoglobin concentration of the sample.

Red Cell Parameters

Traditionally, the red cell parameters were hemoglobin concentration measured by spectrophotometry and the manually measured hematocrit (the percent of the blood volume occupied by RBCs) and red cell count. The mean corpuscular volume (MCV) was calculated by dividing the hematocrit by the red cell count, the mean corpuscular hemoglobin (MCH) by dividing the hemoglobin concentration by the red cell count, and the mean corpuscular hemoglobin concentration (MCHC) by dividing the hemoglobin concentration by the hematocrit. Today, automated cell counters directly measure RBC count, RBC volume, and hemoglobin concentration by using the methods described in the preceding section; MCV and red cell distribution width (RDW) are derived by

analyzing the RBC histogram (Fig. 27.1A). The other parameters are typically calculated: MCH, MCHC, and the hematocrit. Many automated analyzers typically derive hematocrit by multiplying the red cell count with the MCV, making it less reliable and reproducible than hemoglobin concentration when assessing patients with anemia or polycythemia. Because hemoglobin concentration is measured by spectrophotometry, the results are not reliable in cases of increased sample turbidity (i.e., in lipemia or extreme leukocytosis), and should be verified by a manual hematocrit determination.

The MCV is of great utility in evaluating anemias according to red cell size (macrocytic, microcytic, and normocytic), and the RBC count is useful in distinguishing between iron deficiency and thalassemia (decreased in the former and increased in the latter) in patients with low MCV. The MCH represents the average total hemoglobin content of red cells but does not account for the cell size. It can therefore over- or underestimate the average red cell hemoglobin concentration (or the degree of “redness”), which is expressed as the MCHC. MCH and MCHC are seldom useful in clinical practice, although the MCHC is classically increased in hereditary spherocytosis, given the uniform decrease in red cell surface-to-cytoplasm ratio.

The RDW measures the variability in the red cell size of each blood sample, either as RDW-CV (coefficient of variation) or RDW-SD (standard deviation). The former is calculated from the RBC histogram by dividing the SD of MCV with the MCV, and multiplying the product with 100 to express a percentage (the normal range is approximately 11.0% to 15.0%). The latter is an actual measure of the RBC histogram curve width expressed in femtoliters (fL; normal range is 39 to 46 fL). Elevated RDW (CV or SD) serves as a more reliable screening method for anisocytosis (presence of many red cells of different sizes) than the inspection of the blood film for a casual observer.³ However, when markedly elevated, inspection of the blood film should always be performed. RDW is sensitive to the presence of small subpopulations of large or small red cells, and therefore better than the MCV for early detection of nutritional deficiencies. A low MCV and a high RDW are most likely to be due to iron deficiency anemia. RDW often remains normal in milder forms of thalassemia, and the combination of a high or normal RBC count, low MCV, and a normal RDW is a common pattern in thalassemia trait.⁴ One caveat is that RDW-CV is dependent on the MCV, which may produce falsely higher or lower results in cases with low and high MCV, respectively. RDW-SD, on the other hand, is independent of MCV and therefore more accurate in assessing variability in cell size. The use of the RDW has had limited acceptance in the classification of anemias because of unreliable results in complex settings.

Reticulocytes are immediate precursors of mature RBCs that still contain

intracellular RNA, giving them a reticular appearance when viewed microscopically using supravital stains. The RNA usually disappears within a day after reticulocyte release into the peripheral blood. Quantitation of the reticulocyte count by flow cytometry in the automated cell counters has largely replaced the microscopic examination of the peripheral smear, which is not as precise. However, automatic quantification is vulnerable to interference from intracellular organisms, basophilic stippling, and other artifacts. Therefore, microscopic inspection of a standard blood film is warranted whenever the reticulocyte count seems inappropriate for a given clinical scenario. The absolute reticulocyte count ($\% \text{ reticulocytes} \times \text{RBC count} / 100$) is more useful in assessing the marrow reticulocyte production than the simple percentage of reticulocytes, which is affected by a change in total number of circulating RBCs. However, because a highly stimulated marrow produces larger, more immature reticulocytes (termed stress reticulocytes),⁵ which live longer than their mature counterparts, the absolute reticulocyte count may overestimate the true rate of reticulocyte production in these clinical situations. In such cases, the reticulocyte production index (RPI) can be used for a more accurate estimate. To calculate RPI, the percentage of reticulocytes is first multiplied with the ratio of patient's hematocrit and normal hematocrit (usually 45) and then divided with the maturation correction index, which accounts for increased number of stress reticulocytes in more severe cases of anemia. RPI higher than 2% generally indicates adequate bone marrow response to hemolysis or bleeding. Based on the increased RNA content of immature reticulocytes, some automated counters can detect these subpopulations as the immature reticulocyte fraction (IRF), which may be used as an indicator of early response to erythropoiesis-stimulating agents or marrow recovery after chemotherapy or stem cell transplantation.⁶ An increase in reticulocyte count is usually seen 3 to 5 days after the acute bone marrow stimulation. Special automated counters are also capable of selectively measuring the hemoglobin content of reticulocytes, which diminishes rapidly as accessible iron stores are exhausted, making it useful for detecting early iron deficiency.⁶

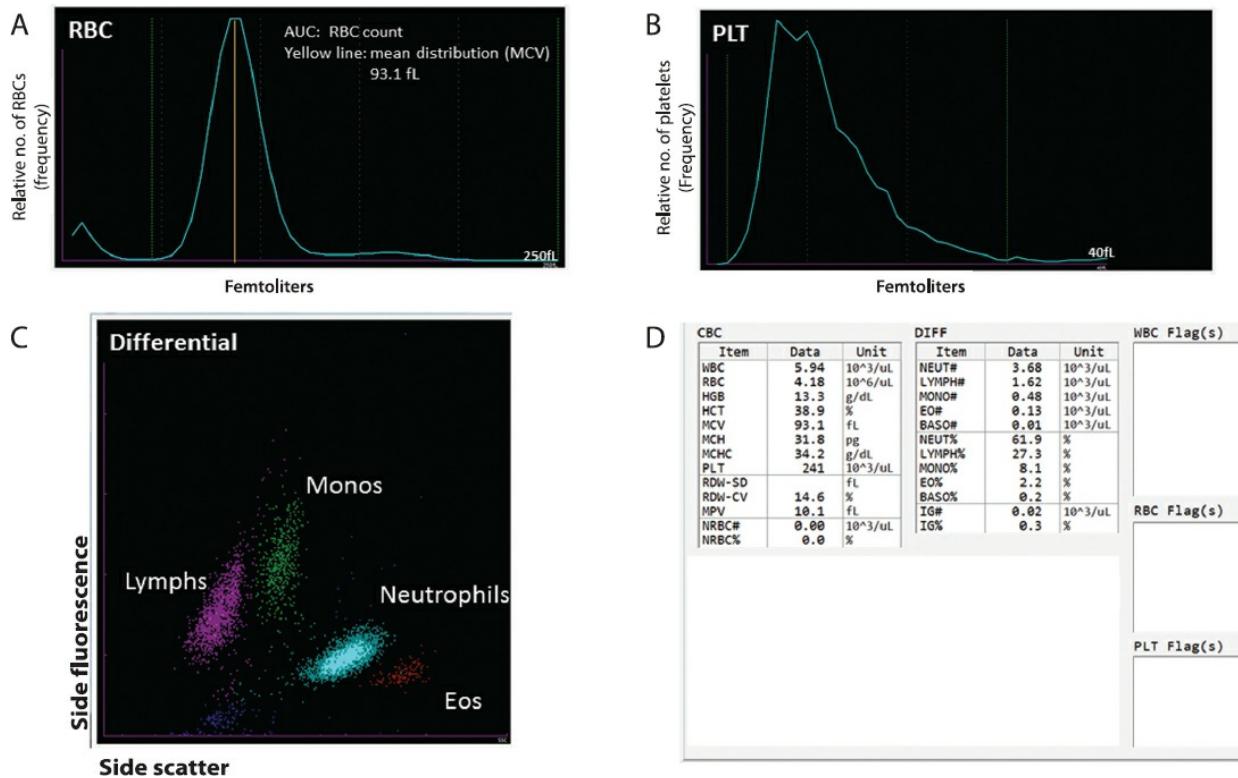


FIGURE 27.1 Histograms, scatterplot, and differential generated by Sysmex automated hematology analyzer. **(A)** RBC histogram showing the relative number of red cells on the y-axis and the size in fL on the x-axis. AUC represents the total RBC count. The yellow line shows the mean distribution on the RBC histogram and corresponds to the MCV. **(B)** Platelet histogram showing the number of platelets on the y-axis and the size in fL on the x-axis. AUC represents the platelet count. **(C)** WBC scatterplot demonstrating separation of major white blood cell populations based on fluorescence (y-axis, proportional to the nucleic acid content of the cell) and complexity of the internal structure, that is, granularity (x-axis). **(D)** Differential readout for a normal blood sample for which no flags are generated.

AUC, area under the curve; CBC, complete blood count; Eos, eosinophils; fL, femtoliters; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PLT, platelet(s); RBC, red blood cell(s); RDW-CV, red cell distribution width (coefficient of variation); RDW-SD, red cell distribution width (standard deviation); WBC, white blood count.

White Blood Cell Parameters

The automated WBC counting by flow cytometry is faster and usually more accurate than the older manual methods, particularly for leukopenic patients with WBC counts as low as 100 cells per μL . Automated leukocyte differentials are accurate when white

cell morphology is normal, but not entirely reliable in instances where abnormal cells are present. Most modern hematology analyzers can detect abnormal cell populations (blasts, lymphoma cells, etc.) and “flag” the sample, alerting the technologist to review the peripheral blood smear and perform a manual differential. However, in some cases, abnormal cells (e.g., small blasts) can be enumerated as normal white cells without flagging. Hence, the WBC differential of any patient with high suspicion of a hematological problem should always be evaluated on the peripheral smear. In neutropenic patients, the automated absolute neutrophil count is a reliable and important tool in evaluating the risk of infection, grading drug toxicity, and assessing recovery from hematopoietic stem cell transplantation.

Platelets

Automated counters are more precise than older manual methods in monitoring thrombocytopenia, specifically for counts less than 10,000 platelets per μL . They mostly employ electrical impedance-based methods, although newer machines can also utilize fluorescent flow cytometry, particularly when counting severely thrombocytopenic samples.

The mean platelet volume (MPV), routinely measured by automated analyzers, has modest value as a measure of increased platelet turnover and activation, relying on the fact that younger platelets are larger than the older ones. However, the MPV may increase rapidly within the first 2 hours after sample collection because of swelling in ethylenediaminetetraacetic acid (EDTA) solution. Moreover, the reference standards are not adequate for comparing values from different institutions.⁷

Some automated counters can measure the RNA content of newly released platelets, expressed as the IPF or absolute immature platelet number ($\% \text{IPF} \times \text{the platelet count}/100$). These measurements may be useful clinical parameters in assessing increased thrombopoiesis in idiopathic thrombocytopenic purpura (ITP) and bone marrow recovery following hematopoietic transplantation.^{8,9}

Sources of Artifact

Clinically relevant artifacts at the point of sample collection may occur due to clot formation or dilution of blood with intravenous fluid, both more commonly seen with samples drawn from indwelling catheters.^{10,11} Although most automated cell counters are equipped to detect clots, some may not be detected, resulting in falsely lower platelet and WBC counts. In contrast, dilution with fluids is generally apparent by a change affecting all the cell lines.

Artifacts may also be introduced during storage of blood samples. When stored at room temperature, absolute RBC, WBC, and platelet counts are stable for up to 3 days, but the red cell MCV increases within 24 hours due to cell swelling, similar to platelet

MPV, resulting in associated increases in the hematocrit and RDW.¹² After 2 days, the relative proportion of monocytes on the WBC differential decreases, with relative increase in leukocyte, lymphocyte, and eosinophil counts.¹²

Red cell agglutination within the test tube (caused by cold or warm reactive antibodies or rouleaux formation) and extremely small or fragmented red cells can artifactually reduce the red cell count, while extreme leukocytosis, particularly with WBC counts of more than 500,000/ μL , can falsely increase the RBC count and calculated hematocrit.¹¹ Falsely decreased platelet counts (pseudothrombocytopenia), caused by EDTA-induced platelet clumping and satellitism (a phenomenon where platelets cluster around the neutrophils), may occur in up to 0.1% of normal samples and up to 15% of patients referred for evaluation of a low platelet count.¹³ In most cases, it can be eliminated by collecting samples into a tube containing citrate anticoagulant; the measured platelet count should be multiplied by 1.1 because of the dilution with sodium citrate. If the clumping persists, presence of cold platelet agglutinins should be suspected. Platelet clumping may sometimes also falsely elevate the WBC count, while small white or red cell fragments, protein precipitates, or circulating fungi may artifactually increase the platelet count.^{10,11,14}

Peripheral Blood Film

Review of the peripheral blood smear stained with either Wright, Romanowsky, or Giemsa stain is commonly used by laboratory personnel to verify abnormalities “flagged” by the automated counters, or by hematologists when the automated results require verification (Fig. 27.2). Blood films can be analyzed directly, using conventional microscopy, or indirectly, using advanced scanning and optical recognition technology in automated digital morphology instruments (i.e., Cellavision, Figs. 27.3 and 27.4). In the latter, the automated camera photographs a preset number (up to 400) of distinct WBCs on the smear, which a complex software program then sorts into different morphologic groups, providing results within minutes. The laboratory technician, hematologist, or pathologist verifies the results on the computer screen and reassigns the cells into morphological categories as needed. In general, the differential generated from a digital morphology instrument is equivalent to the manual differential on a microscope, with the advantage of storing the images for future reassessment by a hematologist or pathologist. As these systems typically image the cells in central areas of the smear (with more preserved morphology), there is a potential of missing the rare instances when large abnormal cells cluster at the periphery of the smear.

Although examination of the peripheral smear is not always necessary in simple anemias due to nutritional deficiencies, it is indicated in cases that do not respond to

therapy, anemias without apparent cause, or if hemolysis, marrow fibrosis or tumor invasion are suspected. Identification of fragmented red cells (associated with microangiopathic hemolytic anemia), spherocytes, sickle cells, or intracellular inclusions or parasites can only be made by examining the peripheral smear.

Review of granulocyte morphology from the peripheral smear has traditionally been superior to older automated methods for evaluation of a left myeloid shift (as seen in early response to stress or infection). However, newer automated instruments can detect and enumerate these IGS and generate white cell differential counts with great accuracy. Blood films remain superior to automatic methods in evaluating morphological WBC abnormalities, such as the presence of blasts, hypogranulated and hypersegmented granulocytes, Pelger–Huet cells (bilobated neutrophils), toxic granulations, and Döhle bodies (ribosomal aggregates seen in instances of increased bone marrow stress). If more than 20% of WBCs on a smear are smudge cells (remnants of cells without any identifiable cellular structures), shear artifact is suspected; blood smears should then be manually performed using an albumin suspension and analyzed on a conventional microscope or automated digital morphology system. If smudge cells persist, chronic lymphocytic leukemia (CLL) is suspected. Of note, RBC morphology may be altered on albumin prepared smears.

Routine inspection of blood smears may reveal platelet clumping sufficient to cause artifactual thrombocytopenia, as well as morphologic platelet abnormalities such as hypogranularity (as in gray platelet syndrome or myelodysplasia) or the presence of very large/ giant platelets (in inherited syndromes such as Bernard–Soulier disease and May–Hegglin anomaly). The automated platelet count can be manually estimated on the peripheral blood smear by counting the average number of platelets present in 10 high power ($1,000\times$) fields and multiplying it by 15,000.

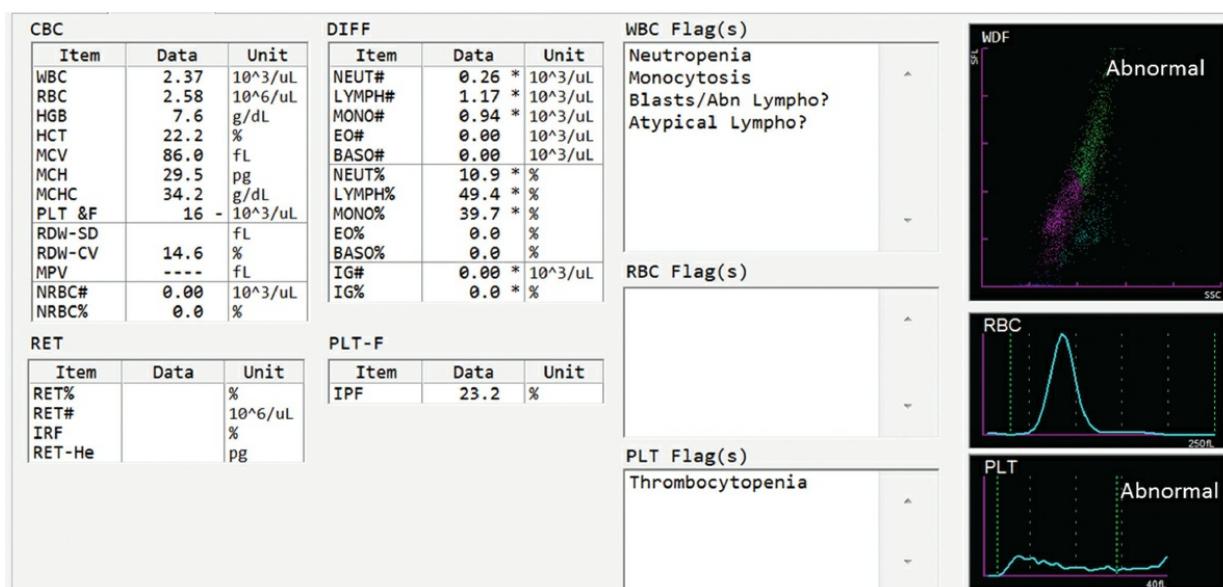


FIGURE 27.2 Analysis of abnormal blood sample from a patient with relapsed acute myeloid leukemia by Sysmex XN automated hematology analyzer. This sample was flagged for manual review to evaluate for blasts versus atypical lymphocytes versus possible monocytosis (WBC flag, upper middle panel) and thrombocytopenia (PLT flag, lower middle panel). The asterisks (*) indicates that the automated differential is unreliable and that a manual review/differential is required. Note that while the hemoglobin and RBC counts are low, the RBC distribution in the RBC histogram appears relatively normal, consistent with the normal MCV and RDW. The same sample that was analyzed by the Cellavision (see Fig. 27.4), which showed increased number of blasts.

IRF, immature reticulocyte fraction; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cell(s); PLT, platelet(s); RDW-CV, red cell distribution width (coefficient of variation); RDW-SD, red cell distribution width (standard deviation); WBC, white blood count.

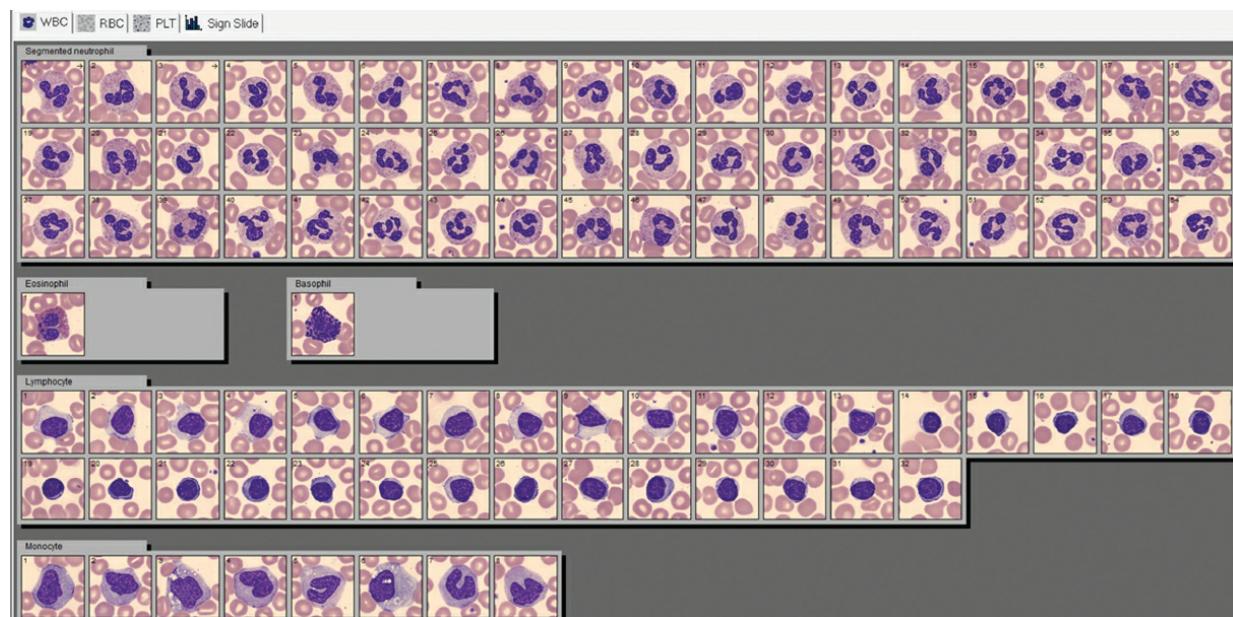


FIGURE 27.3 Peripheral blood differential from normal sample generated on an automated digital morphology instrument (CellaVision). The instrument scans the peripheral blood film, locates cells using optical recognition technology, captures their images, and pre-classifies them into morphologic categories (i.e., segmented neutrophils, eosinophils, basophils, lymphocytes, and monocytes). The trained technologist, hematologist, or pathologist reviews, reclassifies cells as necessary and verifies the differential. An electronic record of

the results can be saved for future reference.

PLT, platelet(s); RBC, red blood cell(s); WBC, white blood count.

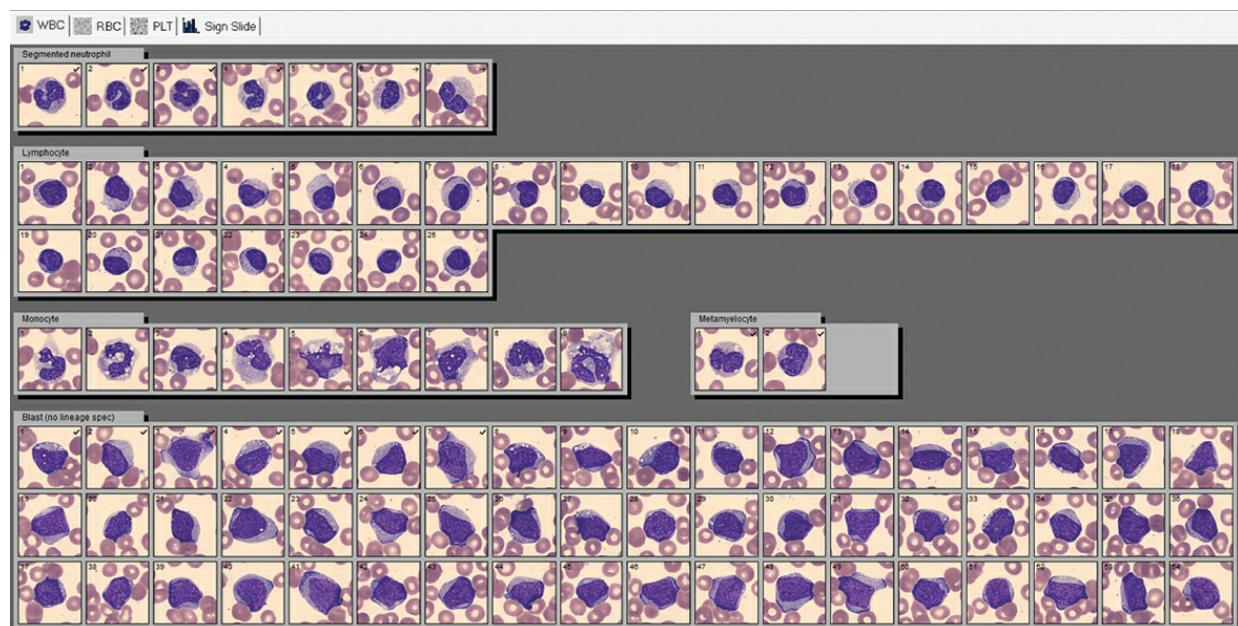


FIGURE 27.4 Peripheral blood differential of a patient with relapsed acute myeloid leukemia with circulating blasts, generated on an automated digital morphology instrument (CellaVision). The WBCs were pre-classified into lineages by the software but required review and final reclassification of a subset of cells by a trained hematology technologist. Reclassification is easily performed by “dragging and dropping” cells into the proper category with automatic real-time updated calculation of the differential. Note this is the same sample analyzed by the automated hematology analyzer in [Fig. 27.2](#).

PLT, platelet(s); RBC, red blood cell(s); WBC, white blood count.

Bone Marrow Aspirate/ Biopsy

Indications

Examination of the bone marrow aspirate and biopsy is important for establishing the cause of severe, persistent or treatment-refractory anemia, thrombocytopenia or granulocytopenia, or in cases of suspected aplastic anemia, myelodysplastic syndrome (MDS), myeloproliferative disorders, leukemia, or another primary marrow disease. Bone marrow aspirates can also be cultured to identify infectious agents such as mycobacteria and fungi. It can be used for diagnosing disorders such as Gaucher disease or amyloidosis, although less invasive tests are more useful for these pathologies.

Risks

Bone marrow aspirate and biopsy can be performed with minimal discomfort using local anesthesia and is generally very safe when performed by an experienced operator. Infiltration of the skin surface and periosteum with several milliliters of lidocaine provides sufficient anesthesia for most patients; conscious sedation should be reserved when significant discomfort is anticipated due to severe apprehension. Thrombocytopenia or antiplatelet therapy are not contraindications, provided adequate landmarks are identified and sufficient pressure is applied to the site after the procedure; some patients with dysfunctional platelets may require platelet transfusion for bleeding control. Anticoagulation should be discontinued or held for an adequate time prior to the procedure, and coagulation factor replacement utilized for patients with serious bleeding diathesis.

Hemorrhage and local infection occur rarely. In one study of more than 50,000 biopsies, there were 14 cases of serious hemorrhage with 6 instances requiring transfusion and one death.¹⁵ Infection can be prevented by the use of careful sterile technique and proper local care, even in patients with compromised immune function. Rare cases when infections do occur can be managed with antibiotics.

Technique

In adults, the posterior iliac crest is the site of choice for most hematologists; it can be located by direct palpation with the patient in the prone or lateral decubitus position, and generally provides adequate core and aspirate samples. The anterior iliac crest is a reasonable alternative when obesity, local irradiation, or skin conditions preclude a posterior approach. Sternal aspiration may be justified when iliac approach is not possible, but it cannot be used to obtain a core biopsy specimen. It may be less well accepted by patients and is more vulnerable to complications, such as rare traumatic penetration or fracture of the sternum with damage to underlying structures, particularly in patients with multiple myeloma or bone invasion by underlying malignancy.

Bone Marrow Aspirate

The bone marrow aspirate is obtained by advancing an obturator-fitted needle through the bone cortex into the medullary space and using the negative pressure to withdraw cells into a syringe after removal of the obturator. Once their presence is determined by visual inspection, individual marrow particles from the first “pull” are spread on a glass slide or cover slip and stained immediately for morphological diagnosis (Wright or Wright–Giemsa stains are used to examine the cellular elements, and Prussian blue stain for detecting iron, as discussed subsequently). Subsequent “pulls” are used for ancillary testing, including flow cytometry, cytogenetics, fluorescent in situ hybridization (FISH), and molecular testing. The clotted portion of an aspirate can be

fixed, sectioned, and stained in the same way as the bone marrow biopsy. In some patients, the marrow may be inaspirable due to hypercellularity, hypocellularity, fibrosis, or metastatic cancer cells. Aspirates are less reliable than biopsies in detecting marrow involvement with metastatic tumor, lymphoma, myelofibrosis, granulomas, or space-occupying lesions.

Bone Marrow Biopsy

It is almost always possible to obtain a biopsy specimen (or core), even when hematopoietic cells have been totally replaced by fibrous tissue or tumor. The core is obtained by further advancing the biopsy needle through the medullary space, using the same puncture site as the aspirate in some instances. This technique is convenient for the operator and patient, but may be associated with artifactual hypocellularity, hemorrhage, and distorted architecture.¹⁶ These artifacts may be reduced by obtaining a larger core sample size (1.5 to 2.5 cm) or changing the location of the biopsy. The core is ejected from the needle by using a metal probe, fixed, decalcified, sectioned, and stained with hematoxylin and eosin and special histochemical (reticulin, collagen, and iron) and immunohistochemical stains. Hematoxylin and eosin-stained biopsy sections show less cytoplasmic and nuclear detail than Wright or Giemsa-stained aspirate films, but provide other essential information about marrow architecture and cellularity.

Marrow Cellularity

Marrow cellularity should be estimated from a large bone marrow core biopsy specimen. Because it decreases with age; normal percent cellularity can be estimated by subtracting patient age from 100 ($\pm 10\%$). Inappropriately low cellularity suggests marrow damage or bone marrow failure, and high cellularity indicates a proliferative disorder, dysplasia, stress reaction, or use of growth factors.

Megakaryocyte Number and Appearance

A normocellular biopsy and aspirate specimen should contain multiple megakaryocytes per low power (100 \times) field. An increase in megakaryocytes is consistent with increased platelet turnover secondary to peripheral destruction, inflammation, iron deficiency, or myeloproliferative/ myelodysplastic disorders. Reduced megakaryocyte numbers may reflect a primary marrow disease (such as aplastic anemia or amegakaryocytic thrombocytopenia) or suppression secondary to chemotherapy. Normal megakaryocytes appear as large cells with three or more connected nuclei. Increased number of smaller megakaryocytes with less than three nuclear lobes indicates a left shift in megakaryocyte maturity due to increased platelet turnover or dysplasia. Megakaryocytes with a single nucleus or multiple small separated nuclei and mature cytoplasm are particularly suggestive of myelodysplasia.

Myeloid and Erythroid Cells

The myeloid to erythroid (M : E) ratio is determined by counting 300 to 500 cells from a marrow aspirate sample; it varies from 1 : 1 to approximately 3 : 1 in healthy adults. Erythroid hyperplasia, characterized by a low M : E ratio, suggests an erythroid response to hemolytic or nutritional megaloblastic anemia, myelodysplasia, or erythropoietin administration. Erythroid hypoplasia, characterized by high M : E ratio, may indicate red cell aplasia, decreased erythropoiesis due to autoimmunity, B19 parvovirus infection, medication side effects or major-ABO-incompatible hematopoietic stem cell transplantation. Myeloid hyperplasia (high M : E ratio) may be seen in response to physiologic stress, infection, exogenous growth factors, or in myeloproliferative disorders. Myeloid hypoplasia (low M : E ratio) with a “maturation arrest” (absence of myeloid precursors beyond the promyelocyte or myelocyte stage) may reflect drug-induced or autoimmune agranulocytosis.

Disruption of the normal maturation sequence, presence of excess numbers of blasts, or dysplastic changes affecting at least two major hematopoietic cell lines (erythroid, myeloid, or megakaryocytic) suggests MDS or leukemia.

Lymphocytes and Plasma Cells

There is a substantial variation in the number of normal bone marrow lymphocytes, which may be distributed diffusely or in well-defined lymphoid aggregates. Benign lymphoid aggregates, seen in aspirates or biopsies, typically contain more T than B cells on immunohistochemical stains; B-cell-rich collections are more likely due to clonal B cell disorders. Paratrabecular lymphoid aggregates in biopsies are seen in follicular lymphoma.

Plasma cells usually constitute less than 2% of marrow cells; increases may be noted in liver disease, inflammatory conditions, HIV infection, benign monoclonal gammopathy, and multiple myeloma. Distinguishing reactive from neoplastic plasma cells in a routine aspirate film may be difficult, as reactive plasma cells may occupy 20% to 30% of the marrow, and hypocellular marrows are often rich in plasma cells. Reactive plasma cells are typically small, uniform in size, and have mature nuclei. Presence of large plasma cells with immature nuclei, extensive multinuclearity, conspicuous variations in cell size, or plasma cell aggregates of more than 5 to 10 cells are highly suggestive of plasma cell neoplasm. Immunohistochemical or *in situ* hybridization staining for intracellular kappa and lambda light chains can be helpful in evaluating light chain restriction indicative of a monoclonal process, although serum studies may be sufficient to confirm clonality (see the following). The presence of an interstitial infiltrate with concentrated collections of plasmacytoid lymphocytes and plasma cells suggest Waldenström macroglobulinemia or lymphoplasmacytic lymphoma

if light chain restriction is demonstrated. Molecular and flow cytometric studies for establishing T-and B-cell clonality and distinguishing reactive from clonal or neoplastic lymphoid populations are discussed in [Chapter 28](#).

Other Cells Anomalously Present in the Marrow

Malignant cells of epithelial or mesenchymal origin form tight clumps of unusually large cells with a very high nuclear–cytoplasmic ratio. Such clumps aspirate poorly and are often rare on the aspirate smears. Therefore, when screening for malignant cells, the whole slide should be scanned at low power, including the leading edge of the film. Tumor cells are often readily identified in marrow biopsies, and specialized immunohistochemical stains can help identify the site of origin.

Bone Marrow Iron Stores

The Prussian blue stain, which specifically binds to iron, is necessary to examine bone marrow iron stores and presence of ringed sideroblasts. Stainable marrow iron confirms the presence of iron stores but does not indicate that these could be mobilized for effective erythropoiesis, as seen in chronic inflammation due to elevated hepcidin levels. Of note, with markedly increased iron stores, yellow granules of hemosiderin may be seen even in routinely stained or unstained aspirate and biopsy preparations. In contrast, the absence of stainable iron suggests depleted iron stores, although the sensitivity of this method depends on the sample size. For maximal yield, at least seven separate particles on aspirate smear should be evaluated.¹⁷ False negative results are seen due to hypocellularity, technical error, or presence of cellular antigens binding to dye-labeled antibodies.¹⁸ Iron stain of the biopsy is usually less reliable than that of the aspirate because of fixation artifact.

Ringed sideroblasts are erythroid precursors containing coarse iron granules surrounding at least half the nuclear circumference due to accumulation of iron within mitochondria. They are always abnormal, implicating anomalous porphyrin synthesis secondary to a congenital abnormality, pyridoxine or copper deficiency, zinc ingestion, exposure to toxins (such as alcohol or lead) and medications, or myelodysplasia.

Additional Studies

Molecular diagnostic studies such as cytogenetics and FISH, immunohistochemical stains, molecular analysis of T and B cell receptor rearrangements and flow cytometry, are discussed in greater detail throughout the Handbook.

SERUM TESTS TO EVALUATE NUTRITIONAL AND HYPOPROLIFERATIVE ANEMIAS

Serum Iron and Total Binding Capacity Assays

Serum iron is measured by automated chemical assays after the dissociation from transferrin.¹⁹ Total iron-binding capacity (TIBC), which mainly describes binding to transferrin, used to be measured by the addition of excess iron to the sample, removing the unbound fraction by absorption, dissociating the bound fraction and measuring it with the serum iron assay. In many laboratories, unsaturated iron-binding capacity (UIBC; the fraction of TIBC unbound to iron) is now measured by automated chemistry analyzers using spectrophotometric methods, and the TIBC is calculated.¹⁹ The percent saturation of transferrin by iron is calculated by dividing the serum iron by TIBC and then multiplying by 100.

There is a marked diurnal variation of serum iron concentration in healthy people. The highest levels are usually in the morning; by midnight, serum iron levels may fall into the iron deficiency range. Iron levels also vary with the menstrual cycle, with a 10% to 30% increase pre-menstruation and a similar decrease at the time of menstruation.¹⁹

Iron deficiency is characterized by low serum iron, elevated serum TIBC, and low iron saturation. In anemia of inflammation/ chronic disease, serum iron levels also fall below normal range, but because TIBC also decreases, iron saturation may remain normal. However, these parameters frequently overlap with those found in iron deficiency anemia, making it difficult to distinguish between the two entities.²⁰ The high TIBC levels (more than 300 µg/ dL) may indicate uncomplicated iron deficiency, but when iron depletion and inflammation coexist, the TIBC is frequently low, and serum ferritin and possibly transferrin receptor assays become necessary to pinpoint the diagnosis²⁰ (see subsequently). In patients with confirmed iron deficiency, a rise in serum iron to levels greater than 100 mcg/ mL 1 to 2 hours after ingestion of 325 mg of ferrous sulfate indicate appropriate bioavailability and normal small bowel absorption.²⁰ This absorption test is useful in patients who do not appear to respond to oral iron tablets.

Elevated serum iron levels and iron saturation occur in both inherited and acquired iron overload states, such as hemochromatosis and transfusion hemosiderosis in patients with severe hemolytic anemias (Table 27.1).²¹ Elevated serum iron levels can also occur because of impaired iron reutilization, as seen in pure red cell aplasia, aplastic anemia, and nutritional megaloblastic anemias, or in severe hepatocyte injury, such as fulminant or chronic hepatitis, particularly hepatitis C. Measurement of serum iron can be falsely elevated in hemolyzed specimens containing free hemoglobin, and for many hours after transfusion of older units of RBCs, which contain cells damaged in storage.²²

Soluble Transferrin Receptor

Soluble transferrin receptor (STR), which is measured by “sandwich type” enzyme-linked automatic immunoassays,^{23,24} is endogenously produced by enzymatic cleavage of the transferrin receptor from the surface of iron-utilizing cells. STR levels are elevated in states with increased demand on available iron, such as increased erythropoiesis (hemolytic anemias, megaloblastic anemia, thalassemia) and in iron deficiency anemia. Elevations observed in some patients with anemia of inflammation likely reflect limited availability of functional iron pools for erythropoiesis rather than depleted stores.²⁵ STR levels are decreased in reduced erythropoiesis (aplastic anemia, anemia of inflammation, and renal insufficiency). Although the assay is reported to distinguish iron deficiency from the anemia of chronic inflammation/ disease,^{20,23} the overlap in STR levels is often noted, and the STR to serum ferritin ratio may be more useful in distinguishing between the two. The STR/ log ferritin index was reported to diagnose iron deficiency in 25% of patients with anemia of inflammation and ferritin levels >100 ng/ mL.²³

Table 27.1 Causes of High Serum Fe and Fe Saturation

Iron Overload States

Genetic hemochromatosis
(HFE, TfR, HJV, hepcidin, ferroportin mutations)

Transfusion hemosiderosis

Impaired Reutilization of Iron

Pure red cell aplasia
Aplastic anemia
Ineffective erythropoiesis
 Nutritional megaloblastic anemias
 Sideroblastic anemias
 Thalassemia intermedia and major
Congenital dyserythropoietic anemias

Unknown or Multiple Mechanisms

Hepatitis C
Liver cirrhosis
Chronic alcoholism

HFE, human hemochromatosis protein; HJV, hemojuvelin; TfR, transferrin receptor.

Serum Ferritin

Serum ferritin can be measured by a variety of immunoassays,¹⁹ and it is the most useful

at the extremes: low values (<15 ng/ mL) are very sensitive to depletion of body iron stores, and very high levels (>1,000 ng/ mL) usually indicate iron overload states. Because serum ferritin is also an acute phase reactant, patients who have both iron deficiency and anemia of inflammation or liver disease often have serum ferritin levels in the normal range, usually between 40 and 70 ng/ mL.²⁰ In one study, less than 7% of anemic patients with depleted iron stores had a serum ferritin level of 100 ng/ mL.²⁶

Very high levels of ferritin with elevated serum iron usually indicate iron overload states (as mentioned previously). High levels without the elevated serum iron are often observed in severe inflammatory/ infectious states, such as disseminated fungal or mycobacterial disease, malignancies or hemophagocytocytic lymphohystiocytosis (formerly known as macrophage activation syndrome; Table 27.2).^{27,28} In the presence of concurrent inflammation or liver disease, ferritin levels may not accurately gauge the response to iron chelation therapy in patients with transfusion hemosiderosis and very high ferritin levels (>1,000 ng/ mL).

Serum Vitamin B₁₂ (Cobalamin)

Serum vitamin B₁₂ is generally measured by an enzyme-linked competitive assay based on binding to the intrinsic factor.¹⁹ Serum levels below 100 pg/ mL are almost invariably associated with cellular B₁₂ deficiency.²⁹ Fifty percent of patients with B₁₂ levels between 100 and 200, 10% with levels between 200 and 300, and 0.1% with levels above 300 pg/ mL will have deficiency at the cellular level, as indicated by elevated serum methylmalonic acid (MMA). Low serum B₁₂ levels in patients with normal MMA levels may indicate either early depletion of B₁₂ stores (without cellular deficiency) or reduced levels of transcobalamin I, the major serum cobalamin-binding protein. In case of the latter, parenteral B₁₂ repletion may fail to restore normal serum B₁₂ levels. Acquired transcobalamin I deficiency occurs in disorders with severely decreased number of myeloid cells, which are the major site of transcobalamin I production (i.e., aplastic anemia). Patients with myeloma or HIV infection also frequently have unexplained low levels of serum B₁₂, possibly due to reduced myeloid cell mass. Elevated serum B₁₂ levels are seen after administration of parenteral vitamin B₁₂, intrinsic release of B₁₂ from the liver stores due to hepatic necrosis, or because of increased transcobalamin I levels, as seen in myeloproliferative disorders, particularly untreated chronic myeloid leukemia.¹⁹

Table 27.2 Causes of Hyperferritinemia Without Increased Serum Fe Saturation

Inflammation
Infection

Metabolic syndrome

Malignancy

Hemophagocytic lymphohistiocytosis

Ferroportin mutations (classical type)

Hyperferritinemia-cataract syndrome (L ferritin gene [FTL] mutation)

Parenteral iron treatment of iron-refractory iron deficiency anemia (TEMPRSS6 mutation)

Serum Methylmalonic Acid

The metabolites of certain amino acids, cholesterol, and odd-chain fatty acids in human cells are shuffled into Krebs cycle via conversion of methylmalonyl-CoA (MMA) coupled with coenzyme A) into succinyl-CoA by methylmalonyl-CoA mutase, a vitamin B₁₂-dependent enzyme. Inherited deficiency of this enzyme (methylmalonic acidemia) or severe vitamin B₁₂ deficiency will cause a block in the pathway and increased levels of serum MMA, which can be assayed by gas–liquid chromatography or mass spectrometry.¹⁹ Elevated MMA levels (in blood or urine) with low cobalamin level indicate cellular B₁₂ deficiency; the diagnosis can be confirmed by normalization of serum MMA levels after initiation of B₁₂ treatment. Normal MMA levels and low serum B₁₂ without macrocytosis or anemia indicate early depletion of B₁₂ stores without cellular deficiency. Elevated serum MMA levels in the absence of B₁₂ deficiency are seen in renal insufficiency, due to reduced MMA excretion, and in methylmalonic academia.

Serum Homocysteine

In human cells, L-homocysteine (a nonprotein amino acid) is either recycled into L-methionine with help of vitamin B₆ or converted into S-adenosyl methionine, an important methyl group donor in a variety of synthetic reactions, with help of tetrahydrofolate and vitamin B₁₂. Therefore, deficiency of folic acid, vitamin B₁₂, or vitamin B₆ will cause increased levels of serum homocysteine, which can be measured by a number of methods.¹⁹ Cellular B₁₂ deficiency usually elevates both MMA and homocysteine levels, but in 5% of B₁₂-deficient patients, only the latter will be elevated. Other causes of elevated homocysteine include renal insufficiency and inherited abnormalities in the metabolism. In clinical practice, it is not cost-effective to use homocysteine in assessment of B₁₂ or folate deficiency; the levels are more often obtained for assessment of arterial or venous hypercoagulability risk (see Chapter 22).

Serum Intrinsic Factor Antibody Assay

This assay is highly specific for diagnosis of vitamin B₁₂ malabsorption due to

autoimmune depletion of intrinsic factor (pernicious anemia), but the sensitivity of the assay is less than 50%.

Serum and Red Cell Folate Assays

Serum and red cell folate levels are determined by a competitive receptor-binding assay.³⁰ Serum levels reflect recent dietary intake, while red cell levels reflect the extent of body folate stores at the time of red cell formation. Reduced red cell folate levels are found with both folate and B₁₂ deficiency because B₁₂ is required for cellular uptake of folate. Reduced serum levels, however, indicate a negative folate intake/utilization balance at the time of blood sampling, but do not necessarily indicate diminished folate stores. Because cereal grain products have been fortified with folate to reduce the risk of fetal neural tube defects, folate deficiency in the United States has become extremely rare, even in nutritionally deficient and alcoholic individuals. It is likely that the assessment of serum and red cell folate levels in patients with anemia is no longer cost-effective, and should be limited to those suspected of having malabsorption. Elevated serum folate is found in B₁₂ deficiency (due to reduced uptake by RBC precursors) and following treatment with folic acid. Elevated red cell folate could be seen with increased serum folate, as the former is measured from hemolysate prepared from whole blood.

Serum Erythropoietin

Marked elevation of serum erythropoietin level (more than 500 to 1,000 U/ mL) occurs with bone marrow failure, and usually predicts failure of recombinant erythropoietin therapy. Erythropoietin levels are commonly low (under 100 U/ mL) in anemic patients with renal insufficiency, malignancy, or inflammation. Erythropoietin assays are useful for establishing the cause of erythrocytosis: patients with secondary polycythemia frequently have erythropoietin within the normal range, which often becomes elevated following phlebotomy treatment, while patients with polycythemia vera present with decreased erythropoietin levels.

TESTS FOR THE EVALUATION OF ABNORMAL HEMOGLOBINS AND HEMOLYTIC ANEMIAS

Hemoglobin Electrophoresis

In large clinical laboratories, hemoglobin electrophoresis has been largely replaced by high-performance liquid chromatography (HPLC), isoelectric focusing (IEF), and capillary electrophoresis.³¹ Smaller clinical laboratories may still continue to use conventional electrophoretic methods to differentiate and quantitate normal and

abnormal hemoglobins (Hbs).

Electrophoresis in an alkaline environment (cellulose acetate or agarose gel electrophoresis at pH of 8.6) can readily identify Hbs A, S, C, F, and A₂. Levels of Hbs A, S, and C are quantified by densitometry (Fig. 27.5), a method measuring the amount of light absorbed by hemoglobin bands on a gel, whereas Hbs F and A₂ are too small to be accurately measured by this assay. On alkaline electrophoresis, a number of Hbs D and G comigrate with Hb S, while Hb E and Hb O Arab comigrate with C. Electrophoresis in acidic environment (acid citrate agar electrophoresis at pH of 6.0) can distinguish Hbs D and G from Hb S, and Hbs E and O Arab from Hb C, and it is therefore routinely used to confirm presence of Hbs S and C.³¹

It should be noted that when two major Hbs are diagnosed, the convention is to list the one with the greater percentage first. Therefore, patients with sickle cell or Hb C trait are described as having Hb AS or Hb AC, respectively. A result of 80% S and 20% A, for instance, is denoted as Hb SA, indicating either recent blood transfusion (admixture with normal hemoglobin) or diagnosis of Hb S-β⁺ thalassemia, in which residual expression of thalassemic β-globin allele enables production of small amounts of normal hemoglobin.

High-Performance Liquid Chromatography and Isoelectric Focusing

Because of the greater power in hemoglobin separation as compared with alkaline or acid agar electrophoresis, HPLC and IEF are increasingly used as screening methods for abnormal hemoglobins, as well as quantification of Hbs A₂ and F.¹⁹ In HPLC, a hemoglobin suspension (diluted plasma) is passed through a column containing porous material with different affinities for hemoglobin molecules based on their distinct physical properties (usually electrical charge). The instrument measures the time needed for hemoglobin fractions to leave the column (termed retention time), which is specific for each hemoglobin type, and assays their concentration with spectrophotometry. The results are then plotted in a display called a chromatogram, which combines the information about the types of hemoglobin present and their relative amounts. IEF, on the other hand, is a type of electrophoresis where proteins travel in an electric field through a gel containing a fixed pH gradient. Different hemoglobins are identified based on their unique isoelectric points (pH values at which they lose the net electrical charge): once a hemoglobin molecule reaches a section of the gel with pH corresponding to its isoelectric point, it stops migrating in the electric field.

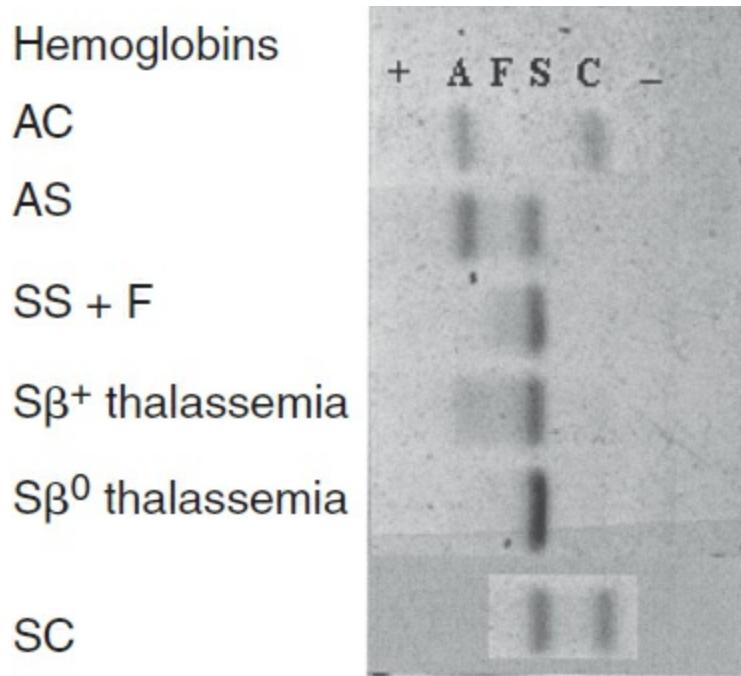


FIGURE 27.5 Hemoglobin electrophoresis, cellulose acetate, pH 8.6.

Sickle Solubility Test

Insolubility of deoxygenated Hb S in concentrated phosphate buffer can be used to confirm the presence of Hb S on electrophoresis. Because it cannot distinguish between sickle trait and sickle cell disease, this test is not useful for diagnosing sickle cell disease in clinical settings. The solubility test has largely replaced microscopic sickle cell prep, which also does not distinguish sickle cell trait from the disease.

Hb A₂ Quantitation

Hb A₂, which consists of two α and two δ subunits, is best measured by HPLC or capillary microelectrophoresis.³¹ Elevated Hb A₂ levels (above 3.5%) will generally confirm a diagnosis of β -thalassemia trait, although the concomitant presence of iron deficiency may lower the Hb A₂ level into the normal range. Patients with α -or $\delta\beta$ -thalassemia will have normal levels of Hb A₂.³¹ The diagnosis and screening for thalassemias are discussed in [Chapter 4](#).

Hb F Quantitation and Hb F Cells

Quantitation of Hb F is important in diagnosis of thalassemias and assessing the therapeutic responses to hydroxyurea in patients with sickle cell disease. To quantitate the percentage of Hb F, HPLC-based methods have largely replaced the traditional alkali-denaturation test. Hb F can also be measured immunologically to determine the amount of Hb F in RBCs and identify high Hb F-containing cells (“F cells”). A uniform

distribution of Hb F is found in the red cells of patients with hereditary persistence of fetal hemoglobin (see [Chapter 4](#)).

Tests for Unstable Hemoglobins

Some unstable hemoglobins such as Hb Zurich and Hb Kohn are recognized based on precipitation when exposed to heat (50°C) or 17% isopropanol. Unstable hemoglobins may also be detected by the formation of Heinz bodies (denatured hemoglobin) in intact RBCs that were exposed to oxidizing conditions, after incubating them with supravital stains such as brilliant cresyl blue or new methylene blue (see [Chapter 3](#)).

Glucose-6-Phosphate Dehydrogenase

Glucose-6-phosphate dehydrogenase (G6PD), the product of G6PD gene on the X chromosome, is required to maintain sufficient cytoplasmic levels of glutathione to protect the RBCs from oxidant injury. Moderate or severe G6PD deficiency will lead to either chronic or intermittent (oxidant-induced) hemolysis, as discussed in [Chapter 3](#). A common screening test for G6PD deficiency is the fluorescent spot test, which exploits the G6PD-catalyzed conversion of NADP to intrinsically fluorescent NADPH—the latter will be decreased in enzyme-deficient RBCs. Because reticulocytes usually have much higher quantities of G6PD than mature red cells, deficiency may not be diagnosed in the acute setting due to compensatory reticulocytosis and destruction of the older RBCs. In such instances, testing should be repeated several weeks after resolution of the hemolytic episode. Female heterozygotes also occasionally escape detection by screening tests, if the mutated copy of the gene is on the inactive X chromosome.³² For definite diagnosis of G6PD deficiency, genetic testing should be utilized. During episodes of acute hemolysis, Heinz bodies (denatured hemoglobin) may be detected in red cells on smears stained with supravital dyes.

Serum Haptoglobin

Haptoglobin is a serum protein that acts as free hemoglobin scavenger; it can be assayed by either nephelometric or turbidometric method (the former measures the scatter of a light beam that is directed through a sample, whereas the latter measures the amount of light that is absorbed).¹⁹ In vivo hemolysis of as little as 50 mL of RBCs completely depletes the blood of haptoglobin, because its complex with hemoglobin is cleared by the reticuloendothelial system in less than 30 minutes. In the absence of continuing hemolysis, restoration to normal levels will take at least 5 days. Haptoglobin level may be increased in presence of acute inflammation, as it is also an acute phase reactant. Decreased levels can be seen in patients with severe liver disease, due to decreased synthesis, and occasionally in patients with a genetic predisposition for low haptoglobin

levels.

Urine Hemosiderin

In patients with chronic intravascular hemolysis such as paroxysmal nocturnal hemoglobinuria or cardiac valve hemolysis, renal excretion of hemoglobin leads to accumulation of hemosiderin in the renal tubular cells. After staining the urine sediment with Prussian blue, microscopic evaluation of the sediment will demonstrate blue-stained particles in renal casts, indicating the presence of iron.

HEMOSTASIS AND COAGULATION ASSAYS

Automated Coagulation Analyzers

Automated coagulation analyzers are based on either mechanical or turbidometric methods.³³ Blood is collected into a tube prefilled with anticoagulant (sodium citrate) in 9:1 blood-to-anticoagulant ratio, centrifuged and loaded into the automatic analyzer, where the sample is incubated at 37°C. Most coagulation parameters (prothrombin time [PT], activated partial thromboplastin time [aPTT], mixing studies, thrombin time [TT], fibrinogen, and individual factors) are measured using the mechanical (chronometric) method: a specific reagent is added to a cuvette containing anticoagulated plasma sample and a small metallic sphere that oscillates in the magnetic field; the time needed for oscillations to stop (indicating clot formation) is measured. To obtain an aPTT, for example, the instrument will add calcium, a phospholipid source termed partial thromboplastin, and a surface-activating agent (i.e., silica) to the sample, and measure aPTT as time (in seconds) required for sphere oscillations to cease. This method is not influenced by sample hemolysis. D-dimers, on the other hand, are measured by turbidometric method, where increased clotting of the sample causes decreased light permeability. Fibrin degradation products (FDPs), which are similar to D-dimers but lack a fibrin cross-link, are still measured manually by an agglutination assay.

Activated Partial Thromboplastin Time

aPTT is sensitive to functional deficiencies of factors XI, X, IX, VIII, V, prothrombin (II), and fibrinogen (I), with the caveat that mild reductions in factor VIII (<30%) and fibrinogen (>100 mg/ dL) may remain undetected.³² Very rare deficiencies of factor XII, prekallikrein, and high-molecular-weight kininogen will also prolong the aPTT, but these are usually not associated with bleeding. Prolongation of the aPTT can also occur due to the presence of lupus anticoagulant and less commonly due to factor-specific antibodies, usually against factor VIII in patients with hemophilia. Factor deficiencies can be distinguished from factor-directed antibodies by performing a mixing study with normal plasma, as discussed in Chapter 20. Spurious elevations of aPTT can occur in

cases of aberrant plasma-to-citrate ratio (due to poorly filled tubes or high hematocrits), delays in delivery of samples to the laboratory, or contamination with intravenous fluids or heparin.

Previously, aPTT was commonly used to monitor the effect of unfractionated heparin (UH) therapy. Because of significant variability in sensitivity of available aPTT reagents to heparin, it was necessary to generate a standard curve in each clinical laboratory, comparing the aPTT with anti-Xa levels in a group of fully heparinized patients. Although one study suggested that aPTT test to control ratio of 2.0 to 3.0 is generally a good target range for therapeutic heparin levels,³⁴ many laboratories now monitor UH therapy solely by anti-Xa levels. The aPTT is not sensitive to low-molecular-weight heparins (LMWHs); when monitoring of the latter is indicated, for example in patients with renal insufficiency, morbid obesity, or pregnancy, an anti-Xa assay must be used. aPTT can be prolonged in patients treated with direct thrombin and factor Xa inhibitors (termed novel anticoagulants), but the levels do not correlate with the effectiveness of therapy.

Anti-Xa Assay

Anti-Xa assay can be used to measure the effect of both LMWH and UH therapy; it has virtually replaced aPTT in monitoring of the latter. In this assay, a combination of antithrombin and excess factor Xa is first added to a plasma sample from a heparin-treated patient. Antithrombin forms complexes with UH or LMWH and inactivates a fraction of factor Xa; the remaining factor Xa then cleaves an artificial substrate, releasing a chromogenic substance that is measured by light absorbance. As the absorbance is inversely correlated with the amount of heparin in the sample, it is converted to express anti-Xa levels: higher levels indicate higher inhibition with heparin. The therapeutic anti-Xa range is usually 0.3 to 0.7 IU/ mL for patients treated with UH, and 0.5 to 1.2 IU/ mL for patients treated with LMWH; the level should be checked 4 hours after LMWH administration. Of note, a narrower range is allowed for UH because of its additional ability to inhibit thrombin, which is not reflected in anti-Xa assay. Anti-Xa levels are not reliable for monitoring the effect of novel anticoagulants.

Prothrombin Time

PT assay utilizes the addition of thromboplastin (a combination of calcium, recombinant or crude tissue factor [factor III], and phospholipids) to an anticoagulated plasma sample for detection factor VII, X, V, prothrombin, and fibrinogen deficiencies.³³ Isolated inherited deficiencies and autoantibodies against these factors are rare; prolonged PT much more often indicates liver dysfunction, vitamin K deficiency,

warfarin therapy, or presence of lupus anticoagulants. PT can be spuriously elevated in some instances causing increased aPTT (as mentioned previously).

The international normalized ratio (INR) has been useful in standardizing the control of warfarin therapy.³³ The INR is the ratio of the patient PT to the mean normal PT raised to the power of the International Sensitivity Index (ISI) of the thromboplastin reagent utilized. Commercial thromboplastins are calibrated and given an ISI value, which reflects their sensitivity to warfarinized plasma. It may be misleading to use the INR to describe the prolongation of the PT in a patient not receiving warfarin, particularly in patients with liver disease.³³

Thrombin Time

TT assay measures the time to form a clot after addition of exogenous thrombin to the sample; it is prolonged in hypofibrinogenemia, dysfibrinogenemia, and presence of paraproteins and heparin.³³ Heparin contamination can be excluded by measuring reptilase time, which is prolonged in similar instances as TT but is insensitive to heparin. Dysfibrinogenemia may be confirmed by comparing the antigenic level of fibrinogen with that determined by the TT assay.³³

Specific Factor Assays

Most specific factor assays are based on the ability of patient plasma to correct clotting times of specific factor-deficient plasma in aPTT-or PT-based assays (see [Chapter 20](#)). Of note, deficiency of factor XIII, which is important to cross-link fibrin monomers, is not associated with a prolonged aPTT, PT, or TT. It is measured immunologically or by the solubility of the clot formed in urea.³³

Fibrinogen

Fibrinogen levels are routinely determined by using a TT-based assay, but chemical or immunologic methods are also available.³³ As fibrinogen is an acute phase reactant, levels are frequently raised in patients with inflammation and malignancy. Decreased levels are found in disseminated intravascular coagulation, hemophagocytic syndrome, advanced liver disease, treatment with asparaginase, or rarely due to an inherited deficiency.

D-Dimers

D-dimers are fibrin degradation products (FDPs) that retain a cross link between the two fibrin units after the blood clot has been digested by plasmin. Elevated levels indicate extensive local (deep vein thrombosis, pulmonary emboli, pneumonia, postoperative states) or disseminated intravascular coagulation (DIC). A negative D-

dimer assay is helpful in ruling out thrombosis,^{35,36} whereas the positive value has a poor specificity for the latter, particularly in the presence of comorbid conditions such as infection, inflammation, or malignancy. A positive D-dimer level can be used to predict recurrence of thrombosis after discontinuing anticoagulation,³⁷ but the factors such as age and sex should also be considered.³⁸

Tests of Platelet Function

The bleeding time was frequently used in the past to screen for suspected platelet dysfunction, but given the problems with its reproducibility, it has been replaced with platelet aggregation assays and automated methods relying on mechanical changes induced by ex vivo platelet clumping.

Platelet aggregation in response to epinephrine, ADP, and collagen assessed by light transmission or impedance aggregometry is the most effective method of testing for platelet dysfunction, but the availability of these assays is often limited. They require the patient to be present at the testing laboratory, because samples must be tested rapidly after venipuncture in order to prevent in vitro platelet activation and spurious results. Platelet aggregation assays are discussed in detail in [Chapter 21](#).

In the last decade, automated instruments have been developed to rapidly screen for platelet function abnormalities³³ and monitoring antiplatelet therapy.³⁹ PFA-100, the earliest instrument and the subject of most studies, aspirates the platelets at high shear rate through an aperture coated with collagen and an activator (either epinephrine or ADP), and measures the time needed for platelets to aggregate and obstruct further flow (the “closure” time). Once medications have been excluded as the cause of closure time prolongation, 90% of abnormalities will be due to von Willebrand disease (VWD) and 10% due to other platelet defects, making it an useful screening method for moderate and severe VWD and monitoring the effect of desmopressin therapy.³⁹ The usefulness in milder VWD and rarer platelet functional defects has been questioned.^{40,41} Abnormal findings will need to be confirmed by specific testing for VWD disease including VWD antigen, ristocetin cofactor assay, and multimer analysis.^{40,41} The automated screening methods may also be of use in patients who might have acquired platelet dysfunction because of severe renal insufficiency, myeloproliferative disorders, or medications.³³

Tests for Hypercoagulability

Functional assays for the endogenous anticoagulant proteins—antithrombin, protein C, and protein S— are more sensitive for detecting deficiencies than the antigen-based assays; they should not be performed in the setting of acute venous thromboembolism, as falsely lower levels may be found due to their utilization. On the other hand, molecular assays for inherited causes of hypercoagulability—factor V Leiden and prothrombin

GP20210A mutations—will not be affected in acute thromboembolism. These assays, as well as testing for the antiphospholipid antibody syndrome, are discussed in greater detail in Chapter 22.

Tests for Hyperfibrinolysis

Direct immunochemical assays for proteins regulating clot degradation (i.e., alpha-2-antiplasmin and plasminogen activator inhibitor I) have largely replaced the euglobulin clot lysis as a method of measuring the fibrinolytic activity of patient's plasma. In this assay, patient's plasma was first acidified to prepare euglobulin, a plasma precipitate containing fibrinogen, plasminogen, and plasminogen inhibitor. The sample was then clotted with thrombin, and the time required for degradation of the formed clot was measured. Abnormally short lysis time was indicative of hyperfibrinolysis (as seen in inherited deficiency of aforementioned regulators or, more commonly, in severe liver disease), but was also present in cases of poor clot formation due to hypofibrinogenemia.

TESTS FOR EVALUATION OF PATIENTS WITH HEMATOLOGIC MALIGNANCIES

Serum Protein Electrophoresis

Based on their mass and electric charge, serum proteins migrate at different paces when placed in the electric field, as done in agarose gel or automated capillary zone electrophoresis.¹⁹ In the former, normal plasma proteins form six different fractions; albumin, α_1 , α_2 , and two β globulins appear as five discrete bands, and γ globulins migrate as a more diffuse and heterogeneous zone (Fig. 27.6 and Table 27.3). The concentration of the proteins in each section of the gel can be determined by densitometry.

Monoclonal immunoglobulins (M proteins or M paraproteins) form discrete bands (“spikes”) in the β or γ globulin regions, making electrophoresis essential in distinguishing reactive (polyclonal) from monoclonal hypergammaglobulinemias. IgM and IgA paraproteins are more likely to be found close to the β globulin region; IgG paraproteins may be located in any area of the β and γ globulin zones. False-negative results may occur because of lower sensitivity of the assay (agarose gel is worse than capillary zone electrophoresis, particularly for small paraproteins),⁴² or because atypical mobility of paraproteins due to temperature, pH, or unknown factors. False-positive results are seen with formation of artifacts, more frequently in agarose gel electrophoresis, and after administration of monoclonal antibodies (i.e., anti-CD38 antibody, daratumumab). To confirm that the suspected monoclonal band indeed

contains only a single heavy chain and a single light chain, immunofixation studies are needed (see the following discussion).

Most monoclonal proteins with serum concentrations below 3 g/dL are not associated with clinical or pathologic evidence of malignancy, and they are referred to as monoclonal gammopathy of unknown significance (MGUS). Occasional individuals exhibit two monoclonal proteins (“diclonal” gammopathy), which may represent the products of two separate clones. If both bands contain the same heavy and light chain, the two immunoglobulins may originate from a single clone despite differing electrophoretic mobilities, possibly due to multimer formation. Patients with marked polyclonal gammopathy, for example, due to HIV infection or liver disease, may have multiple small discrete bands termed oligoclonal gammopathy. M paraproteins concentrations greater than 3.0 g/dL usually reflect the presence of IgG or IgA multiple myeloma or Waldenström macroglobulinemia. The concentration of the serum (or urine) M protein is a marker of tumor burden and can be used for serial monitoring and assessing response to therapy. For this determination to be reliable, the M paraprotein must be quantitated separately from polyclonal immunoglobulins.

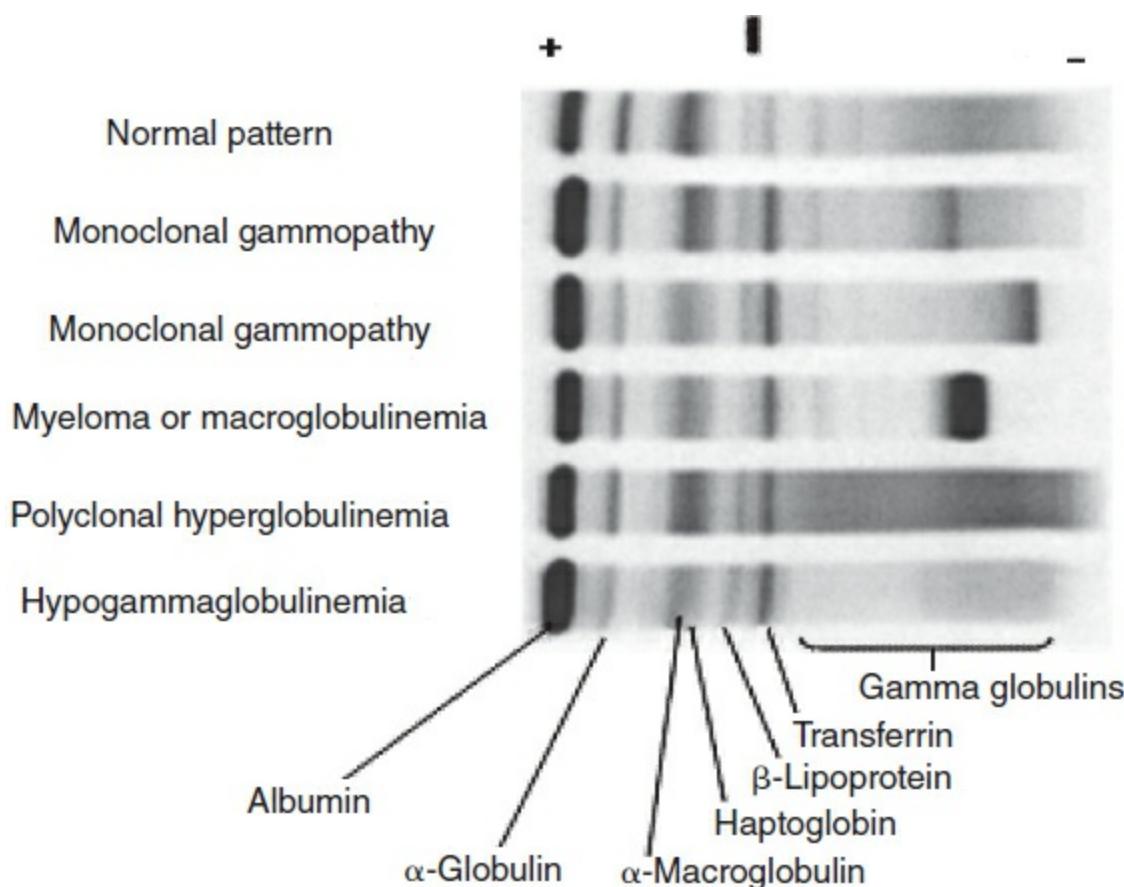


FIGURE 27.6 Serum protein electrophoresis.

Table 27.3 Plasma Protein Migration Patterns on Standard Protein Electrophoresis

Albumin Zone
Albumin
Alpha₁ Zone
Alpha ₁ -antitrypsin
Alpha ₁ -lipoproteins (high-density lipoproteins, HDLs)
Alpha₂ Zone
Alpha ₂ -macroglobulin
Haptoglobin
Ceruloplasmin
Beta Zone
β-Lipoprotein (low-density lipoprotein)
Transferrin
C3 (complement)
Gamma Zone
Fibrinogen (in incompletely clotted specimens)
IgA
IgM
IgG

Ig, immunoglobulin.

In case of light chain disease, free light chains are seen only on serum electrophoresis in myeloma patients with severe renal failure (due to impaired secretion) or in instances where the light chains form spontaneous tetramers too large for renal clearance. Otherwise, the presence of light chains needs to be ruled out by immunofixation.

Urine Protein Electrophoresis

Urinary light-chain excretion must be evaluated when plasma cell dyscrasia is suspected. Approximately 15% of myeloma patients excrete monoclonal light chains in the urine (Bence-Jones proteinuria) without any detectable M protein in the serum. For screening, a random urine specimen is concentrated before electrophoresis. Discrete bands are then assayed by immunofixation to confirm whether they represent free light chains or intact monoclonal immunoglobulins (due to “overflow” of the serum M protein). In patients with urinary paraprotein excretion, serial 24-hour urine collections are useful in monitoring tumor burden and response to therapy.

Immunofixation

Immunofixation is used to determine composition and confirm monoclonal origin of bands identified on the serum protein electrophoresis. First, protein electrophoresis is performed in several replicates (usually six) to separate the bands. Then, antibodies against the heavy (γ , α , and μ) and light (κ and λ) chains are layered separately on each replicate (the remaining replicate is stained with protein-binding dye and used as a control). If there is a monoclonal immunoglobulin, immunofixation bands will form with antibodies against one heavy and/or one light-chain type (Fig. 27.7). If only free light chain is detected, light-chain disease or the uncommon IgD and IgE myelomas should be suspected (anti-IgD and IgE are routinely not applied in immunofixation).

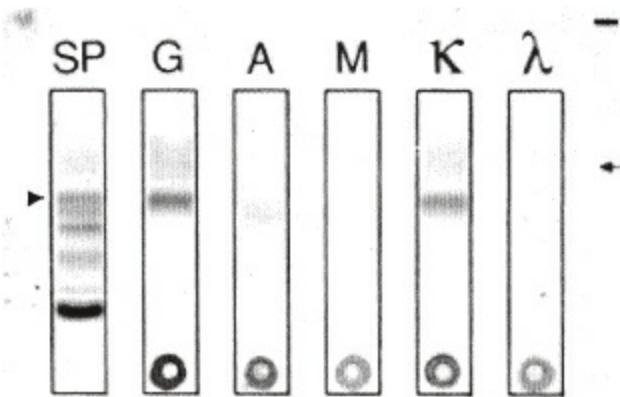


FIGURE 27.7 Immunofixation illustrating an IgG kappa monoclonal protein. The arrowhead points to the putative monoclonal band in the serum protein electrophoresis (SP lane) stained with a protein-binding dye. Staining in the remaining lanes indicates the presence of products reacting with specific antibodies directed against IgG (G), IgA (A), and IgM (M) heavy chains, and Ig kappa (κ) and lambda (λ) light chains. Small arrow on the right indicates origin.

Ig, immunoglobulin.

Serum Free Light Chains

Concentrations of the free serum κ and λ light chains can be determined nephelometrically, by using antibodies specific to light chain epitopes that are exposed in free but hidden in heavy-chain bound molecules. Because of its superior convenience and comparable sensitivity to urine electrophoresis, this assay is being frequently used in diagnosis, prognosis, and therapeutic response monitoring in light-chain myeloma, amyloidosis, and smoldering multiple myeloma.^{43–45} In cases of stable monoclonal gammopathy, the concentration of serum free light chains over time has a greater variability than the M paraprotein level and therefore may be less useful in detecting progression in these patients.⁴⁶

Quantitative Serum Immunoglobulins

Concentrations of serum immunoglobulins are measured with automated nephelometric or turbidometric immunoassays. Despite being useful for quantitating normal immunoglobulins, they are not accurate when measuring paraproteins. For example, multimer-forming IgA and pentamer-forming IgM paraproteins have a propensity to disassociate into smaller molecules, therefore producing erroneous results.

Serum Cryoglobulins

Serum cryoglobulins are immunoglobulins that precipitate when the temperature is reduced below 37°C, causing obstruction of peripheral small blood vessel flow in vivo. When testing for serum cryoglobulins, it is critical that the blood is drawn into a preheated syringe or tube, transported to the laboratory at 37°C and kept at this temperature until the serum is separated from the clot. The serum is then refrigerated at 4°C and examined after 24 hours. A precipitate that dissolves when the tube is rewarmed to 37°C indicates the presence of a cryoglobulin. Redissolved cryoprecipitate is then separated on electrophoresis and subjected to immunofixation, which reveals the culprit immunoglobulin. Cryoglobulinemia may be due to (1) a monoclonal immunoglobulin, usually IgM, (2) a monoclonal IgM with rheumatoid activity binding to polyclonal IgG (“mixed cryoglobulinemia”), or (3) polyclonal IgM bound to polyclonal IgG. The latter two are associated with a variety of lymphoproliferative or autoimmune disorders and infections, particularly hepatitis C.⁴⁷

Serum Viscosity

Serum viscosity may be measured manually with Ostwald viscosimeter, an instrument that compares the time required for serum and water to flow through a capillary tube at 37°C. The normal serum is 1.4 to 1.8 times more viscous than water; hyperviscosity is generally symptomatic when the relative viscosity exceeds 6, but may occur with levels as low as 3 or 4. Automated capillary viscosimeters are used by reference laboratories.

Serum β_2 -Microglobulin

Serum β_2 -microglobulin is a small protein non-covalently linked with class I human leukocyte antigen (HLA) molecules; it can be measured by nephelometric immunoassays. Elevated serum levels may be found in inflammatory conditions, renal failure (due to impaired excretion), and in lymphoid and plasma cell malignancies, where it is used as an important marker. Serum levels are not useful to follow chemotherapy responsiveness in myeloma because of the lack of specificity but may predict early relapse following autologous transplantation.

Serum Lactate Dehydrogenase

Lactate dehydrogenase (LDH), an enzyme that converts lactate to pyruvate, is expressed in virtually all the cells of human body. There are five distinct isoenzymes, based on different ratios of M and H subunits, with identical function but different distribution; distinguishing between these is seldom useful in hematology practice. Elevated serum levels, which can be measured by an automated enzyme activity-based assay, are seen in tissue damage regardless of etiology, as the enzyme is released from necrotic cells into the circulation. In hematology, marked elevations are usually noted with intravascular or intramarrow hemolysis, hemophagocytic lymphohistiocytosis, and tumor lysis syndrome. LDH elevation is also seen in variety of non-hematological disorders: myocardial infarction, heart failure, rhabdomyolysis, liver damage, and severe inflammation/ infection.

Serum Uric Acid

Uric acid, the end product of purine nucleotide metabolism in humans, is produced in a variety of cells and cleared by the kidneys. Elevated serum levels (hyperuricemia), as measured by an automated enzymatic assay, are seen with either increased uric acid production (increased cell turnover, as in tumor lysis syndrome) or impaired renal clearance (kidney disease and certain medications). Decreased serum levels (hypouricemia) may occur in Bence-Jones proteinuria (due to a proximal tubular defect in uric acid absorption) and in SIADH (syndrome of inappropriate ADH secretion).

References

1. Wintrobe MW. The principles and technic of blood examination. In: Wintrobe MW, ed. Clinical Hematology. Philadelphia, PA: Lea and Febiger; 1942:177–214.
2. Bourner G, Dhaliwal J, Sumner J. Performance evaluation of the latest fully automated hematology analyzers in a large, commercial laboratory setting: a 4-way, side-by-side study. *Lab Hematol.* 2005;11(4):285–297.
3. Simel DL, Halvorsen RA Jr, Feussner JR. Erythrocyte anisocytosis. Visual inspection of blood films vs automated analysis of red blood cell distribution width. *Arch Intern Med.* 1988;148(4):822–824.
4. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. *Blood.* 1979;53(2):288–293.
5. Crouch JY, Kaplow LS. Relationship of reticulocyte age to polychromasia, shift cells, and shift reticulocytes. *Arch Pathol Lab Med.* 1985;109(4):325–329.
6. Piva E, Brugnara C, Chiandetti L, et al. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. *Clin Chem Lab Med.* 2010;48:1369–1380.
7. Thompson CB, Diaz DD, Quinn PG, et al. The role of anticoagulation in the

- measurement of platelet volumes. *Am J Clin Pathol.* 1983;80(3):327–332.
- 8. Briggs C, Kunka S, Hart D, et al. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. *Br J Haematol.* 2004;126:93–99.
 - 9. Barsam SJ, Psaila B, Forestier M, et al. Platelet production and platelet destruction: assessing mechanisms of treatment effect in immune thrombocytopenia. *Blood.* 2011;117:5723–5732.
 - 10. Zandecki M, Genevieve F, Gerard J, et al. Spurious counts and spurious results on haematology analysers, a review. Part I: platelets. *Int J Lab Hematol.* 2007;29:4–20.
 - 11. Zandecki M, Genevieve F, Gerard J, et al. Spurious counts and spurious results on haematology analysers, a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices, and reticulocytes. *Int J Lab Hematol.* 2007;29:4–20.
 - 12. Gulati GL, Hyland LJ, Kocher W, et al. Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature. *Arch Pathol Lab Med.* 2002;126(3):336–342.
 - 13. Silvestri F, Virgolini L, Savignano C, et al. Incidence and diagnosis of EDTA-dependent pseudothrombocytopenia in a consecutive outpatient population referred for isolated thrombocytopenia. *Vox Sang.* 1995;68(1):35–39.
 - 14. Latif S, Veillon DM, Brown D, et al. Spurious automated platelet count. Enumeration of yeast forms as platelets by the cell-DYN 4000. *Am J Clin Pathol.* 2003;120:882–885.
 - 15. Bain BJ. Bone marrow biopsy morbidity and mortality. *Br J Haematol.* 2003;121(6):949–951.
 - 16. Islam A. Bone marrow aspiration before bone marrow core biopsy using the same bone marrow biopsy needle: a good or bad practice? *J Clin Pathol.* 2007;26:212–215.
 - 17. Hughes DA, Stuart-Smith SE, Bain BJ. How should stainable iron in bone marrow films be assessed? *J Clin Pathol.* 2004;57(10):1038–1040.
 - 18. Barron BA, Hoyer JD, Tefferi A. A bone marrow report of absent stainable iron is not diagnostic of iron deficiency. *Ann Hematol.* 2001;80(3):166–169.
 - 19. Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 5th ed. St. Louis: Elsevier Saunders; 2012.
 - 20. Cook JD. Diagnosis and management of Iron deficiency. *Best Pract Res Clin Haematol.* 2005;18:319–332.
 - 21. Finberg KE. Unraveling mechanisms regulating systemic iron homeostasis. *Hematology Am Soc Hematol Educ Program.* 2011;2011:532–537.
 - 22. Hod EA, Brittenham GM, Billote GB, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. *Blood.* 2011;118:6675–6682.
 - 23. Skikne BS, Punnonen K, Caldron PH, et al. Improved differential diagnosis of

- anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the sTfR/ log ferritin index. *Am J Hematol.* 2011;86:923–927.
24. Pfeiffer CM, Cook JD, Mei Z, et al. Evaluation of an automated soluble transferrin receptor (sTfR) assay on the Roche Hitachi analyzer and its comparison to two ELISA assays. *Clin Chim Acta.* 2007;382:112–116.
 25. Siebert S, Williams BD, Henley R, et al. Single value of serum transferrin receptor is not diagnostic for the absence of iron stores in anaemic patients with rheumatoid arthritis. *Clin Lab Haematol.* 2003;25:155–160.
 26. Guyatt GH, Patterson C, Ali M. Diagnosis of iron deficiency anemia in the elderly. *Am J Med.* 1990;88:205–209.
 27. Roetto A, Bosio S, Gramalglia E. Pathogenesis of hyperferritinemia cataract syndrome. *Blood Cells Mol Dis.* 2002;29:532–535.
 28. Brudevold R, Hole T, Hammerstrom J. Hyperferritinemia is associated with insulin resistance and fatty liver in patients without iron overload. *PLoS ONE.* 2008;3:e3547.
 29. Stabler SP, Allen RH, Savage DG, et al. Clinical spectrum and diagnosis of cobalamin deficiency. *Blood.* 1990;76:871–881.
 30. Nguyen D, Diamond LW, Braylan RC. Approach to flow cytometry: general considerations. In: Nguyen D, Diamond LW, Braylan RC, eds. *Flow Cytometry in Hematopathology. A Visual Approach to Data Analysis and Interpretation*. 2nd ed. Totowa, NJ: Humana Press; 2007:2–9.
 31. Chui DHK, Steinberg, MH. Laboratory diagnosis of hemoglobinopathies and thalassemias. In: Hoffman R, Benz E, Shattil S, et al., eds. *Hematology: Basic Principles and Practices*. Philadelphia, PA: Elsevier, Churchill Livingstone; 2009.
 32. Beutler E. Glucose-6-phosphate dehydrogenase deficiency. A historical perspective. *Blood.* 2008;111:16–24.
 33. Schmaier AH. Laboratory evaluation of hemostatic disorders. In: Hoffman R, Benz E, Shattil S, et al., eds. *Hematology: Basic Principles and Practices*. Philadelphia, PA: Elsevier, Churchill Livingstone; 2009.
 34. Bates SM, Weitz JI, Johnston M, et al. Use of a fixed activated partial thromboplastin time ratio to establish a therapeutic range for unfractionated heparin. *Arch Intern Med.* 2001;161(3):385–391.
 35. Rathbun SW, Whitsett TL, Raskob GE. Exclusion of first-episode deep-vein thrombosis after-hours using D-Dimer. *Blood Coagul Fibrinolysis.* 2007;16(8):795–800.
 36. Bates SM, Jaeschke R, Stevens SM, et al. Diagnosis of DVT: antithrombotic therapy and prevention of thrombosis. 9th ed: American College of Chest Physicians evidence based clinical practice guidelines. *Chest.* 2012;141(2 suppl.):e351S–418S.

37. Palareti G, Cosmi B, Legnani C, et al. D-Dimer testing to determine the duration of anticoagulation therapy. *N Engl J Med.* 2006;355:1780–1790.
38. Baglin T, Palmer CR, Luddington R, et al. Unprovoked recurrent venous thrombosis: prediction by D-Dimer and clinical risk factors. *J Thromb Haemost.* 2008;66:577–582.
39. Chen F, Maridakis V, O'Neill EA, et al. A randomized clinical trial comparing point-of-care platelet function assays and bleeding time in healthy subjects treated with aspirin or clopidogrel. *Platelets.* 2012;23:249–258.
40. Favaloro EJ. Clinical utility of the PFA-100R. *Semin Thromb Hemost.* 2008;34:709–733.
41. Hayward CP, Harrison P, Cattaneo M, et al. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. *J Thromb Haemostas.* 2006;4:312–319.
42. Bossuyt X, Marien G. False-negative results in detection of monoclonal proteins by capillary zone electrophoresis: a prospective study. *Clin Chem.* 2001;47:1477–1479.
43. Bradwell AR, Carr-Smith HD, Mead GP, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem.* 2001;47(4):673–680.
44. Bradwell AR, Carr-Smith HD, Mead GP, et al. Serum test for assessment of patients with Bence Jones myeloma. *Lancet.* 2003;361(9356):489–491.
45. Kyle RA, Rajkumar SV. Monoclonal gammopathy of unknown significance and smouldering multiple myeloma: emphasis on risk factors for progression. *Br J Haematol.* 2007;139:730–743.
46. Katzmann JA, Snyder MR, Rajkumar SV, et al. Long-term biological variation of serum protein electrophoresis M-spike, urine M-spike, and monoclonal serum free light chain quantification: implications for monitoring monoclonal gammopathies. *Clin Chem.* 2011;57:1687–1692.
47. Ferri C, Zignego AL, Pileri SA. Cryoglobulinemia. In: Young NS, Gershon SL, High KA, eds. *Clinical Hematology.* Mosby Elsevier, Philadelphia PA; 2006:625–636.

Basic Principles and Clinical Applications of Flow Cytometry

Thomas A. Fleisher and Raul C. Braylan

Flow cytometry is a technology used routinely in most hematology laboratories. Its entry into the mainstream of clinical laboratory analysis has been aided by the increasing availability of monoclonal antibodies that define cell surface and intracellular proteins as markers of cell lineage, differentiation, activation, and other biologic properties. Instrument design advances have yielded benchtop cytometers with fixed optics that, when linked with new developments in fluorochrome chemistry, enable a wide range of clinical applications. In addition, proficiency testing is now available in support of these clinical applications through the College of American Pathologists as mandated by the Clinical Laboratory Improvement Amendment of 1988. The major advantage that flow cytometry provides is its capacity to assess multiple measurements on large numbers of individual cells. Flow cytometric studies have extended the understanding of hematopoietic cell development, differentiation, activation, and apoptosis. In addition, they have provided important information regarding hematologic malignancies, insight into reconstitution after stem cell transplantation, and understanding of cell abnormalities that result in immune or hematologic deficiencies. As such, flow cytometry plays an important role in the diagnosis, characterization, and monitoring of a number of hematologic disorders.

The basic design of a flow cytometer involves four major elements: Optics, fluidics, electronics, and a computer equipped with specialized software.^{1,2} The optical system utilizes one or more light sources, typically one or several lasers that produce monochromatic light and serve as the excitation beams. At the opposite side of the optical bench, light generated from the cells that have intersected the excitation beam is filtered, reflected by dichroic mirrors set in fixed locations, and finally collected by linked photodetectors to allow quantitation of the emitted light at specific wavelengths.

To ensure that all cells analyzed experience consistent exposure to the excitation beam, the fluidic system must maintain the cells in a consistent location as they move sequentially through the beam. To accomplish this, the cell suspension is injected into a flowing stream of sheath fluid that hydrodynamically focuses the inner stream of cells within the outer sheath fluid stream.¹ The intersection of the cells with the excitation light beam(s) produces characteristic light scatter (nonfluorescent) signals; additional fluorescent signals are generated by fluorochromes that typically are linked to specific reagents that bind antigens present on or within the cells of interest. The various light signals (parameters) are collected by the optical bench, while instrument design determines the number of parameters collected per cell. The two reagent-independent (nonfluorescent) parameters are forward-angle light scatter, as a marker of cell size, and side-angle light scatter, as an index of cellular regularity/ granularity. The combination of these two parameters allows for an approximate discrimination among the three major types of leukocytes, as well as evaluation of red blood cells and platelets in whole blood samples.³

The fluorescent data collected by a flow cytometer are the result of either cell surface or intracellular binding of antibodies or other specific ligands conjugated directly to fluorochromes or detected with secondary reagents conjugated to fluorochromes, as well as reagents that are inherently fluorescent. Fluorochromes are excited by light of a defined wavelength and emit light of lower energy (longer wavelength). There are currently different fluorochromes used in clinical flow cytometry, including fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, and allophycocyanin and many others that can be excited by newly introduced lasers. Combinations of two fluorochromes linked to each other have been developed; they depend on the transfer of energy from the first fluorochrome to excite the second fluorochrome. These tandem fluorochromes extend the range of emission wavelengths available from one excitation beam. The availability of multiple fluorochromes that absorb light of the same wavelength but emit light at different wavelengths means that multiple reagents can be used simultaneously with a single light source to yield a multicolor (polychromatic) study. One or more additional light sources are present in most current clinical instruments to extend the range of multicolor studies. Extended multicolor studies require complex color compensation and data management processes that typically involve sequential data evaluation.

The clinical application of flow cytometry in hematology saw its earliest use as a supplement to the morphologic classification of leukemias and lymphomas, affording not only lineage information but also the state of differentiation and/ or maturation,^{4,5} cell growth, and apoptosis.⁶ In addition, flow cytometry provided the best prognosticator in HIV infection based on absolute CD4 T-cell numbers.⁷ More recently, flow cytometry

has proven important in characterizing hematopoietic stem cells, detecting minimal residual neoplastic disease, defining immune deficiencies, identifying certain red blood cell-related disorders, measuring the number of contaminating leukocytes in plasma and red blood cell transfusions, evaluating platelets, and characterizing other blood cells.^{8–12} Flow cytometry also can be used to look inside the cell as well as to evaluate cell surface characteristics. Fixation and permeabilization facilitate intracellular entry of reagents to determine specific proteins and to assess functional characteristics.¹³ This chapter is directed at basic concepts of flow cytometry including data presentation and interpretation followed by a brief review of applications for hematologists.

DATA PRESENTATION AND INTERPRETATION

Flow cytometers generally yield graphic displays of the cell frequency versus the light intensity for one or more parameters by means of specialized computer software. [Figure 28.1](#) shows a single-parameter histogram that reflects the quantitative distribution of cells (y-axis) versus light signal strength (x-axis). Alternatively, the signal intensity of two correlated parameters can be plotted versus cell frequency; the latter displayed as dot density (dot plot, [Fig. 28.2A](#)) or a series of concentric lines (contour plot, [Fig. 28.2B](#)). When measuring multiple parameters (as colors), the data are typically evaluated by using dual-parameter displays. Depending on the application, 10,000 to 100,000 events are collected to provide sufficient numbers of cells for meaningful data relative to the subpopulations of interest. However, when the cell or cells of interest are infrequent, such as evaluating hematopoietic stem cells ($CD34^+$) in peripheral blood or detecting minimal residual disease (MRD) in leukemia, substantially larger total numbers of cells must be collected.^{8,14}

Distinguishing a positive signal is usually based on defining the background signals by evaluating either unstained cells (no monoclonal antibody added) or cells that have been incubated with a fluorochrome-conjugated irrelevant antibody. By convention, the negative–positive discriminator is defined by the intensity of a signal that includes 99% (or 98%) of all cells based on one of the background conditions described earlier; cells that emit a signal above this discriminator are scored as positive for binding by the specific reagent(s) added to the cell suspension. This approach applies to well-defined populations with homogeneous antigen expression, but modifications or alternative interpretations may be necessary when the cell populations analyzed are heterogeneous or express dim fluorescence, which can lead to rather arbitrary calculations of percentages of positive cells.

The data generated by the computer are only as good as the instrument capabilities, settings, reagents, and cell preparation used. To prevent reporting invalid data, certain standards must be met.¹⁵ First, optimal instrument performance is integral and depends

on a quality control program utilizing specialized software and methods. The use of validated reagents is also part of good laboratory practices, while the quality of the cell preparation can be assessed using the nonfluorescent parameters, forward-and side-angle light scatter, to confirm the population of interest. Each major blood cell type has distinctive features in this scatter plot. Platelets are obviously smaller than all other blood cells and heterogeneous in size, characteristics that can be confirmed in comparison to red blood cells. Erythrocytes have a characteristic appearance based on forward-and side-angle light scatter (Fig. 28.3A), which partially overlaps with that of lymphocytes. However, because of the large difference in frequency of circulating erythrocytes and leukocytes, there is no practical concern of lymphocytes contaminating erythrocytes (a collection of 10,000 erythrocytes normally include less than 20 lymphocytes). In contrast, erythrocytes make evaluation of lymphocytes virtually impossible. In part because of this reason, the study of lymphocytes in a whole blood sample or other specimens containing a significant amount of blood typically involves a red blood cell lysis step to eliminate erythrocytes (Fig. 28.3B). Following successful red cell lysis, a three-part differential is observed in peripheral blood with normal lymphocytes representing the smallest (forward-angle light scatter) and most regular/agranular (side-angle scatter) cells, while granulocytes are slightly larger (higher forward-angle scatter) and show substantial granularity (high side-angle scatter), and monocytes fall between these two cell types (Fig. 28.3B). The two less prevalent granulocyte types differ in their location on a scatter plot, with eosinophils typically falling within the granulocyte population while basophils overlap with lymphocytes. Hematopoietic stem cells are normally found in the lymphocyte area of the scatter plot. It is important to recognize that the cell relationships noted previously do not necessarily apply in cases of hematopoietic malignancy because neoplastic cells may exhibit altered light scatter properties or appear as a distinct population, separate from normal elements. Current standard practice for identifying the major leukocyte types, and particularly lymphocytes, includes using the pan-leukocyte monoclonal antibody CD45, alone or in combination with the monocyte-specific antibody CD14. CD45 is usually included in each staining combination as a specific lymphocyte identifier (characteristically bright staining) when red cell lysis is inadequate (Fig. 28.4) or substantial numbers of non-lymphocytes or debris contaminate the lymphocyte gate.¹⁶ Malignant cells also may differ from their normal counterparts in their staining characteristics with various reagents, including CD45 expression (Figs. 28.5 and 28.6). The correlated analysis of side scatter and CD45 with or without appropriate sequential gating of subpopulations of interest is extremely helpful in recognizing hematopoietic and lymphoid neoplasia.¹⁷

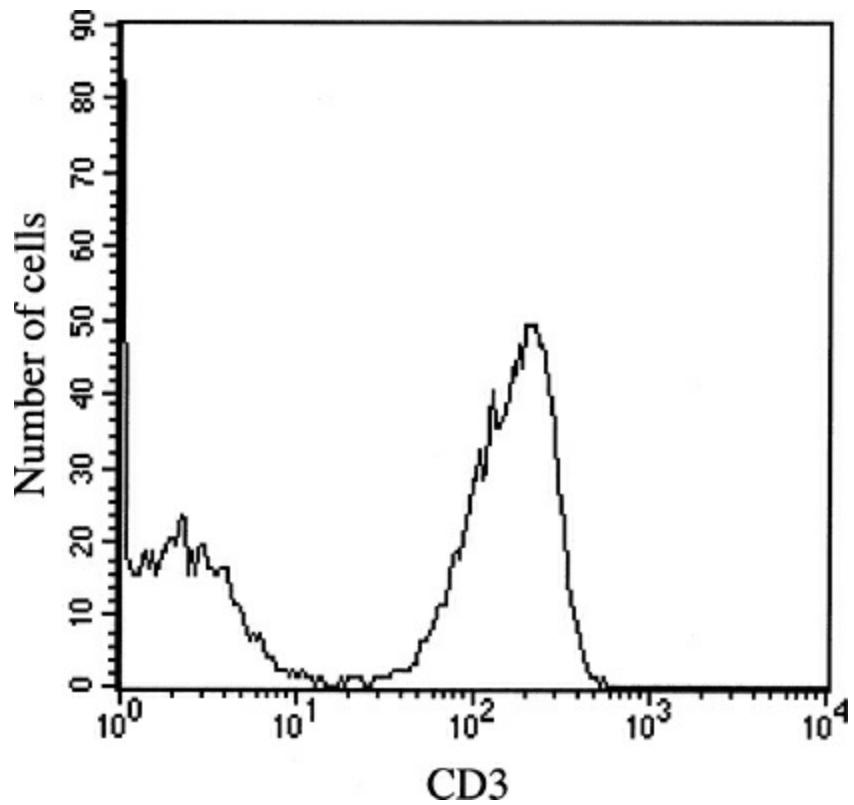


FIGURE 28.1 Single-parameter histogram, a distribution plot of CD3 fluorescence intensity (x-axis) versus number of events/ cells (y-axis) evaluating lymphocytes.

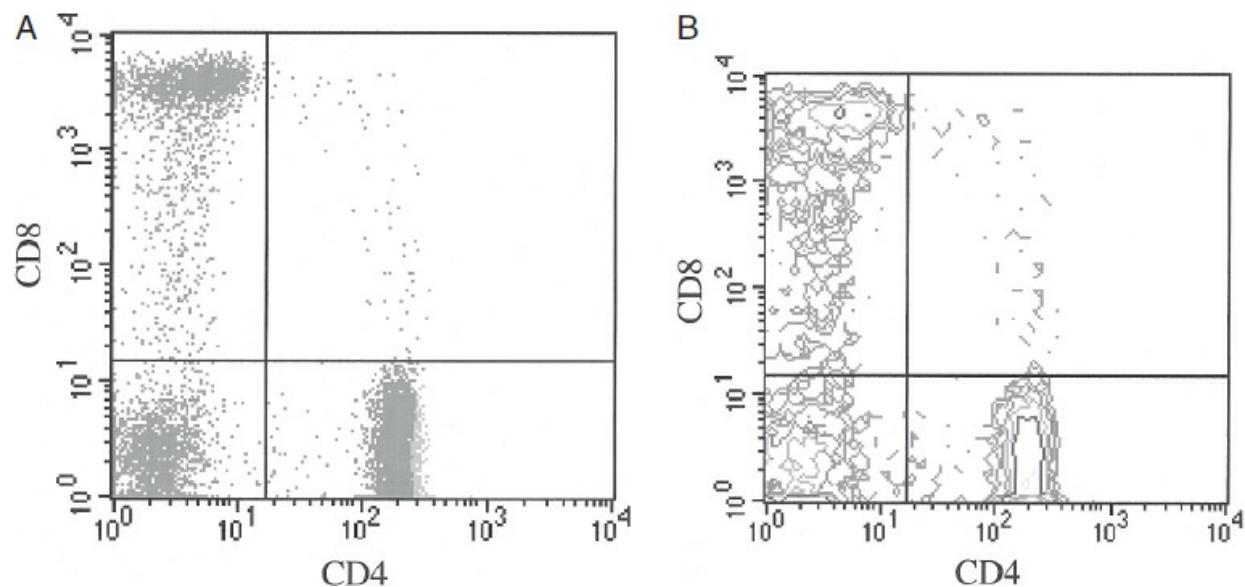
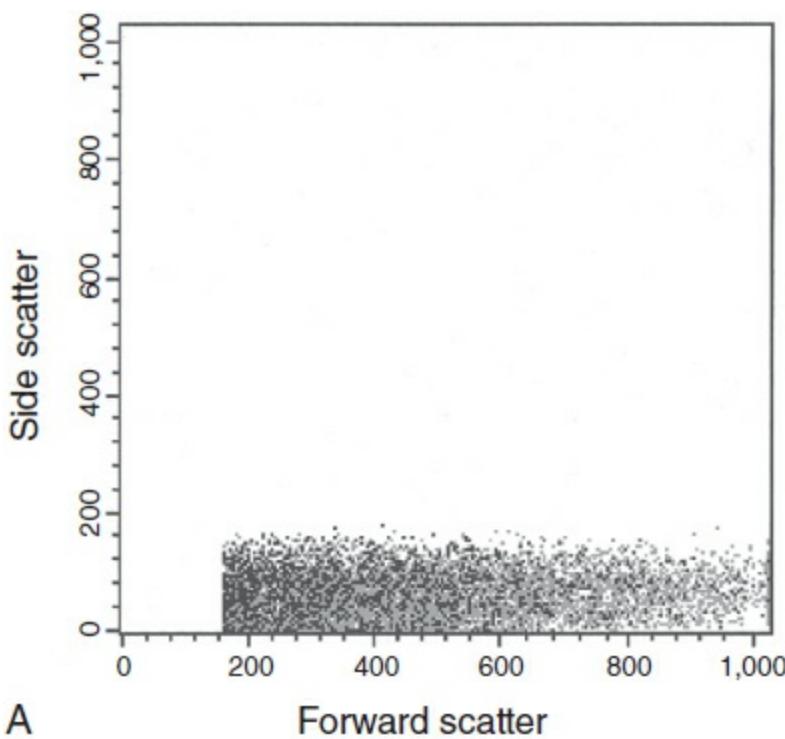
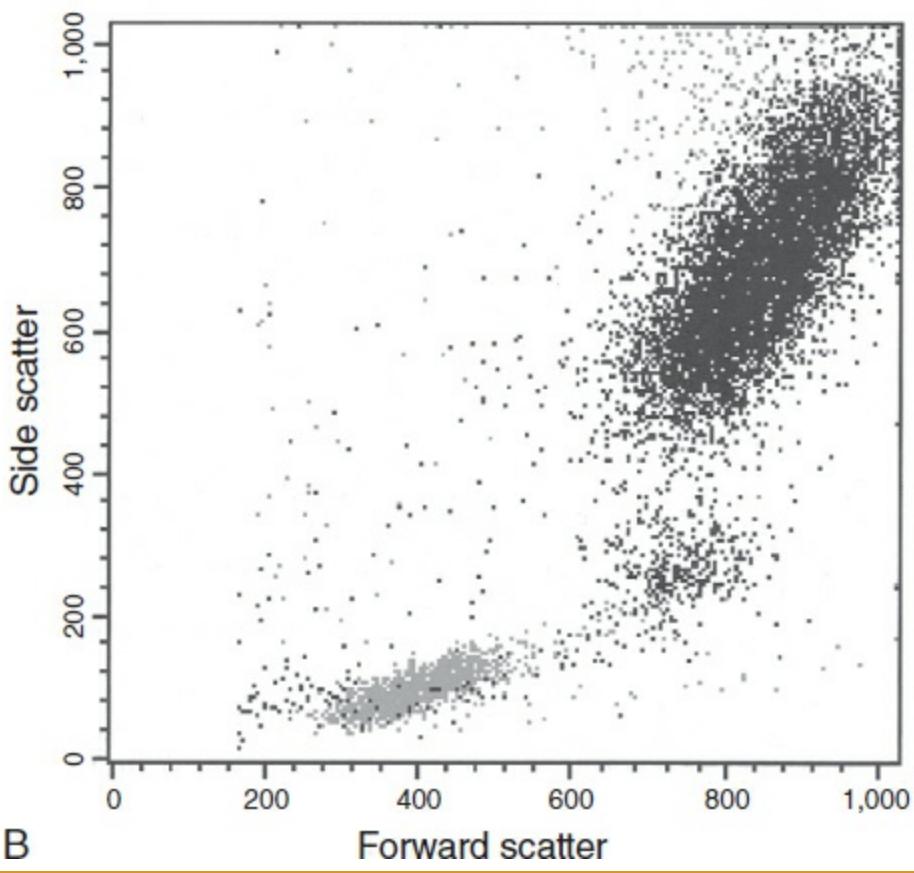


FIGURE 28.2 (A) Dot plot of two-color (CD4 and CD8) staining evaluating lymphocytes. Frequency of events is reflected by the number of dots. **(B)** Contour plot of two-color (CD4 and CD8) staining evaluating lymphocytes. Frequency of events is reflected by the

contour levels.



A Forward scatter



B Forward scatter

FIGURE 28.3 (A) Dot plot of forward scatter (x-axis) versus side

scatter (y-axis) on non-lysed whole blood sample. (B) Dot plot of forward scatter (x-axis) versus side scatter (y-axis) on lysed whole blood sample demonstrating a three-part leukocyte differential: lymphocytes, monocytes, and granulocytes.

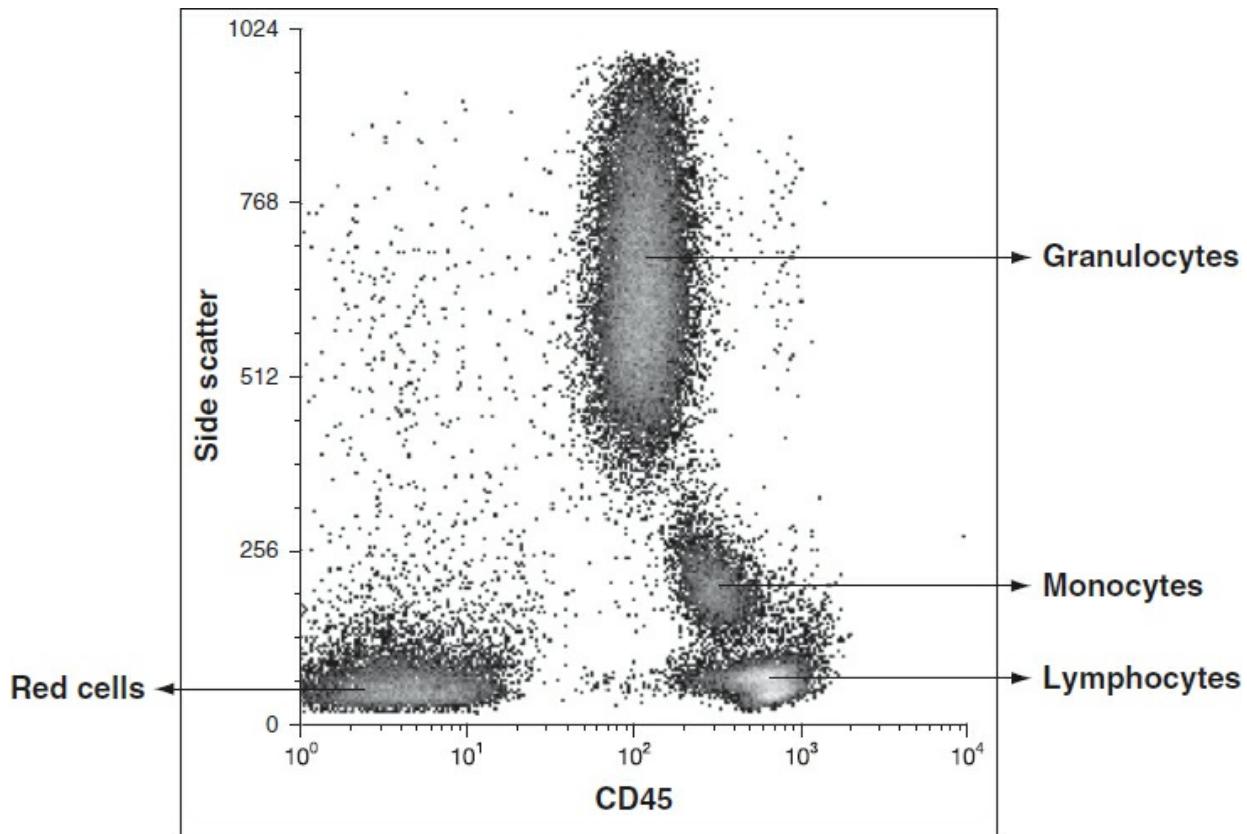


FIGURE 28.4 Side scatter and CD45 analysis of normal peripheral blood demonstrates distinct clusters of granulocytes, monocytes, lymphocytes, and residual red cells.

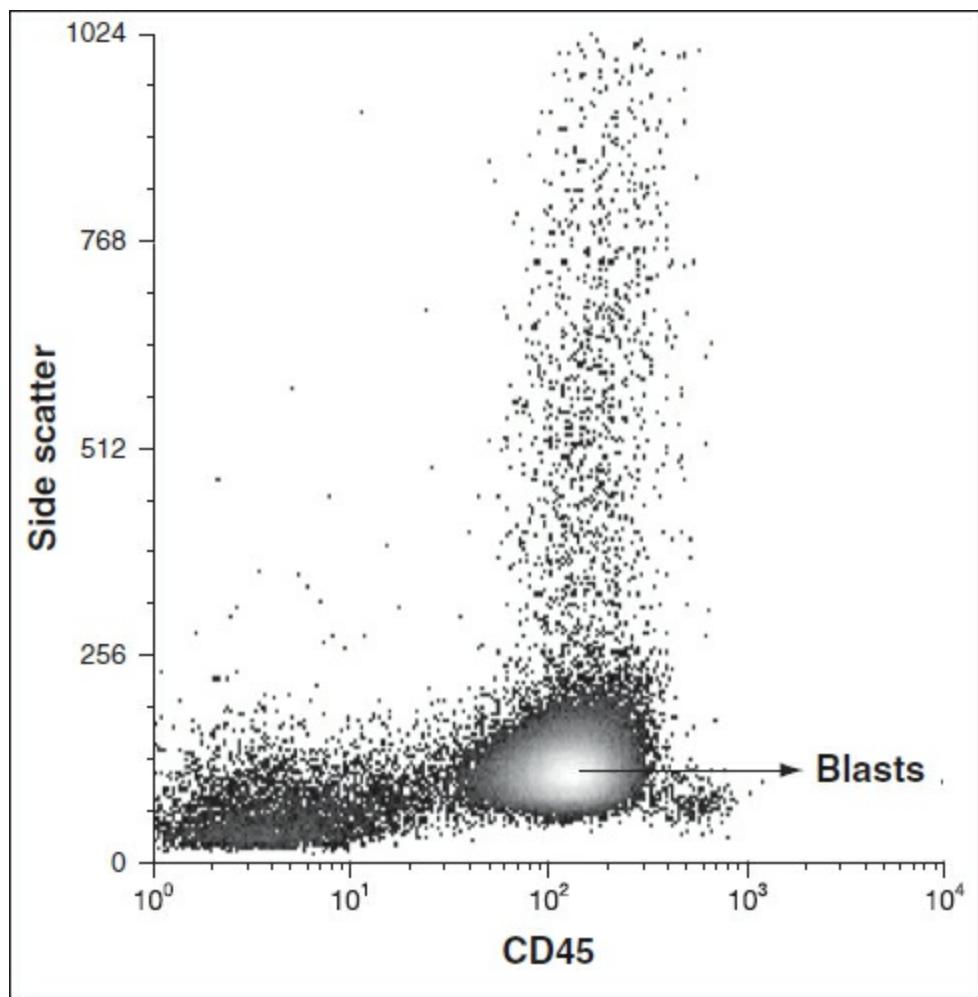


FIGURE 28.5 Analysis of side scatter and CD45 of peripheral blood from a patient with acute myeloid leukemia demonstrates characteristic blasts (myeloblasts).

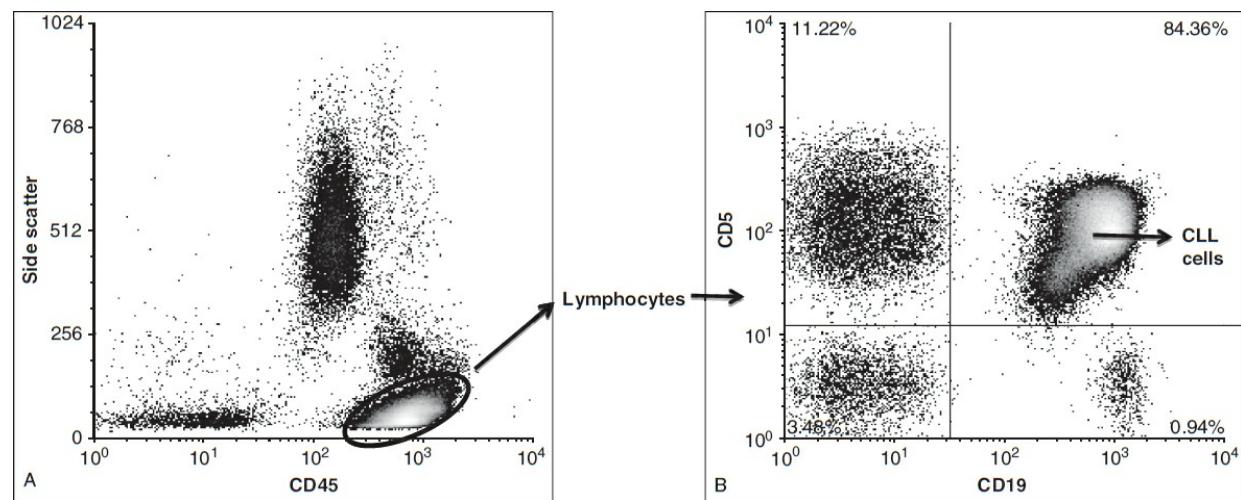


FIGURE 28.6 (A) Side scatter and CD45 analysis of peripheral blood from a patient with chronic lymphocytic leukemia shows increased

lymphocytes. (B) A lymphocyte gate (oval selection) is used for further analysis that demonstrates that the most lymphocytes abnormally coexpress CD19 and CD5, which is a typical feature of chronic lymphocytic leukemia (CLL).

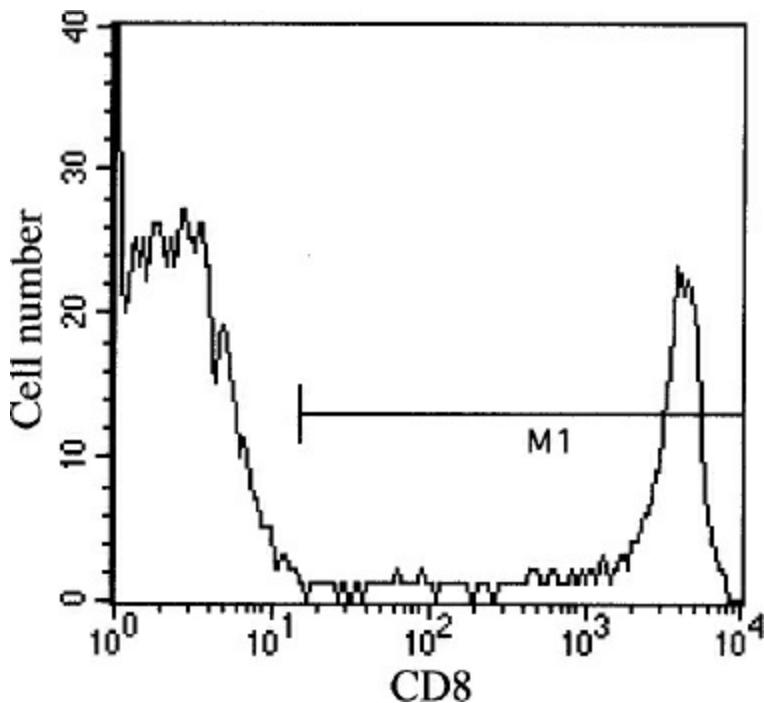


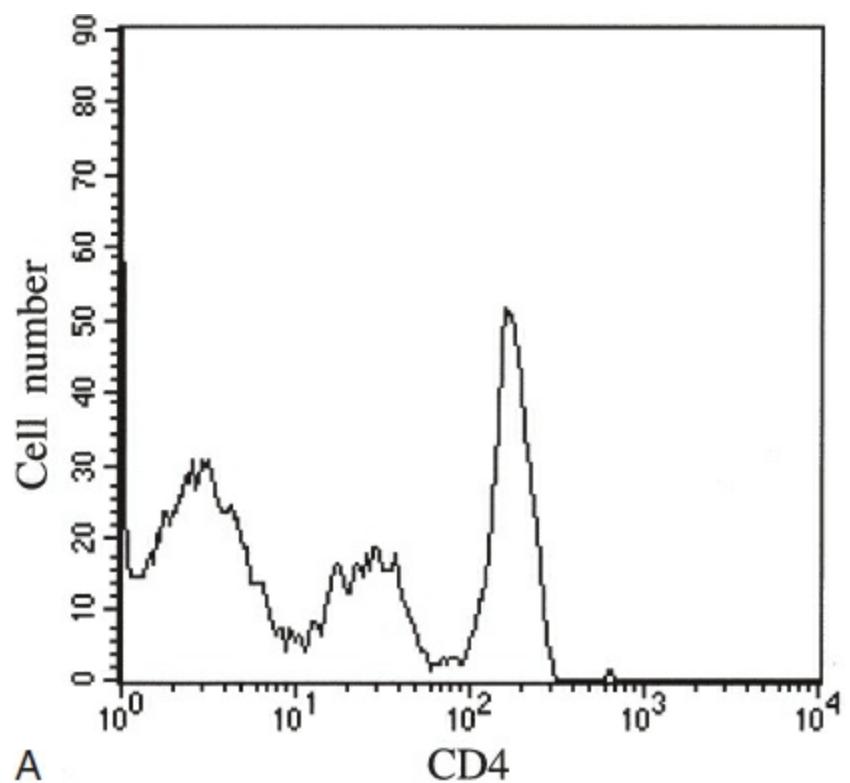
FIGURE 28.7 CD8 histogram evaluating lymphocytes.

The interpretation of fluorescent data based on antibody binding reflects the biology of the particular cognate cell surface protein. When the monoclonal reagent identifies exclusively one cell population, data interpretation is unambiguous, as for the pan-T-cell marker CD3 shown in [Figure 28.1](#). In this example, when the evaluation is confined to lymphocytes using a lymphocyte gate, there clearly are two populations, CD3⁻ cells, including B and natural killer (NK) lymphocytes, and CD3⁺ T cells. In other situations, biologic variability in surface protein expression impacts data interpretation; examples are shown in [Figures 28.7](#) and [28.8](#). In both histograms, there are at least three cell populations: Cells negative for the marker, cells showing intermediate fluorescence, and cells that have bright fluorescence. In [Figure 28.7](#), the CD8 intermediate cells are predominantly NK cells, while the bright staining cells are primarily CD8⁺ T cells. In [Figure 28.8A](#), the CD4 intermediate staining cells are monocytes, while the bright staining cells are T cells, with the former being present only in very small numbers with proper gating on lymphocytes ([Fig. 28.8B](#)). The finding of low-density CD4 expression on monocytes helps to explain HIV infection of this cell lineage.

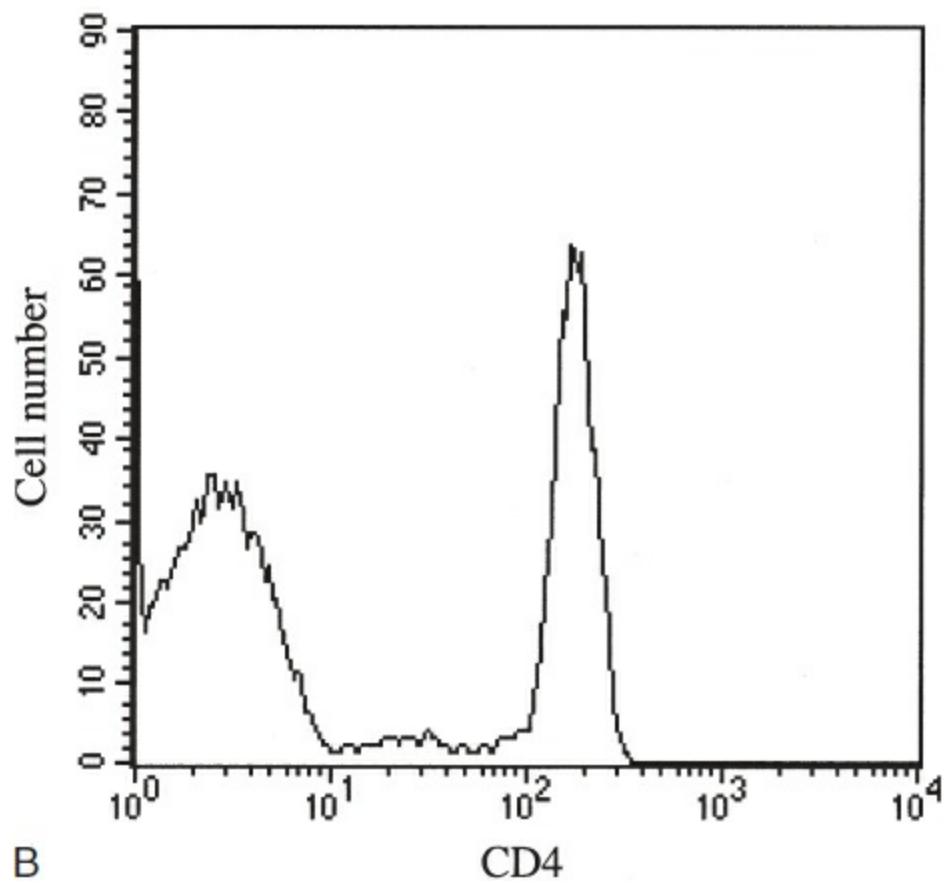
Many monoclonal antibodies, individually or in combination, can serve to

distinguish cells of a specific lineage (Table 28.1), and characteristic binding features can be used to direct a flow cytometry study to a specific cell population. As mentioned earlier, nonfluorescent parameters of forward-angle and side-angle scatter help distinguish among lymphocytes, monocytes, granulocytes, and platelets.³ Within the granulocyte population, neutrophils and eosinophils can be discriminated by the differential expression of the complement receptor CD16: Neutrophils stain for CD16 while eosinophils are negative.¹¹ Cells of the erythroid lineage can be identified based on the expression of glycophorin. Within the lymphocyte population, lineage-specific antibodies differentiate various populations and subpopulations. Hematopoietic stem cells can be identified by the expression of the cell surface protein, CD34, a valuable marker that has enabled evaluation and ex vivo isolation of bone marrow or mobilized circulating hematopoietic stem cells for transplantation.⁸

Many of the monoclonal reagents used to evaluate hematopoietic elements detect antigens that are not exclusively expressed on one specific cell type, and interpretation of data must incorporate knowledge of different surface protein expression patterns. A combination of additional antibodies often clarifies the relative expression of different antigens on specific cell populations. Cell surface proteins may be altered under different circumstances during the life cycle of a cell, including preferential expression early and/or late during differentiation, expression in response to cell activation, and/or in various states of cell-specific function. Protein upregulation implies a range of expression that could include cells transforming from negative to clearly positive, depending on the temporal pattern of expression. For example, the α chain of the interleukin-2 receptor (CD25) shows such a pattern on T cells (Fig. 28.9), but the interpretation of CD25 expression as an activation marker has been complicated by the identification of T regulatory cells among CD25 expressing CD4 $^{+}$ T cells.¹⁸ When the interpretation of positive and negative is visually less clear, consistent interpretation criteria are crucial for valid comparison of data among different studies. In some circumstances, isoforms of a specific protein are differentially expressed, and cells may express one or the other isoform or both (Fig. 28.10).



A



B

FIGURE 28.8 (A) CD4 histogram evaluating mononuclear cells (lymphocytes and monocytes). (B) CD4 histogram evaluating only

lymphocytes.

As mentioned earlier, sometimes the use of percentage positive for a specific marker is misleading, as shown in [Figure 28.11](#), where the histogram for the unstained cells overlaps significantly with that of stained cells. The histogram overlay demonstrates that there is a shift in the stained cells that would not be adequately reflected by simply scoring cells as positive or negative. Currently, many laboratories typically note the geometric mean channel (GMC) fluorescence of the unstained and stained cells and then report the cells to be positive for the specific marker with an increased fluorescence of x-fold over background (based on the quotient of the GMC-stained cells divided by the GMC of unstained or irrelevant antibody-treated cells). These considerations are particularly relevant for many markers used to evaluate malignant cells. In fact, consensus groups have repeatedly emphasized that reporting percentage values when interpreting results in hematopoietic malignancies is generally unsatisfactory.[19,20](#) These values may not be sufficiently informative to allow the detection of neoplastic cells and cannot adequately describe the phenotype of the malignant cells. For this reason, it is recommended that interpretation of flow cytometric results in hematopoietic and lymphoid malignancies be based on the visual examination of the plots for each of the antibodies used, and that the results be primarily descriptive, in a manner similar to the microscopic evaluation of cells or tissues. Numerical values are only used to indicate the fraction of neoplastic cells or other well-defined cell populations present in the sample.

Table 28.1 Commonly Used Leukocyte Antigens Used in Clinical Flow Cytometry Based on CD Designation

- CD1a: Cortical thymocytes, dendritic cells, Langerhans cells
- CD2: T cells, thymocytes, NK-cell subset
- CD3: T cells, thymocytes
- CD4: T-cell subset, thymocyte subset, monocytes/ macrophages
- CD5: T cells, B-cell subset
- CD7: Thymocytes, T cells, NK cells, early myeloid cells
- CD8: T-cell subset, thymocyte subset, NK-cell subset
- CD10: Early B cell, neutrophils, bone marrow stromal cells
- CD11b: Monocytes, granulocytes, NK cells
- CD11c: Myeloid cells, monocytes
- CD13: Myelomonocytic cells
- CD14: Monocytes, myelomonocytic cells
- CD15: Granulocytes, monocytes, endothelial cells

CD16: NK cells, granulocytes, macrophages
CD19: B cells (from pre-B-cell stage to plasma cells)
CD20: Mature B cells
CD21: Mature B cells, follicular dendritic cells
CD22: Mature B cells
CD23: Activated B cells
CD25: Activated T cells, activated B cells, regulatory T cells
CD27: Memory B cells
CD30: Activated T, B, NK cells, monocytes, Reed Sternberg cells
CD33: Myeloid cells, myeloid progenitor cells, monocytes
CD34: Hematopoietic precursor cells, capillary endothelium
CD36: Platelets, monocytes/ macrophages
CD38: Most thymocytes, activated T cells, B-cell precursors, germinal center B cells, plasma cells, myeloid cells, monocytes, NK cells
CD41: Megakaryocytes, platelets
CD42b: Megakaryocytes, platelets
CD45: Leukocytes
CD45RA: T-cell (naïve) subsets, B cells, monocytes
CD45RO: T-cell (memory) subsets, B-cell subsets, monocytes/ macrophages
CD56: NK cells, NK T cells
CD57: NK cells, T-cell subsets, B cells, monocytes
CD61: Megakaryocyte platelets, megakaryocytes, macrophages
CD64: Mature neutrophils, monocytes
CD71: Erythroid precursors, proliferative cells
CD79a: B cells
CD95 (Fas): Lymphocytes (upregulated after activation), monocytes, neutrophils
CD103: Intestinal epithelial T lymphocytes
CD117: Myeloid blast cells, mast cells
CD138: Epithelial cells, plasma cells
CD235a (glycophorin A): Erythroid cells

CD, cluster of differentiation; NK, natural killer.

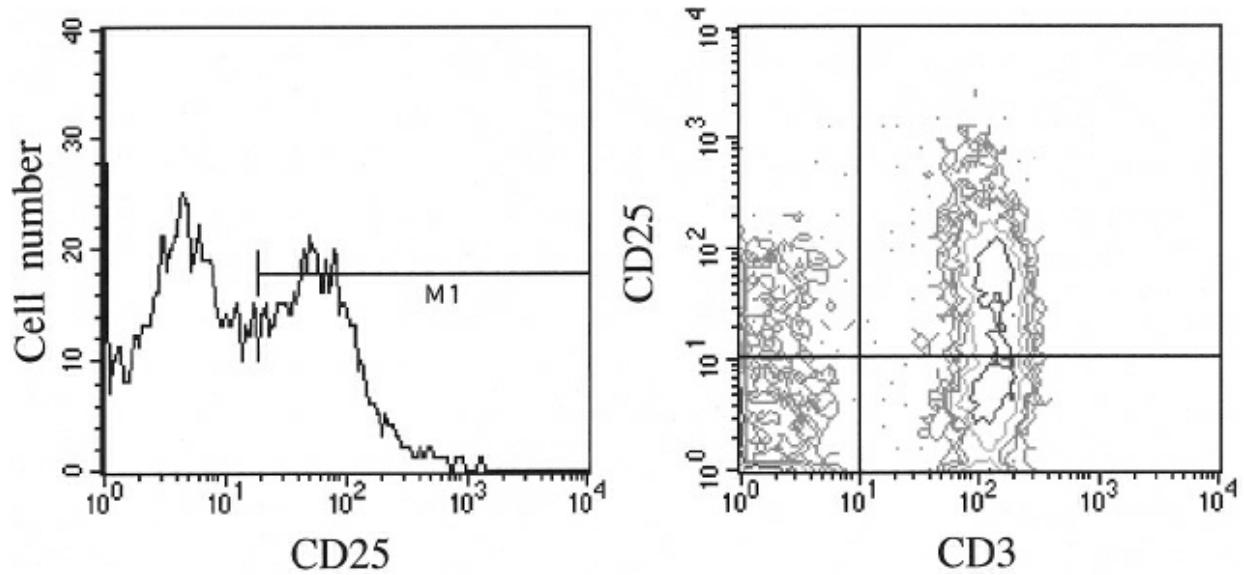


FIGURE 28.9 Single-parameter histogram of CD25 expression on lymphocytes (left panel) and contour plot of CD25 (y-axis) and CD3 (x-axis) expression.

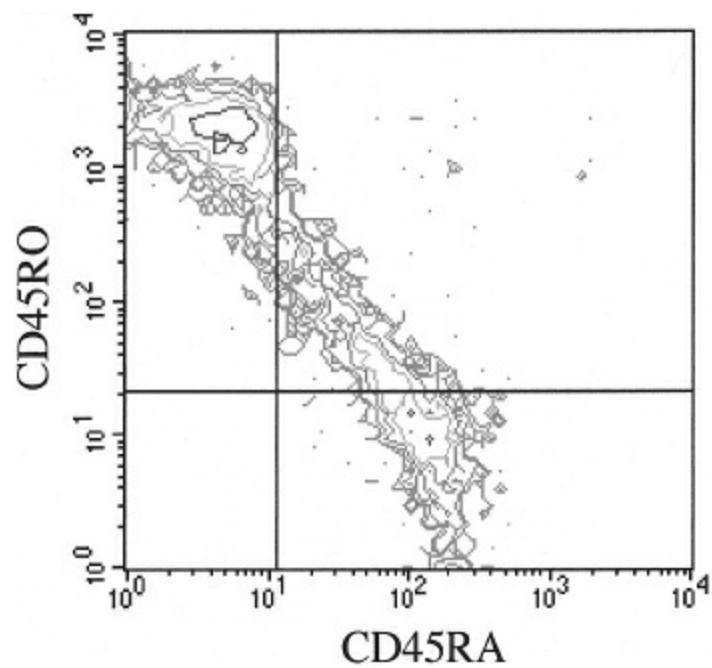


FIGURE 28.10 Contour plot of CD45RA and CD45RO expression on CD4⁺ T cells.

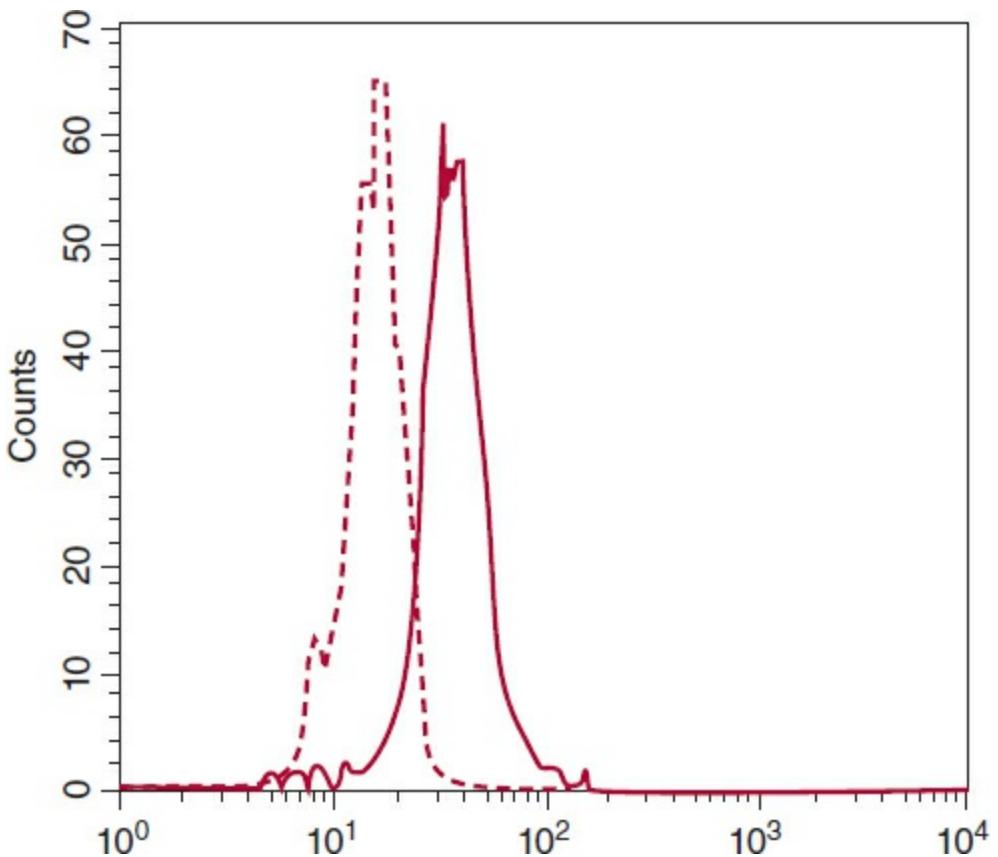


FIGURE 28.11 Overlapping control and positively stained histograms.

Flow cytometry has also been applied to investigate intracellular characteristics and, specifically, proteins that may only be detected intracellularly¹³ or as well as those that ultimately are also expressed on the cell surface. In addition, there are a series of reagents that bind to DNA and allow assessment of cell cycle status.²¹ More recently, intracellular flow cytometry has been applied to measure some functional properties of cells, including the detection of intracellular cytokines following cell stimulation and cell-activation-specific processes such as calcium flux, pH changes, and phosphorylation of intracellular signaling proteins,¹³ but these applications currently have limited applications in routine clinical laboratory practice. Certain intracellular proteins that are not expressed on the cell surface serve diagnostic or prognostic purposes in malignant conditions and are often used clinically, such as terminal deoxynucleotidyl transferase,²² Bcl-2,^{23–25} ZAP-70,²⁶ and myeloperoxidase.²⁷

APPLICATIONS OF FLOW CYTOMETRY IN HEMATOLOGY

Flow cytometry is of value in the evaluation of numerous hematologic conditions, but no other diseases have benefited more from the use of this technology than hematopoietic and lymphoid neoplasias. Flow cytometry has revolutionized the manner in which we

diagnose, classify, and monitor acute leukemia or lymphoproliferative disorders, and it is rare now that patients with these disorders are treated without including the flow cytometric data. The technology is rapid, quantitative, and can analyze simultaneously multiple antigens in a large number of cells, allowing the easy detection, characterization, and enumeration of malignant cells, even when admixed with normal elements. The ability of flow cytometry to recognize malignancy is based on its capacity to distinguish differences in antigen expression between normal and neoplastic cells. Normal hematopoietic cells originate from a stem cell in the marrow that subsequently gives rise to a progeny of different cell lineages. These cell progenitors traverse various developmental stages and ultimately evolve into mature elements in the circulation and other peripheral organs. As the hematopoietic cells develop and differentiate, they undergo changes in their surface or intracellular antigenic profile that is characteristic of their lineage and differentiation stage. Hematopoietic malignancies are clonal cell populations that express similar antigens to those of their nonneoplastic counterparts but usually with a different expression pattern that is unique for each type of neoplasia. This antigen expression can be increased, decreased, absent, asynchronous, or may be of a different cell lineage. Thus, knowledge of the immunophenotype of a cell together with its physical properties revealed by light scatter signals allows determination of not only their lineage and developmental stage but also, in most instances, their normal or neoplastic nature. Furthermore, in T-or B-cell lymphoproliferative disorders, the clonal nature of the lymphocytes can be established by recognizing the restriction of expression of immunoglobulin light chains²⁸ or T-cell receptor beta chains.²⁹ The identification of monoclonal lymphoid expansions simultaneously with other informative antigens has proven extremely useful in the differential diagnosis between benign and malignant lymphoid disorders³⁰ not only in marrow and blood³¹ but also in lymph nodes³² and extranodal lymphoid sites.³³ In conjunction with cytologic examination, this technology is especially helpful in samples obtained from fine needle aspirates.^{34–36}

The utilization of flow cytometry has recently extended to the assessment of myelodysplasia³⁷ and has also been found to be useful in plasma cell disorders.³⁸ In the latter, this analysis has relevance in the differential diagnosis between myeloma and other plasma cell disorders, and also provides prognostic utility based on the identification of high-risk monoclonal gammopathy of undetermined significance and smoldering myeloma.³⁹

Flow cytometry is a useful tool for MRD detection, which refers to the identification and quantitation of low levels of malignant cells following treatment.⁴⁰ Broader capabilities provided by technical advances, a larger selection of antibodies and fluorochromes, and new analytical approaches have resulted in an increased sensitivity

in the identification of malignant cells. This identification is possible due to the antigenic expression and/ or physical properties of neoplastic cells that are different from those of their normal counterparts. Depending on the particular case, detection sensitivities of .01% or higher can be achieved using this technology, rivaling those based on the polymerase chain reaction. Flow-based MRD assays are used in monitoring leukemias after initiation of treatment to identify patients with resistant disease^{41,42} who are at a high risk for relapse, and also to recognize good responders who may benefit from reduced toxicity therapy. Although genetic abnormalities in acute leukemic cells can be recognized by molecular genetic techniques, flow cytometry is an excellent alternative in cases where genetic markers are absent. Although usually most MRD studies have been performed in acute leukemia patients during early treatment, MRD testing may also be useful in later stages in other diseases such as chronic lymphocytic leukemia^{31,43} and multiple myeloma⁴⁴ in which response duration may be more relevant than early response. Despite the remarkable results obtained by individual laboratories with MRD testing by flow cytometry, there remains high variability in sensitivity and reproducibility of this assay across laboratories due to lack of uniformity in methodology and data interpretation. In addition, it is possible that modern molecular techniques such as next-generation sequencing that are more sensitive and easier to standardize, may substitute for flow cytometry in the routine assessment of MRD in the future.

Many applications in nonmalignant diseases are also routinely performed in the clinical laboratory. This technology remains a critical tool in monitoring disease progression and therapy in HIV infection.⁷ There are specific lymphocyte findings characteristic of primary immunodeficiencies, including loss of cell populations or subpopulations, the absence of specific cell surface or intracellular proteins, and changes in normal immunologic processes that can be detected by flow cytometry (e.g., the development of memory T cells and/ or B cells).¹³ Assessment of specific lymphocyte populations and subpopulations is being studied in various disorders characterized by inflammation, with particular attention to the expression of activation markers. Reconstitution of the immune system following intensive chemotherapy and hematopoietic stem cell transplantation can be monitored by flow cytometry, an approach that is even more significant due to the recent focus on immunotherapy and vaccines in experimental treatment protocols for malignancies.

Assessment of leukocytes other than lymphocytes is emerging in the clinical laboratory. Monocytes can be evaluated to define deficiencies associated with defective monocyte surface receptor expression.¹³ Granulocyte expression of critical adhesion molecules and their capacity to generate reactive oxygen species can be assessed by flow cytometry.¹³ In addition, granulocyte-specific autoantibodies can be detected.

Flow cytometric methods are being used to characterize eosinophils in settings of increased production such as the hypereosinophilia syndrome,¹⁶ and basophils have been studied for intracellular cytokine production, as well as activation of antigen expression ex vivo following specific antigen exposure.

Hematopoietic stem cell identification is dependent on flow cytometry, which is routinely used to characterize and quantitate stem cells in the transplantation setting, generally based on CD34 expression together with other cell surface markers.⁸ Separation techniques to purify stem cells from either bone marrow or peripheral blood typically utilize CD34 selection methods, and patients are followed by flow cytometric testing posttransplantation to assess donor cell engraftment and in certain settings, donor–host cell chimerism.

The evaluation of erythrocytes by flow cytometry has been applied to cell surface proteins, autoantibodies in hemolytic anemia, and the detection of F-cells in fetomaternal hemorrhage and sickle cell anemia.^{9,45} The detection of glycosylphosphatidylinositol-anchored proteins on erythrocytes and leukocytes by flow cytometry is now the favored method to accurately diagnose paroxysmal nocturnal hemoglobinuria.⁴⁶

Flow cytometric evaluation of platelets has been described as a method to study these cells in whole blood, thus eliminating the need for platelet isolation and minimizing cell manipulation.¹⁰ This approach allows for the detection of platelet-associated immunoglobulin, assessment for states of platelet activation and aggregation, and detection of reticulated platelets. Flow cytometry is also a rapid and useful method to detect platelet defects in structural or functional glycoproteins, such as the abnormal expression of GpIIb/ IIIa in Glanzmann thrombasthenia and GpIb in Bernard–Soulier disease.¹⁰

SUMMARY

Flow cytometry has evolved as integral in the laboratory assessment of many hematologic disorders. This technology provides a powerful tool to assess simultaneously cell surface and intracellular characteristics. The increasing range of reagents and expanded understanding of cell biology mean that flow cytometry play an even larger role in the clinical evaluation of various cellular components of the hematologic system, both in benign and malignant conditions.

References

1. Givan AL. Principles of flow cytometry: an overview. *Methods Cell Biol.* 2001;63:19–50.
2. McCoy JP Jr. Basic principles of flow cytometry. *Hematol Oncol Clin North Am.*

2002;16:229–243.

3. Loken MR, Brosnan JM, Bach BA, et al. Establishing optimal lymphocyte gates for immunophenotyping by flow cytometry. *Cytometry*. 1990;11:453–459.
4. Szczepanski T, van der Velden V, van Dongen JJ. Flow-cytometric immunophenotyping of normal and malignant lymphocytes. *Clin Chem Lab Med*. 2006;44:775–796.
5. Kussick SJ, Wood BL. Using 4-color flow cytometry to identify abnormal myeloid populations. *Arch Pathol Lab Med*. 2003;127:1140–1147.
6. Darzynkiewicz Z, Bedner E, Smolewski P. Flow cytometry in analysis of cell cycle and apoptosis. *Semin Hematol*. 2001;38:179–193.
7. Mandy F, Nicholson J, Autran B, et al. T-cell subset counting and the fight against AIDS: reflections over a 20-year struggle. *Cytometry*. 2002;50:39–45.
8. Gratama JW, Kraan J, Keeney M, et al. Validation of the single-platform ISHAGE method for CD34⁺. Hematopoietic stem and progenitor cell enumeration in an international multicenter study. *Cytotherapy*. 2003;5:55–65.
9. Davis BH. Diagnostic utility of red cell flow cytometric analysis. *Clin Lab Med*. 2001;21:829–840.
10. Linden MD, Frelinger AL III, Barnard MR, et al. Application of flow cytometry to platelet disorders. *Semin Thromb Hemost*. 2004;30:501–511.
11. Gopinath R, Nutman TB. Identification of eosinophils in lysed whole blood using side scatter and CD16 negativity. *Cytometry*. 1997;30:313–316.
12. Khan SS, Solomon MA, McCoy JP Jr. Detection of circulating endothelial cells and endothelial progenitor cells by flow cytometry. *Cytometry B Clin Cytom*. 2005;64:1–8.
13. Bleesing JJ, Fleisher TA. Cell function-based flow cytometry. *Semin Hematol*. 2001;38:169–178.
14. Shulman HM, Wells D, Gooley T, et al. The biologic significance of rare peripheral blasts after hematopoietic cell transplantation is predicted by multidimensional flow cytometry. *Am J Clin Pathol*. 1999;112:513–523.
15. Perfetto SP, Ambrozak D, Nguyen R, et al. Quality assurance for polychromatic flow cytometry. *Nat Protoc*. 2006;1:1522–1530.
16. Schnizlein-Bick CT, Mandy FF, O’Gorman MR, et al. Use of CD45 gating in three and four-color flow cytometric immunophenotyping: guideline from the National Institute of Allergy and Infectious Diseases, Division of AIDS. *Cytometry*. 2002;50:46–52.
17. Borowitz MJ, Guenther KL, Shults KE, et al. Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. *Am J Clin Pathol*. 1993;100:534–540.
18. Raimondi G, Turner MS, Thomson AW, et al. Naturally occurring regulatory T

- cells: recent insights in health and disease. *Crit Rev Immunol*. 2007;27:61–95.
- 19. Braylan RC, Atwater SK, Diamond L, et al. U.S.-Canadian Consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: data reporting. *Cytometry*. 1997;30:245–248.
 - 20. Wood BL, Arroz M, Barnett D, et al. 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry B Clin Cytom*. 2007;72(Suppl 1):S14–S22.
 - 21. Darzynkiewicz Z, Bedner E, Smolewski P. Flow cytometry in analysis of cell cycle and apoptosis. *Semin Hematol*. 2001;38:179–193.
 - 22. Farahat N, Lens D, Morilla R, et al. Differential TdT expression in acute leukemia by flow cytometry: a quantitative study. *Leukemia*. 1995;9:583–587.
 - 23. Cook JR, Craig FE, Swerdlow SH. Bcl-2 expression by multicolor flow cytometric analysis assists in the diagnosis of follicular lymphoma in lymph node and bone marrow. *Am J Clin Pathol*. 2003;119:145–151.
 - 24. Cornfield DB, Mitchell DM, Almasri NM, et al. Follicular lymphoma can be distinguished from benign follicular hyperplasia by flow cytometry using simultaneous staining of cytoplasmic bcl-2 and cell surface CD20. *Am J Clin Pathol*. 2000;114:258–263.
 - 25. Laane E, Tani E, Bjorklund E, et al. Flow cytometric immunophenotyping including Bcl-2 detection on fine needle aspirates in the diagnosis of reactive lymphadenopathy and non-Hodgkin's lymphoma. *Cytometry B Clin Cytom*. 2005;64:34–42.
 - 26. Slack GW, Wizniak J, Dabbagh L, et al. Flow cytometric detection of ZAP-70 in chronic lymphocytic leukemia: correlation with immunocytochemistry and Western blot analysis. *Arch Pathol Lab Med*. 2007;131:50–56.
 - 27. Guy J, Antony-Debré I, Benayoun E, et al. Flow cytometry thresholds of myeloperoxidase detection to discriminate between acute lymphoblastic or myeloblastic leukaemia. *Br J Haematol*. 2013;161:551–555.
 - 28. Chizuka A, Kanda Y, Nannya Y, et al. The diagnostic value of kappa/ lambda ratios determined by flow cytometric analysis of biopsy specimens in B-cell lymphoma. *Clin Lab Haematol*. 2002;24:33–36.
 - 29. Beck RC, Stahl S, O'Keefe CL, et al. Detection of mature T-cell leukemias by flow cytometry using anti-T-cell receptor V beta antibodies. *Am J Clin Pathol*. 2003;120:785–794.
 - 30. Weisberger J, Wu CD, Liu Z, et al. Differential diagnosis of malignant lymphomas and related disorders by specific pattern of expression of immunophenotypic markers revealed by multiparameter flow cytometry review. *Int J Oncol*. 2000;17:1165–1177.

31. Sanchez ML, Almeida J, Vidriales B, et al. Incidence of phenotypic aberrations in a series of 467 patients with B chronic lymphoproliferative disorders: basis for the design of specific four-color stainings to be used for minimal residual disease investigation. *Leukemia*. 2002;16:1460–1469.
32. Martinez A, Aymerich M, Castillo M, et al. Routine use of immunophenotype by flow cytometry in tissues with suspected hematological malignancies. *Cytometry B Clin Cytom*. 2003;56:8–15.
33. Almasri NM, Zaer FS, Iturraspe JA, et al. Contribution of flow cytometry to the diagnosis of gastric lymphomas in endoscopic biopsy specimens. *Mod Pathol*. 1997;10:650–656.
34. Barrena S, Almeida J, Del CG-M, et al. Flow cytometry immunophenotyping of fine-needle aspiration specimens: utility in the diagnosis and classification of non-Hodgkin lymphomas. *Histopathology*. 2011;58:906–918.
35. Demurtas A, Accinelli G, Pacchioni D, et al. Utility of flow cytometry immunophenotyping in fine-needle aspirate cytologic diagnosis of non-Hodgkin lymphoma: a series of 252 cases and review of the literature. *Appl Immunohistochem Mol Morphol*. 2010;18:311–322.
36. Meda BA, Buss DH, Woodruff RD, et al. Diagnosis and subclassification of primary and recurrent lymphoma. The usefulness and limitations of combined fine-needle aspiration cytomorphology and flow cytometry. *Am J Clin Pathol*. 2000;113:688–699.
37. Kussick SJ, Fromm JR, Rossini A, et al. Four-color flow cytometry shows strong concordance with bone marrow morphology and cytogenetics in the evaluation for myelodysplasia. *Am J Clin Pathol*. 2005;124:170–181.
38. Paiva B, Almeida J, Perez-Andres M, et al. Utility of flow cytometry immunophenotyping in multiple myeloma and other clonal plasma cell-related disorders. *Cytometry B Clin Cytom*. 2010;78:239–252.
39. Perez-Persona E, Vidriales MB, Mateo G, et al. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. *Blood*. 2007;110:2586–2592.
40. Campana D, Coustan-Smith E. Detection of minimal residual disease in acute leukemia by flow cytometry. *Cytometry* 1999;38:139–152.
41. Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a children's oncology group study. *Blood*. 2008;111:5477–5485.
42. Terwijn M, van Putten WL, Kelder A, et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/ SAKK AML 42A study. *J Clin Oncol*. 2013;31:3889–3897.

43. Rawstron AC, Bottcher S, Letestu R, et al. Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL. *Leukemia*. 2013;27:142–149.
44. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the medical research council myeloma IX study. *J Clin Oncol* 2013;31:2540–2547.
45. Chen JC, Davis BH, Wood B, et al. Multicenter clinical experience with flow cytometric method for fetomaternal hemorrhage detection. *Cytometry*. 2002;50:285–290.
46. Borowitz MJ, Craig FE, Digiuseppe JA, et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. *Cytometry B Clin Cytom*. 2010;78:211–230.

INDEX

Page numbers with “f” and “t” denote figures and tables, respectively.

- Abetalipoproteinemia, 34
- ABL1 oncogene, 181
- Abnormal localization of immature precursors (ALIPs), 93
- ABO incompatibility in transplant settings, 375, 376t
 - major incompatibility, 375
 - minor incompatibility, 375
 - Rh incompatibility, 376
- Absolute iron deficiency, 1, 3–4, 9
 - causes of, 2t
- Absolute neutrophil count (ANC), 126, 142, 177
- Absolute reticulocyte count (ARC), 27, 443
 - normal ranges, 27
- ABVD, 215, 216–217, 217t, 220
- Acalabrutinib, 205
- Acanthocyte, 28t
- Acanthocytosis, 33, 34
- Acetaminophen, 44, 384
- Achlorhydria, 2, 16, 20
- Acquired aplastic anemia, 291
 - clinical features, 68
 - definitive treatments, 72–74
 - diagnosis and differential diagnosis, 69–72
 - etiology, 67–68
 - pathophysiology, 67–68
- Acquired coagulation disorders, 136–139
- Acquired Glanzmann thrombasthenia, 333

- Acquired hemophilia, 316
- Acquired neutropenias, 126, 437
- Acquired prothrombotic disorders, 141–142
 - drug-induced neutropenia, 126
 - immune-related neutropenia, 126–127
 - infection-related neutropenia, 126
- Activated B-cell (ABC) type, 224, 233
- Activated B-cell-like (ABC) gene signature, 224
- Activated partial thromboplastin time (aPTT), 307, 309, 310, 318, 360, 456–457
- Activated protein C resistance (Factor V Leiden), 348–349
- Acute chest syndrome (ACS), 47–48
- Acute graft-versus-host disease, 278–280
 - grading of, 279t
- Acute intermittent porphyria (AIP), 59, 63
- Acute intravascular hemolysis, 27–29, 29f
- Acute lymphoblastic leukemia (ALL), 154, 168, 274, 276
 - allogeneic stem cell transplantation (SCT), 171, 176
 - classification, 169–170
 - clinical features, 168–169
 - CNS-directed therapy, 174
 - dose modification, 175
 - emergency presentations, 169t
 - epidemiology, 168
 - etiology and risk factors, 168
 - evaluations, 177
 - after treatment, 178
 - during treatment, 178
 - late effects, 178
 - extramedullary leukemia, 175
 - French–American–British (FAB) system, 169
 - infant ALL, 173
 - intrathecal chemotherapy, 174t
 - laboratory features, 169
 - management of relapse, 175

- allogeneic stem cell transplantation, 176
 - new treatment approaches, 175
 - Philadelphia-chromosome positive ALL, 173
 - presenting features, 169t
 - prognostic factors, 170–171
 - radiation guidelines, 174
 - risk-group assignment for B-precursor ALL, 173
 - supportive care, 176
 - antiemetics, 176
 - chemotherapy prophylaxis, 177
 - infection prophylaxis, 177
 - nutritional support, 177
 - psychosocial support, 177
 - transfusions, 176–177
 - tumor lysis syndrome, 176
 - T-ALL, 173
 - testicular leukemia, 174
 - treatment, 171–174
 - WHO classification system, 169
- Acute myelogenous leukemia, 90, 142
- Acute myeloid leukemia (AML), 70, 148, 270, 276
 - age-related incidence of, 149f
 - classification, 152–153
 - clinical features, 150–151
 - clinical trials with novel agents in, 163t
 - cytogenetic and molecular abnormalities, risk status based on, 155t
 - diagnostic evaluation, 153–154, 154t
 - epidemiology, 148
 - etiology, 148–150
 - French–American–British (FAB) classification of, 152t
 - laboratory findings, 151–152
 - morphologic diagnosis of, 154
 - pathogenesis, 150
 - posttreatment response criteria in, 164

- prognostic factors, 155–157, 156t
- risk factors for development of, 149t
- treatment, 157
 - of acute promyelocytic leukemia, 161
 - better-risk acute myeloid leukemia, 160
 - hematopoietic growth factors, 163
 - hematopoietic stem cell transplantation, 159–160
 - induction therapy, 158
 - initial management, 157–158
 - intermediate-risk acute myeloid leukemia, 160–161
 - minimal residual disease, evaluation of, 164
 - novel therapeutic targets, 164
 - in older or unfit patients, 161–162
 - poor-risk acute myeloid leukemia, 160
 - postremission treatment, 158–159
 - in pregnancy, 163–164
 - of relapsed and refractory acute myeloid leukemia, 162–163
- World Health Organization classification of, 153t
- Acute promyelocytic leukemia (APL), 151, 156, 161, 324
- Acute splenic sequestration, 49
- ADAMTS-13, 34, 427
 - assays for, 301
- Adaptive immune response, 224, 228
- Adenosine deaminase (ADA), 33
- Adenosine diphosphate (ADP), 32
- Adenosine triphosphate (ATP), 30, 32
- Adult T-cell leukemia/ lymphoma (ATLL), 224, 236
- African Burkitt lymphoma (BL), 224
- African trypanosomiasis (AT), 432
- Agent-specific prophylaxis, 177
- Aggressive B-cell lymphomas, 224, 245–246
- Aggressive natural killer-cell leukemia, 235
- Aggressive non-Hodgkin lymphoma, 268, 277
 - alternative or salvage regimens for, 247t

- standard therapy for, 245t
- Agranulocytosis, 78
 - diagnosis and treatment, 78
 - drugs associated with, 79–80t
 - etiology and pathophysiology, 78
 - immune versus toxic agranulocytosis, 80t
- AIDS-related DLBCL, 224
- AIDS-related lymphoma (ARL)
 - dose-adjusted EPOCH for, 250t
 - treatment considerations, 249–250
- Alanine aminotransferase (ALT), 413
- ALAS2 gene, 62
- Albumin, 29, 386t, 444
- Aldolase A deficiency, 32
- Alemtuzumab, 73, 74, 95, 96, 99, 244
- Alkylating agents, 90, 96, 113, 217, 244, 250
- Allogeneic hematopoietic stem cell transplantation (allo-HSCT), 71, 205, 270
 - alternative donor transplantation, 284
 - from matched unrelated donors, 284
 - from mismatched related donors, 284–285
 - umbilical cord transplants, 285
 - antileukemic potential of, 271
 - complications of, 278
 - conditioning-related toxicities, 278
 - graft-versus-host disease (GVHD), 278–281
 - pulmonary complications, 281
 - indications for, 271
 - infectious complications, 281
 - bacterial infections, 281–282
 - Epstein–Barr Virus–associated lymphoproliferative disorder, 283
 - fungal infections, 282
 - graft failure, 283
 - late sequelae of transplantation, 283
 - viral infections, 282

- planning, 271
 - conditioning regimen, 274
 - conventional/ myeloablative conditioning, 274–275
 - evaluation of transplant recipients, 273
 - identification of suitable donor, 273
 - procurement of hematopoietic stem cells, 274
- preparative regimens commonly used in, 275t
- reduced intensity conditioning HSCT, 283–284
- results of, 275
 - acute lymphoblastic leukemia, 276
 - acute myeloid leukemia (AML), 276
 - aplastic anemia, 277–278
 - chronic myeloid leukemia (CML), 275–276
 - multiple myeloma, 277
 - myelodysplastic syndrome, 276–277
 - non-Hodgkin's lymphoma, 277
- Allogeneic HSCT (allo-HSCT), 159, 159t, 268, 269, 270–271, 274, 275, 276, 277, 278, 285
- Allogeneic stem cell transplantation, 46, 82, 97, 116, 171, 176, 181, 191, 192, 193, 194
 - selecting patients for, 192–193
 - unrelated donor transplants, 192
- Alloimmune neonatal neutropenia, 126, 437
- Alloimmunization, 50, 145, 391
- Allopurinol, 67, 176, 188, 194, 246
- All-trans retinoic acid (ATRA), 161, 164, 324
- Alpha-2-antiplasmin, 311
 - deficiency, 318
- Alternatives to allogeneic blood transfusion, 396, 397t
- AMCA/ ACA, 399t
- American Society for Apheresis (ASFA), 402
- Aminocaproic acid, 96, 140, 315, 316, 327
- δ-Aminolevulinic acid dehydratase (ALAD) deficiency, 59
- δ-Aminolevulinic acid dehydratase deficiency porphyria (ADP), 63

- › δ-Aminolevulinic acid synthase (ALAS), 60
- › Anagrelide, 113, 116, 348
- › Anaphylactic transfusion reactions, 392
- › Anaplastic large cell lymphoma (ALCL)
 - anaplastic lymphoma kinase-negative, 238
 - anaplastic lymphoma kinase-positive, 238
- › Anaplastic lymphoma kinase-positive (ALK⁺), 238
 - large B-cell lymphoma, 234
- › Ancylostoma duodenale, 432
- › Androgens, 83
- › Anemia, 1, 131, 132t. See also Aplastic anemia (AA); Fanconi anemia (FA); Hemolytic anemia; Iron deficiency; Sickle cell anemia
 - of chronic inflammatory states, 4
 - macrocytic, 135–136
 - in malaria, 431t
 - microcytic, 131–134, 132t
 - normocytic, 134–135
 - nutritional causes of, 12
 - in pregnancy, 424
 - recommendations for anemia screening, 134t
 - of rheumatoid arthritis, 9
 - treatment of, 5
 - associated with chronic inflammatory diseases, 9
 - dietary iron, 5
 - intravenous iron therapy, 7, 8t
 - oral iron therapy, 5–6, 6t
 - in patients with advanced malignancy, 8–9
 - red blood cell transfusion, 8
- › Anemia of chronic disease (ACD), 3, 4
- › Angioimmunoblastic T-cell lymphoma (AITL), 238
- › Angiotensin-converting enzyme (ACE) inhibitors, 49
- › Anti-activated factor (FXa), 429
- › Anticoagulation, consultations in, 356
 - antiphospholipid syndrome, 366

- inferior vena cava (IVC) filters, indications for, 362–363
- new anticoagulants, 366–369
- postthrombotic syndrome (PTS)
 - management of, 366
 - prevention, 367t
 - recognition of, 365
- venous thromboembolism (VTE), prophylaxis and treatment of, 356
 - anticoagulation options in cancer patients, 361–362
 - in cancer patients receiving chemotherapy, hormonal, and/ or anti-angiogenic treatment, 358–359
 - in cancer patients undergoing surgical intervention, 357–358
 - in cancer patients who are immobilized/ hospitalized, 359
 - general approach in treating VTE in cancer patients, 360–361
 - in patients with brain metastases and primary brain tumors, 360
 - thrombosis and venous access devices, 362
 - TROUSSEAU's syndrome, treatment of, 360
- venous thromboembolism in pregnancy, 363
 - diagnosis of, 363–364
 - management, 364–365
- Anti-D, 294, 425
- Antidepressants, 44
- Antiemetics, 44, 176
- Antifibrinolytic agents, 327
- Anti-FVIII immunoglobulin G (IgG) antibodies, 316
- Antigen-negative blood, 373
- Antiglobulin test, 373, 374
- Anti-parietal cell antibodies, 20
- Antiphospholipid antibodies (APAs), 342
- Antiphospholipid antibody syndrome (APA/ APS), 345–346, 361, 363, 366
- Antithrombin (antithrombin III), deficiency of, 349
- Antithymocyte globulin (ATG), 73, 95, 277
- Anti-Xa assay, 310, 457
- Antral gastritis, 15
- Apheresis, 402

- Apixaban, 368–369
- Aplastic anemia (AA), 66, 277–278
 - acquired, 67
 - clinical features, 68
 - definitive treatments, 71–74
 - diagnosis and differential diagnosis, 68–71
 - etiology and pathophysiology, 67–68
 - constitutional, 66
 - diseases easily confused with, 70t
 - treatment of, 75f
- Ardeparin, 358
- Argatroban, 344, 345, 429
- Arginine (Normosang), 63
- Arsenic trioxide (ATO), 161
- Aspergillus fungal infections, 278
- Aspergillus nidulans, 124
- Aspirin, 110, 113, 331, 348, 430
- ASXL1 gene, 156t
- Ataxia telangiectasia, 238
- ATRX gene, 92
- Atypical hemolytic uremic syndrome (aHUS), 427
- Auer rods, 154
- Autoimmune atrophic gastritis, 15
- Autoimmune hematologic manifestations, 208
- Autoimmune hemolytic anemia (AIHA), 199, 401
 - cold agglutinin syndrome, 402
 - paroxysmal cold hemoglobinuria (PCH), 402
 - warm autoimmune hemolytic anemia, 401
- Autoimmune lymphoproliferative syndrome (ALPS), 144, 238
- Autoimmune neutropenia (AIN), 437–438
 - of infancy/ childhood, 127
- Autologous-hematopoietic stem cell transplantation (auto-HSCT), 268
 - general considerations, 268–269
 - in hematologic malignancies, 269–270

- results of, 269–270
 - in solid tumors, 270
 - Autologous stem cell transplantation (ASCT), 218, 231, 243, 249, 257, 260
 - Automated coagulation analyzers, 456
 - Automated complete blood counters, 440–441
 - 5-Aza-2'-deoxyazacitidine, 98
 - 5-Azacitidine, 98
 - Azacitidine, 98
-
- Bacteremia, 50
 - Bacteria in the gut of herbivorous animals, 12
 - Bacterial contamination and sepsis, 393–394
 - Bacterial overgrowth in small bowel, 15
 - Band 3, 33
 - Bariatric surgery, 14
 - Basophilic stippling, 28t
 - B-cell acute lymphoblastic leukemia, 246
 - B-cell immunophenotype, 229t
 - B-cell lymphoma, 235
 - aggressive, 224, 245–246
 - molecular characteristics of, 227t
 - B-cell neoplasms, 228
 - B-cell receptor (BCR), 198
 - BCR-ABL1 translocation, molecular basis of chronic myelogenous leukemia in, 182
 - Bendamustine and rituximab (BR), 203
 - Benign ethnic neutropenia (BEN), 129
 - Benzodiazepines, 44, 63
 - Bernard–Soulier syndrome (BSS), 140, 305, 334
 - Beta-2-microglobulin (B2M), 200
 - Bethesda assay, 310
 - Bethesda units (BUs), 310
 - Bevacizumab, 346, 358
 - Bexarotene, 250, 358, 360
 - Binet classification, 200

- › 1,3-Biphosphoglycerate, 32
- › Bivalirudin, 345
- › Bleeding, 136, 137t
 - acquired coagulation disorders, 136–139
 - inherited factor deficiencies, 139
 - platelet disorders, 139–140
- › Bleeding time (BT), 310, 458
- › Bleomycin, 217, 220
- › Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1102 phase III trial, 97
- › Blood eosinophilia, 433
- › Blood pressures in SCD, 47
- › Blood transfusion, 373
 - ABO incompatibility in transplant settings, 375, 376t
 - major incompatibility, 375
 - minor incompatibility, 375
 - Rh incompatibility, 376
 - antiglobulin test, 374
 - blood cell antigens, 373
 - blood compatibility, 374–375
 - blood components and transfusion therapy, 376–377
 - blood derivatives, 385, 386–387t
 - coagulation factor preparations, 388t
 - corrected count increment (CCI), 381
 - Cryoprecipitate (cryo), 383–384
 - derivative and recombinant coagulation factors, 389
 - fresh-frozen plasma (FFP), 383
 - granulocytes, 382–383
 - hematopoietic stem and progenitor cells, 384–385
 - immunohematologic disorders, 400
 - autoimmune hemolytic anemias (AIHA), 401–402
 - dysproteinemias, 406
 - hemolytic disease of the newborn (HDN), 400
 - maternal immune thrombocytopenic purpura (maternal ITP), 401

- neonatal alloimmune thrombocytopenia (NAIT), 400–401
- therapeutic apheresis in the management of, 402–405
- thrombotic microangiopathies, 406
- indications for additional modifications of cellular blood components, 379–380t
- major blood groups, laboratory determination of, 373–374
 - forward grouping, 373
 - reverse grouping, 374
- platelet refractoriness, 381–382
- platelets, 378, 381
- prothrombin complex concentrate (PCCs), 385
- red blood cells (RBCs), 378
- Rh immune globulin (RhIg), 385, 389
- storage and infusion of blood products, 377
- transfusion reactions and adverse sequelae, 389, 390t
 - acute hemolytic transfusion reaction, 390–391
 - alternatives to allogeneic blood transfusion, 396, 397t
 - anaphylactic transfusion reactions, 392
 - bacterial contamination and sepsis, 393–394
 - causes of hemolysis, 391–392
 - delayed hemolytic transfusion reaction (DHTR), 391
 - disseminated intravascular coagulation (DIC), 400
 - febrile nonhemolytic transfusion reactions (FNHTR), 395
 - hemolysis associated with passive antibody infusion, 391
 - hemosiderosis, 395
 - hypotension associated with transfusion, 395
 - massive transfusion, 396–400
 - mild allergic/ urticarial transfusion reactions, 394
 - pharmacologic hemostatic agents, 398–399t
 - posttransfusion purpura (PTP), 394–395
 - sickle cell hemolytic transfusion syndrome, 391
 - transfusion-associated circulatory overload (TACO), 393
 - transfusion-associated graft-versus-host disease (TA-GVHD), 393
 - transfusion-related acute lung injury (TRALI), 392–393
 - transfusion-transmitted infections, 396

- whole blood, 377
- Blue sclerae, 1
- Bone marrow (BM) failure syndromes, 66
 - acquired, 66–67
 - acquired aplastic anemia, 67
 - clinical features, 68
 - definitive treatments, 72–74
 - diagnosis and differential diagnosis, 69–71
 - etiology and pathophysiology, 67–68
 - agranulocytosis, 78
 - diagnosis and treatment, 78
 - etiology and pathophysiology, 78
 - congenital amegakaryocytic thrombocytopenia, 86
 - constitutional, 78, 81–82t
 - Diamond–Blackfan anemia, 84
 - hematologic findings, 85
 - treatment, 85
 - dyskeratosis congenita, 83
 - diagnostic testing for aplastic anemia with short telomeres, 84
 - Fanconi anemia, 79
 - androgens, 83
 - clinical features, 80
 - diagnostic tests, 80
 - hematologic presentations and cancer predisposition, 80–82
 - hematopoietic growth factors, 83
 - human papillomavirus vaccination (HPV), 83
 - stem cell transplantation (SCT) and supportive care, 82–83
 - paroxysmal nocturnal hemoglobinuria (PNH), 74
 - clinical features, 76
 - diagnosis, 76
 - etiology and pathophysiology, 76
 - treatment, 76
 - pure red cell aplasia (PRCA), 77
 - clinical features and diagnosis, 77

- etiology and pathophysiology, 77
- treatment, 78
- › severe congenital neutropenia and cyclic neutropenia, 86
- › Shwachman–Diamond syndrome, 85
 - clinical diagnostic criteria, 85–86
 - treatment, 86
- › Venn diagram of the relationship among, 67f
- › Bone marrow aspirate/ biopsy, 448
 - bone marrow biopsy, 449
 - bone marrow iron stores, 450
 - cells anomalously present in the marrow, 450
 - indications, 448
 - lymphocytes and plasma cells, 449–450
 - marrow cellularity, 449
 - megakaryocyte number and appearance, 449
 - myeloid and erythroid cells, 449
 - risks, 448
 - technique, 448
- › Bone marrow biopsy, 93
- › Bone marrow failure, 361
 - syndromes, 135
 - thrombocytopenia, 290–291
- › Bone marrow hematopoiesis, 105f
- › Bone marrow transplantation, 35
 - Gratwohl score for predicting outcome after, 193
 - versus immunosuppression, 72t
- › Bone morphogenetic protein (BMP), 410
- › Bortezomib, 245, 257, 259–260, 261t, 262, 263, 269
- › B-precursor acute lymphoblastic leukemia, risk-group assignment in, 171t
- › B-Prolymphocytic leukemia (B-PLL), 230
- › BRAFV600E mutation, 230
- › Breakpoint cluster region (BCR), 181
- › Breast cancer, 270
- › Brentuximab, 219, 237

- › Bruton tyrosine kinase (BTK) inhibitor, 205, 208
- › Budd–Chiari syndrome (BCS), 77, 342
- › Burkholderia cepacia, 124
- › Burkitt leukemia, 170
- › Burkitt-Like Lymphoma With 11q Aberration
- › Burkitt lymphoma (BL), 234–235, 246
- › Burst forming units-erythrocyte derived (BFU-E-derived) colony, 106
- › Busulfan, 116, 274, 348
- › Butorphaol, 44
- › ¹⁴C serotonin, 362
- › C282Y, 409
 - homozygosity, 413, 416
 - mutation, 410
- › Cachexia, 68
- › Calcium dependent ileal membrane antagonism, 15
- › Calreticulin gene (CALR), 104, 111
- › Candida albicans infections, 124
- › Candida fungal infections, 278
- › Candida glabrata, 124
- › Cardiac bypass, 333
- › Cardiovascular manifestations, 47
- › Carfilzomib, 260, 261t, 262, 269
- › Caveat, 20
- › CD3, 213
- › CD7, 213
- › CD8, 471
- › CD15, 213
- › CD18 protein antigen, 121–123
- › CD19, 205, 213
- › CD20, 213, 220
- › CD30, 213, 220
- › CD34 cell, 67
- › CD38, 202, 263

- › CD45, 468
- › CD49d, 200, 202
- › CD59, 76
- › CD79a, 213
- › CEBP^Abiallelic gene, 156t
- › Cellavision, 444, 446f
- › Cells anomalously present in the marrow, 450
- › Central venous catheter (CVC), 140, 141
- › Cephalosporin, 49
- › Chelation, iron overload and, 54–55
- › Chemoimmunotherapy (CIT), 203–205
- › Chemotherapy-induced anemia, ESA for, 8–9
- › Chemotherapy-induced thrombocytopenia, 361
- › Childhood hematologic diseases, 131
 - anemia, 131, 132t
 - macrocytic anemias, 135–136
 - microcytic anemias, 131–134, 132t
 - normocytic anemias, 134–135
 - bleeding, 136, 137t
 - acquired coagulation disorders, 136–139
 - inherited factor deficiencies, 139
 - platelet disorders, 139–140
 - hematologic manifestations of systemic conditions, 143–144
 - leukocytosis, 143
 - neutropenia, 142–143
 - normal hematologic parameters in children, 132t
 - thrombosis, 140
 - acquired prothrombotic disorders, 141–142
 - congenital prothrombotic disorders, 140–141
 - transfusion support, 144
 - specialized blood products to prevent toxicities, 145
- › Chimeric antigen receptors (CARs), 175
- › Chlorambucil, 203, 205, 348
- › Chloromas, 194

- › Chlorosis, 1
- › CHOP, 243, 245
- › Chronic alcoholics, 12
- › Chronic granulomatous disease (CGD), 33, 124–125
- › Chronic GVHD, 280–281
- › Chronic HCV infection, 421t
- › Chronic idiopathic neutropenia, 128
- › Chronic idiopathic unconjugated hyperbilirubinemia, 24
- › Chronic inflammatory diseases
 - treatment of anemia associated with, 9
- › Chronic kidney disease (CKD)
 - ESA administration in patients with, 4
 - intravenous (IV) iron therapy, 7
- › Chronic lymphocytic leukemia (CLL), 198, 277
 - background, 198
 - chemoimmunotherapy options in, 204t
 - clinical manifestations, 198–199
 - complications of chronic lymphocytic leukemia and their treatment, 208
 - autoimmune manifestations, 208
 - infections, 208–209
 - Richter's transformation, 209
 - diagnosis, imaging, and laboratory studies, 199
 - bone marrow biopsy, 200
 - complete blood count and blood smear, 199
 - computed tomography and positron emission tomography, 200
 - immunophenotyping and flow cytometry, 199
 - lymph node biopsy, 200
 - other laboratory evaluations, 199–200
 - differential diagnosis, 200
 - etiology and pathogenesis, 198
 - fluorescence in situ hybridization (FISH), 201
 - immunophenotype of B-cell CLL, 201t
 - natural history, staging, and prognosis, 200
 - CD38, 202

- CD49d, 202
- cytogenetics, 201–202
- immunoglobulin variable region heavy chain gene mutation, 201
- lymphocyte doubling time (LDT), 202
- somatic mutations, 202
- ZAP-70, 202
- Rai Clinical Staging System, 201t
- treatment, 202
 - assessing response to therapy, 203
 - cellular therapies, 205
 - chemoimmunotherapy (CIT), 203–205
 - combination of small molecules and conventional agents, 208
 - individualized therapy and predictive biomarkers, 208
 - paradigm of watchful waiting, 202–203
 - small molecules, 205
 - unique aspects of targeted agents in chronic lymphocytic leukemia, 205–208
- treatment trials using small molecules in, 206–207t
- Chronic lymphocytic leukemia (CLL), 92, 205–208, 230, 271, 359, 444
- Chronic lymphoproliferative disorders of natural killer cells, 235
- Chronic myelogenous leukemia (CML), 104, 181, 271
 - accelerated phase (AP), 182, 186
 - blastic phase (BP), 182, 186
 - characterization of, 187
 - chronic phase, 185–186
 - commonly used drugs to treat, 190t
 - course and clonal evolution of, 186f
 - diagnosis, 183, 185f
 - blood count, 183
 - blood film, 183
 - bone marrow, 183
 - chromosome analysis, 183
 - differential diagnosis, 184
 - molecular diagnosis, 183–184
 - disease monitoring in, 190f

- epidemiology, 181–182
- evolving standard of care, 195
 - treatment-free remission, 195
 - tyrosine kinase inhibitors, trigger to change, 195
 - tyrosine kinase inhibitors as first-line treatment, 195
- pathophysiology, 182
 - BCR-ABL1 oncprotein, 182
 - clonal dominance, 182
 - genomic instability, 182
 - leukemic hematopoiesis, 182
- presentation, 182
 - classic presentation, 182
 - rare presentations, 183
 - typical presentation in the developed world, 183
- prognostic factors, 187–188
- special issues in CML management, 194
 - chloromas, 194
 - leukostasis, 194
 - myelofibrosis, 194
 - pregnancy in CML, 194
 - psychological response of patients with CML, 194–195
 - splenic infarcts, 194
- treatment, 188
 - of accelerated phase, 193
 - allogeneic stem cell transplantation, 191–193
 - of blastic phase, 193
 - of newly diagnosed CML in chronic phase, 188–191
- treatment algorithm for, 189f
- Chronic myeloid leukemia (CML), 275–276
- Chronic myelomonocytic leukemia (CMML), 95, 104, 117
 - clinical features, 117
 - diagnostic testing, 117
 - epidemiology, 117
 - pathophysiology, 117

- staging and prognostic features, 117–118
- treatment, 118
- › Chronic myelomonocytic leukemia with eosinophilia, 435
- › Chédiak–Higashi syndrome (CHS), 125, 142
- › Circulating transferrin receptors, 3
- › Citrate, 304, 403
- › Cladribine, 244
- › Classical Hodgkin lymphoma (CHL), 212
- › Clofarabine, 162
- › Clonal cytopenias of undetermined significance (CCUS), 93
- › Clonal eosinophilic disorders, 433
 - acute leukemias, 435
 - chronic myelomonocytic leukemia with eosinophilia, 435
 - subtypes, 435–436
 - FIP1L1-PDGFR- α hypereosinophilic disorders, 436
 - idiopathic hypereosinophilic syndrome, 436
 - T-cell clonal disorders, 434
- › Clonal hematopoiesis and myelodysplastic syndrome, 91f
- › Clonal hematopoiesis of indeterminate potential (CHIP), 92
- › Clonal hypereosinophilic disorders, 435
- › Clopidogrel, 333
- › Clostridium perfringens, 27
- › Coagulation, 307
 - coagulation cascade, 308, 309f
 - coagulation factors, 308
 - common coagulation tests, 308–310
 - differential diagnosis of abnormal coagulation tests, 311
 - conditions associated with a prolonged aPTT, 316–317
 - conditions associated with a prolonged PT, 317–318
 - conditions associated with bleeding and normal coagulation tests, 318–319
 - conditions associated with prolonged aPTT and PT, 318
 - specialized coagulation tests, 310–311
- › Coagulation factor IX, 388t
- › Coagulation factor preparations, 388t

- › Coagulation factor replacement products, 314
- › Cobalamin. See Vitamin B₁₂
- › CODOX-M regimen, 248t
- › Cold agglutinin syndrome, 402
- › Collagen, heritable defects of, 318
- › Collagen vascular diseases, 144
- › Colony forming units-erythrocyte-derived (CFU-E-derived) colony, 106
- › Colony-stimulating factor (CSF), 158, 163, 239
- › Combination antiretroviral therapy (cART), 223
- › Common variable immunodeficiency (CVID), 238
- › Complete blood count (CBC), 27, 41, 199, 440
 - automated complete blood counters, 440–441
 - platelets, 443
 - red cell parameters, 441–443
 - sources of artifact, 443–444
 - white blood cell parameters, 443
- › Complete remission (CR), 148, 157
- › Complete response (CR), 256
- › Compression ultrasonography (CU), 429
- › Computed tomography (CT), 200, 240, 360
- › Computed tomography pulmonary angiography (CTPA), 363–364, 429
- › Computed tomography venography (CTV), 340
- › Congenital amegakaryocytic thrombocytopenia, 86, 140, 291
- › Congenital disorders of signal transduction, 336
- › Congenital erythropoietic porphyria (CEP), 63
- › Congenital FVIII deficiency, 313
- › Congenital neutropenias, 127–128, 436–437
- › Congenital prothrombotic disorders, 140–141
- › Conjugated Estrogens, 398t
- › Constitutional bone marrow failure syndromes, 78
- › Consultative hematology, 424
 - clonal eosinophilic disorders, 433
 - acute leukemias, 435
 - chronic myelomonocytic leukemia with eosinophilia, 435

- FIP1L1-PDGFR- α hypereosinophilic disorders, 436
- idiopathic hypereosinophilic syndrome, 436
- T-cell clonal disorders, 434
- hematologic complications of pregnancy, 424
 - anemia, 424
 - sickle cell disease, 424–425
 - thrombocytopenia, 425
 - venous thromboembolism (VTE), 428
- neutropenia, 436
 - extrinsic disorders, 437
 - intrinsic disorders, 436
- tropical disease, manifestations of, 431
 - malaria, 431
- › Coombs test, 135, 373
- › Copper deficiency, 4
- › Cord blood, 285, 385
- › Corrected count increment (CCI), 381
- › Corticosteroids, 73, 76, 127, 140, 250
 - for idiopathic hypereosinophilic syndrome, 436
 - for ITP in pregnant patients, 425
- › Coulter principle, 440
- › C-reactive protein (CRP), 134, 359
- › Crohn's disease, 124
- › Cryoglobulinemia, 461
- › Cryohemolysis test, 34
- › Cryoprecipitate, 145, 323, 383–384
- › CT pulmonary angiography (CTPA), 363
- › Cutaneous T-cell lymphoma (CTCL), 236
- › CXCR4 chemokine receptor, 129
- › Cyclic neutropenia (CN), 86, 142, 437
- › Cyclic thrombocytopenia, 292
- › Cyclin D1 overexpression, 232
- › Cyclophosphamide, 127, 172t, 173t, 203, 217, 250, 257, 261t, 262, 269
- › Cyclosporine (CsA), 73, 74, 96, 277

- › Cytarabine, 158, 172t, 173t
- › Cytochrome c mutations, 336
- › Cytogenetics, 201–202, 448
- › Cytomegalovirus, 74, 282
- › Cytopenias, 96, 110
 - differential diagnosis of, 71f
- › Cytoskeleton proteins, 33
- › Cytotoxic chemotherapy, 96
- › Cytotoxic lymphocytes (CTLs), 67

- › Dabigatran, 367, 368
- › Dactylitis, 50, 135
- › Dalteparin, 344, 346, 358
- › Dalteparin sodium, 351
- › Danazol, 73
- › Daratumumab, 263, 269
- › Dasatinib, 175, 188, 193, 195
- › Daunorubicin, 158
- › DDAVP, 327, 333, 399t
- › D-dimer, 341–342, 351, 458
- › Decitabine, 98
- › Deep venous thrombosis (DVT), 338, 358, 360, 363
 - imaging of, 340
 - inferior vena cava filters in the treatment of, 351
 - in pregnancy, 428, 429
 - and pulmonary embolism, 338–340
 - in sites other than the distal veins of the lower extremities, 342–343
- › Deferasirox, 55, 424
- › Deferiprone, 55, 418
- › Deferoxamine, 54, 424
- › Deferoxamine and/ or deferiprone, 418
- › Del 17p/ 11q, 202
- › Delayed hemolytic transfusion reaction (DHTR), 391
- › Denileukin diftitox, 250

- › Derivative and recombinant coagulation factors, 389
- › Desmopressin, 139, 458
- › Dexamethasone, 172t, 173t, 253, 259, 260, 261t, 262, 263, 293
- › Diamond–Blackfan anemia (DBA), 77, 84, 135
 - hematologic findings, 85
 - treatment, 85
- › Diamond–Blackfan syndrome, 134
- › Diepoxybutane (DEB), 136
- › Dietary iron, 2, 5
- › Diferric transferrin, 3, 410
- › Diffuse intravascular coagulation (DIC), 427–428
- › Diffuse large B-cell lymphoma (DLBCL), 223, 224, 233, 234, 239, 245
- › Dihydroxyacetone phosphate, 32
- › Dilute prothrombin, 345
- › Dilute Russell viper venom time (DRVVT), 345
- › Dimethyl sulfoxide (DMSO), 384
- › 2,3-Diphosphoglycerate (2,3-DPG), 32
- › 2,3-Diphosphoglycerate mutase (DPGM) deficiencies, 32
- › Diphyllobothrium latum, 14
- › Dipyridamole, 333
- › Direct antiglobulin test (DAT), 33, 199, 369, 373
- › Direct thrombin inhibitor (DTI), 296, 318, 362, 367
- › Disorders of Hemostasis I. See Coagulation
- › Disorders of hemostasis II, 321
 - disseminated intravascular coagulation (DIC), 321
 - conditions associated with, 322t
 - diagnosis, 322–323
 - ISTH diagnostic scoring system for, 322t
 - pathophysiology, 321
 - presentation, 321
 - treatment, 323–324
 - qualitative platelet disorders, 328
 - acquired disorders, 331–333
 - inherited disorders, 333–336

- platelet function testing, 329–330
- review of hemostasis and the role of platelet biochemistry, 329
- von Willebrand disease (VWD)
 - diagnosis, 326–327
 - epidemiology, 324
 - laboratory evaluation of, 326t
 - pathophysiology and classification, 324–325
 - pregnancy and, 328
 - presentation, 326
 - treatment, 327, 328t
- › Disseminated intravascular coagulation (DIC), 134, 139, 151, 157, 321, 400
 - conditions associated with, 322t
 - diagnosis, 322–323
 - ISTH diagnostic scoring system for, 322t
 - pathophysiology, 321
 - presentation, 321
 - thrombocytopenia in, 301–302
 - treatment, 323–324
- › Diuretics, 145, 193
- › DNA methyltransferase inhibitors, 98–99
- › DNMT3A gene, 92, 156t
- › Donath-Landsteiner antibody, 402
- › Donor–host killer immunoglobulin-like receptor (KIR) incompatibility, 285
- › Down syndrome, 168, 175
- › Doxorubicin, 172t, 173t, 217, 250t, 257, 261t, 269
- › Drug-induced isolated neutropenia, mechanisms of, 439t
- › Drug-induced ITP (DITP), 304
- › Drug-induced neutropenia, 126, 438–439
- › Drug-induced thrombocytopenia, 304
- › Drug-induced thrombotic microangiopathies, 304
- › Duffy antigen and receptor for chemokine (DARC), 129
- › Duodenal-type follicular lymphoma, 232
- › Dynamic International Prognostic Scoring Scale (DIPSS) score, 116
- › Dyserythropoietic anemias, 421t

- › Dyskeratosis congenita (DKC), 66, 83, 84, 290
- › Dysproteinemias, 406

- › EBV⁺ DLBCL, NOS, 234
- › Eculizumab, 35, 77, 301, 347
- › Edoxaban (Savaysa), 369
- › Ehlers–Danlos syndrome, 318, 335
- › Elliptocyte, 28t
- › Elotuzumab, 263, 269
- › Eltrombopag, 73, 97, 294, 335
- › Embden–Meyerhof pathway, 30
- › Endogenous erythropoietin stimulation, 4
- › End-organ damage associated with hypereosinophilia, 433t
- › End-stage renal disease (ESRD), 8
- › Enolase, 32
- › Enoxaparin, 344, 346, 357t, 358, 359, 361, 368
- › Enteropathy-associated T-cell lymphoma (EATL), 236
- › Enzyme deficiencies, 29–30, 134
- › Enzyme-linked immunosorbent assay (ELISA), 295, 341, 361
- › EORTC trial, 98
- › Eosin-5'-maleimide-binding (EMA) test, 34
- › Eosinophilia, 432, 433
 - diseases commonly associated with, 434t
- › Eosinophils, 433, 434
- › Epsilon aminocaproic acid (Amicar), 327
- › Epstein–Barr virus (EBV), 67, 74, 143, 212, 224
- › Epstein–Barr Virus (EBV)–associated lymphoproliferative disorder, 283
- › Eptifibatide, 331
- › Erwinia Asparaginase, 172t
- › Erythrocytapheresis/ red cell exchange, 404
- › Erythrocytes, 23, 30, 53, 466–467
 - morphology and associated pathology, 28t
- › Erythrocyte sedimentation rate (ESR), 134
- › Erythroferrone (ERFE), 410

- › Erythroid cytoskeleton abnormalities, 29
- › Erythroid enzymopathies, 29–30
 - adenosine deaminase (ADA), 33
 - glutathione metabolism, enzymes involved in, 30–31
 - glycolysis, enzymes involved in, 31–32
 - heme oxygenase-1, 33
 - lecithin cholesterol acyltransferase (LCAT), 33
 - pyrimidine 5' nucleotidase deficiency, 32–33
- › Erythroid hyperplasia, 3
- › Erythroid membrane defects, 33–34
- › Erythropoiesis, 1, 3, 27, 35
 - iron-deficient, 3
 - Erythropoietic activity, 4
 - Erythropoietic protoporphyrina (EPP), 62, 64–65
 - Erythropoietin (EPO), 8, 9, 23, 55, 96
 - assays, 453
 - production, conditions related to, 109t
 - receptor signaling, 104, 116
 - Erythropoietin-stimulating agent (ESA), 4
 - treatment, in patients with CKD/ ESRD, 8
- › Escherichia coli, 124
 - E. coli 0157:H7, 299, 301
- › Essential thrombocythosis (ET), 104, 111
 - clinical features, 112
 - diagnostic testing, 112
 - epidemiology, 111
 - pathophysiology, 111–112
 - treatment, 112–113, 114t
- › Ethylenediaminetetraacetic acid (EDTA), 303, 443
- › Etoposide, 158, 173t, 217, 219, 250t
- › Etrombopag, 96
- › Euglobulin clot lysis time (ECLT), 311
- › European Collaboration on Low-Dose Aspirin in PV (ECLAP) trial, 110
- › Extracorporeal circulation-related thrombocytopenia, 302

- › Extracorporeal photochemotherapy, 404
- › Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), 231
- › Extranodal natural killer/ T-cell lymphoma, nasal type, 236
- › Extraosseous plasmacytoma, 231
- › Extravascular hemolysis, 23, 431
- › Eye disease, in patients with SCD, 47

- › Factor deficiencies, 383, 456
- › Factor VII concentrate, 388t
- › Factor VIII, 359
 - deficiency, 139
- › Factor IX, 388t
 - deficiency, 139
- › Factor Xa, 369
- › Factor XI deficiency, 316
- › Factor XIII deficiency, 318
- › Familial hemophagocytic lymphohistiocytosis, 305
- › Fanconi anemia (FA), 79, 90, 136, 290
 - androgens, 83
 - clinical features, 80
 - diagnostic tests, 82
 - hematologic presentations and cancer predisposition, 80–82
 - hematopoietic growth factors, 83
 - human papillomavirus vaccination (HPV), 83
 - stem cell transplantation and supportive care, 82–83
- › Favism, 30
- › Febrile nonhemolytic transfusion reactions (FNHTR), 395
- › Felty's syndrome, 438
- › Feraheme, 8t
- › Ferric iron, 2
- › Ferric reductase, 2
- › Ferritin screening, 416
- › Ferrlecit, 7, 8t
- › Ferroportin, 2–3, 420

- › Ferrous iron, 2–3
- › Fetal death syndrome, 428
- › Fetal hemoglobin induction, 55
- › Fibrinogen, 387t, 457–458
- › Fibrinolysis inhibitors, 323–324
- › Fibrinolytic therapy, 350
 - for pulmonary embolism, 352
- › Fibrin split products (FDPs), 458
- › Fibrotic transformation, 110
- › Fifth Disease. See Parvovirus infection
- › Filamin A disorders, 335
- › Filgrastim, 250t
- › FIP1L1-PDGFR- α hypereosinophilic disorders, 436
- › Fish tapeworm, 14
- › Flow cytometry, 93, 199, 440, 448, 465, 477
 - applications in hematology, 475–476
 - commonly used leukocyte antigens used in, 473t
 - data presentation and interpretation, 465–475
 - evaluation of erythrocytes by, 476
 - evaluation of platelets, 476
- › FLT3-ITD gene, 156t
- › Fludarabine, 203, 439
- › Fluorescence in situ hybridization (FISH), 93, 183, 201, 448
- › Fluorodeoxyglucose-positron emission tomography (FDG-PET), 239
- › Folate, 12, 35
 - biology and dosing, 13t
 - daily adult requirement for, 12
 - deficiency of, 12, 19–20, 135, 361, 424
 - response to correction of, 21
 - metabolic pathways involving, 13f
 - metabolic roles of, 12–14
 - patient populations at risk, 16
 - shortage of, 12
 - treatment/ response, 20–21

- › Folic acid deficiency
 - causes of, 15
 - clinical presentation, 16, 16t
 - and cooked vegetables, 15
 - development of, 15–16
 - and fresh vegetables, 15
 - laboratory evaluation, 17
 - hematologic abnormalities, 17
 - serum methylmalonic acid and homocysteine, 19
 - serum vitamin concentrations, 17–19
 - therapeutic trial, 19
 - laboratory tests for, 18t
- › Follicular and mantle cell prognostic index, 242
- › Follicular lymphoma (FL), 232
- › Follicular T-cell lymphoma, 238
- › Fondaparinux, 344, 350, 351, 356, 357t, 361, 429
- › Forward-directed scatter (FSC), 440–441
- › Free erythrocyte protoporphyrin (FEP), 133
- › French–American–British (FAB) classification of acute myeloid leukemia, 152t
- › Fresh-frozen plasma (FFP), 139, 145, 301, 316, 323, 383
- › Fructose-1,6-bisphosphate, 32
- › Fructose-6-phosphate, 32
- › Functional iron deficiency, 1, 4–5
 - treatment of, 8
- › Gall bladder problems with sickle cell disease, 48–49
- › Gamma herpes viruses, 224
- › Gastrointestinal consultation, 6
- › Gaucher cell, 144
- › Gelatinase, 121
- › Gene expression profiling (GEP), 224, 238
- › Genetics-based discoveries, 23
- › Gene transcription signatures, 223
- › Genotype screening, 416

- › German Hodgkin Study Group (GHSG), 216
- › Germ cell tumors, auto-HSCT, 270
- › Germinal center B-cells (GCB), 232, 233
- › Germ-line GATA2 gene mutations, 66
- › Gestational thrombocytopenia, 304
- › Gilbert's syndrome, 24, 49
- › Glanzmann thrombasthenia (GT), 140, 335
- › α-Globin chains, 38
- › α-Globin gene deletion haplotypes, 38
- › α-Globin structural variants, 38
- › Glucocorticoids, 279
- › Glucose-6-phosphate dehydrogenase (G6PD) deficiency, 24, 29, 30, 32, 134, 455
 - common drugs and chemicals to avoid in patients with, 31t
- › γ-Glutamylcysteine synthetase, 31
- › Glutathione metabolism, enzymes involved in, 30–31
- › Glutathione peroxidase (GSH-Px), 31
- › Glutathione reductase, 31
- › Glutathione reduction, 30
- › Glutathione synthetase deficiency, 31
- › Glyceraldehyde-3-phosphate, 32
- › Glycolysis (Embden–Meyerhof pathway), 30
 - enzymes involved in, 31–32
- › Glycosylphosphatidylinositol-anchored proteins, 29
- › Glycosylphosphoinositol anchor (GPI), 76
- › Golgi-staining patterns, 213
- › Graduated compression stocking (GCS), 365
- › Graft failure, 283
- › Graft-versus-host disease (GVHD), 72, 74, 160, 192, 271, 274, 385
- › Graft-versus-leukemia (GVL) effect, 159, 271
- › Graft-versus-tumor (GVT), 268, 271
- › Gram-negative bacterial infections, 126
- › Granulocyte colony stimulating factor (G-CSF), 86, 96, 127, 128, 142, 163, 177, 268, 384
 - G-CSF-mobilized stem cells, 72

- › Granulocyte-macrophage colony-stimulating factor, 163
- › Granulocyte monocyte colony-stimulating factor, 78
- › Granulocytes, 378t, 382–383
- › Gratwohl score for predicting outcome after bone marrow transplantation, 193
- › Gray platelet syndrome, 335
- › Gunther's disease. See Congenital erythropoietic porphyria (CEP)

- › Haemophilus influenzae, 35
- › Hairy cell leukemia (HCL), 230
- › Ham test, 70
- › Hand–foot syndrome, 50
- › Haptoglobin, 456
- › Hashimoto thyroiditis, 231
- › Hb A₂ quantitation, 455
- › Hb Barts, 38
- › HbC, 41
- › Hb Constant Spring, 41
- › HbE, 41
- › Hb F quantitation and Hb F cells, 455
- › Hb H, 38
- › Hb Lepore, 41
- › HbOArab, 41
- › μ-Heavy chain disease (HCD), 231
- › Heinz bodies, 28t, 30, 31
- › Helicobacter pylori, 15
- › Helminth infections, 432
 - and eosinophilia, 433
 - iron deficiency associated with, 433t
- › Hematologic abnormalities, 17, 86
- › Hematologic malignancies, evaluation of, 421t, 459
 - immunofixation, 460–461
 - quantitative serum immunoglobulins, 461
 - serum cryoglobulins, 461
 - serum free light chains, 461

- serum lactate dehydrogenase, 462
- serum protein electrophoresis, 459–460
- serum uric acid, 462
- serum viscosity, 461–462
- serum β 2-microglobulin, 462
- urine protein electrophoresis, 460
- Hematologic manifestations of systemic conditions, 143–144
- Hematopoiesis, 23, 67, 105f
 - clonal, 91
- Hematopoietic growth factors, 83, 163
- Hematopoietic progenitor cells, 384–385
- Hematopoietic stem cell transplantation (HSCT), 55, 71, 72, 76, 158, 159–160, 268
 - allogeneic HSCT. See Allogeneic hematopoietic stem cell transplantation (allo-HSCT)
 - autologous-HSCT. See Autologous-hematopoietic stem cell transplantation (auto-HSCT)
 - indications for, 272t
- Hematopoietic stem cells, 384–385, 466, 468, 471, 476
- Heme, 2, 60
- Heme of meat, 5
- Heme oxygenase-1, 33
- Hemochromatosis, 409
 - clinical features and diagnosis, 412
 - common clinical presentation, 413, 414t
 - new diagnostic definition, 412–413
 - epidemiology, 409
 - genetic basis, 409
 - future challenges, 420
 - hemochromatosis subjects as blood donors, 419–420
 - laboratory testing, 413
 - liver biopsy, 415
 - radiographic and other tests, 415
 - non-HFE iron overload, 420, 421–422t
 - pathophysiology, 410

- hepcidin, 410
- HFE localization and function, 410–411
- iron homeostasis, 410
- iron overload disorders and hepcidin deficiency, 410
- population screening, 415, 417t
 - laboratory screening, 416
 - screening of family members of C282Y homozygotes, 416
- prognosis and response to therapy, 419
- treatment, 416
 - arthritis, endocrine replacement, vaccinations, and cancer surveillance, 418–419
 - dietary and lifestyle counseling, 419
 - evaluation of anemia during phlebotomy therapy, 418
 - guidelines for phlebotomy therapy, 417–418
 - phlebotomy therapy, 416–417
 - use of iron chelators, 418
- Hemodialysis, 301
- Hemoglobin, 4, 9
 - electrophoresis, 454
 - fetal hemoglobin induction, 55
 - mean corpuscular hemoglobin (MCH), 43
 - normal and variant hemoglobin, 39t
 - oxidation of, 30
 - sickle hemoglobin (HbS), 40
 - unstable, tests for, 455
- Hemoglobinopathies, 23, 38, 40, 134
 - HbC, 41
 - Hb Constant Spring, 41
 - HbE, 41
 - Hb Lepore, 41
 - HbOArab, 41
 - sickle cell disease, 40–41
- Hemojuvelin, 410
- Hemolysis
 - associated with passive antibody infusion, 391

- cessation of, 29
 - enzymopathies associated with, 32–33
 - immune-mediated, 23, 33
 - intravascular, 23, 27–29, 76
 - nonimmune, 33
- Hemolysis, elevated liver enzymes, and low platelets syndrome (HELLP), 307, 323–324, 426–427
- Hemolytic anemia, 23
 - acute intravascular hemolysis, 27–29
 - causes of, 23
 - clinical approach to, 24, 26f
 - history, 24–27
 - physical findings/ examination, 27
 - differential diagnosis of, 23–24, 25t
 - enzyme defects in, 29
 - erythrocyte morphology and associated pathology, 28t
 - erythroid enzymopathies, 29–30
 - adenosine deaminase (ADA), 33
 - enzymes involved in glutathione metabolism, 30–31
 - enzymes involved in glycolysis, 31–32
 - heme oxygenase-1, 33
 - lecithin cholesterol acyltransferase (LCAT), 33
 - pyrimidine 5' nucleotidase deficiency, 32–33
 - erythroid membrane defects, 33–34
 - etiology of, 23–24
 - extrinsic causes of, 24f
 - glucose-6-phosphate dehydrogenase (G6PD) deficiency, 30
 - intrinsic causes of, 23, 24f
 - membrane defects in, 29
 - treatment options for, 34–35
 - Hemolytic anemias, abnormal hemoglobins and, 454
 - glucose-6-phosphate dehydrogenase (G6PD), 455
 - Hb A₂ quantitation, 455
 - Hb F quantitation and Hb F cells, 455

- hemoglobin electrophoresis, 454
- high-performance liquid chromatography and isoelectric focusing, 454–455
- serum haptoglobin, 456
- sickle solubility test, 455
- tests for unstable hemoglobins, 455
- urine hemosiderin, 456
- Hemolytic disease of the newborn (HDN), 136, 373, 400
- Hemolytic transfusion reactions, 390–391
- Hemolytic uremic syndrome (HUS), 134, 296, 406, 427
- Hemopexin, 29
- Hemophagocytosis, 305
- Hemophilia, 139
 - hemophilia A and B, 313–316
 - hemophilia C, 316
- Hemophilus influenza, 44
- Hemorrhagic cystitis, 278, 283
- Hemorrhagic disease of the newborn (HDN). See Vitamin K deficiency bleeding (VKDB)
- Hemosiderin, 456
- Hemosiderosis, 395
- Hemostasis and coagulation assays, 456
 - activated partial thromboplastin time, 456–457
 - anti-Xa assay, 457
 - automated coagulation analyzers, 456
 - D-dimers, 458
 - fibrinogen, 457–458
 - prothrombin time (PT), 457
 - specific factor assays, 457
 - tests for hypercoagulability, 458
 - tests for hyperfibrinolysis, 458–459
 - tests of platelet function, 458
 - thrombin time (TT), 457
- Heparin, 295, 317, 323, 343, 347, 350, 361, 456–457
- Heparin-induced thrombocytopenia (HIT), 294–296, 297t, 343–345, 361, 364, 429

- pretest prediction of, 343t
- › Hepatic problems with sickle cell disease, 48–49
- › Hepatic vein thrombosis
 - prevalence of prothrombotic states in, 342t
- › Hepatitis C virus (HCV), 224
- › Hepatoerythropoietic porphyria (HEP), 64
- › Hepatosplenic T-cell lymphoma, 236
- › Hepcidin, 2, 410
 - deficiency, 410
 - iron homeostasis regulation by, 411f
- › Hepcidin levels and iron-related diseases, 5, 5t
- › Hepcidin-producing adenomas, 4
- › Herbivorous animals, bacteria in the gut of, 12
- › Hereditary coproporphyrina, 64
- › Hereditary elliptocytosis (HE), 34
- › Hereditary hemochromatosis (HH). See Hemochromatosis
- › Hereditary hemorrhagic telangiectasia, 318
- › Hereditary persistence of fetal hemoglobin (HPFH), 40
- › Hereditary pyropoikilocytosis, 34
- › Hereditary spherocytosis (HS), 33, 134
 - osmotic fragility test for, 34
- › Hereditary stomatocytosis, 34
- › Hexokinase deficiency, 32
- › Hexose monophosphate shunt, 30
- › HFE-hemochromatosis, 409
 - clinical features and diagnosis, 412–413
 - HFE localization and function, 410–411
 - HFE mutations, 409
- › HHV-8⁺ DLBCL, NOS, 234
- › High-density lipoprotein (HDL) levels, 33
- › High-dose cytarabine (HDAC)-based therapy, 158
- › High-grade B-cell lymphoma
 - not otherwise specified, 235
 - with MYC and BCL2 and/ or BCL5 rearrangements, 235

- › High-performance liquid chromatography (HPLC), 41, 454–455
- › High-throughput technologies, 224
- › HIV-related thrombocytopenia, 304
- › Hodgkin lymphoma (HL), 212, 226t
 - clinical features, 214
 - diagnostic RS cells seen in classic types of, 213f
 - dose intensity and treatment of the elderly patient, 220
 - epidemiology, 212–213
 - future directions, 220
 - histopathologic classifications of, 213–214
 - newly diagnosed classical HL, management of, 215
 - advanced-stage HL, 217–218
 - early-stage HL, 216–217
 - nodular lymphocyte-predominant HL, 219–220
 - pretreatment evaluation and staging, 215
 - prognostic features, 215
 - relapsed and primary refractory classical HL, 218–219
 - signaling pathways, genetics, and tumor microenvironment of, 214
 - staging of, 216t
 - survivorship, 219
- › Homocysteine (Hcy), 19, 19t
- › Homozygous sickle disease (HbSS), 40, 53
- › Horse ATG (hATG), immunosuppression with, 99
- › HPA-1a, 302
- › Human androgen receptor gene locus (HUMARA), 434
- › Human herpes virus-8 (HHV-8), 224
- › Human leukocyte antigen (HLA), 157, 375, 382
- › Human leukocyte antigen (HLA)-matched family members, 72
- › Human leukocyte antigen (HLA)-matched sibling donor, 125, 134
- › Human leukocyte antigen (HLA) typing, 93, 253
- › Human neutrophil antigens (HNA), 382
- › Human papillomavirus vaccination (HPV), 83
- › Human parvovirus B19, 46
- › Human platelet antigens (HPA), 381

- › Human T-lymphotropic virus type 1 (HTLV-1), 224
- › Hydroa vacciniforme-like lymphoproliferative disorder, 236
- › Hydroxyurea (HU), 50, 51, 52, 55, 110, 113, 116, 348
 - clinical use and monitoring of, 52t
- › Hypercoagulability
 - of malignancy, 346–347
 - tests for, 458
- › Hyperfibrinolysis, tests for, 458–459
- › Hyperhomocysteinemia, 349–350
- › Hyper IgM syndrome, 238
- › Hyperleukocytosis, 404
- › Hypermethylation, 98
- › Hyperreactive malarial splenomegaly (HMS), 431
- › Hypersplenism, 24, 139
- › Hypocellular MDS, 95
- › Hypochromia, 38, 39
- › Hypoferrremia, 4
- › Hypogammaglobulinemia, 177
- › Hypomethylating agents (HMAs), 98
 - disease-modifying, 98–99
- › Hyposthenuria, 49
- › Hypotension associated with transfusion, 395
- › Hypotensive reactions, 377
- › Iatrogenic immunodeficiency-associated lymphoproliferative disorders, 239
- › Ibrutinib, 205, 208, 230
- › Idelalisib, 205, 208, 230
- › IDH1 and IDH2 genes, 156t
- › Idiopathic cytopenia of undetermined significance (ICUS), 93
- › Idiopathic dysplasia of uncertain significance (IDUS), 93
- › Idiopathic hypereosinophilic syndrome, 436
- › Idiopathic neutropenia, 128–129
 - chronic, 437
- › Ifosfamide, 173t

- › Imatinib, 116, 188, 193, 194, 195, 436
 - monitoring response to, 188
- › Imatinib mesylate (Gleevec), 175
- › Immature reticulocyte fraction (IRF), 443
- › Immune-based spherocytosis, 33
- › Immune-mediated hemolysis, 23, 33
- › Immune-mediated platelet refractoriness, 381, 382
- › Immune neutropenias, 437–438
- › Immune-related neutropenia, 126–127
- › Immune thrombocytopenia (ITP), 292–294
- › Immune thrombocytopenic purpura (ITP), 139, 140, 145, 227t, 361, 425–426
 - maternal, 401
- › Immunodeficiency-associated lymphoproliferative disorders, 238–239
 - iatrogenic immunodeficiency-associated lymphoproliferative disorders, 239
 - lymphomas associated with HIV Infection, 239
 - lymphoproliferative disease associated with primary immune disorders, 238
 - posttransplant lymphoproliferative disorders, 239
- › Immunofixation, 460–461
- › Immunoglobulin G (IgG), 126, 177, 343
- › Immunoglobulin heavy chain variable (IGHV)-unmutated or minimally mutated B cells, 232
- › Immunoglobulin F (IgE), 434
- › Immunoglobulin M (IgM), 431
 - Ig M spike, 199
 - monoclonal gammopathy of undetermined significance (MGUS), 231
- › Immunoglobulin variable region heavy chain gene (IGHV), 200, 201
- › Immunohematologic disorders, 400
 - autoimmune hemolytic anemias (AIHA), 401–402
 - dysproteinemias, 406
 - hemolytic disease of the newborn (HDN), 400
 - maternal immune thrombocytopenic purpura (maternal ITP), 401
 - neonatal alloimmune thrombocytopenia (NAIT), 400–401
 - therapeutic apheresis in the management of, 402–405
 - thrombotic microangiopathies, 406

- › Immunomodulatory agent (IMiD), 260
 - and immunosuppression, 99
- › Immunophenotyping of maternal and paternal platelets, 140
- › Immunosuppression, 73, 99
- › Incidental thrombocytopenia of pregnancy, 425
- › Indirect antiglobulin test (IAT), 374
- › Indolent B-cell and T-cell lymphomas, 243–244
- › Indolent lymphoma treatment, 244
- › Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract, 236
- › Infection-and sepsis-related thrombocytopenia, 305
- › Infection prophylaxis, 158, 177
- › Infection-related neutropenia, 126
- › Infectious mononucleosis (IM), 143
- › Inferior vena cava (IVC) filters, 360
 - indications for, 362–363
 - in the treatment of deep venous thrombosis/ pulmonary embolus, 351
- › Inflammatory bowel disease (IBD), 7, 9, 15
- › Influenza vaccine, 135
- › Inherited disorders of platelet function, 333
 - congenital disorders of signal transduction, 336
 - cytochrome c mutations, 336
 - giant platelet disorders, 334
 - Bernard–Soulier syndrome (BSS), 334
 - Filamin A disorders, 335
 - gray platelet syndrome, 335
 - May–Hegglin anomaly, 334–335
 - MYH9-related disease, 335
 - Glanzmann thrombasthenia, 335
 - isolated laboratory-specific defects, 336
 - Scott syndrome, 335–336
 - storage pool disease (SPD), 335
- › Inherited factor deficiencies, 139
- › Inherited hypercoagulable states, 348
- › Inherited platelet disorders, 140

- › Inherited thrombophilias, 363
- › Initial cytotoxic induction chemotherapy, 162
- › Injectafer, 8t
- › Innate immune response, 224, 228
- › In situ follicular neoplasia, 232
- › In situ mantle cell neoplasia, 233
- › Integral membrane proteins, 33
- › Interferon (IFN), 116
 - IFN- α , 110, 113, 236
 - IFN- γ , 67
- › Intergroupe Francophone du Myelome/ Dana-Farber Cancer Institute (IFM/ DFCI) trial, 260
- › Interleukin-5 (IL-5), 434
- › International normalized ratio (INR), 308
- › International prognostic index for diffuse large B-cell lymphomas, 242
- › International Prognostic Score (IPS), 218
- › International Prognostic Scoring Scale (IPSS), 116
- › International Sensitivity Index (ISI), 256, 308, 457
- › International Working Group for Myelofibrosis Research and Treatment (IWG-MRT), 116
- › Intracellular flow cytometry, 475
- › Intracellular iron, 1
- › Intracellular oxidative stress, level of, 30
- › Intramuscular B₁₂, 20
- › Intravascular hemolysis, 23, 27–29, 76
- › Intravascular large B-cell lymphoma (IVLBCL), 234
- › Intravenous immunoglobulin (IVIg), 127, 140, 177, 386–387t, 425, 430
- › Intravenous iron therapy, 7, 8t
- › Involved-field radiation therapy (IFRT), 216, 217
- › Iodine-131 tositumomab (Bexxar), 243
- › Ipilimumab, 439
- › Iron
 - average daily requirement of, 1
 - balance, 1–2

- chelators, 2, 418
- deficiency. See Iron deficiency
- dietary, 2, 5
- ferric iron, 2
- ferrous iron, 2–3
- heme iron, 2
- intracellular, 1
- medications malabsorbed when coadministered with, 7t
- medicinal iron, 2
- metabolism, 1–3
- negative iron balance, 3, 4t
- non-erythroid, 1
- parenteral iron, 7, 8, 9
- serum iron, 4
- stainable marrow iron, 3
- storage iron, 3–4
- Iron cycle, 3f
- Iron deficiency, 1, 131, 451
 - absolute iron deficiency, 1, 3–4
 - causes, 1, 2t
 - clinical presentation, 1
 - during pregnancy, 424
 - functional iron deficiency, 1, 4–5
 - treatment of, 8–9
 - hepcidin levels and, 5, 5t
 - response to iron therapy, 8
 - symptoms, 1
 - treatment of anemia, 5
 - associated with chronic inflammatory diseases, 9
 - dietary iron, 5
 - intravenous iron therapy, 7, 8t
 - oral iron therapy, 5–6, 6t
 - in patients with advanced malignancy, 8–9
 - red blood cell transfusion, 8

- › Iron-deficient erythropoiesis, 3
- › Iron dextran, 7
- › Iron homeostasis, 410, 412
 - body iron distribution, 412t
 - regulation of, 411f
- › Iron malabsorption, 6
- › Iron overload, 97
 - and chelation, 54–55
 - disorders
 - classification, 411t
 - factors influencing clinical penetrance, 414t
 - and hepcidin deficiency, 410
- › Iron–porphyrin complex, 2
- › Iron refractory iron deficiency anemia (IRIDA), 4
- › Iron-replacement dose estimates, 7t
- › Iron requirements during pregnancy, 4
- › Iron sequestration syndromes, 4, 8
- › Iron supplements, 6, 133
- › Iron therapy
 - intravenous, 7, 8t
 - oral, 5–6, 6t
 - response to, 8
- › Irradiation
 - thrombocytopenia, 292
- › Isoelectric focusing (IEF), 454–455
- › Isoimmune neonatal neutropenia, 126
- › Isolated laboratory-specific defects, 336
- › Ixazomib, 261t, 262, 263

- › JAK2V617F mutation, 105t, 106, 111, 115
- › JAK/ STAT pathway, 214
- › Janus Kinase 2 (JAK2) V617F, 104
- › Janus kinase inhibitor, 110

- › Kala-Azar. See Visceral leishmaniasis (VL)

- › Kaolin clotting time (KCT), 345
- › Kaposi sarcoma-associated herpes virus (KSHV), 224
- › Karyotyping, 93
- › Kasabach–Merritt syndrome, 139
- › Kell blood group, 33
- › Kidneys, sickle-related manifestations in, 49
- › KIT gene, 156t
- › Klebsiella pneumoniae, 9
- › Koilonychias, 1
- › Kostmann disease, 142
- › Kostmann syndrome, 86, 127–128, 437
- › Lactate dehydrogenase (LDH), 27, 50, 76, 151, 200, 301, 462
- › Lactoferrin, 121
- › Laparoscopic surgery
 - for acquired thrombosis, 347
- › Large B-cell lymphoma with IRF4 rearrangement, 232
- › Large granular lymphocyte (LGL), 127, 437
- › Large granular lymphocytosis/ leukemia, 127
- › Lecithin cholesterol acyltransferase (LCAT), 33
- › Leg ulcers, and SCD, 49–50
- › Leishmania donovani, 431
- › Lenalidomide, 95, 99, 269, 253, 257, 259, 260, 261t, 346, 358
- › Lepirudin, 345, 429
- › Leucovorin, 177
- › Leukemias, 69
 - acute, 435
- › Leukemic hematopoiesis, 182
- › Leukemic myeloblasts, 153
- › Leukocytapheresis (leukapheresis), 404
- › Leukocyte adhesion deficiency (LAD), 121–123
 - leukocyte adhesion deficiency I (LADI), 143
- › Leukocytosis, 40, 107, 115, 143, 404
- › Leukodepletion, 96, 177

- › Leukoerythroblastosis, 115
- › Leukopenia, 115, 134
- › Leukostasis, 151, 194
- › L-homocysteine, 453
- › Liver biopsy, 415
- › Long-chain 3-hydroxyacyl-CoA dehydrogenase, 427
- › Low-molecular-weight heparin (LMWH), 323, 350, 351, 356–357, 361, 429–430, 457
- › Lugano staging system for primary nodal lymphomas, 241t
- › Lupus anticoagulants (LAs), 312–313, 344–345, 360
- › Lymphadenopathy, 68, 199
- › Lymphocyte doubling time (LDT), 202
- › Lymphocyte-rich classic Hodgkin lymphoma (LRCHL), 214
- › Lymphocytes and plasma cells, 449–450
- › Lymphocytic and histiocytic (L&H) cells, 220
- › Lymphoid neoplasms
 - categories of, 224
 - WHO classification of, 225t
- › Lymphomas, 269–270
 - associated with HIV Infection, 239
- › Lymphomatoid granulomatosis, 234
- › Lymphoplasmacytic lymphoma, 231
- › Lymphoproliferative disease (LPD) associated with primary immune disorders, 238
- › Lysosomal storage diseases, 143
- › LYST gene mutations, 125
- › Macrocytic anemias, 135–136
- › Macrocytosis, 17
 - causes of, 18t
- › Magnetic resonance venography (MRV), 340
- › Maintenance, 260–262
- › Major incompatibility, 375
- › Malaria, 431
 - African trypanosomiasis (sleeping sickness), 432
 - causes of anemia in, 431t

- helminth infections, 432
- hematologic manifestations of, 432t
- visceral leishmaniasis (VL), 431
- Malignancy, advanced
 - treatment of anemia in patients with, 8–9
- Malignant leukocytosis, 404
- Mantle cell lymphoma (MCL), 232–233, 245
- Marrow cellularity, 449
- Marrow failure, 76
- Marrow fibrosis, causes of, 116t
- Marrow infiltration
 - thrombocytopenia, 291
- Massive transfusion, 396–400
 - adverse sequelae of, 397
- Matched unrelated donors (MUD), 72
- Maternal immune thrombocytopenic purpura (maternal ITP), 400–401
- Mature B-cell neoplasms, 225–226t, 228–234
- Mature T-and natural killer-cell neoplasms, 226t, 235–238
- May–Hegglin anomaly, 140, 305, 334–335
- McLeod phenotype, 33
- Mean cell volume (MCV), 4, 27, 134
- Mean corpuscular hemoglobin (MCH), 43, 441
- Mean corpuscular hemoglobin concentration (MCHC), 33, 441
- Mean corpuscular volume (MCV), 441
- Mean platelet volume (MPV), 443
- Measurable residual disease (MRD), 160
- Meat, heme of, 5
- Mechlorethamine, vincristine (Oncovin), procarbazine, and prednisone (MOPP), 217, 220
- Medicinal iron, 2
- Megakaryocyte number and appearance, 449
- Megaloblastic hematopoiesis, 12
- Melanomas, 283
- Melphalan, 116, 257, 259

- › Meningococcal vaccine, 77
- › Meperidine, 44, 74
- › Mercaptopurine, 172t, 173t
- › Mesna, 173t, 177
- › Metformin, 15
- › Methemoglobin, 29
- › Methicillin-resistant *S. aureus* liver abscess, 124
- › Methotrexate, 127, 173t, 177, 237, 277
- › 8-Methoxy-psoralen (8-MOP), 404
- › Methylene tetrahydrofolate reductase (MTHFR) gene defects, 349
- › Methylmalonic acid (MMA), 18, 19, 19t, 452, 453
- › Methylprednisolone, 73
- › Methylprednisolone, 279
- › Methyl-tetrahydrofolic acid, 14
- › Microcytes, 4
- › Microcytic anemias, 131–134
- › Microcytosis, 39
- › Mild allergic/ urticarial transfusion reactions, 394
- › Minimal residual disease (MRD), 171, 188–191, 466, 476
 - negativity, 256
- › Minor incompatibility, 375
- › Mixed cellularity classic Hodgkin lymphoma (MCCHL), 214
- › Modern tumor classification, 223
- › Molecular testing, 136, 448
- › Monoclonal antibodies, 175, 262, 471
- › Monoclonal B-cell lymphocytosis (MBL), 199, 230
- › Monoclonal gammopathy of undetermined significance, 231
- › Monoclonal gammopathy of unknown significance (MGUS), 253, 256t, 459
- › Monoclonal immunoglobulins, 231, 459, 460
- › Monofer, 8t
- › Monoferric transferrin, 3
- › MonoMAC syndrome, 66
- › Morphine, 44
- › M paraproteins, 460

- › Mucosal-associated marginal zone (MALT) lymphoma, 231, 244
- › Multicenter Study of Hydroxyurea (MSH), 50, 51
- › Multicentric Castleman disease, 224
- › Multiparametric flow cytometry (MFC), 256
- › Multiple myeloma (MM), 252, 269, 277
 - approach to newly diagnosed multiple myeloma, 257–259
 - bortezomib, 259–260
 - carfilzomib, 260
 - daratumumab, 263
 - diagnostic criteria of, 252
 - elotuzumab, 263
 - epidemiology, 252
 - IMWG response criteria, 258–259t
 - initial evaluation, 253–256
 - ixazomib, 263
 - lenalidomide, 260
 - maintenance, 260–262
 - monoclonal gammopathy of unknown significance (MGUS), 253, 256t
 - panobinostat, 263
 - pathophysiology and genetics, 253
 - pomalidomide, 262
 - primary regimens for treatment of, 261t
 - response criteria, 256–257
 - Revised International Staging System (R-ISS), 257t
 - salvage regimens for treatment of, 262t
 - salvage therapy, 262
 - smoldering MM (SMM), 253, 256t
 - staging, 256
 - transplantation, 260
- › Mutated IGHV genes (M-IGHV), 201
- › Mycosis fungoides (MF), 237
- › Myeloablative hematopoietic stem cell transplantation (HSCT), 55
- › Myeloablative HSCT, 55
- › Myelodysplasia (MDS), 69, 70, 291

- thrombocytopenia, 291
- Myelodysplasia (MDS)/ myeloproliferation, 333
- Myelodysplastic syndromes (MDS), 90, 148, 276–277
 - clinical features, 92–93
 - clonal hematopoiesis and, 91f
 - clonality and molecular features, 91–92
 - diagnostic studies and morphologic classification, 93–94
 - etiology, 90–91
 - investigational therapeutic strategies for, 97t
 - prognostic criteria and clinically relevant subtypes, 94–96
 - recurrent somatic gene mutations in, 92t
 - risk-adjusted schema for management of, 100f
 - standard treatments, 96
 - disease-modifying hypomethylating agents, 98–99
 - immunomodulatory agents and immunosuppression, 99
 - investigational treatments, 99
 - stem cell transplantation, 97–98
 - supportive care, 96–97
 - WHO classification of, 94t
- Myelofibrosis, 70, 194
- Myeloid and erythroid cells, 449
- Myeloid growth factors, 177
- Myeloid neoplasms with germline predisposition, 150t
- Myeloperoxidase (MPO), 121
 - deficiency, 123–124, 348
- Myeloproliferative neoplasms (MPNs), 93, 104
 - bone marrow hematopoiesis, 105f
 - chronic myelomonocytic leukemia (CMML), 117
 - clinical features, 117
 - diagnostic testing, 117
 - epidemiology, 117
 - pathophysiology, 117
 - staging and prognostic features, 117–118
 - treatment, 118

- essential thrombocytosis (ET), 111
 - clinical features, 112
 - diagnostic testing, 112
 - epidemiology, 111
 - pathophysiology, 111–112
 - treatment, 112–113
- high risk, 110–111
- low risk, 110
 - with extreme thrombocytosis, 110
- polycythemia vera (PV), 104
 - clinical features, 106–107
 - diagnostic criteria, 107–108
 - epidemiology, 105
 - pathophysiology, 106
 - staging and prognostic features, 108
 - treatment, 108–110
- primary myelofibrosis (PMF), 113
 - clinical features, 115
 - diagnostic testing, 115
 - epidemiology, 113
 - pathophysiology, 113–115
 - staging and prognostic features, 115–116
 - treatment, 116–117
- Myelosuppressive agents, 113
- MYH9-related disease, 335
- Nadroparin, 358
- Nalbuphine, 44
- National Comprehensive Cancer Network (NCCN), 257
- National Marrow Donor Program (NMDP), 273
- Natural killer (NK) cells, 66
- Naturally occurring antibodies, 373
- Negative iron balance, 3, 4t
- Neisseria meningitidis, 35, 44

- › Neonatal alloimmune thrombocytopenia (NAIT), 139, 140, 302, 400–401
- › Neonatal hyperbilirubinemia, 30
- › Nephrotic syndrome, 345
- › Neurodegeneration, 33
- › Neurovisceral symptoms, 62, 64, 65
- › Neutropenia, 122t, 126, 142–143, 436
 - acquired, 126
 - drug-induced neutropenia, 126
 - immune-related neutropenia, 126–127
 - infection-related neutropenia, 126
 - benign ethnic neutropenia (BEN), 129
 - classification, 437t
 - clinically significant neutropenia, inherited disorders associated with, 128
 - common drugs causing, 127t
 - congenital, 127
 - cyclic neutropenia, 128
 - severe congenital neutropenia, 127–128
 - extrinsic disorders, 437
 - drug-induced neutropenia, 438–439
 - immune neutropenias, 437–438
 - neutropenia associated with autoimmune disorders, 438
 - neutropenia associated with infectious diseases, 438
 - neutropenia associated with large granular lymphocyte syndrome, 438
 - idiopathic neutropenia, 128–129
 - intrinsic disorders, 436
 - acquired neutropenias, 437
 - congenital neutropenias, 436–437
- › Neutropenic fever, 177
- › Neutrophil disorders, 121, 122t
 - chronic granulomatous disease (CGD), 124–125
 - Chédiak–Higashi syndrome (CHS), 125
 - leukocyte adhesion deficiency, 121–123
 - myeloperoxidase deficiency (MPO), 123–124
 - specific granule deficiency (SGD), 125–126

- Neutrophil elastase mutations, 127, 128
- Neutrophilia, 143
- Neutrophils, 17, 96
- Neutrophil secondary granules, 125
- Next-generation molecular sequencing (NGS), 256
- Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, 123, 124
- Niemann–Pick disease, 144
- Nijmegen breakage syndrome, 238
- Nilotinib, 175, 188, 195
- Nivolumab, 219
- Nodal marginal zone lymphoma (NMZL), 231–232
- Nodal peripheral T-cell lymphoma with T follicular helper phenotype, 238
- Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), 212, 213, 219–220
- Nodular sclerosis classic Hodgkin lymphoma (NSCHL), 213
- Nonalcoholic fatty liver disease (NAFLD), 422t
- Non-erythroid iron, 1
- Non-HFE iron overload, 420, 421–422t
- Non-Hodgkin lymphomas (NHLs), 223, 277
 - classification, 224–239
 - B-cell lymphoma, unclassifiable, 235
 - Burkitt lymphoma (BL), 234–235
 - high-grade B-cell lymphoma, not otherwise specified, 235
 - high-grade B-cell lymphoma, with MYC and BCL2 and/ or BCL5 rearrangements, 235
 - immunodeficiency-associated lymphoproliferative disorders, 238–239
 - mature B-cell neoplasms, 228–234
 - mature T-and natural killer-cell neoplasms, 235–238
 - precursor B-and T-cell neoplasms, 228
 - clinical management, 239
 - initial evaluation, 239–240
 - response evaluation following therapy, 240–241
 - staging, 240
 - epidemiology, 223
 - IVAC regimen, 249t

- pathophysiology, 224
- principles of therapy, 243
 - aggressive B-cell lymphomas, 245–246
 - indolent B-cell and T-cell lymphomas, 243–244
- prognostic features, 241–242
- treatment principles, 243
 - AIDS-related lymphoma (systemic) treatment considerations, 249–250
 - Burkitt lymphoma/ B-cell acute lymphoblastic leukemia, 246
 - salvage therapy, 249
 - T-cell lymphoma, 250–251
 - treatment of recurrent and refractory B-cell lymphoma, 246–248
- › Nonimmune hemolysis, 33
- › Non-immune-mediated platelet refractoriness, 381, 382
- › Nonmyeloablative HSCT, 55
- › Non-opioid analgesics, 44
- › Nonsteroidal anti-inflammatory drugs (NSAIDs), 44, 330, 331, 384, 426
- › Non-transferrin bound iron (NTBI), 412
- › Non-valvular atrial fibrillation (NVAF), 367, 369
- › Normocytic anemias, 134–135
- › NPM1 gene, 156t
- › Nutritional and hypoproliferative anemias, evaluation of, 450
 - high serum Fe and Fe saturation, causes of, 451t
 - serum and red cell folate assays, 453
 - serum erythropoietin, 453
 - serum ferritin, 452
 - serum homocysteine, 453
 - serum intrinsic factor antibody assay, 453
 - serum iron and total binding capacity assays, 450–451
 - serum methylmalonic acid, 453
 - serum vitamin B₁₂ (cobalamin), 452
 - soluble transferrin receptor (STR), 451–452
- › Obinutuzumab, 203, 243
- › Obstetrical disseminated intravascular coagulation, causes of, 428t

- › Ofatumumab, 203, 205
 - › Older/ unfit patients, acute myeloid leukemia in, 161–162
 - › Omega-3 fatty acids, 333
 - › Opioid agonists, 44, 45t
 - › Optimal post remission therapy, 161
 - › Oral anticoagulants, 356
 - › Oral iron absorption testing, 6
 - › Oral iron therapy, 5–6, 6t
 - › Osler–Weber–Rendu disease, 318
 - › Osmotic fragility test for HS, 34
 - › Osteogenesis imperfect, 318
 - › Osteomyelitis, 50, 124
 - › Osteonecrosis, 49
 - › Osteosclerosis, 115
 - › Overall response rates (ORRs), 203
 - › Oxymetholone, 84
-
- › Packed red blood cells (PRBCs), 8, 144, 378t
 - › PAI-1 deficiency, 318
 - › Palpable hepatomegaly, 115
 - › Pancreatic enzymes, deficiency of, 15
 - › Pancytopenia, differential diagnosis of, 69
 - › Panobinostat, 263
 - › Parasitemia, 405
 - › Parasites, 28t
 - › Parenteral iron, 7, 8, 9
 - › Paroxysmal cold hemoglobinuria (PCH), 402
 - › Paroxysmal nocturnal hemoglobinuria (PNH), 24, 29, 35, 66, 70, 74, 76, 90, 347
 - clinical features, 76
 - diagnosis, 76
 - etiology and pathophysiology, 76
 - treatment, 76
 - › Partial deficiency of porphobilinogen deaminase (PBGD) activity, 63
 - › Partial response (PR), 256

- › Parvovirus B19, 77, 438
- › Parvovirus infection, 35, 46
- › Patient-controlled delivery (PCA) pumps, 44
- › PAX-5, 213
- › Pediatric-type follicular lymphoma, 232
- › Pegylated L-asparaginase, 172t
- › Pelger–Huët nuclei, 93
- › Penicillin, 44, 46, 54, 135
- › Pentazocine, 44
- › PER977 (Perosphere), 369
- › Periostitis, 115
- › Peripheral arterial disease (PAD), 422t
- › Peripheral blood and bone marrow, cellular analysis of, 440
 - bone marrow aspirate/ biopsy, 448
 - bone marrow biopsy, 449
 - bone marrow iron stores, 450
 - cells anomalously present in the marrow, 450
 - indications, 448
 - lymphocytes and plasma cells, 449–450
 - marrow cellularity, 449
 - megakaryocyte number and appearance, 449
 - myeloid and erythroid cells, 449
 - risks, 448
 - technique, 448
 - complete blood count (CBC), 440
 - automated complete blood counters, 440–441
 - platelets, 443
 - red cell parameters, 441–443
 - sources of artifact, 443–444
 - white blood cell parameters, 443
 - peripheral blood film, 444–448
- › Peripheral blood smear, 41
- › Peripheral blood stem cells (PBSCs), 268, 274, 384, 385
- › Peripheral T-cell lymphoma, not otherwise specified, 237

- › Pernicious anemia, 15, 18, 20
- › PFA-100, 330, 458
- › Phagocytosis of senescent erythrocytes, 1
- › Pharmacologic hemostatic agents, 398–399t
- › Philadelphia (Ph) chromosome, 181, 183, 184, 184f, 188
- › Philadelphia-chromosome positive ALL, 173
- › Phlebotomy therapy, 110, 416–417, 420
 - evaluation of anemia during, 418
 - guidelines for, 417–418
 - indications for, 421–422t
- › Phosphoenolpyruvate, 32
- › Phosphofructokinase deficiency, 32
- › 2-Phosphoglycerate, 32
- › 3-Phosphoglycerate, 32
- › Phosphoglycerate kinase (PGK) deficiency, 29, 32
- › Photopheresis (extracorporeal photochemotherapy), 404
- › Phytates, 2
- › Plasmablastic lymphoma (PBL), 234
- › Plasma, administration per NIH Practice, 378t
- › Plasma cell myeloma, 231
- › Plasmapheresis, 34, 405
- › Plasminogen activator inhibitor (PAI), 311
 - PAI-1, 360
- › Plasmodium malariae, 431
- › Plasmodium Vivax, 431
- › Platelet aggregation studies, 329, 330f
- › Platelet biology, 289
- › Platelet count, 145, 289–290, 291t, 293, 425
- › Platelet COX, 331
- › Platelet derived growth factor (PDGF), 113–115
- › Platelet derived growth factor receptor β (PDGFR β), 117, 435
- › Platelet disorders, 139–140, 305, 334t
- › Platelet dysfunction, substances associated with, 331t
- › Platelet function, tests of, 329–330, 458

- › Platelet function analyzer, 330
- › Platelet GP IIb/ I IIa inhibitors, 331
- › Platelet granules, characteristics of, 329t
- › Plateletpheresis, 113
- › Platelets, 145, 176, 378, 378t, 381, 443
 - refractoriness, 381–382
 - storage granule defects, 140
 - transfusions, 301
 - for disseminated intravascular coagulation (DIC), 323
- › Platelet-type bleeding, 289
- › Platelet-type pseudo-VWD, 140
- › Plummer-Wilson syndrome, 1
- › Pneumococcal polysaccharide immunization, 35
- › Pneumocystis jiroveci pneumonia (PCP), 177
- › Polycythemia rubra vera, 422t
- › Polycythemia vera (PV), 104
 - and apparent polycythemia, 109t
 - clinical features, 106–107
 - diagnostic criteria, 107–108
 - epidemiology, 105
 - pathophysiology, 106
 - properties of agents used to treat, 114t
 - and secondary polycythemia, 109t
 - staging and prognostic features, 108
 - treatment, 108–110, 111f
 - 2016 World Health Organization Criteria for diagnosis of, 108t
- › Polycythemia Vera Study Group (PSVG), 105
- › Polymerase chain reaction (PCR), 183, 185f, 239, 476
 - PCR-based assay, 107
 - PCR-based DNA testing, 43
- › Polymorphonuclear cells (PMNs), 121
- › Pomalidomide, 262, 269
- › Ponatinib, 193
- › Porphyria cutanea tarda (PCT), 59, 64, 421t

- › Porphyrias, 59
 - acute intermittent porphyria (AIP), 63
 - classification, 60, 61f
 - clinical manifestations, 60
 - congenital erythropoietic porphyria (CEP), 63
 - diagnosis, 60–62
 - epidemiology, 59
 - erythropoietic protoporphyrina (EPP), 64–65
 - hepatoerythropoietic porphyria (HEP), 64
 - hereditary coproporphyria, 64
 - pathophysiology, 60
 - porphyria cutanea tarda (PCT), 64
 - variegate porphyria, 64
 - X-linked dominant protoporphyrina, 62
 - δ-aminolevulinic acid dehydratase deficiency porphyria (ADP), 63
- › Portal vein thrombosis, prevalence of prothrombotic states in, 342t
- › Positron emission tomography (PET), 200
- › Postpolycythemic metaplasia (PPMM), 113
- › Postsplenectomy thrombocytosis, 54
- › Postthrombocythemic myeloid metaplasia (PTMM), 113
- › Postthrombotic syndrome (PTS), 338
 - management of, 366
 - prevention, 367t
 - recognition of, 365
 - villalta scale for evaluation of, 339t
- › Posttransfusion purpura (PTP), 302, 394–395
- › Posttransplantation cyclophosphamide (PTCy), 284
- › Posttransplant lymphoproliferative disorders, 239
- › PPF, 386t
- › Prasugrel, 333
- › Pre-B-cell acute lymphoblastic leukemia, common treatment regimens for, 172–173t
- › Precursor B-and T-cell neoplasms, 225t, 228
- › Prednisone, 116, 172t, 173t, 217, 250t, 259, 269, 425, 430
- › Preeclampsia, 426

- › Pregnancy
 - acute myeloid leukemia in, 163–164
 - -associated microangiopathies, 428
 - in chronic myelogenous leukemia, 194
 - iron requirements during, 4
 - and von Willebrand Disease, 328
- › Pregnancy, hematologic complications of, 424
 - anemia, 424
 - sickle cell disease, 424–425
 - thrombocytopenia, 425
 - diffuse intravascular coagulation (DIC), 427–428
 - hemolysis, elevated liver enzymes, and low platelets syndrome (HELLP), 426–427
 - hemolytic uremic syndrome (HUS), 427
 - immune thrombocytopenic purpura (ITP), 425–426
 - incidental thrombocytopenia, 425
 - preeclampsia, 426
 - thrombotic thrombocytopenic purpura (TTP), 427
 - venous thromboembolism (VTE), 428
 - diagnosis, 429
 - prophylactic anticoagulation, 430
 - thrombophilias and recurrent miscarriage, 430
 - treatment, 429–430
- › Pregnant women with SCD, 53
- › Priapism and SCD, 49
- › Primary cutaneous CD30+ T-cell lymphoproliferative disorders (PCALCL), 237
- › Primary cutaneous DLBCL, leg type, 233
- › Primary cutaneous follicle center lymphoma (CFCL), 232
- › Primary DLBCL of the central nervous system, 233
- › Primary effusion lymphomas (PELs), 224, 234
- › Primary hemostasis, 325f, 329
- › Primary mediastinal (thymic) large B-cell lymphoma (PMBL), 234
- › Primary myelofibrosis (PMF), 104
 - clinical features, 115

- diagnostic testing, 115
- epidemiology, 113
- pathophysiology, 113–115
- staging and prognostic features, 115–116
- treatment, 116–117
- Procarbazine, 217
- Progression-free survival (PFS), 203, 217, 233
- Progressive disease (PD), 256, 258
- Pro-inflammatory cytokines, 129
- Prolymphocytic leukemia (PLL), 199
- Protamine, 398t
- Protein C and S deficiencies, 140, 342, 349
- Prothrombin complex concentrate (PCCs), 385
- Prothrombin mutation (G20210A), 349
- Prothrombin time (PT), 308, 457
 - prolonged PT
 - aboratory diagnostic algorithm for, 313f
 - conditions associated with, 317–318
- Proton pump inhibitors, 15
- Protoporphyrina. See Erythropoietic protoporphyrina (EPP)
- Pruritis, 106, 110
- Prussian blue stain, 450
- P-selectin, 356
- Pseudothrombocytopenia, 303–304
- Psoralen combined with UVA (PUVA), 250
- Pulmonary embolism (PE), 338–340
 - ancillary findings diagnostic of, 341t
 - diagnosis, 340–341
 - inferior vena cava filters in the treatment of, 351
- Pulmonary hypertension, 48, 135, 362
- Pure red cell aplasia (PRCA), 67, 77
 - clinical features and diagnosis, 77
 - etiology and pathophysiology, 77
 - treatment, 78

- › Purpura fulminans, 141
- › Pyrimidine 5' nucleotidase deficiency, 32–33
- › Pyrimidine aglycones, 30
- › Pyropoikilocytes, 28t
- › Pyruvate kinase (PK) deficiency, 31–32
- › Qualitative platelet disorders, 305, 328
 - acquired disorders, 331–333
 - inherited disorders, 333
 - congenital disorders of signal transduction, 336
 - cytochrome c mutations, 336
 - giant platelet disorders, 334
 - Glanzmann thrombasthenia, 335
 - isolated laboratory-specific defects, 336
 - Scott syndrome, 335–336
 - storage pool disease (SPD), 335
 - management of, 334t
 - platelet function testing, 329–330
 - review of hemostasis and the role of platelet biochemistry, 329
- › Quantitative serum immunoglobulins, 461
- › Rai classification, 200
- › Rai Clinical Staging System, 201t
- › Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY), 367
- › RAPID trial, 217
- › Rare factor deficiencies, 139
- › R-CHOP, 245t, 246
- › R-DHAP (rituximab plus dexamethasone, high-dose cytarabine, and cisplatin), 245
- › Recombinant and plasma-derived coagulation factors, 389
- › Recombinant factor VII, 388t
- › Recombinant factor VIIa (rVIIa), 323, 335, 388t
- › Recombinant factor IX, 388t
- › Recurrent and refractory B-cell lymphoma, treatment of, 246–248
- › Red blood cells (RBCs), 176–177, 378, 440
 - antigens, 373

- hemolysis, 23
- RBC indices, 131
- transfusion, 8, 34
- Red cell count, 4, 441
- Red cell distribution width (RDW), 4, 27, 43, 133, 441
- Red cell exchange, 404
 - and parasitemia, 405
 - and sickle cell anemia, 404–405
- Red cell folate, 18, 453
- Red cell parameters, 441–443
- Reduced intensity conditioning (RIC), 97, 160, 274
- Reduced intensity stem cell transplantation (RIST), 249
- Reed–Sternberg (RS) cells, 212, 214
- Relapsed acute myeloid leukemia, 445f
- Relapsed and refractory acute myeloid leukemia, treatment of, 162–163
- Reptilase time, 310
- Reticulocyte production index (RPI), 443
- Reticulocytes, 23, 27, 77, 441
 - hemoglobin, 3
 - normal values in newborn infants, 27
- Reticulocytopenia, 134
- Reticulocytosis, 23, 24, 27
- Reticuloendothelial system (RES), 1, 23
- Reviparin, 358
- Revised International Prognostic Scoring System (IPSS-R), 94, 95t
- Revised International Staging System (R-ISS), 256
- RFVIIa, 398t
- Rhabdomyolysis, 53
- Rheumatoid arthritis, 7
 - anemia of, 9
- Rh immune globulin (RhIg), 385, 389
- Rh incompatibility, 376
- Rh-null phenotype, nonimmune hemolysis due to, 33
- Ribosomal protein gene (RPS14), 77

- › Richter's transformation, 209
 - › Rituximab, 175, 203, 205, 208, 230, 232, 233, 243, 270, 283, 294, 425, 439
 - › Rivaroxaban, 310, 350, 368
 - › Romiplostim, 96, 294
 - › Rosenthal syndrome, 316
 - › Rotational thromboelastography (ROTEM), 311
 - › R-proteins, 15
 - › RPS14 gene, 91, 95
 - › RUNX1 gene, 156t
 - › Ruxolitinib, 110
-
- › S-adenosylmethionine, 14
 - › SBDS gene, 142
 - › Schilling test, 20
 - › Schistocyte, 28t, 311
 - › Scott syndrome, 335–336
 - › Scurvy, 319
 - › Secondary erythrocytosis, 422t
 - › Selective estrogen receptor modulators (SERMs), 346
 - › Senescent erythrocytes, phagocytosis of, 1
 - › Septic arthritis, 50
 - › Serotonin reuptake inhibitors (SSRIs), 333
 - › Serratia marcescens, 124
 - › Serum and red cell folate assays, 453
 - › Serum cryoglobulins, 461
 - › Serum erythropoietin, 96, 453
 - › Serum ferritin, 3, 6, 17, 413, 452
 - › Serum free light chains, 461
 - › Serum haptoglobin, 456
 - › Serum homocysteine, 453
 - › Serum immunoglobulins, 199, 461
 - › Serum intrinsic factor antibody assay, 453
 - › Serum iron, 4, 413, 450–451
 - › Serum lactate dehydrogenase, 17, 462

- › Serum methylmalonic acid, 453
 - and homocysteine, 19
- › Serum protein electrophoresis, 459–460
- › Serum-soluble transferrin receptor concentration, 3
- › Serum viscosity, 461–462
- › Serum vitamin B₁₂ (cobalamin), 18, 452
- › Serum vitamin concentrations, 17–19
- › Serum β2-microglobulin, 462
- › Severe combined immunodeficiency, 238
- › Severe congenital neutropenia and cyclic neutropenia, 86
- › SF3B1 mutations, 92
- › Shift cells, 27
- › Shwachman–Diamond syndrome, 85, 142
 - clinical diagnostic criteria, 85–86
 - treatment, 86
- › Sickle cell, 28t
- › Sickle cell anemia, 38
 - anesthesia and surgery, 53
 - cardiovascular manifestations, 47
 - clinical syndromes and treatment of, 44–50
 - contraception and pregnancy, 52–53
 - curative intent, therapy with, 55
 - gene therapy, 55
 - hematopoietic stem cell transplantation (HSCT), 55
 - diagnosis, 41
 - eye disease, 47
 - gall bladder problems in, 48–49, 48t
 - general features of, 39t
 - hematologic characteristics of, 42t
 - hepatic problems in, 48–49, 48t
 - indications for transfusions in SCD, 51t
 - infections, 44–46
 - leg ulcers, 49–50
 - pathophysiology, 38–41

- perioperative considerations, 53t
- priapism, 49
- pulmonary complications, 47
 - acute complications, 47–48
 - chronic complications, 48
- renal abnormalities, 49
- screening, 41
- sickle cell trait (SCT) and disease, 43–44
- skeletal complications, 49–50
- splenic problems in, 48–49, 48t
- stroke, 46–47
- suggested health maintenance schedule, 56t
- therapy, 50
 - drugs, 52
 - HbF induction, 50–52
 - transfusions, 50
- vaso-occlusive episodes (VOE), 44
- Sickle cell disease (SCD), 40–41, 134, 135, 421t
 - in pregnancy, 424–425
- Sickle cell hemolytic transfusion syndrome, 391
- Sickle cell syndromes, 35
- Sickle cell trait (SCT), 53
- Sickle hemoglobin (HbS), 40
- Sickle solubility test, 455
- Side scatter (SSC), 441
- Sjögren syndrome, 231
- Skeletal complications, and SCD, 49–50
- Sleeping sickness. See African trypanosomiasis (AT)
- Slow early responders (SERs), 171
- Small bowel, bacterial overgrowth in, 15
- Small lymphocytic lymphoma (SLL), 199, 230
- Smoldering MM (SMM), 253, 256t
- Sodium lauryl sulfate (SLS), 441
- Solid tumors, 291

- Soliris, 77
- Solitary plasmacytoma of the bone, 231
- Soluble transferrin receptor (STR), 451–452
- Soluble vascular cell adhesion molecule-1 (sVCAM1), 359
- Somatic mutations, 202
- Specific factor assays, 457
- Specific granule deficiency (SGD), 125–126
- Spherocytes, 28t, 33
- Splanchnic vein thrombosis, 343
- Splenectomy, 32, 35, 54, 294, 425
- Splenic B-cell lymphoma/ leukemia, unclassifiable, 231
- Splenic infarcts, 194
- Splenic marginal zone lymphoma, 230
- Splenic problems with sickle cell disease, 48–49
- Splenomegaly, 38, 54, 68, 110, 115, 199
- Splenomegaly, causes of, 303t
- SRSF2, 92
- Stable disease (SD), 256
- Stainable marrow iron, 3
- Standard International Myeloma Workshop Consensus Panel, 256
- Staphylococcus aureus, 123, 124
- Steady-state erythropoiesis, 3
- Stem cell transplantation (SCT), 96, 97–98, 134, 171
 - and supportive care, 82–83
- Steroids, 301, 427
- Stomach, food (protein)-bound vitamin B₁₂ in, 15
- Stomatocyte, 28t
- Storage iron, 3–4
- Storage pool disease (SPD), 335
- Streptococcus pneumoniae, 35, 44, 46
- Stressed erythropoiesis, 27
- Stress erythropoiesis, 8
- Stringent CR (sCR), 256
- Stroke and SCD, 46–47

- Stroke Prevention (STOP) trial, 46
- Stroke With Transfusion Changing to Hydroxyurea (SWiTCH) trial, 46
- Subcutaneous panniculitis-like T-cell lymphoma (SPTCL), 236–237
- Sulfhemoglobin, 30
- Surgery and general anesthesia in SCD, 53
- Sysmex automated hematology analyzer, 442f
- Sysmex XN automated hematology analyzer, 445f
- Systemic EBV⁺ T-cell lymphoma of childhood, 236
- Systemic fat embolism syndrome, 48
- Sézary syndrome (SS), 237

- T-acute lymphoblastic leukemia (ALL), 228
- Tamoxifen, 346, 358
- Tandem auto-HSCT, 269
- Tannins, 2
- Target cell, 28t
- Tarui disease. See Phosphofructokinase deficiency
- T-cell acute lymphoblastic leukemia, common treatment regimens for, 172–173t
- T-cell clonal disorders, 434
- T-cell immunophenotype, 230t
- T-cell large granular lymphocytic leukemia (T-LGL), 235
- T-cell lymphomas, 250–251
 - molecular characteristics of, 228t
- T-cell prolymphocytic leukemia (T-PLL), 235
- T-cell/ histiocyte-rich large B-cell lymphoma, 233
- Telomerase reverse transcriptase (TERT), 84
- Telomere disease, 66
- Telomere length (TL), 66
- TET2 gene, 92, 156t
- T follicular helper (Tfh), 238
- Th2 cytokine pattern type, 124
- Thalassemia intermedia, 39, 41
- Thalassemias, 4, 35, 38, 133, 134, 421t
 - clinical syndromes and treatment of, 53

- fetal hemoglobin induction, 55
- iron overload and chelation, 54–55
- splenomegaly, 54
- transfusions, 54
- diagnosis, 41, 42f
- hematologic characteristics of, 42t
- pathophysiology, 38–40
- prenatal diagnostic schema for, 43f
- α-thalassemia trait and disease, 43
- β-thalassemia trait and major, 43
- α-Thalassemia, 40, 43, 54, 133–134
 - general features of, 39t
 - syndromes, 38
- β-Thalassemia, 39–40, 39t, 40, 41, 43, 54, 55, 133
- Thalassemia syndromes, 133
 - α-Thalassemic syndromes, 38
 - β-Thalassemic syndromes, 39
- Thalidomide, 99, 116, 257, 259, 346, 358
- Therapeutic apheresis, in immunohematologic disorders, 402–405
 - erythrocytapheresis/ red cell exchange, 404
 - leukocytapheresis (leukapheresis), 404
 - photopheresis, 404
 - plasmapheresis, 405
 - red cell exchange and parasitemia, 405
 - red cell exchange and sickle cell anemia, 404–405
 - thrombocytapheresis, 404
- Therapeutic plasma exchange, 405t
- Therapeutic trial, 19
- Therapy-related (or secondary) MDS, 96
- 6-Thioguanine (6-TG), 173t
- Thrombin time (TT), 310, 457
- Thrombocytapheresis, 404
- Thrombocytopenia, 68, 115, 134, 140, 289, 331
 - causes of, 303

- drug-induced thrombocytopenia, 304
- gestational thrombocytopenia, 304
- hemophagocytosis, 305
- HIV-related thrombocytopenia, 304
- infection-and sepsis-related thrombocytopenia, 305
- pseudothrombocytopenia, 303–304
- qualitative platelet disorders, 305
- Wiskott–Aldrich syndrome (WAS), 305
- disorders characterized by decreased production of platelets, 290
 - bone marrow failure, 290–291
 - cyclic thrombocytopenia, 292
 - irradiation and chemotherapy, 292
 - marrow infiltration, 291
 - myelodysplasia, 291
 - nutritional deficiencies, 292
- disorders characterized by increased clearance of platelets, 290t, 292
 - disseminated intravascular coagulation (DIC), 301–302
 - extracorporeal circulation-related thrombocytopenia, 302
 - heparin-induced thrombocytopenia (HIT), 294–296
 - immune thrombocytopenia (ITP), 292–294
 - neonatal alloimmune thrombocytopenia (NAIT), 302
 - posttransfusion purpura (PTP), 302
 - thrombotic microangiopathies (TMAs), 296–301
 - von Willebrand disease (VWD), type 2B, 302
- disorders characterized by increased sequestration of platelets, 303
- drugs associated with, 300t
- etiology and clinical features of, 289–290
- platelet biology, 289
- in pregnancy, 425
 - diffuse intravascular coagulation (DIC), 427–428
 - hemolysis, elevated liver enzymes, and low platelets syndrome (HELLP), 426–427
 - hemolytic uremic syndrome (HUS), 427
 - immune thrombocytopenic purpura (ITP), 425–426

- incidental thrombocytopenia, 425
- preeclampsia, 426
- thrombotic thrombocytopenic purpura (TTP), 427
- › Thrombocytopenia-absent radii (TAR), 140
- › Thrombocytopenia with absent radius (TAR) syndrome, 291
- › Thrombocytosis, 112, 115
 - essential thrombocytosis. See Essential thrombocytosis (ET)
- › Thromboelastography (TEG), 311
- › Thromboembolism, 141
- › Thrombolysis/ thromboembolectomy, 296
- › Thrombomodulin, 359
- › Thrombophilias, 430
- › Thrombopoietin, 96, 97
- › Thrombosis, 140
 - acquired prothrombotic disorders, 141–142
 - associated with antiphospholipid antibodies, 345
 - congenital prothrombotic disorders, 140–141
 - and venous access devices, 362
- › Thrombotic microangiopathies (TMAs), 296–301, 406
 - drug-induced, 304
- › Thrombotic thrombocytopenic purpura (TTP), 27, 34, 134, 296, 299, 361, 406, 427
- › Thymidylate synthesis, 12
- › Thymoma, 77
- › Thymus and activation-regulated chemokine (TARC), 434
- › Thyroiditis, 15
- › Ticlopidine, 333
- › Tinzaparin, 344, 358
- › Tissue injury, excess iron and, 412
- › Tissue neutropenia, 123
- › Tissue plasminogen activator (t-PA), 46, 362
- › Tissue thromboplastin inhibition (TTI) test, 345
- › T-large granular lymphocytosis (T-LGL) lymphoproliferative disorders, 90
- › Tositumomab, 243
- › Total body irradiation (TBI), 173, 274

- › Total iron-binding capacity (TIBC), 133, 413, 450–451
- › Total skin electron beam therapy (TSEBT), 250
- › TP53 gene, 156t
- › Transcobalamin 1, 19
- › Transcobalamin 2, 19
- › Transcranial Doppler ultrasound (TCD), 46, 135
- › Transferrin, 2, 3, 4
- › Transferrin saturation (TS), 4, 413, 416
- › Transforming growth factor (TGF) β , 115
- › Transfusion dosing in pediatrics, 144t
- › Transfusion reactions and adverse sequelae, 389, 390t
 - acute hemolytic transfusion reaction, 390–391
 - alternatives to allogeneic blood transfusion, 396, 397t
 - anaphylactic transfusion reactions, 392
 - bacterial contamination and sepsis, 393–394
 - causes of hemolysis, 391–392
 - delayed hemolytic transfusion reaction (DHTR), 391
 - disseminated intravascular coagulation (DIC), 400
 - febrile nonhemolytic transfusion reactions (FNHTR), 395
 - hemolysis associated with passive antibody infusion, 391
 - hemosiderosis, 395
 - hypotension associated with transfusion, 395
 - massive transfusion, 396–400
 - mild allergic/ urticarial transfusion reactions, 394
 - pharmacologic hemostatic agents, 398–399t
 - posttransfusion purpura (PTP), 394–395
 - sickle cell hemolytic transfusion syndrome, 391
 - transfusion-associated circulatory overload (TACO), 393
 - transfusion-associated graft-versus-host disease (TA-GVHD), 393
 - transfusion-related acute lung injury (TRALI), 392–393
 - transfusion-transmitted infections, 396
- › Transfusion-related acute lung injury (TRALI), 392–393
- › Transfusions, 34, 144, 176–177
 - and iron chelation, 54

- specialized blood products to prevent toxicities, 145
- › Transient erythroblastopenia of childhood (TEC), 134
- › Transplant-related mortality (TRM), 159, 192
- › Trimethoprim/ sulfamethoxazole (TMP/ SMX), 123
- › Triosephosphate isomerase (TPI) deficiency, 32
- › Trisomy 21, 168
- › Tropical disease, manifestations of, 431–432
- › Tropical spastic paraparesis (TSP), 236
- › Trouseau syndrome, 324, 346
- › TROUSSEAU's syndrome, treatment of, 360
- › Trypanosoma brucei gambiense, 432
- › Trypanosoma brucei rhodesiense, 432
- › Tumor lysis syndrome (TLS), 176, 205
- › Tumor necrosis factor α (TNF α), 67, 359
- › Tyrosine kinase inhibitors (TKIs), 181, 184, 186, 188, 191–194
 - as first-line treatment, 195
 - optimal/ failure of response to, 191
 - trigger to change, 195
- › Ultrasonography, for imaging of deep venous thrombosis, 340
- › Ultraviolet (UV) light phototherapy, 237, 250
- › Umbilical cord transplants, 285
- › Unbound iron capacity (UIBC), 413
- › Unfractionated heparin (UFH), 343, 356, 359, 360, 429–430
- › Unmutated IGHV genes (U-IGHV), 201
- › Uric acid, 462
- › Uridine 5' monophosphate hydrolase, 32
- › Urine hemosiderin, 29, 456
- › Urine protein electrophoresis, 460
- › Variegate porphyria, 64
- › Vaso-occlusive crises, 49, 135
- › Vaso-occlusive episodes (VOE) and SCD, 44
- › Venetoclax, 205, 208
- › Venofer, 8t

- Venography, 340
- Veno-occlusive disease (VOD), 274
- Venous thromboembolism (VTE), 140, 324, 338, 356
 - acquired thrombophilic states, 343
 - activated protein C resistance (Factor V Leiden), 348–349
 - antiphospholipid antibody syndrome (APA), 345–346
 - deficiencies of proteins S and C and antithrombin (antithrombin III), 349
 - heparin-induced thrombocytopenia (HIT), 343
 - heparin-induced thrombocytopenia with thrombosis, 343–345
 - hypercoagulability of malignancy, 346–347
 - hyperhomocysteinemia, 349–350
 - inherited hypercoagulable states, 348
 - myeloproliferative disorders (MPDs), 348
 - nephrotic syndrome, 345
 - paroxysmal nocturnal hemoglobinuria (PNH), 347
 - prothrombin mutation (G20210A), 349
 - surgery as a risk for acquired thrombosis, 347
 - deep venous thrombosis (DVT)
 - imaging of, 340
 - and pulmonary embolism, 338–340
 - in sites other than the distal veins of the lower extremities, 342–343
 - laboratory diagnosis of, 341–342
 - in pregnancy
 - diagnosis of, 363–364, 429
 - management, 364–365
 - prophylactic anticoagulation, 430
 - thrombophilias and recurrent miscarriage, 430
 - treatment, 429–430
 - prophylaxis, 356
 - in cancer patients receiving chemotherapy, hormonal, and/ or anti-angiogenic treatment, 358–359
 - in cancer patients undergoing surgical intervention, 357–358
 - in cancer patients who are immobilized/ hospitalized, 359
 - in patients with brain metastases and primary brain tumors, 360

- pulmonary embolism (PE)
 - diagnosis, 340–341
 - signs and symptoms of, 339t
 - treatment, 350
 - anticoagulation options in cancer patients, 361–362
 - extended treatment to prevent recurrent VTE, 351
 - fibrinolytic therapy, 352
 - general approach in treating VTE in cancer patients, 360–361
 - inferior vena cava filters in the treatment of deep venous thrombosis or pulmonary embolus, 351
 - initial treatment for acute VTE, 350–351
 - in patients with primary brain tumors or brain metastases, 360
 - prophylaxis, 350
 - thrombosis and venous access devices, 362
 - TROUSSEAU's syndrome, 360
- Venous thrombosis, 76
- Ventilation/ perfusion (V/ Q) lung scan, 363
- Vincristine, 172t, 173t, 217, 250t, 257, 269
- Visceral leishmaniasis (VL), 431
- Visual impairment, in patients with SCD, 47
- Vitamin B₁₂, 12
 - biology and dosing, 13t
 - clinical presentation, 16, 16t
 - daily adult requirement for, 12
 - deficiency of, 19–20, 135, 361
 - causes of, 14t
 - development of, 14–15
 - laboratory tests for, 18t
 - response to correction of, 21
 - and HIV infection, 15
 - laboratory evaluation, 17
 - hematologic abnormalities, 17
 - serum methylmalonic acid and homocysteine, 19
 - serum vitamin concentrations, 17–19

- therapeutic trial, 19
- metabolic pathways involving, 13f
- metabolic roles of, 12–14
- pancreatic enzymes, deficiency of, 15
- patient populations at risk, 16
- repletion, 20
- shortage of, 12
- small bowel, bacterial overgrowth in, 15
- in stomach, 15
- treatment/ response, 20–21
- Vitamin K, 136, 398t
 - Vitamin K antagonists (VKAs), 141t, 341
 - Vitamin K deficiency bleeding (VKDB), 136
 - Vitamin requirements, sources, and stores, 12
- Von Willebrand disease (VWD), 139, 307, 458
 - diagnosis, 326–327
 - epidemiology, 324
 - laboratory evaluation of, 326t
 - pathophysiology and classification, 324–325
 - pregnancy and, 328
 - presentation, 326
 - treatment, 327, 328t
 - type 1, 324
 - type 2, 324–325
 - type 2B, 302
 - type 3, 325
- Von Willebrand factor (VWF), 34, 139, 296, 324, 427
 - multimer analysis, 327f
 - replacement products, 327
- Von Willebrand syndrome, 110, 113
- Waldenström macroglobulinemia, 231, 460
- Warfarin, 296, 317, 346, 350, 367
- Warfarin-induced skin necrosis, 141

- › Warm autoimmune hemolytic anemia, [401](#)
- › Warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (WHIM) syndrome, [128](#)
- › White blood cell parameters, [443](#)
- › White blood cells (WBCs), [143](#), [145](#), [440](#)
- › Whole blood, [377](#), [378t](#)
- › Wiskott–Aldrich syndrome (WAS), [128](#), [140](#), [238](#), [305](#)
- › WT1 gene, [156t](#)
- › X-linked dominant protoporphyrina (XLDPP), [60](#), [62](#)
- › X-linked lymphoproliferative disorder, [238](#)
- › X-linked recessive inheritance, [307](#)
- › X-linked thrombocytopenia, [140](#), [305](#)
- › Yersinia enterocolitica, [9](#)
- › Yttrium 90-ibritumomab tiuxetan (Zevalin), [243](#)
- › ZAP-70, [202](#)
- › Zidovudine, [236](#)
- › Zollinger–Ellison syndrome, [15](#)