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SECOND EDITION

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Preface



THIS SECOND EDITION OF *PRACTICAL GUIDE to Transfusion Medicine* contains significantly updated coverage of key topics, such as noninfectious adverse effects of transfusion, transfusion-transmitted infectious diseases, and indications for transfusion, in which there have been a number of exciting and challenging developments during the 6 years since the first edition was published. We have also revised and updated the information on blood group antigens, blood substitutes (with focus on developments in the area of hemoglobin-based oxygen substitutes), and plasma derivatives. Finally, the chapter on compatibility testing has been expanded to cover selection of blood components for transfusion, which had been detailed in later chapters in the first edition. What has not changed is the focus providing scientific information in a manner that highlights its clinical relevance.

Practical Guide to Transfusion Medicine, as its name implies, is intended to provide practical guidance to those involved in the clinical aspects of transfusing blood, particularly in the hospital setting. It addresses two very different populations. First, it serves as an introduction to blood banking for the clinicians who order blood components and the nurses who administer them—ie, it is the book we wish we had when we were on the wards, in the emergency room, or in the operating room. Second, this volume is intended for those in training to become blood bankers, both physicians and medical technologists—ie, it is the book we wish had in medical school, residency, and fellowship and the book we would like our residents, medical technology students, and new technologists to have.

While no book can be all things to all people, we tried wherever possible to include a level of detail that would make this volume a useful resource for our own personal use, as well. Likewise, in areas of controversy, we have attempted not only to present our points of view, but also to include enough of the background data to provide a window into the thought process that led us to a particular conclusion.

In order to facilitate assimilation of key concepts and to alert readers to potentially risky actions, we have designed a system of marginal notes to accompany the text. Marginal notes fall into one of five categories:



Key concept: This icon defines a pivotal concept around which much of the discussion on the page centers.

Example: If you lack an antigen, then you are able to make alloantibody against that antigen. If an antigen is present, you cannot make alloantibody to that antigen.



Quick Summary: Quick summary notes provide a synopsis of the material in a given section, usually in more detail than is included with key concepts.

Example: In addition to exposure through transfusion and fetomaternal hemorrhage, sensitization to red cell antigens can occur through sharing of blood-contaminated needles by intravenous drug users or by the exchange of blood between two “blood brothers.”



Hot Tip: Hot tips are very short “pearls” that provide information that is readily memorized and probably worth committing to memory.

Example: One unit of Red Blood Cells should raise an adult's hemoglobin by 1 g/dL or hematocrit by 3%.



Caution: Caution notes are analogous to storm watches or yellow traffic lights. They alert the reader to situations that are potentially serious, but not always so.

Example: Group O-negative, uncrossmatched RBC units are not entirely without risk, because you do not know whether the patient has any other red cell antibodies (risk: approximately 1 in 1000).



Warning: Warnings, on the other hand, are analogous to storm warnings or red lights. They contain information that, if unheeded, is likely to result in untoward consequences.

Example: Platelets are contraindicated in patients with TTP.

In short, in this book we have aimed to provide a distillate of the current body of knowledge, presenting the material in a conversational style using language comprehensible to the nonspecialist and highlighting key ideas, facts, and conclusions in the form of marginal notes. If we have succeeded in our goals, the reader who completes this volume should have a solid basis on which to make intelligent transfusion decisions as well a good foundation for further reading. If we can convince the reader that, rather than being frightening, transfusion medicine is fascinating and fun, then we will have achieved our highest goal, which is to prove by example the statement "medicine is a whole lot more enjoyable when you know what you are doing."

Marian Petrides, MD
Gary Stack, MD, PhD
Laura Cooling, MD, MS
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About the Authors



Marian Petrides, MD, is associate professor of clinical pathology and medical director of the transfusion service and coagulation laboratory at the University of Missouri School of Medicine/University of Missouri Health Care in Columbia, MO. Dr. Petrides received her AB magna cum laude in history and science from Harvard University and her MD from Dartmouth Medical School. Following an internship in internal medicine at Rutgers University, she practiced primary care and emergency medicine for 15 years. She completed a residency in clinical pathology at Dartmouth-Hitchcock Medical Center and fellowship in transfusion medicine at Yale-New Haven Hospital. She has been practicing academic transfusion medicine since then, and is board-certified in clinical pathology and blood banking/transfusion medicine.

Dr. Petrides is a fellow of the College of American Pathologists and an active member of AABB. Her AABB activities include serving on the Annual Meeting Planning Committee, lecturing at the Annual Meeting, reviewing for *TRANSFUSION*, and authoring book chapters. She is keenly interested in computer technology, and is the software developer and primary author of the AABB Press CD-ROM *Transfusion Medicine Interactive: A Case-Study Approach*.

Gary Stack, MD, PhD, is associate professor of laboratory medicine at the Yale University School of Medicine, New Haven, CT and chief of the pathology and laboratory medicine service of the Veterans Administration Connecticut Healthcare System, West Haven, CT.

Dr. Stack received his MD from Johns Hopkins University and served his residency at Yale-New Haven Hospital, where he also completed a fellowship in transfusion medicine. He holds BS and PhD degrees in biochemistry from the Universities of Maryland and Wisconsin, respectively, and completed a fellowship in molecular biology at the Institut de Chimie Biologique in Strasbourg, France. Dr. Stack is board-certified in clinical pathology and blood banking/transfusion medicine, is a member of the AABB, and is a fellow of the College of American Pathologists.

Laura Cooling, MD, MS, is clinical professor of pathology at the University of Michigan in Ann Arbor. She is also associate medical director of transfusion medicine and director of the progenitor cell processing laboratory at the University of Michigan Hospitals. Dr. Cooling received her MD and MS from the University of Iowa in Iowa City and completed her residency in laboratory medicine and a fellowship in transfusion medicine at the University of Iowa Hospitals and Clinics. She is board-certified in clinical pathology and transfusion medicine.

Dr. Cooling has received numerous grants and research awards, primarily for work in her areas of focus—glycobiology and blood group serology. She has published a variety of articles and abstracts in peer-reviewed journals and contributed chapters in multi-authored books. She is a contributor to many professional society communications vehicles, including *AABB News*.

An active AABB member, Dr. Cooling serves on the Scientific Section Coordinating Committee, the Annual Meeting Planning Committee, and the Selection of Abstracts Program Unit. She is a frequent invited speaker before various groups, including the AABB and the Michigan Association of Blood Banks (MABB). She currently serves on the MABB's Board of Directors and is its president-elect.

Lou Ann (Lanne) Young Maes, MD, is currently associate professor in the Department of Pathology at the University of Illinois at Chicago (UIC). She is also medical director of the SBB graduate program at the UIC College of Health-Related Professions. In addition to hospital appointments at UIC earlier in her service there, Dr. Maes has held hospital, administrative, and faculty appointments at the Medical University of South Carolina and the University of Arkansas for Medical Sciences.

She received her MD at the University of Arkansas for Medical Sciences, and remained there for her internship, a postdoctoral research fellowship, and residency. She completed a fellowship in transfusion medicine at the Univer-

sity of Iowa Hospitals and Clinics. She is board-certified in transfusion medicine and clinical pathology.

Dr. Maes is an active volunteer in several professional groups, including the National Marrow Donor Program, the American Society for Clinical Pathology, the American Society for Apheresis, and AABB. Her activities for these and other groups include service on committees, peer-review of abstracts and manuscripts for publication, and directing continuing education sessions at national meetings.

In addition to teaching others in the classroom and through long-distance learning, Dr. Maes enjoys her work on clinical research. Some of her most recent grants have focused on embryonic stem cell research, sickle cell disease, macular degeneration, thrombotic thrombocytopenic purpura, oral candidasis, and the development of an animal model to study oral cancers.

1

Blood Component Preparation

MARIAN PETRIDES, MD



ANY DISCUSSION OF THE COLLECTION AND processing of blood and blood components benefits from review of the methods used to screen donors in order to minimize the risk of infectious disease transmission. Donors are questioned to identify those who have engaged in high-risk behaviors that are likely to make them more prone to contract certain viral infections [especially human immunodeficiency virus (HIV) and hepatitis C virus (HCV)]. Donors are also questioned about travel to areas where malaria is endemic and about history of babesiosis and Chagas' disease because each of the intracellular parasites associated with those diseases can be transmitted by transfusion.

The donated blood is screened for the following infectious disease markers:

- HIV antibody and antigen (anti-HIV-1, anti-HIV-2) and HIV-1 nucleic acid testing (NAT)
- Hepatitis B surface antigen (HBsAg)
- Hepatitis B core antibody (anti-HBc, or "anti-core")
- Hepatitis C antibody (anti-HCV) and HCV NAT
- Human T-cell lymphotropic virus antibody (anti-HTLV-I and -II)
- Serologic test for syphilis



Donated blood is screened for:

- HIV (antibody and NAT)
- HTLV
- Hepatitis B (antigen and anti-core)
- Hepatitis C (antibody and NAT)

Syphilis (serologic test)

At the time of publication, an ELISA test for Chagas' disease had just been licensed. While testing for Chagas' disease is not yet mandated, it likely will be in the near future.



Screening tests are not available for:

Malaria
Babesiosis
Variant CJD

so donor history alone must be relied on.

Donors with positive test results, a positive history, or both are not permitted to donate blood. Note: Nucleic acid testing for HCV and HIV using the polymerase chain reaction (PCR) is performed on all donor samples, either as single tests or as a multiplex NAT test for HIV-1 and HCV simultaneously. In addition, NAT testing for West Nile virus (WNV) is performed on donor samples during endemic periods. Chapter 7: Adverse Effects of Transfusion discusses HIV, HCV, and WNV testing in more detail.

Because of growing concerns about the possibility of blood-borne transmission of Creutzfeldt-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (vCJD), American donors at particular risk of exposure as a result of travel or residency in areas with bovine spongiform encephalopathy (BSE) epidemics are deferred, as are donors who have received pituitary-derived human growth hormone, bovine insulin since 1980, or dura mater transplants. Also deferred are donors with blood relatives diagnosed with CJD unless the donor can be demonstrated by genetic testing to lack abnormal prion genes. Chapter 7: Adverse Effects of Transfusion discusses donor deferral for CJD/vCJD as well as potential screening tests that are under development.

Finally, since 2003, blood banks and transfusion services have been required to use methods to limit and detect bacterial contamination in all platelet components. Chapter 2: Blood Component Storage discusses in more detail the implications of that change on the storage of platelets.



Whole blood is collected from the donor and centrifuged ex vivo to produce:

Red Blood Cells
Platelets
FFP

Component Preparation

Most blood collected in the United States today is collected as whole blood (450-500 mL) and processed into one or more of the following components: Red Blood Cells (RBCs), Fresh Frozen Plasma (FFP), and Platelets. These components can also be collected separately by apheresis, which takes advantage of centrifugation to separate the elements, thereby allowing removal and collection of the desired component while the remainder of the whole blood is returned to the donor. Recent developments in the field of apheresis permit leukocyte reduction of the collected component in the process of collection, obviating the need for leukocyte filtration after collection.

Apheresis is discussed in detail in Chapter 14; thus, the remainder of this chapter is devoted to discussion of the components prepared from whole blood. It is important to

note, however, that the requirement for testing to limit the risk of bacterial contamination has resulted in a shift toward increased collection of platelets by apheresis rather than preparation from whole blood because, among other reasons, testing of a single apheresis unit is more economical financially and in terms of loss of product than testing of the six individual whole-blood-derived platelet concentrates that make up a platelet pool. Indeed, in some regions of the United States, the only platelet products collected are apheresis units.

Collection of whole blood with postcollection processing into components remains the mainstay of blood collection because it requires far less time on the part of the blood donor and uses less complex, more portable equipment for collection. Blood is collected in either 450-mL or 500-mL bags containing approximately 63 mL or 70 mL of anticoagulant/preservative solution, respectively. The anticoagulant most commonly used is either citrate-phosphate-dextrose (CPD) or citrate-phosphate-dextrose-adenine (CPDA). CPD is chosen if the resultant RBC unit is destined to be an additive solution unit, whereas a unit that will not receive additive solution is collected into a bag that contains CPDA.

The volume of blood drawn is limited to a maximum of 15% of the donor's estimated blood volume, including samples. Collection from donors weighing less than 50 kg (110 pounds) must be proportionately smaller. When 300–404 mL are drawn, the same volume of anticoagulant/preservative solution may be used, but the unit must be labeled "Low-Volume Unit: ___ mL RBCs." Volumes under 300 mL require proportionate reduction in the amount of anticoagulant/preservative solution.

The most commonly used blood collection bag systems are composed of three bags: a primary bag containing the anticoagulant/preservative solution into which the whole blood is drawn, a satellite bag containing an additive solution designed to prolong red cell survival and function, and an empty satellite bag. If only whole blood is desired, as is often the case with autologous donations, a single bag containing anticoagulant/preservative solution may be used. Rarely, double bags are used when collection of platelet concentrates derived from whole blood is not required.

After collection, the triple bag is centrifuged at 2000 rpm (2000 $\times g$) for 3 minutes in a "soft spin" that separates red cells from platelet-rich plasma (PRP). The PRP is expressed into the satellite bag by using a plasma expander (Fig 1-1), and additive solution is added to the RBC bag,



Blood components (RBCs, platelets, FFP, and Cryoprecipitated AHF) are anticoagulated with citrate, which is rapidly metabolized by the recipient's liver after transfusion.



RBC units contain 150 to 230 mL of red cells.



Figure 1-1. Plasma expressor.



Additive solution red cell units have a lower hematocrit (55-60%) than do units without AS (70%).



Platelets remain functional for 7 days after collection. However, until recently, concerns about bacterial contamination forced reduction of platelet shelf life to 5 days. The FDA recently approved a collection bag and test system combination for 7-day storage.

which is clamped, removed steriley, and placed in refrigerated storage (1-6 C) for up to 42 days. RBCs with additive solution contain approximately 30-40 mL of CPD plasma, 100 mL of additive solution, and 150-230 mL of red cells for a hematocrit of 55% to 60%. If additive solution is not used, the same volume of RBCs can be stored in approximately 60-70 mL of anticoagulant/preservative solution (hematocrit 70%) for 35 days if CPDA-1 solution is used or for 21 days if a solution lacking additional adenine is used [ie, CPD or acid-citrate-dextrose (ACD)].

Meanwhile, the PRP is spun a second time in a faster and longer “hard spin” (5000 rpm for 5 minutes), and the supernatant platelet-reduced plasma is expressed, leaving a concentrate of platelets suspended in approximately 50 mL of plasma. This platelet concentrate is “rested” for 1 hour, then put on a platelet rotator that provides gentle agitation of the platelets, and stored at room temperature (20-24 C) for up to 5 days.

Although platelets have been demonstrated to remain viable under those storage conditions for as long as 7 days, the 5-day storage interval is prescribed because storage at room temperature for longer periods is associated with an unacceptably high rate of bacterial contamination. However, now that collection facilities and transfusion services are testing platelets for bacterial contamination before issue, it has become possible for the Food and Drug Admin-

istration (FDA) to approve one combination of collection bag and test system for 7-day storage of platelets.

There will still be some red cells and white cells in the platelet suspension thus produced, but the platelet concentrate should not be bloody. A crossmatch to ensure compatibility of this small volume of red cells with the intended recipient's serum is not necessary unless the estimated red cell content exceeds 2 mL, as would be the case with a grossly bloody platelet concentrate. Generally, platelet concentrates are pooled immediately before transfusion, in pool sizes ranging from 4 units to 10 units, but most commonly in 6 units. Six-unit platelet pools are generally regarded as being therapeutically equivalent to a single unit of apheresis platelets.



Unless the red cell content of a platelet unit exceeds 2 mL (grossly bloody unit), a crossmatch of the recipient's serum and the donor's red cells is not required before platelet transfusion.

Fresh Frozen Plasma and Cryoprecipitated AHF

The expressed platelet-poor plasma is frozen (-18°C or colder) and can be stored at that temperature for up to 12 months after phlebotomy. Freezing must take place within 8 hours of phlebotomy to ensure preservation of the labile coagulation factors (Factor V and Factor VIII). This plasma, called Fresh Frozen Plasma, is a source of coagulation factors, both labile and stable, including fibrinogen. Plasma may be frozen more than 8 hours after phlebotomy, but this plasma will be deficient in the labile factors to a greater or lesser degree, depending on the time interval. Such plasma is labeled "Plasma Frozen within 24 Hours After Phlebotomy."

If Cryoprecipitated AHF (cryo), which provides fibrinogen and von Willebrand factor in a concentrated form, is to be prepared from FFP, the FFP is left frozen for 24 hours and then thawed in a refrigerator. After thawing to the slush stage, the FFP is spun at 4200 rpm for 10 minutes in a refrigerated (4°C) centrifuge. The supernatant plasma is removed, leaving the cryo in 5-15 mL of plasma. The cryo is then frozen and stored at -18°C or colder for up to 12 months. The cryosupernatant plasma (officially designated "Plasma Cryoprecipitate Reduced," but commonly referred to as "cryo-poor plasma") is also refrozen and stored at -18°C or colder for up to 12 months. Plasma Cryoprecipitate Reduced is used primarily for plasma exchange in thrombotic thrombocytopenic purpura (TTP). However, it remains unclear whether using Plasma Cryoprecipitate Re-



FFP contains 1 U/mL of all coagulation factors (pro- and anticoagulant) and 2-4 mg fibrinogen/mL.



Cryo contains fibrinogen and vWF in concentrated form (5-15 mL volume per cryo unit).



After thawing:

FFP and Plasma Cryoprecipitate Reduced are stored refrigerated for up to 24 hours.

Cryo is stored at room temperature for a maximum of 4 hours.

duced instead of FFP in the treatment of TTP provides any real advantage.

Before FFP, Plasma Cryoprecipitate Reduced, or cryo can be used for transfusion, the components must be thawed in a waterbath at 30-37 C. After thawing, FFP and Plasma Cryoprecipitate Reduced can be stored refrigerated for up to 24 hours. Cryo, however, is stored at room temperature after thawing and outdates in 4 hours.

Plasma Derivatives

Instead of being stored as FFP, plasma from whole blood units (as well as plasma obtained by plasmapheresis) can be frozen and sent to fractionation facilities where it can be processed into plasma derivatives such as albumin, intravenous immune globulin (IVIG), and factor concentrates, most notably Factor VIII concentrate and Factor IX concentrate. The FFP that goes into those products is thawed at the fractionation center and pooled into large (thousands of units) lots.

The pooled plasma is fractionated using a technique known as Cohn fractionation, which involves cold ethanol precipitation, and further processed into the product of choice (see Table 13-1). Processing involves a purification step that selectively enriches the concentration of the desired factor in the purified product. One common method of purification involves passing the fractionated plasma over an immunoaffinity column impregnated with antibody directed against the factor desired. This technique causes the desired factor to adhere to the column, while the remaining plasma passes through and is discarded. An elution step frees the desired factor from the column and allows collection of a purified concentrate consisting almost exclusively of the target factor. Ion-exchange chromatography columns and heparin-sepharose affinity columns are among the alternatives that can be used, depending on the factor desired. The resultant concentrate is then lyophilized (freeze-dried) and must be reconstituted before use.

It is important to note, in this context, that certain Factor VIII preparations are purified using an immunoaffinity column directed against von Willebrand factor (vWF), which travels in a complex with Factor VIII procoagulant, rather than a column that selects for Factor VIII per se. Concentrates purified in this fashion, most notably Humate-P (CSL Behring, King of Prussia, PA) and Alphanate (Grifols USA,



Humate-P and Alphanate brands of Factor VIII concentrate contain vWF and can be used to treat vWD.

Los Angeles, CA), will contain significant amounts of vWF in addition to Factor VIII and, thus, can be used as a specific factor concentrate for treatment of von Willebrand disease (vWD). Factor VIII concentrates that are not purified by a method that uses a vWF capture antibody, however, will not contain significant quantities of vWF and can be used to treat only hemophilia A, not vWD.

In the past, infectious disease transmission was a problem with pooled plasma derivatives, most notably transmission of HIV in Factor VIII concentrates. Risk of virus transmission varies considerably depending on the fraction involved, with fractions that contain coagulation factors posing higher risk than the immunoglobulin fractions. All plasma derivatives are now subjected to a virus inactivation step (eg, pasteurization, solvent/detergent treatment, viral clearance filtration) as a routine part of the manufacturing process.

Because the causative agent of CJD/vCJD is not yet certain, no one can be positive that infection is not transmissible by pooled plasma products. Indeed, there have been several large-scale recalls of plasma derivatives in Canada because of CJD in a donor who contributed to the pool from which those derivatives were made. To date (early 2007), no vCJD cases have been associated with plasma products, although there have been four transfusion-transmitted cases of vCJD with cellular blood components.

So, although pooled plasma derivatives appear to be virtually risk-free with respect to transmission of known viral diseases, they should be used as cautiously as any other biologically derived product (or any other drug for that matter)—that is, only when clearly indicated and only in the minimal effective dose.

Table 1-1 summarizes the common blood components. Chapter 13 discusses plasma derivatives in detail.



To date, classic CJD has never been transmitted by blood components. However, there have been four cases of transfusion-associated vCJD with cellular blood components. No vCJD cases have been associated with plasma products.

Leukocyte Reduction at the Time of Collection (Prestorage Leukocyte Reduction)

Leukocyte reduction of cellular blood components is considered desirable for a number of reasons; in many cases, prestorage leukocyte reduction has been shown to be more effective than bedside leukocyte reduction by filtration. Thus, there is a growing movement in the United States toward universal leukocyte reduction at the time of collection. Such a policy has already been implemented in

Table 1-1. Summary of Common Blood Components

	Definition	Volume	Composition (per unit)	Shelf Life	Optimal Storage Conditions	Subcomponents
Whole Blood (WB)	Blood collected in an anticoagulant/preservative solution and not processed further	1 unit = 450 mL ± 45 mL blood, plus 63 mL anticoagulant/preservative (typically CPDA-1)*	Provides RBCs, plasma proteins, variable amounts of stable clotting factors depending on shelf-life of unit; volume expansion; about 10^8 leukocytes; no functional platelets except early in storage	35 days using CPDA-1	1-6 °C	
Red Blood Cells (RBCs)	Predominantly available as Additive Solution Red Blood Cells (AS-1 Red Blood Cells)	1 unit ≈ 320 mL	~180 mL red blood cells (hematocrit 55-60%) ~40 mL plasma with anticoagulant (CPD) 100 mL additive solution (900 mg NaCl; 27 mg adenine; 2.2 g dextrose; 750 mg mannitol) ~ 10^8 leukocytes	42 days	1-6 °C	Leukocyte-reduced Irradiated Washed Frozen-deglycerolized CMV-seronegative Split unit/reduced volume
Platelet Concentrates	Platelets separated from a whole blood unit by differential centrifugation	~50 mL	$\geq 5.5 \times 10^{10}$ total platelets ~50 mL plasma ~ 10^7 leukocytes ≤ 0.5 mL RBCs	5 days	20-24 °C with agitation	Irradiated Washed CMV-seronegative Reduced volume

Apheresis Platelets	Platelets collected from a single donor by apheresis	Approximately 300 mL	$\geq 3.0 \times 10^{11}$ total platelets Approximately 300 mL plasma <2 mL RBCs Most units leukocyte-reduced at collection. If so, $<5 \times 10^6$ leukocytes	5 days	20-24 C with agitation
Fresh Frozen Plasma (FFP)	Plasma separated by centrifugation from a whole blood unit and frozen within 8 hours of donation	~200 mL	Stable and labile coagulation factors (1 unit of each clotting factor and 2-4 mg fibrinogen per mL of plasma) Other plasma proteins (including albumin) About 20% of total volume is CPD anticoagulant solution	1 year	-18 C or colder
Cryoprecipitated AHF (Cryo)	Cold insoluble proteins precipitated from one unit of FFP when it is thawed in refrigerator	10-15 mL	200-250 mg fibrinogen (minimum of 150 mg required by regulations) 80-120 units of Factor VIII (minimum of 80 units is required by regulations) von Willebrand factor Factor XII Fibronectin	1 year	-18 C or colder

*500-mL bags are also available and hold proportionately more blood and anticoagulant/preservative. CMV = cytomegalovirus.
(Courtesy Gary Stack, MD, PhD, with modification.)



Potential benefits of leukocyte reduction include decreases in:

- Risk of CMV, HTLV, and EBV
- HLA alloimmunization
- Platelet refractoriness
- Febrile nonhemolytic reactions
- TRIM (transfusion-related immunomodulation)

the United Kingdom and Canada, largely in an attempt to reduce the risk of transfusion transmission of vCJD because such transmission appears to be leukocyte dependent.

Leukocyte reduction significantly decreases the risk of transmission of infectious agents that are obligate intracellular (intraleukocyte) pathogens, most notably cytomegalovirus (CMV) but also HTLV and Epstein-Barr virus. A 1995 study found that the risk of CMV transmission was comparable in marrow transplant patients who received leukocyte-reduced cellular blood components and in similar patients who were transfused with CMV-seronegative components. The question of whether leukocyte reduction alone suffices to reduce CMV risk remains controversial; nevertheless, it is clear that leukocyte reduction does significantly reduce the risk of CMV transmission. CMV risk-reduction strategies are discussed in more detail in Chapter 7: Adverse Effects of Transfusion.

Alloimmunization to HLA antigens and subsequent development of platelet refractoriness are also significantly reduced by leukocyte reduction. The incidence of febrile nonhemolytic transfusion reactions is likewise diminished, but not eliminated altogether, by leukocyte reduction. In addition, there is growing interest in the possibility that leukocyte reduction might also decrease the immunomodulatory effects of blood transfusion and, thus, might be of benefit in reducing the risk of posttransfusion bacterial infection and tumor recurrence.

Prestorage leukocyte reduction can be accomplished in several ways. First, apheresis equipment that removes leukocytes from cellular blood components in the process of collection is used widely. For components derived from whole blood, collection bags that incorporate a built-in leukocyte-reduction filter are available. It is also possible to use a sterile connecting device to “dock” a leukocyte filter to a bag of RBCs or platelets to accomplish leukocyte reduction immediately after whole blood is processed into components. Generally, this latter method is regarded as cost prohibitive for platelet concentrates because it would add considerably to the cost of a pool of platelet concentrates.

The advantages of prestorage leukocyte reduction include ensuring consistent quality of leukocyte reduction and removing leukocytes by means of prestorage filtration before they can release their activation products (cytokines) into the stored product. Although leukocyte reduction filters are capable of removing some cytokines and some of the white cell stroma that gets released by rupture



Prestorage leukocyte reduction is more effective than bedside filtration because it

- Reduces leukocytes before they can be activated
- Provides better quality control
- Avoids (rare) reactions to bedside filters

of leukocytes during storage, elimination of leukocytes before storage remains more effective in eliminating both cytokine and stromal contamination. Furthermore, leukocyte filtration at the bedside is often performed by relatively inexperienced personnel who may be unaware that 1) the filter must be perfectly vertical to function properly, 2) the blood component should not be forced through the filter under pressure, and 3) the filter should not be flushed after filtration. In addition, the occasional patient reaction to bedside filtration makes it preferable to filter cellular components immediately after collection and before storage.

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2

Blood Component Storage

MARIAN PETRIDES, MD



THE CONSTITUENTS OF BLOOD REQUIRE vastly different storage conditions; that fact is a driving force behind the separation of whole blood into components, making it reasonable to review the optimal conditions for storage of red cells, platelets, granulocytes, plasma, and Cryoprecipitated AHF (cryo). In the case of cellular components, storage must preserve not only function but also viability. Plasma and plasma-derived components such as cryo, though, must be stored in a fashion designed to preserve the function of proteins, most notably coagulation factors.

Before discussion of the specific storage requirements for individual components, a few general observations are in order. First, processing of whole blood into components must be completed within 8 hours because that is the time frame for freezing of Fresh Frozen Plasma (FFP) to ensure preservation of all coagulation factors. Second, once the closed collection system is breached, as it is when platelets and cryo are pooled or when red cells are washed, maximal allowed storage times are dictated by the temperature at which the component is stored after processing. The allowable postprocessing interval is primarily a reflection of the risk of growth of bacterial contaminants. Thus, units of Platelets and Cryoprecipitated AHF that are stored at room temperature after pooling outdate in 4 hours, whereas Red Blood Cell (RBC) units that are either washed or deglycerolized in an open system may be stored at refrigerator temperature for up to 24 hours.



Once the seal on a unit is breached for processing, the unit's allowable storage time is:

- 4 hours if stored at room temperature
- 24 hours if refrigerated

RBC Storage



2,3-DPG is critical to the ability of red cells to deliver oxygen to tissues.

RBC storage has two objectives: maintenance of red cell viability, which is measured by survival 24 hours after transfusion, and preservation of red cell function, which is reflected by the ability of transfused cells to deliver oxygen to tissues normally. RBC units are stored at refrigerator temperature (1-6 C) for as long as 42 days, depending on the preservative solution. Longer-term storage (up to 10 years) is possible if the RBCs are frozen in glycerol and maintained at temperatures below -65 C (if 40% glycerol is used) or -120 C (with 20% glycerol).

Oxygen release at the tissue level is largely dependent on intraerythrocyte 2,3-diphosphoglycerate (2,3-DPG) levels. Decreased 2,3-DPG results in increased hemoglobin affinity for oxygen, which translates into decreased oxygen delivery to tissues (ie, shift of the oxyhemoglobin dissociation curve to the left). Also, 2,3-DPG levels fall linearly after 2 weeks of storage, but once a unit is transfused, the transfused cells regenerate 2,3-DPG rapidly, thereby regaining about half within 12 hours and returning to full levels within 24 hours after transfusion.

The simplest of red cell storage solutions is ACD, or acid-citrate-dextrose, in which the citrate functions as an anticoagulant that is metabolized rapidly by the recipient after transfusion. The dextrose provides an energy source, and the acid pH prevents caramelization during storage, in addition to somewhat improving red cell survival. ACD is used as an anticoagulant during apheresis but is rarely used as a storage solution.

The addition of phosphate to storage solutions (CPD, or citrate-phosphate-dextrose) primarily improves red cell function by diminishing the loss of 2,3-DPG, which is critical to the ability of red cells to deliver oxygen efficiently. The addition of phosphate may also confer a slight survival advantage. Because adenine is rapidly deaminated to inosine on storage, an additional source of adenine enhances red cell survival, making CPDA (citrate-phosphate-dextrose-adenine) preferable to CPD. Current blood collection practices most commonly collect whole blood into a CPDA solution. If no further processing takes place, red cells can be stored in CPDA at 1-6 C for up to 35 days with acceptable posttransfusion viability and function.

Additive solutions containing saline, adenine, dextrose, and mannitol are capable of increasing the storage interval by 1 week, to 42 days. If those solutions are to be used, more plasma is expressed during processing, leaving about



2,3-DPG levels fall with storage but return to normal within 24 hours after transfusion.



Citrate anticoagulant is rapidly metabolized after transfusion.

30 mL of plasma instead of 70 mL, to which 100 mL of additive solution is added. As a consequence, the volume of an additive solution unit is 50 mL to 70 mL greater than a CPDA unit, but with a somewhat lower hematocrit (about 70-80% for CPDA; 55-60% for additive solution). Hematocrits in excess of 70% to 80% are not acceptable for prolonged storage because the units involved would contain insufficient preservative. Additive solutions must be added within 72 hours after phlebotomy to maximize the allowable storage interval.

One easy way to remember the constituents of additive solutions is to note that they are all variants on an early additive called SAGMAN, an acronym for saline, adenine, glucose (dextrose), and mannitol. Several additive solutions exist, including AS-1, or Adsol, which contains about twice as much dextrose as SAGMAN but is otherwise similar in composition; AS-3, or Nutricel, which is phosphate enriched; and AS-5, or Opticel, which contains slightly more adenine.

Rejuvenating Solutions for RBCs

Although not widely used because of cost, solutions that are capable of restoring depleted adenosine triphosphate (ATP) and 2,3-DPG levels in stored RBCs are available. Such rejuvenating solutions contain pyruvate, inosine, phosphate, and adenine and can be used to rejuvenate stored RBCs for up to 3 days after outdate. RBCs thus rejuvenated can be either glycerolized and frozen for prolonged storage or stored refrigerated for up to 24 hours. Inosine in the rejuvenating solution can be toxic to the recipient; thus, rejuvenated RBCs must be washed before they are transfused. Obviously, if rejuvenated RBCs are frozen, the deglycerolization that accompanies thawing will also remove inosine.

Frozen Storage of RBCs

To permit frozen storage of RBCs, glycerol is used as a cryopreservative, which allows freezing at a slow controlled rate without damage to the red cells. In the absence of a cryopreservative, slow freezing of cells results in the freezing of extracellular water before intracellular water, which in turn results in dehydration of the cell on freezing. Such intracellular hypertonicity not only results in significant cell damage but also leaves the cell at risk for tonic lysis on thawing. Rapid freezing, in contrast, damages cells by creating intracellular ice crystals.



Red cells stored in:
ACD or CPD outdate in 21 days
CPDA outdate in 35 days
Additive solution outdate in 42 days



RBCs can be rejuvenated using a special solution for up to 3 days past their outdate.



Frozen RBCs must be washed to remove glycerol after thawing. This process takes 1 to 2 hours.

Glycerol is chosen as a cryopreservative for red cells because its molecules are small enough to readily penetrate the red cell. Once inside the cell, glycerol provides an osmotic force that counteracts the osmotic gradient caused by extracellular formation of ice during the freezing process. Because glycerol is hypertonic to blood, it must be added gradually to allow equilibration with red cells. Likewise, after thawing at 37 C, RBCs that have been frozen in glycerol are washed in saline solutions of progressively decreasing tonicity, beginning with 12% saline and ending with normal (0.9%) saline. Only then can these frozen, thawed, and then deglycerolized RBCs be safely transfused.



Platelets that degranulate cannot resynthesize granules because they lack RNA.

Platelet Storage

As with RBCs, not only must transfused platelets be viable following transfusion, but also they must retain normal function. Platelets do not contain RNA, so once they have been activated and have undergone granule release, they are incapable of resynthesizing granules. Platelets are particularly susceptible to damage if improperly stored because improper storage can decrease platelet viability and may also result in platelet activation with consequent loss of function. Platelet activation may even affect platelet recovery because resultant adhesion molecules increase the rate at which activated platelets are cleared from the circulation. In fact, even platelets properly stored at 20-24 C have diminished posttransfusion recovery and decreased survival when compared with fresh platelets.

Temperature, pH, and gas exchange are all critical to platelet storage. Platelets damaged by cold lose their discoid shape and become spherical; thus, it is critical to ensure that platelets are stored at room temperature (20-24 C). It is possible to roughly assess the retention of discoid shape by observing the shimmering or swirling behavior of a platelet unit.

On storage, platelets rapidly deplete their glycogen and begin generating lactate, resulting in decreased pH. Even at intermediate pH levels (6.2-6.8), platelets demonstrate some changes, but those changes are reversible. However, platelets subjected to a pH below 6.2 undergo irreversible swelling, agglutination, and lysis. Consequently, the current standard for platelet storage specifies a pH greater than 6.2 at the end of allowable storage.



Platelet activation during storage results in loss of function (in-vitro degranulation) and may also reduce posttransfusion platelet recovery.

Platelets require storage in a gas-permeable bag for two reasons: to ensure proper oxygenation and to facilitate removal of carbon dioxide resulting from buffering of lactic acid produced on storage. Not only must the bag be gas permeable, but also the platelets must be agitated constantly and gently to facilitate gas exchange across the bag. Recent studies have shown, however, that it is possible to discontinue agitation for as long as 24 hours without harm. That margin allows for shipment of units over long distances.

Platelets remain viable for up to 7 days after collection, but the current requirement for a maximum storage interval of 5 days has resulted from observation of increased rates of clinically significant bacterial contamination when platelets are stored for more than 5 days. As with red cell 2,3-DPG, which is rapidly reconstituted after transfusion, there is evidence that at least partial recovery from the platelet storage lesion occurs once platelets are transfused.

Since 2003, blood banks and transfusion services in the United States have been required to use methods to limit and detect bacterial contamination in all platelet components. This requirement has two implications for storage of platelets. First, because collection and transfusion services are testing for bacterial contamination before issue of platelet units, it becomes theoretically possible to return to 7-day storage of platelets. Indeed, late in 2005, the Food and Drug Administration (FDA) approved one storage bag and test system combination for 7-day storage. However, only this one system has been approved, so not all platelet components are yet approved for 7-day storage.

One unfortunate consequence of the requirement for preissue bacteria detection is that the cost of such testing caused several blood centers to discontinue or markedly reduce production of whole-blood-derived platelet concentrates. However, also late in 2005, the FDA approved a prestorage pooling bag and test system combination that permits prestorage pooling of whole-blood-derived platelet concentrates, enabling a single test for bacterial contamination to be performed on the pool instead of six tests on individual concentrates.



Platelets remain viable for 7 days if properly stored. However, this does not necessarily mean platelet units can be stored for 7 days.

The allowable storage interval for most platelet units is 5 days because of the risk of bacterial contamination during extended room temperature storage.

Platelet units are now tested (usually by culture) to limit bacterial contamination. At the time of this writing (early 2007) the FDA had approved only one manufacturer's storage bag and test system combination for 7-day storage.



FFP contains significant amounts (1 U/mL) of all coagulation factors, both pro- and anticoagulant. This includes 2-4 mg/mL of fibrinogen.

Fresh Frozen Plasma

When frozen within 8 hours of collection, plasma can be stored for up to 1 year at -18°C or colder and still retain

significant amounts of all of the major procoagulant and anticoagulant clotting factors. Alternatively, FFP can be retained at -65 C or colder for up to 7 years. Although most coagulation proteins are stable at refrigerator temperatures (1-6 C), frozen storage is particularly important to the preservation of the labile coagulation factors—Factor V and Factor VIII—whose levels diminish rapidly even in the refrigerator. This propensity to diminish explains why FFP must be stored frozen and why it may be stored for only 24 hours in the refrigerator once thawed.

Several other plasma preparations also carry FDA approval for replacement of coagulation factors other than Factors V and VIII. They include “Plasma Frozen within 24 Hours After Phlebotomy” and plasma that has been separated from whole blood no more than 5 days after the expiration date of the whole blood (known as either “Plasma,” if stored frozen, or “Liquid Plasma,” if stored at refrigerator temperature). None of those plasma preparations should be used for the replacement of Factor V or Factor VIII.

However, a recent paper comparing levels of a variety of coagulation factors, including the labile factors (Factor V and Factor VIII) and fibrinogen, documented Factor VIII activity levels ranging from 50% to 76% at 2 days and from 43% to 66% at 3 days after thawing. The observed variation in levels correlated with the blood group, with group A having the highest levels and group B FFP having the lowest levels. Likewise, all other factors, including Factor V, remained at 75% or higher at 2 days and at 71% or higher at 3 days after thawing. Fibrinogen levels did not decrease with prolonged storage. These results have led a number of transfusion services to retain thawed but untransfused plasma for up to 2 or even 3 days after thawing, allowing it to be issued as “Thawed Plasma” instead of Fresh Frozen Plasma. Thawed Plasma is used in a manner similar to FFP for the treatment of deficiencies of multiple coagulation factors, for emergent reversal of warfarin, and as replacement plasma for thrombotic thrombocytopenic purpura. This practice not only reduces wastage of plasma components but also provides plasma in emergent situations, avoiding the time ordinarily required to thaw a frozen unit or units.



If not frozen within 8 hours of collection, plasma will have reduced levels of the labile coagulation factors (V and VIII).



Cryoprecipitated AHF contains significant amounts of fibrinogen, Factor VIII, and von Willebrand factor, but it is transfused almost exclusively to replace fibrinogen.

Cryoprecipitated AHF

The cold-insoluble portion of FFP contains significant amounts of fibrinogen, Factor VIII, and von Willebrand factor. Cryo-

Table 2-1. Component Storage Summary

Component	Storage Temperature	Duration of Storage	Preparation Required	Postpreparation Storage	Notes
Red Blood Cells, Additive solution [eg, AS-1 (Acsol), AS-3 (NutriCell)]	Refrigerator 1-6 C	Up to 42 days	None		Transport temperature 1-10 C.
Red Blood Cells, CPDA	Refrigerator 1-6 C	Up to 35 days	None		Transport temperature 1-10 C.
Red Blood Cells, ACD, CPD, CP2D	Refrigerator 1-6 C	Up to 21 days	None		Transport temperature 1-10 C.
Frozen Red Blood Cells In 20% glycerol In 40% glycerol	Frozen -120 C or below -65 C or below	10 years 10 years	Must be deglycer- olized Must be deglycer- olized	Following deglycer- olization, may be stored refriger- ated (1-6 C) for up to 24 hours.	Following deglycer- olization, product is essentially a washed red cell unit. Transport at 20-24 C. May be without agitation for up to 24 hours.
Platelets	Room temperature (20-24 C) Requires gentle agitation	Up to 5 days	Pool before issue Typical pool size 4-10 units	Must be infused within 4 hours of pooling	(continued)

Table 2-1. Component Storage Summary (continued)

Component	Storage Temperature	Duration of Storage	Preparation Required	Postpreparation Storage	Notes
Apheresis Platelets	Room temperature 20-24 C Requires gentle agitation	Up to 5 days	None	Not applicable	Transport at 20-24 C. May be without agitation for up to 24 hours.
Fresh Frozen Plasma	Frozen -18 C or colder or -65 C or colder	Up to 12 months Up to 7 years	Thaw at 30-37 C before use	May be stored refrigerated (1-6 C) for up to 24 hours after thawing.	Placed in freezer within 8 hours of collection (6 hours if collected in ACD).
Plasma Frozen within 24 hours After Phlebotomy	Frozen -18 C or colder or -65 C or colder	Up to 12 months Up to 7 years	Thaw at 30-37 C before use	May be stored refrigerated (1-6 C) for up to 24 hours after thawing.	Placed in freezer within 24 hours of collection. Contains reduced levels of labile coagulation factors (Factors V and VIII).
Plasma Cryoprecipitate Reduced	Frozen -18 C or colder	Up to 12 months	Thaw at 30-37 C before use	May be stored refrigerated (1-6 C) for up to 24 hours after thawing.	Open system or pooled: transfuse within 4 hours. Single unit: transfuse within 6 hours.
Cryoprecipitated AHF	Frozen -18 C or colder	Up to 12 months	Thaw at 30-37 C before pooling	Usually pooled before issue. Store at room temperature (20-24 C) after thawing.	Open system or pooled: transfuse within 4 hours. Single unit: transfuse within 6 hours.

precipitated AHF, or cryo, is prepared by thawing FFP at 1-6 C in a refrigerator and then centrifuging it at the same temperature. Once separated, the cryoprecipitate is again frozen and can be stored at -18 C for up to 1 year. As with FFP, cryo is thawed at 37 C before use, but, unlike FFP, it is stored at room temperature after thawing. Thus, although thawed FFP outdates after 24 hours of refrigerated storage, cryo must be pooled and transfused within 4 hours of thawing.

General Storage Considerations

All blood components must be stored in equipment designed to ensure maintenance of proper thermal conditions. (Component storage conditions are summarized in Table 2-1.) Refrigerators, freezers, and platelet incubators should have a means of continuous temperature monitoring that records the storage temperature at intervals of 4 hours or less. Temperature records must be maintained for at least 5 years.

Refrigerators and freezers must also be equipped with an alarm system that provides sufficient warning to permit removal of blood components to a proper storage environment in the event of failure. In other words, the alarm should trigger before the temperature rises (or falls) to an unacceptable level. Obviously, provision must be made for the alarm to be monitored by personnel capable of responding to it immediately. Whenever possible, there should be an independent emergency power source available, or, if this source is not feasible, appropriate measures for alternative storage during an emergency must be in place.



When used for blood component storage, freezers, refrigerators, and platelet incubators must be continuously monitored for temperatures. Storage temperatures must be recorded at intervals of 4 hours or less.

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3

Pretransfusion Compatibility Testing

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BLOOD GROUP SEROLOGY STUDIES 1) THE antigenic substances on the surface of red cells that may be recognized as foreign if transfused into a recipient who is not antigenically identical to the donor and 2) the antibodies those substances stimulate and then react with. The only commonplace setting in which a recipient is likely to be identical to the donor is if the recipient and donor are identical twins, so, in the majority of cases, transfused blood is potentially capable of inducing an antibody response in the recipient.

The expression of blood group antigens is controlled by genes. In some cases, genetic control is direct, as in protein antigens such as Rh, Kidd, Kell, and Duffy. In other cases, including carbohydrate antigens such as ABO and Lewis, genes control the expression of an enzyme that is responsible for attaching a sugar that confers antigenic specificity to a precursor carbohydrate backbone.

Blood group antigens are expressed codominantly, and the genes for some are so closely linked on their respective chromosomes that they are almost always inherited together as a haplotype. This linkage is true of the Rh group antigens C/c and E/e and of the HLA antigens.

If an individual lacks a particular antigen on his or her red cells, the individual is capable of producing an antibody to that antigen if the antigen is present on red cells received in a blood transfusion, through fetomaternal hem-



Protein antigens such as Rh group, Kidd, Kell, and Duffy antigens are under direct genetic control. For carbohydrate antigens, genes control the expression of an enzyme that attaches the immuno-dominant sugar to a precursor backbone.



IgM antibodies can cause direct agglutination on in-vitro tests, whereas IgG antibodies usually require the use of antihuman globulin (Coombs) reagent to permit bridging across red cells and, thus, agglutination.



IgG antibodies are more likely to be active at body temperatures (37 C) and, thus, are usually more clinically significant than IgM antibodies, which tend to be reactive in the cold (room temperature or below).



Many terms are used interchangeably:

- Indirect antiglobulin test
- IAT
- Indirect Coombs test
- Antibody screen
- Direct antiglobulin test
- DAT
- Direct Coombs test
- Antihuman globulin (AHG)
- Coombs reagent
- Antiglobulin reagent
- AHG phase (of antibody screen or ID panel)
- IAT phase
- Coombs phase

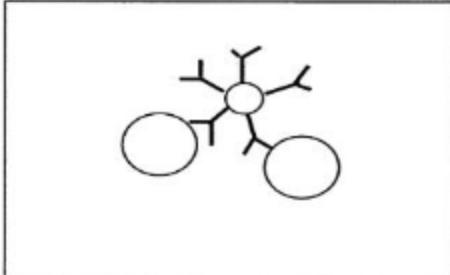
orrhage, or through other exposure to allogeneic blood. Conversely, if the antigen is present on a person's red cells, he or she will not form alloantibody against that antigen. (Autoantibodies are a different matter.)

Some blood group antigens tend to elicit an antibody response that is primarily IgM, and some predominantly stimulate production of IgG antibodies. Whether the immune response is primarily IgM or mainly IgG determines the reaction characteristics of the antibody in compatibility testing. The nature of the antibody response also has significant implications in determining the severity of the reaction if antigen-positive blood is transfused into a recipient who is capable of forming (or who has already formed) antibody directed against that particular antigen.

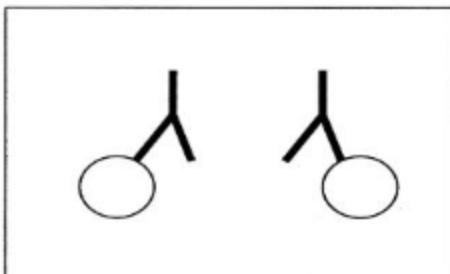
The structure of the antibodies themselves plays a significant role in determining how they react in in-vitro testing. IgM antibodies are pentamers consisting of five immunoglobulin subunits, each carrying a pair of Fab fragments and, therefore, having a total of 10 potential antigen-binding sites. IgG antibodies, in contrast, are considerably smaller, consisting of a single immunoglobulin subunit and having only two Fab fragments to act as antigen-binding sites. In addition, a single IgM molecule can bridge the distance between two red cells, which are ordinarily held at a distance by ionic forces termed the "zeta potential," and can directly cause agglutination. IgG molecules, however, cannot ordinarily bridge the distance between red cells; thus, they require the presence of an antihuman globulin (AHG) reagent to participate in the bridging. AHG, often referred to as the Coombs reagent, may consist of rabbit antibody directed against human IgG or monoclonal anti-IgG. (See Fig 3-1.)

IgM antibodies are more efficient at both binding and activating complement. Consequently, one might expect IgM antibodies to be more likely to cause intravascular hemolysis. However, IgM antibodies also tend to be reactive primarily at room temperature or below (20-24 C) and only infrequently at body temperature (37 C). Thus, with the exception of ABO antibodies (which are usually a mixture of IgG and IgM and are reactive across a wide thermal range, from room temperature to 37 C), IgM antibodies that are active in vitro only, but not at body temperature, are unlikely to cause major intravascular hemolysis in vivo.

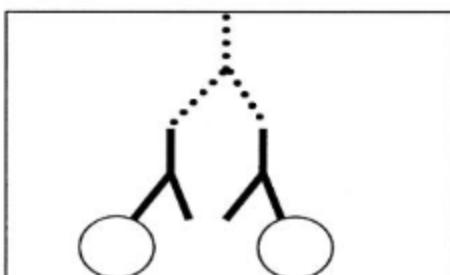
Sometimes IgM antibodies are referred to as "complete" antibodies, meaning they can cause agglutination without requiring antiglobulin reagent, whereas IgG antibodies are called "incomplete," meaning they are incapable of caus-



A single IgM molecule is capable of bridging two red cells, causing their direct agglutination.



A single IgG molecule alone is often incapable of bridging two red cells.



Addition of the antihuman globulin reagent (anti-IgG), depicted with dashed lines, allows bridging between two IgG-sensitized red cells, producing agglutination.

Figure 3-1. Diagram of the function of the antihuman globulin reagent.

ing agglutination in the absence of antiglobulin reagent. Those two terms are not particularly helpful because they imply that IgG antibodies are somehow defective in structure, which they really are not.

In the absence of activation of complement, binding of antibody to red cells (Fab fragment of antibody binding to antigen on red cell surface) results in extravascular clearance of the red cells because the Fc portion of the bound antibody gets recognized by macrophage/monocytes in the reticuloendothelial system (RES). If complement is bound to the antigen-antibody complex but not activated, this action further facilitates clearance of the antibody-coated red cells by the RES. This action is true whether the antibody is IgM or IgG.

IgG antibodies, unlike their IgM counterparts, tend to be reactive at physiologic temperatures and may bind and even activate complement in some cases. Furthermore, because they are small, IgG antibodies can cross the placenta from mother to fetus and can enter the circulation of the fetus, causing hemolytic disease of the fetus and newborn (HDFN) in the fetus or the neonate. Thus, IgG antibodies tend to be more of a concern than are IgM antibodies in non-ABO blood group serology. The most clinically important red cell antibodies (ABO, Rh, Kidd, Kell, and Duffy) all have a significant IgG component.

Not all red cell antigens are equally immunogenic; some are potent immunogens and some rarely elicit an antibody response. Likewise, some patients tend to be antibody "responders," whereas others require far more antigenic stimulus to evoke an antibody response.

Sensitizing exposure to red cell antigens can occur by transfusion of blood or other cellular blood components, such as platelets or granulocytes, or during pregnancy through fetomaternal hemorrhage. Hemorrhage from the fetus to the mother most commonly occurs at the time of delivery, miscarriage, or abortion. In addition, invasive procedures during pregnancy, such as amniocentesis, also carry a risk of significant fetomaternal hemorrhage. Rarely, exposure to red cell antigens can occur by other means, such as the sharing of blood-contaminated needles by intravenous drug users or by the exchange of blood between two "blood brothers."

Transfusion history can sometimes be misleading because, in exceptional situations such as those noted, a patient may appear to have no history of allogeneic exposure when such exposure did take place. Thus, although it is important to obtain a transfusion history to determine whether a



The most clinically important antibodies are those directed against antigens in the ABO, Rh, Kell, Kidd, and Duffy blood groups. All of these have a significant IgG component.



In addition to exposure through transfusion and fetomaternal hemorrhage, sensitization to red cell antigens can occur through sharing of blood-contaminated needles by intravenous drug users or by the exchange of blood between two "blood brothers."

patient is likely to have a red cell alloantibody, those other factors must also be considered.

Some red cell antibodies can occur without apparent sensitizing exposure. They are termed "naturally occurring" or "non-red-cell-stimulated" antibodies, which is a misnomer, because most, if not all, cases do involve some form of sensitizing exposure. Such sensitizing exposure may not be to red cells per se, but rather to plant or bacteria antigens so similar to the red cell antigen that the exposure elicits an antibody response that is directed against the red cell antigenic component. The most important of the naturally occurring antibodies are anti-A and anti-B, which are directed at the ABO blood group antigens.

Compatibility Testing

From a practical standpoint, pretransfusion compatibility testing begins with the type and screen procedure. For simplicity's sake, the term "serum" is used, but it is important to recognize that recipient plasma is an acceptable alternative sample, except where specifically noted. Issues related to the proper identification of samples submitted for testing are covered in Chapter 4: Carbohydrate Blood Group Antigens and Collections.

The type and screen procedure begins with determining the recipient's ABO group and Rh type, followed by a screening procedure involving two or three red cell antigens to detect the presence of non-ABO blood group antibodies. If an antibody is detected by the antibody detection test (also called antibody screen test), an antibody identification panel must be performed to determine the specificity of that antibody. Once the antibody is identified, Red Blood Cell (RBC) units of the appropriate ABO group and Rh type are screened for the corresponding antigen, and units that are negative for that antigen are crossmatched with the recipient's serum to ensure compatibility.

Rarely, the antigen-negative unit will appear to be crossmatch-incompatible with the recipient's serum, alerting the transfusion service technologist to the possible presence of an additional antibody, usually directed against a low-incidence antigen. If an antigen-negative unit is unexpectedly crossmatch-incompatible with the intended recipient, further antibody identification testing, most often in the form of a selected red cell panel, will be required. For example,



The type and screen procedure involves determining the recipient's ABO and Rh(D) type followed by a two- or three-cell screening panel to determine whether any unexpected antibodies are present in the recipient serum. If an antibody is found, it must be identified using an antibody identification panel.



When a clinically significant antibody is detected and identified, antigen-negative units must be found and then crossmatched with the recipient's serum.

if a recipient is known to have an antibody to an antigen in the Kell system, a panel of Kell-negative reagent red cells would be used to help delineate the nature of the unexpected antibody.

ABO Typing

ABO typing takes advantage of the fact that an individual who lacks a given ABO antigen will demonstrate the presence of antibody directed against the ABO antigen that is lacking. Table 3-1 summarizes the antibodies expected on the basis of the patient's ABO blood group.

Forward Typing



ABO forward typing determines which ABO antigens are present on the recipient's red cells, whereas reverse typing looks for the presence of corresponding antibodies in the recipient's serum.

The forward (or front) type tests the recipient's red cells with reagent anti-A and anti-B to answer the question, "What ABO antigens do these red cells have?"

A drop of recipient red cells is mixed with one drop of commercial anti-A in one tube and with one drop of commercial anti-B in a second tube. The tubes are centrifuged briefly (approximately 15 seconds) to drive the cells closer together and, thus, to facilitate bridging by antibody. The resultant red cell button is gently resuspended and observed for agglutination. Red cells that are agglutinated by anti-A bear the A antigen, those that are agglutinated by anti-B bear the B antigen, and those that are agglutinated by neither are group O.

The strength of the agglutination observed is graded on a scale from 0 (no agglutination) to 4+ (a large clump in a clear background devoid of unagglutinated cells). (See the left side of Fig 3-2.)

Table 3-1. Relationship between Patient ABO Group and Antibodies in Serum

Antigens on Red Cells	Antibodies Present in Serum
A	Anti-B
B	Anti-A
AB	Neither
O	Both anti-A and anti-B

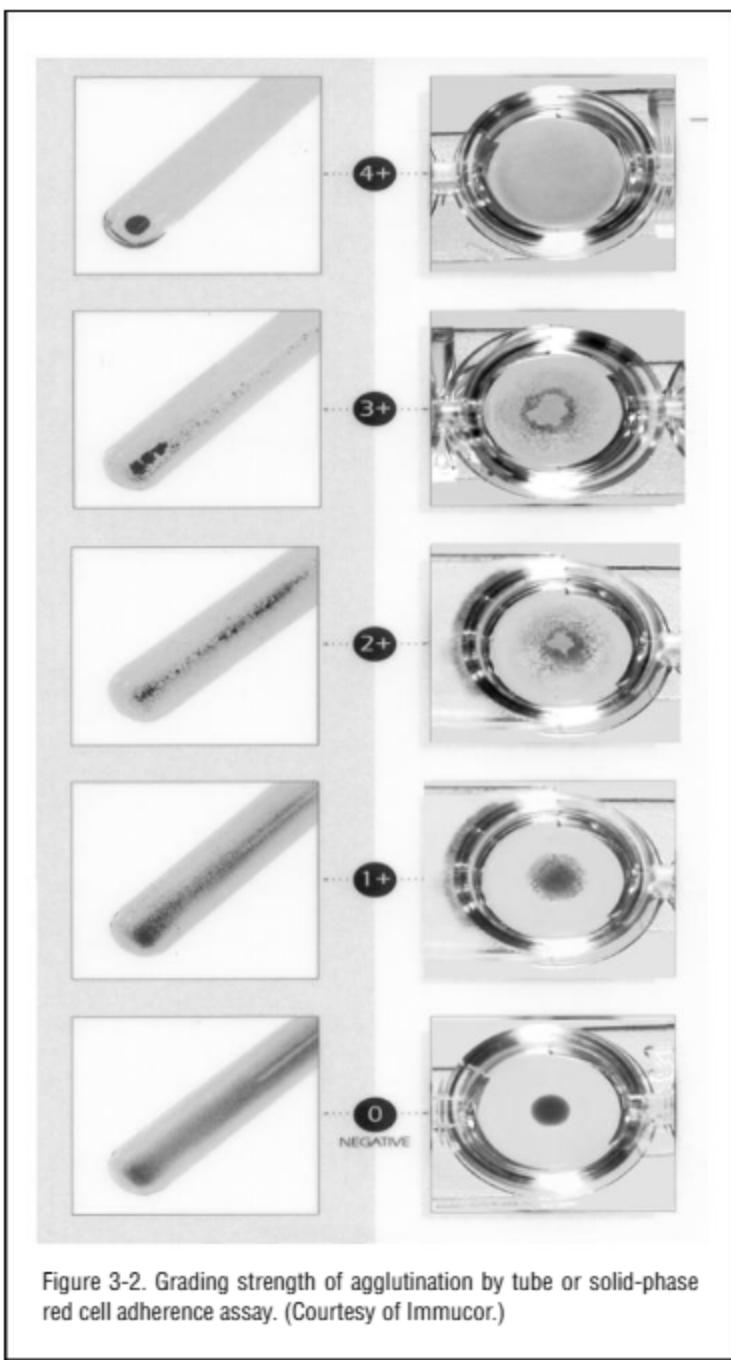


Figure 3-2. Grading strength of agglutination by tube or solid-phase red cell adherence assay. (Courtesy of Immucor.)

Reverse, or Back, Typing

The reverse (or back) type, in contrast, tests the recipient's serum with reagent red cells to determine what antibodies are present in the patient's serum. This test is used to confirm (or more correctly act as a control for) the forward type. An individual who is group A should have anti-B but

not anti-A in his or her serum, whereas a group B patient should have anti-A but not anti-B. A group O patient should have both anti-A and anti-B, and a group AB individual should demonstrate the presence of neither anti-A nor anti-B.

Reverse typing uses two drops of recipient serum or plasma mixed with a drop of reagent A₁ red cells in one tube and reagent B red cells in a second tube. The same centrifugation and resuspension steps are used to determine whether agglutination has taken place. A₁ cells are chosen because they exhibit the strongest expression of the A antigen.

Forward and Reverse Typing Discrepancies



When a discrepancy exists between the forward and reverse type, the discrepancy must be resolved before ABO-group-specific blood is issued for transfusion. In an emergency, group O blood of appropriate D type should be used until the recipient's ABO group can be definitively determined.

Whenever there is a discrepancy between the forward and reverse type, it is important to determine the cause of the discrepancy before transfusing the patient with type-specific RBCs. In an emergency, if the discrepancy cannot readily be resolved, group O Rh-compatible RBCs should be issued until the recipient's ABO group can be ascertained.

The most common cause of ABO forward and reverse discrepancies is a patient who belongs to a subgroup of A and who has formed anti-A₁. Approximately 80% of group A patients are group A₁ (and 80% of group AB patients are A₁B); the vast majority of the remainder are group A₂ (or A₂B). The A transferase of group A₂ individuals is considerably less efficient at converting H antigen to A antigen than is the A transferase of A₁ individuals, meaning that A₂ individuals produce quantitatively less A antigen. See Chapter 4 for more information on how A and B antigens are produced by the action of transferases on a carbohydrate precursor.

In addition, there are qualitative differences between the A antigen of group A₁ and A₂ individuals, meaning that some A₂ individuals are capable of making anti-A₁, which is capable of agglutinating A₁ red cells. Fortunately, only a small percentage of group A₂ individuals (perhaps 1 in 10, or only about 2% of all A patients) make anti-A₁. Anti-A₁ is a far less potent antibody than anti-A and is generally considered clinically significant only when it demonstrates broad thermal amplitude, reacting not only at room temperature but also at 37 C. Rarely, examples of broadly reactive anti-A₁ have resulted in significant hemolysis of A₁ cells; consequently, patients with known anti-A₁, particularly of broad thermal amplitude, should be transfused

with A₁-negative blood (or, alternatively, with group O blood, which will also be A₁ negative).

Other causes for ABO forward and reverse typing discrepancies are listed in Table 3-2.

Rh Typing

The terms "Rh positive" and "Rh negative" refer to the presence or absence of the D antigen, respectively. To make that determination, one drop of recipient red cells is mixed with one drop of reagent anti-D, centrifuged, and resuspended. Red cells that are agglutinated by anti-D bear the D antigen and are termed Rh positive, whereas red cells that do not agglutinate in the presence of anti-D are Rh negative. Reverse typing is not performed for Rh because anti-D will



There are multiple antigens in the Rh system. The terms Rh-positive and Rh-negative refer specifically to the presence (Rh-positive) or absence (Rh-negative) of the D antigen.

Table 3-2. Causes of ABO Forward and Reverse Typing Discrepancies

Additional or Unexplained Reactivity on Forward Typing:

- Acquired antigens, eg, acquired B antigen in group A individual
- Nonspecific agglutination (eg, Wharton's jelly, silica glass tubes)
- Polyagglutinable red cells
- Recent transfusion (mixed field)
- Marrow or progenitor cell recipient
- Sensitized red cells (under some circumstances)

Additional or Unexplained Reactivity on Reverse Typing:

- Another alloantibody having immediate-spin reactivity (IgM antibody)
- Autoantibody (IgM in class)
- Passive transfer of antibody due to recent transfusion (eg, transfusion of out-of-group platelets) or treatment with derivatives such as intravenous immunoglobulin (IVIG)
- Rouleaux
- Subgroup (especially subgroup of A)

Lack of Expected Reactivity on Forward Typing:

- Antigen altered by disease (eg, leukemia)
- Large-volume transfusion of group O red cells
- Chimera
- Weak subgroup antigen that reacts poorly with reagent anti-A or anti-B
- Neonate (ABO antigen expression not fully developed at birth)

Lack of Expected Reactivity on Reverse Typing:

- Chimera
- Transplant patient
- Deterioration of antigen expression on reagent red cells with storage
- Hypogammaglobulinemia
- Neonate (little or no antibody produced during first 4-6 months of life; reverse typing is typically omitted during first 4 months for this reason)

be found only in Rh-negative patients who have been exposed to Rh-positive blood, and such exposure is unlikely. (Anti-D is not a naturally occurring antibody.) If anti-D is unexpectedly present in the recipient's plasma, that antibody will be detected in the antibody screen test.

There is no built-in control for Rh typing as there is for ABO typing, so it is important to perform an Rh control to ensure that an individual who types Rh positive is truly positive. Thus, when there are no negative reactions (sample tests as AB positive), one should perform a "D-control," substituting either saline or 6% albumin (to mimic the diluent in anti-A and anti-B reagents) for the D antisera. This "D-control" should test as D-negative if the test is valid. A positive "D-control" means the AB-positive result is suspect and requires further evaluation.

Testing for Weak D



Some individuals have either weak expression of the D antigen (weak D) or express only some of the epitopes of the D antigen (partial D). These weakly expressed D antigens may not be detected at immediate spin, but may require antiglobulin phase testing.



Testing for the presence of weak D need be performed only when a person is to be the donor, intentional or unintentional, of RBCs to another individual. Obviously, this would include all blood donors and also apparently Rh-negative infants born to Rh-negative mothers.

All of the testing thus far described for ABO and Rh typing is performed at "immediate spin"; in other words, it does not involve an incubation step, nor does it involve the addition of an antiglobulin reagent. There are, however, some individuals whose expression of the D antigen is weak (weak D) and who will appear falsely Rh negative on immediate-spin testing. Ordinarily, this scenario presents no problem because an Rh-positive recipient who tests falsely Rh negative would receive Rh-negative blood, which would cause him or her no problem. However, if this individual were to be the donor of blood transfused to a truly Rh-negative recipient, the weak-D antigen could potentially elicit the production of anti-D.

Thus, additional testing to prove that a sample that appears to be Rh negative is not weakly Rh positive is required only for blood donors and for infants born to Rh-negative mothers who are candidates for Rh Immune Globulin prophylaxis. Testing for weak D consists of immediate-spin testing, followed by incubation at 37°C for 15 to 30 minutes, then a wash step and addition of the antiglobulin reagent.

The distinction between "weak D" and "partial D" is covered in Chapter 5.

Antibody Detection Test

The antibody detection test is used to detect the presence of unexpected alloantibody directed against non-ABO blood group antigens (eg, Kidd, Kell, and Duffy) in the

patient's serum. The patient's serum (or plasma) is tested using a panel of two or three group O red cells of known antigenic composition. Group O red cells are used to avoid interference by anti-A and anti-B present in the recipient's serum. The following sections describe testing as it is performed in test tubes, which is the least common denominator for antibody testing because it can be performed in small hospital laboratories where the testing volume does not justify alternative technologies. Alternatives to tube testing are discussed near the end of this chapter.

Sometimes, the term "antibody detection test" is used synonymously with the term "indirect antiglobulin test" (IAT). It must be remembered, however, that the same indirect antiglobulin methodology is used in antibody identification testing, antigen typing, and full crossmatches.

In any event, the term "indirect" reflects the fact that an IAT detects free unbound antibodies in the recipient serum, whereas the direct antiglobulin test (DAT) detects antibodies bound directly to the recipient's red cells *in vivo*. Unlike the DAT, an IAT requires a sensitization step in which the antibody is bound *in vitro* to the reagent red cells, followed by the detection of agglutination resulting from this *in-vitro* binding.

The antibody detection procedure has three distinct phases: immediate spin, 37°C, and antiglobulin.

Immediate-Spin Phase

In the first step, a drop of reagent red cell suspension from each of three screening red cell vials is added to a separate test tube and mixed with two drops of patient plasma (or serum). In the past, a fourth tube containing patient red cells and patient serum was used as an autocontrol, but this practice has been largely abandoned as part of the antibody screen test and is reserved for use only when antibody identification is required.

The tubes are then centrifuged briefly (15 seconds) to facilitate antibody-mediated agglutination. The resultant cell button is gently resuspended and observed for hemolysis or agglutination. This is the "immediate-spin" reading, and it detects antibodies reactive at room temperature (so-called cold antibodies or cold-reactive antibodies), which are predominantly IgM class.

37°C Phase

After an immediate-spin reading, a potentiator such as low-ionic-strength saline (LISS) may be added and the tubes



An indirect antiglobulin test detects the presence of antibody in a person's plasma. The direct antiglobulin test looks for antibody bound *in vivo* directly onto red cells in the recipient's circulation.



Antibody detection tests and antibody identification tests involve three phases:

- Immediate spin
- Incubation at 37°C with or without potentiator
- Antihuman globulin testing

incubated at 37°C. The duration of incubation is determined by which potentiator is used. The manufacturer's package insert will state the minimum to maximum incubation time required for sufficient antibody uptake. In the absence of LISS or another potentiator, 30- to 60-minute incubation is required. When 37°C incubation is completed, the tubes are spun and read again.

Antiglobulin Phase



The antiglobulin reagent, which is also known as AHG or antihuman globulin, consists of rabbit antibody directed against human IgG. In some cases, it may be polyclonal with specificity for both human IgG (anti-IgG) and complement (anti-c3d).

At this point, the tubes are washed three to four times with saline, in an automated cell washer when available, to remove any unbound globulins, and the antiglobulin reagent (rabbit antibody directed against the Fc portion of human IgG) is added to each tube. The tubes are spun and read a final time. Some centers read IAT phase reactions under the microscope as well as macroscopically, although most centers have eliminated microscopic reading.

Some centers continue to use a "polyspecific" antiglobulin reagent, which is a mixture of antibody directed against human IgG (anti-IgG) and antibody directed against complement (anti-C3d). In the past, polyspecific antiglobulin reagent was preferred because some antibodies, most notably those in the Kidd system, were detectable only by the presence of bound complement. However, many centers now use anti-IgG antiglobulin only, relying on the increased sensitivity afforded by the addition of LISS or other potentiators.

Antibody Identification



If an antibody is detected, its specificity must be determined using an antibody identification panel.

If agglutination, hemolysis, or both are detected in the antibody detection test, the antibody must be identified and the antigen-negative RBC units must be selected for transfusion. The first step is the testing of a reagent panel for antibody identification.

Antibody Identification Testing

The reagent red cell panel is similar to the antibody detection test but contains 10 to 16 different group O samples of known antigenic composition. The technique used in antibody identification is identical to that used in detecting unexpected antibodies with one exception: a reagent red cell panel includes an autocontrol, in which patient red cells are tested with patient serum or plasma, whereas the antibody detection test typically does not contain an autocontrol.

In patients without clinical evidence of immune hemolysis and with a negative antibody detection test result, the likelihood of discovering a significant yet subclinical antibody through the autocontrol is minuscule. As a consequence, in most centers, the autocontrol is not performed as part of the antibody detection test, but only when the antibody screen test detects an antibody and when an antibody identification panel test is performed.

A positive autocontrol is usually followed by a DAT to ensure that the agglutination observed when the recipient's red cells are mixed with his or her own serum (or plasma) is antibody-mediated and not the result of nonimmunologic clumping.

Patient Phenotype

The final step in antibody identification is to phenotype the patient for the corresponding antigen to confirm that he or she is capable of making the alloantibody. The recipient should be antigen-negative for the antigen corresponding to the identified antibody. Accurate phenotypes may not be possible to obtain if a patient has been transfused recently because a false-positive phenotype may result from antigen-positive donor cells mixed with recipient antigen-negative cells. Likewise, when tests are performed using reagents that require reading at IAT, a recipient who has a positive DAT may yield a false-positive phenotype. Unless the bound antibody causing the positive DAT is dissociated before phenotyping, that bound antibody may be mistaken for antibody bound during the 37°C incubation step.

Recently transfused sickle cell anemia patients are an exception, in that it is possible to obtain an accurate phenotype on these individuals by first lysing any non-hemoglobin SS or SC red cells with hypotonic saline washes, leaving only autologous cells to be phenotyped.

Appendix 3-1 illustrates how to identify antibodies with a commercial reagent panel.



An autocontrol (recipient serum mixed *in vitro* with recipient red cells) need be performed only when there is demonstrable evidence of circulating antibody, ie, only as part of antibody identification testing.

When the autocontrol is positive, a DAT should be performed to determine whether agglutination of the autocontrol was, indeed, immunologic in origin.



Before an antibody can be definitively identified in a patient's serum, the patient must be shown to lack the corresponding antigen and, thus, to be capable of producing alloantibody against that antigen.

Component Selection

RBC Components

RBC units selected for transfusion must be ABO-compatible with the intended recipient and lack the antigenic target of any clinically significant antibody identified in the recipient's plasma at any time, either past or present. Table 3-3 summarizes which RBC and plasma-containing units

Table 3-3. Summary of Safe Transfusion Practice

Recipient Blood Group	Red Cells to Transfuse	Plasma to Transfuse
A	A or O	A or AB
B	B or O	B or AB
AB	A, B, AB, or O	AB
O	Only O	O, A, B, or AB

Note: If whole blood is to be transfused, it must be ABO-identical with the intended recipient because it contains not only red cells but also a significant amount of plasma. Fortunately in the United States, the use of whole blood is confined almost exclusively to autologous transfusion, in which case the units will be ABO-identical.



Rh-negative recipients should receive D-negative (Rh-negative) RBCs even if they do not have anti-D in their sera. This is to avoid sensitizing exposure that might result in formation of anti-D.

are acceptable based on the recipient's ABO group. "Universal donor" red cells are group O, whereas "universal donor" plasma is group AB.

Whenever possible, Rh-negative (D-negative) individuals should receive Rh-negative RBC units, even if they have never produced anti-D. This precaution is taken to avoid exposure to D-positive RBCs, which could result in the development of anti-D in the weeks to months after transfusions. There are two reasons for taking that precaution. First is the potency of the D antigen as an immunogen. Exposure to the D antigen has classically been estimated to result in the development of anti-D in more than 80% of Rh-negative recipients so exposed, although more recent data suggest that the actual rate of alloimmunization may be lower—in the range of 30% to 40%. Second, the rationale for prophylactically selecting Rh-negative RBC units for Rh-negative patients who lack anti-D is even more compelling in females of childbearing potential. In that group, exposure to the D antigen with the development of anti-D can result in HDFN should the woman in question subsequently become pregnant with an Rh-positive fetus. Strategies for dealing with the emergency crossover of Rh-negative patients to Rh-positive RBCs are discussed at length in Chapter 12, and HDFN is discussed in Chapter 11.

If no antibody has been detected by the antibody screen test and the patient has no history of red cell alloantibody, random units of the appropriate ABO and Rh type may be



A recipient with a history of a clinically significant antibody must receive antigen-negative blood even if the antibody is no longer detectable.

selected for crossmatching with the patient's serum (or plasma). If, however, the patient has a history of alloantibody, antigen-negative units must be selected whether or not the antibody is detectable. Antigen-negative units are also required if the patient has an alloantibody, regardless of whether it was previously identified.

Granulocytes, Plasma, Platelets, and Cryoprecipitate

Because they contain significant numbers of red cells, granulocytes for transfusion must be ABO-compatible with the antibodies present in the recipient's plasma, and the unit must undergo a red cell crossmatch with recipient serum/plasma.

Platelet units, in contrast, do not require crossmatch as long as there are fewer than 2 mL of red cells in the unit. If more than 2 mL of red cells are present in a platelet product, the RBCs in the unit must be ABO-compatible with the recipient plasma, and an RBC crossmatch is required.

Fresh Frozen Plasma (FFP) for transfusion must be ABO-compatible with recipient plasma (see Table 3-3). For Cryoprecipitated AHF and platelets, however, ABO compatibility (plasma compatibility), although preferred, may be dispensed with, if clinically necessary. In the case of Cryoprecipitated AHF, the volume of plasma in a pool of cryo (50-150 mL in a 10-unit pool) is sufficiently diluted into a recipient's much larger plasma volume so as to be unlikely to cause problems. This rationale also applies to single doses of platelets because one apheresis unit or six pooled whole-blood-derived platelet concentrates contain approximately 300 mL of plasma, compared with an average adult plasma volume of approximately 3000 mL.

One must be particularly careful, however, in giving group O platelets to a recipient who is group A because, occasionally, donors will have a very potent anti-A that is capable of causing hemolysis of recipient red cells when passively transfused into a group A recipient. Similar considerations apply when giving group O platelets to a group B recipient. When transfusion of ABO-identical platelets is impossible because of supply limitations, transfusion of group A platelets to a group B recipient, or vice versa, is preferable to the use of group O platelets. When group O platelets are to be used for non-group-O recipients, some centers will measure anti-A, anti-B, or both titers in the donor and permit the use of plasma-incompatible platelets only when incompatible antibody is present in low titer. Unfortunately, no consensus has been reached yet about



For Cryoprecipitated AHF and platelets, ABO compatibility (plasma compatibility) with recipient RBCs is preferred but not absolutely required.



Transfusion of group O platelets to a non-group-O recipient should be avoided, if possible, because some group O donors have high-titer anti-A and/or anti-B.

what constitutes a critical titer in such a setting. Alternative or adjunct measures that may be used include volume reduction of the platelet unit or machine-washed, saline-resuspended platelets.

Granulocyte products and platelet units containing greater than 2 mL of red cells must be crossmatched with recipient serum/plasma, but crossmatch is not required for FFP, Cryoprecipitated AHF, or platelets lacking significant numbers of red cells, which constitute the vast majority of platelet units.

Platelets themselves do not express Rh antigens, but each platelet unit contains a small number of red cells (0.3-0.5 mL per whole-blood-derived platelet concentrate and 0.0002 to 0.007 mL per apheresis platelet unit). Thus, if Rh-positive platelets are transfused to an Rh-negative recipient, the potential exists that the red cells contained in the unit might stimulate the recipient to produce anti-D. Although the risk of alloimmunization appears to be lower in immunosuppressed patients, it is nonetheless advisable to give an Rh-negative recipient who receives Rh-positive platelets a dose of either the intramuscular (IM) or intravenous (IV) preparation of Rh Immune Globulin (RhIG) within 72 hours of transfusion. Given that a 300- μ g dose of RhIG protects against 15 mL of red cells and has a half-life of approximately 3 weeks, a single 300- μ g dose should cover five to six doses of platelets, each of which contains no more than 2 mL of red cells. A 50- μ g "mini-dose" of IM RhIG should suffice to cover a single Rh-positive apheresis platelet unit.



Rh Immune Globulin should be considered when Rh-positive platelets are transfused to an Rh-negative recipient who does not already have anti-D in order to prevent Rh sensitization of the recipient by red cells in the platelet unit.



Crossmatch testing mixes recipient serum with donor red cells, to detect agglutination. When no agglutination is seen, the donor unit is termed "crossmatch-compatible."

Crossmatch

Serologic Crossmatch

The crossmatch involves testing the patient's serum (or plasma) with donor red cells taken from a segment attached to the selected RBC unit.

If no antibody is detectable and the patient has no history of antibody, an abbreviated crossmatch may be performed: donor red cells and patient serum (or plasma) are mixed, centrifuged, and subjected to an immediate-spin reading. In such a case, the immediate-spin crossmatch serves as one final check to be certain that the unit is ABO-compatible with the intended recipient. If the immediate-spin crossmatch demonstrates no agglutination, the cross-

match is deemed compatible, and the unit is identified as safe to transfuse to that patient.

However, whenever there is a history of antibody, if there is antibody detectable in the testing of the patient's current sample, or both, a full crossmatch is required—meaning that the crossmatch must be carried through all three phases and that no agglutination or hemolysis has been detected at any phase. If no hemolysis or agglutination occurs on full crossmatch, the unit is considered crossmatch-compatible and may be issued for transfusion. Any evidence of agglutination or hemolysis at any phase renders the unit crossmatch-incompatible and, thus, unsafe for transfusion. Furthermore, the finding of crossmatch incompatibility between an antigen-negative unit and the recipient's serum (or plasma) should result in further investigation aimed at identifying the antibody responsible for the incompatibility.

One question that is frequently asked is this: "Why not just do a crossmatch and skip the antibody screen test?" Doing so might result in missing a weak antibody directed against an antigen expressed in single dose (often referred to as heterozygous expression) on the donor red cells. The combination of weak antibody and weak antigen expression might yield a false-negative test result that implies the unit is compatible when it is not. The antibody screen test, though, is specifically designed to use red cells that are selected to show double dose (homozygous) expression of almost all major antigens.

Another common question is this: "Why even bother with a crossmatch?" As mentioned previously, the crossmatch is used to detect an unexpected antibody directed against a low-incidence antigen present on the cells in the donor RBC unit but not on any of the reagent cells in the screen test or antibody panel. Furthermore, the immediate-spin phase of the crossmatch provides an additional confirmation of the ABO compatibility of the unit being crossmatched.

Electronic Crossmatch

If a transfusion service has a computer system approved by the Food and Drug Administration for the performance of electronic crossmatches and if the system has been validated on site, that computer system can be used in lieu of the immediate-spin crossmatch to detect ABO incompatibility. Electronic, or computer, crossmatch is permissible only if 1) the recipient's ABO group has been determined



When a patient has no history of clinically significant alloantibody and no currently detectable antibody on the screening test, an abbreviated (immediate-spin only) crossmatch may be performed safely. In all other situations, the crossmatch must be carried through to the antiglobulin phase.



Antibody screening is crucial for detecting weak antibodies that might be missed on crossmatch alone.

Crossmatches, on the other hand, are important for detecting antibody directed against low-incidence antigens that may be present on donor red cells but not expressed on any of the red cells on the antibody screen and/or antibody identification panel.



An electronic (computer) crossmatch may be substituted for an immediate-spin crossmatch if both of the following conditions apply.

The recipient's ABO group has been determined on two separate occasions. The computer uses logic to alert the user to ABO incompatibility between the unit selected and the intended recipient.

either on two separate occasions (one of which may be historical) or by retesting the same sample, and 2) the computer system contains logic to alert the user to ABO discrepancies between the donor unit and the recipient.

The second stipulation is required because manual, direct ABO verification (as compared with immediate-spin crossmatch) may not be sufficiently reliable. The addition of a computerized crosscheck appears to enhance accuracy further, to the point where one study involving the crossmatch of nearly 30,000 RBC units found no incidents of major ABO discrepancy.

Frequency of Repeat Testing

AABB standards require testing of a patient sample obtained within 3 days before transfusion for ABO, Rh, and unexpected antibodies but only if the recipient has been pregnant or transfused within the preceding 3 months or if the history is uncertain or unavailable. The same level of currency is not required for patients who can be determined not to have been pregnant or transfused in the last 3 months and who, additionally, have a negative antibody screen and no history of antibody. In such patients, the sample on which a pretransfusion antibody screen has been performed can be stored until the immediate preoperative period and used for crossmatch, if needed.

Many institutions permit storage of pretransfusion samples for as long as 30 to 45 days when those conditions are met. The sample is drawn and tested several weeks preoperatively and stored until the day of surgery. An identification band is made at the time of the initial phlebotomy and is either affixed to the patient at that time or kept with the patient's chart to be placed on the patient's arm when he or she returns for surgery. If the initial antibody screen is negative, the patient is interviewed either on the evening before or on the morning of surgery. Assuming there is no history of transfusion or pregnancy in the preceding 3 months, the transfusion service is notified and will perform any necessary crossmatches using the stored sample. However, if the antibody screen is positive or if there is a history of recent transfusion or pregnancy, a fresh sample and full workup are required within 3 days of the date of surgery. This system is more time efficient both for the patient and for transfusion service personnel.



When a patient has been transfused or pregnant within the last 3 months, repeat testing is required every 3 days (day of draw is day 0).

Longer periods (up to 30-45 days) between collection of the pretransfusion sample and the intended transfusion date are permissible when the patient has no past or present evidence of antibody and has not been recently pregnant or transfused.

Direct Antiglobulin Test

The direct antiglobulin test, previously known as the direct Coombs test, detects the presence of antibody bound *in vivo* to red cells in the patient's circulation. If the patient has not been transfused within the past 3 months, the bound antibody will almost certainly be an autoantibody bound to the patient's own cells. If, however, the patient has recently been transfused, antibody detected by the DAT may be either alloantibody bound to circulating donor cells or autoantibody bound to patient cells (as well as to donor cells).

The DAT is used as a part of pretransfusion compatibility testing to determine whether a positive autocontrol is the result of antibody-mediated agglutination or the result of nonimmunologic clumping. It is also used in the investigation of suspected transfusion reactions, in evaluation of newborn cord blood to detect possible HDFN, and in the workup of suspected autoimmune hemolytic anemia.

The DAT is performed in the same manner as the final stages of an IAT (antibody screen/panel) but uses the patient's own cells rather than reagent red cells. Patient red cells in suspension are washed to remove unbound globulins and mixed with antiglobulin reagent, spun, and read immediately. If a polyclonal antiglobulin reagent is used, the mixture is allowed to sit for 5 minutes at room temperature, followed by a second spin and reading to detect weak reactions due to complement.

There are three common types of antiglobulin reagents used in the DAT: anti-IgG (the same reagent used in an IAT), anticomplement (anti-C3d), and polyclonal antiglobulin (a mixture of anti-IgG and anti-C3d). Some centers perform a screening test by using polyclonal antiglobulin, and only those red cells that test positive with polyclonal antiglobulin are tested to determine the nature of the bound globulin (IgG vs C3d, or both). Bound IgG is best detected immediately after the addition of antiglobulin reagents, whereas the 5-minute incubation at room temperature facilitates the detection of bound complement (C3d).

The finding of a positive DAT should trigger obtaining additional medical history, including transfusion history, with particular attention to transfusion during the preceding 3 months; drug history, because many drugs can cause a positive DAT; and history of autoimmune disease.

If the DAT is positive with anti-IgG, regardless of whether there is reactivity with anti-C3d, an elution procedure should be performed to dissociate the antibody from



In a recently transfused patient, it is important to recognize that a positive DAT (and positive autocontrol) may be due to alloantibody coating the transfused donor red cells. A positive autocontrol should not be ascribed to autoantibody until this possibility has been excluded, either by a reliable history of no recent transfusion or by further testing in the laboratory.



When a recipient has a positive autocontrol, a DAT should be performed to determine whether the observed agglutination on the autocontrol is, indeed, immunologically mediated.

When the DAT is positive with anti-IgG reagent, an elution should be performed, to remove the bound IgG antibody from the red cells, and the eluate tested against an antibody identification panel, in an attempt to identify the bound antibody.

the red cells and to allow the performance of antibody identification on the eluted antibody. This step is particularly important when there is a history of recent transfusion because the bound antibody might be an alloantibody bound to transfused red cells in the recipient's circulation rather than being an autoantibody. Antibody elution is also an important part of the evaluation of a patient who is exhibiting clinical signs and symptoms of hemolysis to confirm or rule out immune-mediated causes, such as acute or delayed alloimmune hemolysis as well as autoimmune hemolytic anemia. See Chapter 7: Adverse Effects of Transfusion for more details.

Antibody Elution



Many different methods can be used to dissociate (elute) bound antibody from the surface of red cells, but the most commonly used technique is the acid elution, which uses acidic glycine.

A number of techniques, including heating or freezing and then thawing the red cells, have been used to dissociate (elute) bound antibody from the surface of the cells *in vitro*. Probably the most commonly used elution procedure is rapid acid elution in which red cells coated with (IgG) antibody are treated with an acidic glycine solution to dissociate the antibody. The resulting solution (called the eluate) is buffered and subjected to a reagent red cell panel to identify the antibody that was bound to the cell surface. Elution is rarely performed unless the DAT shows reactivity with anti-IgG because the IgM antibodies responsible for C3d binding will have already dissociated and will not be detectable in the eluate.

Adsorption Procedures

It may be difficult or even impossible to determine whether an alloantibody is present when the recipient also has an autoantibody coating his or her red cells because most autoantibodies are panagglutinins (which agglutinate all panel cells). In that case, an adsorption procedure must be performed to remove the autoantibody from the recipient's serum, which, one hopes, will leave behind any alloantibody.

If the recipient has not been recently transfused, his or her autologous red cells can be treated to remove bound autoantibody and used to adsorb autoantibody from the serum. The reagent often used to treat autologous red cells before the adsorption of serum is a mixture of dithiothreitol and papain, a mixture often called ZZAP. Following

treatment with ZZAP, the treated red cells are allowed to incubate with the patient's serum for up to 1 hour, and the resultant adsorbed serum is tested against a panel of red cells for antibody identification. Often, a single adsorption is unable to remove sufficient autoantibody, in which case several (up to six) serial adsorptions are performed on the same serum sample by using different aliquots of ZZAP-treated autologous red cells. After serial adsorptions, the adsorbed serum can be tested with a panel for antibody identification. Although the use of an adsorbed sample for crossmatching may assist in locating the least incompatible units for a patient requiring transfusion, the results of the crossmatch performed with neat plasma should also be performed and recorded. Alternatively, if the choice is made not to perform a neat crossmatch, it must be made clear to the transfusing physician that the units provided are not crossmatch-compatible in the conventional sense of the term, but are, rather, "least incompatible." The strength of reactivity with the least incompatible red cells may be useful for the clinicians as they evaluate the risks of transfusion in these difficult cases.

In the case of recently transfused recipients with autoantibodies, autologous adsorption is contraindicated because the circulating red cells are a mixture of allogeneic and autologous cells and thus are capable of adsorbing alloantibody as well as autoantibody. In that situation, a panel of specially selected allogeneic red cells can be treated with ZZAP and used to perform differential adsorptions (also known as allogeneic adsorptions). This panel is selected to ensure that each major alloantibody is left behind by at least one of the adsorbing red cells—for example, a Jk(a-) adsorbing cell could be relied on to leave anti-Jk^a behind in the adsorbed serum. Interpretation of differential adsorptions is complex; therefore, this procedure is generally performed only by high-volume transfusion services and immunohematology reference laboratories.



When the recipient has a broadly reactive antibody (panagglutinin) that appears to be an autoantibody, it is necessary to perform adsorption procedures to be certain that there is no underlying alloantibody present but masked by the autoantibody.

In patients who have not been recently transfused, adsorption may be performed using autologous red cells (autoadsorption). Recently transfused patients, however, require use of a more complex technique using allogeneic red cells of known antigenic composition to perform the adsorption, a procedure known as allogeneic or differential adsorption.

Other Special Techniques

Enzyme Treatment and Neutralizations

Treatment of reagent red cells (panel or screen cells) with proteolytic enzymes such as ficin or papain before use in antibody identification may facilitate antibody differentia-



Proteolytic enzyme treatment can be used to remove antigens in the MNS and Duffy systems from reagent RBCs in order to facilitate antibody identification. Following enzyme treatment, antibodies directed against MNS and Duffy antigens will no longer be detected because the red cells no longer contain these antigens. Little-s is only variably responsive to enzyme treatment.



Lewis antibodies can be neutralized by saliva obtained from Lewis antigen-positive individuals.

Echinococcus hydatid cyst fluid or albumin from pigeons and turtle doves will neutralize anti-P1.

tion. Certain antigens, most notably M, N, S, and the Duffy antigens, are destroyed by enzyme treatment. Thus, enzyme treatment of reagent red cells before reaction with recipient serum would be expected to eliminate reactivity caused by antibodies directed against those antigens. Most other antigen-antibody reactions are either unaffected or enhanced by enzyme treatment. Reactivity of Rh antibodies and antibodies that are predominantly cold-reactive, such as those in the Lewis system, may be enhanced by enzyme treatment.

Table 3-4 lists the major antigens that are usually destroyed by an enzyme (ficin or papain) and those that tend to be enhanced by an enzyme.

Neutralization of Recipient Serum

By allowing patient serum to react with certain neutralizing substances, specific antibodies may be removed from the serum, allowing either verification of the identity of the neutralized antibody or detection of additional underlying antibody specificities. A commonly used neutralization procedure is Lewis antibody neutralization by saliva from Lewis antigen-positive individuals. P₁ antibody can be neutralized by fluid derived from echinococcus hydatid cysts, although commercial P1-neutralizing substance is more often obtained from avian sources (ie, the egg whites of pigeons or turtle doves).

Use of Chemical Compounds

Antibody identification can be aided with the use of chemical compounds such as chloroquine and dithiothreitol. Table 3-5 describes some of those treatments.

Table 3-4. Response to Enzyme Treatment

Inactivated	M, N, S (s less reliably) Fy ^a and Fy ^b
Enhanced	Rh and Kidd systems (most) Lewis, P1, I, other cold antibodies
Destroyed by enzyme on most occasions	Ch, Rg, Yt ^a , Mg, JMH, Pr, Tn, Mi ^a /Vw, Cl ^a , Je ^a , Ny ^a , In ^b , some Ge, most En ^a

Table 3-5. Other Special Treatments**Chloroquine**

Removes HLA Class I antigens from red cells and platelets
Removes bound IgG antibody in 80% of cases

Dithiothreitol

Treatment of serum abolishes:
Ability of IgM antibodies to agglutinate red cells
Ability of IgM antibodies to bind complement
Treatment of red cells destroys:
Kell antigens, Lutheran antigens
Antigens that evoke antibodies that are classed by reactivity, ie, high-titer, low-avidity

2-ME (2-mercaptoethanol)

Treatment of serum abolishes:
Ability of IgM antibodies to agglutinate red cells
Ability of IgM antibodies to bind complement

AET (2-aminoethylisothiouronium)

Destroys Kell antigens, Lutheran antigens

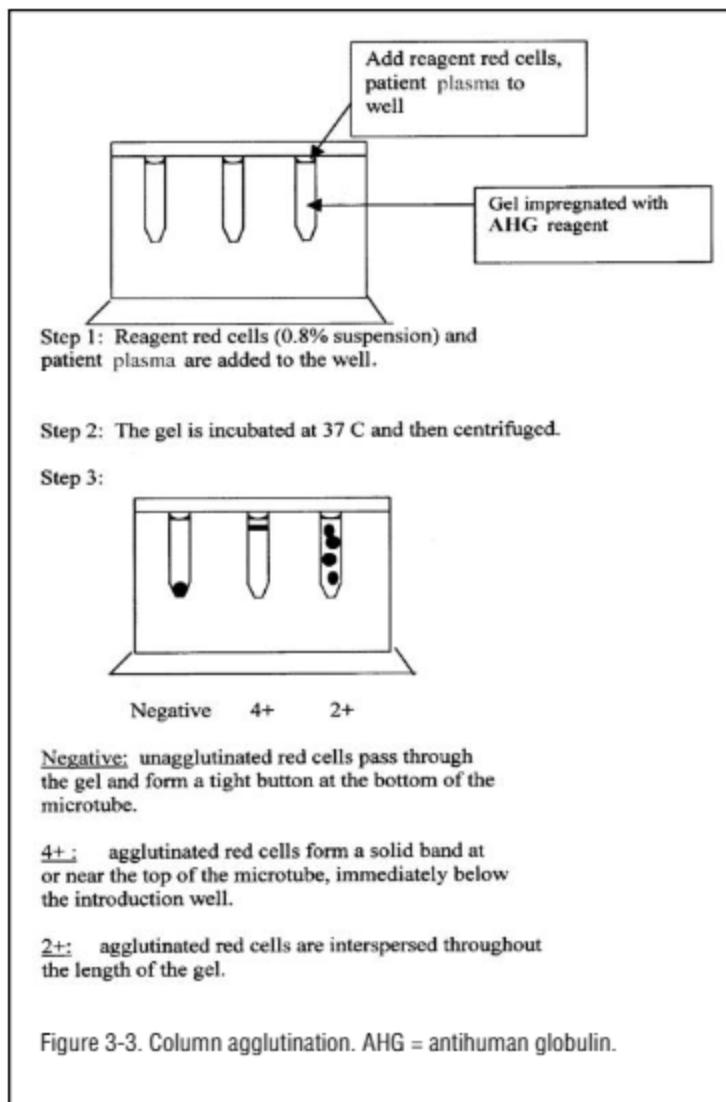
Alternatives to Tube Testing

Although test tube-based serology is the tried-and-true method, two newer technologies are finding increasing favor, especially in high-volume laboratories.

Column (Gel) Agglutination

The first of the alternative technologies uses a dextran acrylamide gel with pores large enough to permit unagglutinated red cells to pass during centrifugation but small enough to trap agglutinated red cells. When used for antibody identification, the gel is impregnated by the manufacturer with antigen reagent. (See Fig 3-3.)

Patient plasma and reagent red cells, both in microliter quantities, are measured by using a micropipette and are placed in a well at the top of the gel, where they are allowed to incubate. The gel is then centrifuged, at which time unagglutinated red cells pass through to get to the bottom, whereas agglutinated cells are trapped in the gel matrix. The larger the clumps, the closer the agglutinate stays to the top of the gel, near where the sample was originally introduced. Thus, reactivity in the gel can be graded in a fashion similar to that used in grading tube testing.



Advantages of gel testing include less subjective interpretation and the ability to retain gels for several hours to allow consultation on problematic results.

Disadvantages of gel testing include inability to confirm visually whether reagents or serum have been added properly.

Hemolysis can be detected by observing the fluid in the well. It is also possible to detect mixed-field agglutination in the gel itself, reflected as two distinct layers of red cells—the slower moving layer representing agglutinated cells and the other, unagglutinated cells.

Advantages of the gel system include less subjective grading and the ability to retain gels for several hours, allowing consultation with a more experienced technologist or supervisor. Disadvantages include the fact that there is no way to readily determine whether reagents, serum, or both have been added to the well because the volumes are so small.

Solid-Phase Red Cell Adherence Assay

The solid-phase red cell adherence (SPRCA) test systems use microtiter plates coated with stroma from reagent red cells. Patient plasma and LISS are added to the red-cell-coated wells. Although the system is designed to use reagent red cells provided by the manufacturer, it is nonetheless possible to perform the coating process in the transfusion service laboratory. Indeed, that alternative is utilized in the preparation of the autocontrol. (See Fig 3-4.)

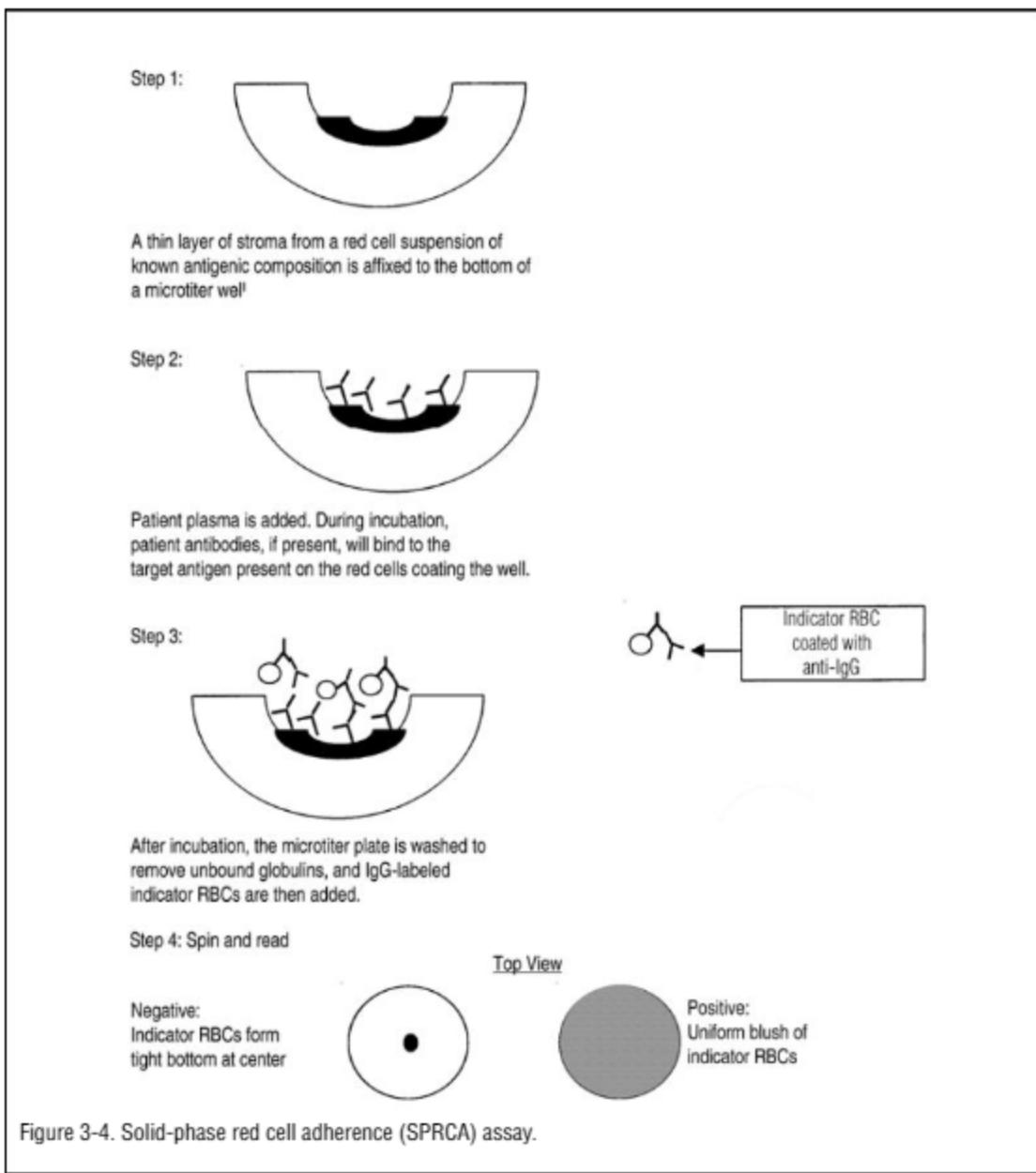


Figure 3-4. Solid-phase red cell adherence (SPRCA) assay.

After the addition of patient plasma to the coated microtiter wells, the plasma-containing plate is incubated for 15 to 60 minutes to allow any antibody present in the patient plasma to bind with membrane antigens present on the red cell stroma lining the well. Unbound immunoglobulins are washed from the wells, and a suspension of indicator red cells coated with anti-IgG is added to each well. At this point, the plate is centrifuged to allow binding between the anti-IgG-coated indicator red cells and any patient antibodies that have bound to the reagent red cells lining the wells.



A negative result on SPRCA testing shows a tight button of indicator red cells at the bottom of the test well, whereas a positive test shows a uniform blush. This is precisely the opposite of results seen in traditional tube testing.

Interpretation of SPRCA testing results takes adjustment on the part of personnel performing the test. A negative test will present with a tight button of indicator cells at the bottom of the well, whereas a positive test will show a uniform blush throughout the well, precisely the opposite of the results seen in traditional tube testing. (See the right side of Fig 3-2.)

A similar methodology is available to perform screening for HLA and platelet-specific antibodies and for platelet crossmatching. In those instances, the stroma lining the wells is derived from reagent platelets (for the platelet antibody screen) or from donor platelets (for platelet crossmatching).

Compatibility Testing for Platelets and Plasma Products



When Whole Blood is to be transfused instead of RBCs and FFP, the Whole Blood must be ABO identical with the recipient, because it will contain significant amounts of donor plasma in addition to donor red cells.

Although plasma products and Cryoprecipitated AHF should be ABO compatible with the recipient's red cells, minor crossmatch of donor plasma with recipient red cells is not required.

Whole Blood, however, must be ABO identical with the recipient because it contains a significant amount of donor plasma in addition to donor red cells. Major crossmatch (recipient serum and donor cells) is required, but minor crossmatch (recipient cells and donor plasma) is not a necessity.

For platelet transfusion, ABO compatibility of the plasma in the unit is preferred but not absolutely required because the volume of plasma transfused is small relative to the total plasma volume of the recipient. The only exception is in infants in whom the volume of ABO-incompatible plasma infused must be limited because of the small total plasma volume. See the section above titled "Component

Selection" for more details concerning the use of out-of-group platelets.

A small number of red cells are in any platelet unit, whether whole-blood-derived or collected by apheresis, but crossmatch is not required unless the volume of red cells exceeds 2 mL.

Although it is safe to give Rh-positive platelets to Rh-negative recipients, the amount of red cells, albeit minuscule, may be sufficient to stimulate the production of anti-D. Thus, when Rh-negative patients receive Rh-positive platelets, it is advisable to provide immunoprophylaxis with RhIG to prevent sensitization of the recipient to the D antigen even for recipients who are not females of child-bearing potential. As with pregnant mothers, RhIG can be given any time within 72 hours of exposure. A single 300- μ g vial will treat 15 mL of red cell exposure (or 30 mL whole blood), which equates to several platelet doses. For patients who cannot tolerate intramuscular injection, an intravenous preparation is available (WinRho SDF, Baxter Healthcare Corp., Deerfield, IL).

Transfused granulocytes also contain a significant number of red cells, so they must be ABO and Rh compatible with the recipient and must be crossmatch compatible with recipient serum (or plasma).



Although ABO compatibility of the plasma accompanying a platelet unit with the recipient is desirable, it is not absolutely required, except when the recipient is an infant.



Rh-positive platelet units can be safely transfused to Rh-negative patients. However, because there are a small number of Rh-positive red cells in the unit, it is advisable to provide RhIG prophylaxis to the recipient within 72 hours, if possible. A single 300 μ g vial of RhIG will cover several (5-6) platelet doses.

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Appendix 3-1. Method for Reagent RBC Panel Interpretation

Prepare the Panel for Interpretation

1. Highlight nonreactive rows.



The antibody identification process involves four major stages:

- Review patient history
- Eliminate the presence of other antibodies (rule-outs)
- Confirm identification of suspected antibody—three antigen-positive and three antigen-negative panel cells (or statistical equivalent)
- Phenotype to confirm recipient can make antibody in question

On the phenotype sheets provided with reagent red cell panels and reagent red cell screens, the column headings correspond to the series of antigens for which each red cell on the panel has been tested. (See Fig 3-5.) Each row contains the phenotype for a single red cell donor, with + indicating that the cell bears the antigen depicted in that column and a 0 indicating the absence of the antigen.

Using a highlighter, highlight every row that is completely nonreactive ("negative"), meaning that it shows no agglutination (or hemolysis) in any phase of testing when the patient's plasma or serum is tested with the RBC. (See Fig 3-6.)

Note that in the following figures, positive rows are depicted by a single plus sign (+) for simplicity of

VIAL	Rh - Hu		Kell		Duffy		Lewis		P		MN		Lutheran		Xg		PATIENT'S SERUM TEST RESULTS TEST METHODS											
	D	C	c	E	s	f	V	C	K	k	Kp	Kp	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Jd	M	N	S	Le ^a	Le ^b	Le ^c	Le ^d	IS	37°C	AHG	
1	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	1	00+			
2	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	2	000			
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	3	000			
4	+	0	+	0	+	+	0	0	+	0	+	0	+	0	0	0	0	+	+	0	+	0	+	4	000			
5	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	0	+	5	00+			
6	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	0	+	6	00+			
7	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	0	+	7	000			
8	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	0	0	+	0	+	0	+	8	00+			
9	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	0	0	+	0	+	0	+	9	00+			
10	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	10	000			
11	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	0	0	+	0	+	0	+	11	000			
																									Autod control	12	000	

Figure 3-5. Panel ready for interpretation.

VIAL	Rh - Hr										Kell			Duffy		Kidd		Levats		p		MN		Lutheran		Xg		PATIENT'S SERUM TEST RESULTS TEST METHODS			
	D	C	c	E	e	f	V	CW	K	k	Kp ^a	Kp ^b	J ^a	J ^b	PY ^a	PY ^b	Jk ^a	Jk ^b	L ^a	L ^b	P ₁	M	N	S	s	Lw ^a	Lw ^b	Xg ^a	Xg ^b	IAT	37C
1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	0	+	+	+	1	OO+		
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	2	OOO			
3	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	3	OOO			
4	+	0	+	0	+	+	+	0	0	+	0	+	0	0	+	0	0	0	0	+	+	0	+	0	+	0	4	OOO			
5	0	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	0	0	+	0	+	+	5	OO+		
6	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	+	6	OO+			
7	0	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	0	+	+	+	0	0	+	0	+	0	7	OOO			
8	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	8	OO+			
9	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	+	0	+	+	9	OO+		
10	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	+	+	+	+	10	OOO		
10C	+	0	+	0	+	+	0	0	0	+	0	+	0	0	+	0	0	+	0	+	+	0	0	+	0	+	0	10C	OOO		
																										Autodcontrpl	=	OOO			

Figure 3-6. Panel with phenotypes of nonreactive RBCs highlighted.

explanation, but, on actual panels, the reactivity is graded on a scale from w+ to 4+ and recorded as such.

2. Observe the pattern of reactivity.

Observe the pattern of reactivity at the far right, making a mental note of any variability in either reaction strength or phases of reactivity that might suggest the presence of more than one antibody. For example, a mixture of an Rh system antibody plus a Kell system antibody might show some cells (rows) reactive at 37 C as well as at indirect antiglobulin test (IAT), whereas other rows are reactive only at IAT.

3. Note whether the autocontrol is positive or negative.

Observe the results for the autocontrol, noting whether it is positive or negative. Interpretation of this result is discussed later.

Interpret the Panel Reactivity

1. Scan each column for a (+) in a highlighted row.

Beginning at the left, scan down each column until a (+) in a highlighted (nonreactive) row is found.

2. Check to see if the expression of that antigen is double dose ("homozygous") or single dose ("heterozygous").



Double-dose or "homozygous" rule-outs are particularly important when an antibody is known to show "dosage," ie, stronger reactivity with cells exhibiting double-dose expression of the cognate antigen and weak-to-absent reactivity with cells having only heterozygous antigen expression.

Once a (+) has been found in the column, check the column for the antithetical antigen to see if it also has a (+). If that antigen is also expressed, the exclusion is by means of a heterozygous genotype; continue down the column until a homozygous genotype for the exclusion is reached, ie, one for which the antithetical antigen is absent—indicated by a (0). If a homozygous exclusion cannot be found, further testing (selected cell panel) may be required.

Table 3-6 contains a list of antithetical pairs. Note that some antigens found on the panel sheet are not present in the table. In such a case, there is no antithetical antigen present on the panel, in which case a single (+) in a nonreactive row can be regarded as homozygous.

In general, it is desirable to have homozygous exclusions, particularly for antigens in the Rh, Kidd, Duffy, and MNS systems. The Kell system is no less important, but antigen frequencies are such that it may be difficult to find homozygous exclusions for K. In that case, and in similar cases, several heterozygous exclusions may suffice. This conclusion is particularly true when other evidence clearly points to a specific antibody.

Table 3-6. Antithetical Pairs Found on Panel Sheets

System	Antigen	Antithetical Antigen	Notes
Rh	C	c	D has no allele
	E	e	
Kell	K	k	
	Kp ^a	Kp ^b	Kp ^a very low incidence
	Js ^a	Js ^b	Js ^a very low incidence
Duffy	Fy ^a	Fy ^b	
Kidd	Jk ^a	Jk ^b	
MNS	M	N	
	S	s	
Lutheran	Lu ^a	Lu ^b	Lu ^a very low incidence

Likewise, it may be impossible to exclude Kp^a, Js^a in the Kell system, and Lu^a in the Lutheran system because they are such low-incidence antigens that often there is not even one antigen-positive red cell on the panel. Although the antigenic specificity cannot be excluded in such cases, there is generally no reason to suspect its presence because it would not account for any of the reactive rows (red cells). However, in the rare instance when one of those three antigens is present on a red cell in a reactive (positive) row, it may be necessary to test additional selected cells to exclude the antibody.

3. Mark exclusions at the top of each column.

Decide upon a method that can be applied consistently to every panel and use that method to mark each column. One method is to circle all antigens for which there is no exclusion at all, to mark an X through antigens that have homozygous (double dose) exclusions, and to place a single slash (/) through those with heterozygous exclusions. It is also helpful to note the number of heterozygous exclusions (eg, marking 2 H above the column if there are two heterozygous exclusions). This method is demonstrated in the following example. (See Fig 3-7.)

VIAL	Rh - Hr		Kell		Duffy	Kidd	Lewis	P	MN	Suth- aran	Eg	PATIENT'S SERUM TEST RESULTS TEST METHODS		
												IS	37°C	AHG
1	+	+	0	0	+	0	0	+	0	+	0	+	+	+
2	+	+	0	0	+	0	+	0	+	0	+	0	+	+
3	+	0	+	0	0	0	0	+	0	+	0	+	+	3
4	+	0	+	0	+	+	0	+	0	0	+	+	+	0
5	0	+	+	0	+	0	+	0	+	0	0	+	0	+
6	0	0	+	+	+	0	0	+	0	+	0	0	+	*
7	0	0	+	0	+	+	0	+	0	+	+	+	0	7
8	0	0	+	0	+	0	0	+	0	+	0	0	+	*
9	0	0	+	0	+	0	0	+	0	+	0	0	+	*
10	0	0	+	+	+	0	0	+	0	+	+	+	+	10
11	+	0	+	+	0	0	+	0	+	0	+	+	0	10
														Autogcontrol
														nc
														000

Figure 3-7. Panel with exclusions marked across the top.

4. Mark possible specificities (rule-ins).

For each circled column (each column corresponding to an antigenic specificity that cannot be excluded), draw a box around all of the (+) marks present in non-highlighted (reactive) rows. The marking will facilitate your seeing which antigen best matches the observed antibody reactivity. It will also facilitate your recognizing the possible presence of a second antibody hidden behind the reactivity of a more broadly reactive antibody. (See Fig 3-8.)

5. Verify the tentative identification.

At this point, it should be apparent what antibody or antibodies appear to be present. All that remains is to verify that there are enough positive and negative reactions to give reasonable statistical probability that the antibody identification is correct. The usual rule-of-thumb is that one needs three appropriate positive reactions and three appropriate negative reactions to confirm the identity of a given antibody. In reality, there are a number of other combinations of negative and positive results that will give a comparable level of statistical certainty in antibody identification. (See Table 3-7 for probability values.)

The antibody identified in this example is anti-Fy^a.

VIAL	PATIENT'S SERUM TEST RESULTS TEST METHODS										
	Rh - Hr	Kell	Duffy	Kidd	Lewis	P ^a	MN	Luthe-	Xg		
										IS 37°C	
1	+	+	0	0	+	0	0	+	+	1	OO+
2	+	+	0	0	+	0	+	0	+	2	OOO
3	+	0	+	0	0	0	0	+	0	3	OOO
4	+	0	+	0	+	0	0	+	0	4	OOO
5	0	+	0	+	0	0	0	+	0	5	OO+
6	0	0	+	+	0	0	+	0	0	6	OO+
7	0	0	+	0	+	0	0	+	0	7	OOO
8	0	0	+	0	+	0	0	+	0	8	OO+
9	0	0	+	0	+	0	0	+	0	9	OO+
10	0	0	+	+	0	0	+	0	+	10	OOO
11	+	0	+	+	0	0	+	0	+	11	OOO
											Autogontrol PC OOO

Figure 3-8. Panel with exclusions and all positive reactions accounted for by a single pattern match.

Table 3-7. Probability Values

No. Tested	No. Positive	No. Negative	p (Fisher)	p (Harris and Hochman)
5	3	2	0.100	0.035
6	4	2	0.067	0.022
6	3	3	0.050	0.016
7	5	2	0.048	0.015
7	4	3	0.029	0.008
8	7	1	0.125	0.049
8	6	2	0.036	0.011
8	5	3	0.018	0.005
8	4	4	0.014	0.004
9	8	1	0.111	0.043
9	7	2	0.028	0.008
9	6	3	0.012	0.003
10	9	1	0.100	0.039
10	8	2	0.022	0.007
10	7	3	0.008	0.002
10	6	4	0.005	0.001
10	5	5	0.004	0.001

6. Confirm that the patient is capable of making this antibody.

The final step in antibody identification is to phenotype the patient to be certain that he or she is capable of making the identified alloantibody. A recipient who is positive for a given antigen is incapable of making an alloantibody against that antigen. (Autoantibodies are another matter.) Sometimes, antigen typing the patient, assuming he or she has had no recent transfusion, can help provide additional exclusions when more than one specificity remains possible after routine evaluation of a panel.

Interpret the Autocontrol

If the autocontrol is negative, there is no indication that the patient's serum agglutinates the red cells in his or her cir-

culation. If an antibody has been identified in the steps above, no further action is required.

If the autocontrol is positive, two steps should be taken: perform a DAT and obtain a patient history if the DAT is positive.

A DAT determines whether there is evidence of an immunologic cause for the observed reactivity of the autocontrol. If the DAT is negative, a clinically significant immunologic basis for the positive autocontrol is unlikely and further evaluation is unnecessary.

If the DAT is positive, however, the patient history should be obtained, with particular attention to whether the patient has been transfused in the last 3 months. If there is a history of recent transfusion, the positive autocontrol might be the result of either an alloantibody agglutinating transfused cells that remain in the patient's circulation or an autoantibody reacting with the recipient's own cells. In the absence of a history of recent transfusion, an autoantibody is the likely cause.

Plan a Further Workup, if Indicated

If an antibody is identified and if the exclusions eliminate all other common red cell antigens from involvement, no further evaluation may be necessary. If, however, the DAT is positive with anti-IgG, an elution and perhaps adsorptions may be indicated. If some exclusions remain to be completed, selected red cell panels, chemically modified panels (ie, enzymes, dithiothreitol, polyethylene glycol), or both, may be necessary.

A Semantic Aside

Most people refer to exclusions (rule-outs) as homozygous or heterozygous; such references are not strictly correct. Only genes are homozygous or heterozygous; the antigens found on red cells are directly or indirectly gene products, not the genes themselves. Because the terms "homozygous" and "heterozygous" are commonly used and more euphonious, they are used in the preceding discussion. However, one should be aware that the more correct terms are "double dose" (instead of homozygous) and "single dose" (rather than heterozygous) expression.

4

Carbohydrate Blood Group Antigens and Collections

LAURA COOLING, MD, MS



SEVERAL BLOOD GROUP ANTIGENS, INCLUDING ABO, are actually small carbohydrate or sugar epitopes on proteins and membrane lipids. Unlike the protein antigens described in the next chapter, these antigens are posttranslational modifications under the control of several enzymes, known as glycosyltransferases, which are present in the golgi apparatus. The antigens are not red-cell-specific—they are present on many cells and tissues and, therefore, are referred to as “histo-blood group antigens” in some texts. In addition to playing an important role in transfusion medicine, these antigens are a factor in organ transplantation, cell development, cancer, and infectious disease.

ABO Blood Group System

Summary Overview

ABO is the single most important blood group system in transfusion and transplantation medicine and is the foundation for all pretransfusion testing in the blood bank. ABO antibodies are highly significant and are capable of

causing severe acute hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN). In organ transplantation, ABO incompatibility can result in acute humoral rejection and organ loss. Because of the clinical importance of ABO, ABO typing and a check for ABO compatibility are always required before blood is dispensed and transfused. Similar safety measures are required in solid organ transplantation. Discrepancies in ABO typing require laboratory and clinical investigation (see Chapter 3: Pretransfusion Compatibility Testing).



When determining a patient's ABO group,

The red cells are phenotyped with anti-A and anti-B (forward type). The plasma or serum is tested for the presence of antibodies against A and B antigens (reverse type).

Phenotypes

The ABO system consists of two autosomal codominant antigens (A and B) and four major phenotypes: A, B, AB, and O. Group O (no A or B) is an autosomal-recessive, null phenotype caused by the inheritance of two amorph ABO genes. The ABO system is unique among the blood groups in that both the red cells and the plasma or serum are typed to determine an individual's ABO type. In the blood bank, red cells are screened for the presence of A and B antigens with monoclonal A and B antibodies (cell grouping or forward type). In addition, the patient's plasma or serum is tested against group A₁ and B red cells for the presence or absence of A and B antibodies, termed isoagglutinins, against A or B antigens (serum grouping or reverse type). The results of forward and reverse typing should inversely complement one another (Table 4-1). For example, a group O individual who lacks both A and B antigens on red cells should have both anti-A and anti-B in his or her plasma or serum. The presence of mixed cell populations as well as weak, missing, or unexpected addi-

Table 4-1. ABO Phenotypes and Genotypes

ABO Type	Antigens on RBCs (Forward Type)	Antibodies in Serum (Reverse Type)	Possible Genotype
O	(H)	Anti-A, -B, -A,B	OO
A	A	Anti-B	AA or AO
B	B	Anti-A	BB or BO
AB	AB	None	AB or <i>cis</i> -AB

tional reactions requires laboratory and clinical investigation before an ABO type can be concluded. Please see sections on ABO subgroups and ABO typing discrepancies below and in Chapter 3.

There are ethnic differences in the distribution of ABO types (see Table 4-2). Group A and group O are the most common phenotypes in US and European donors, whereas group AB is the least common. Group O is the overwhelming predominant blood type in Native Americans. Presence of the group O phenotype is part of the genetic evidence used by anthropologists to support human migration from Asia and colonization by those humans of the North American continent. Historians have also tried linking ABO types with pandemics, such as plague and smallpox, in which certain ABO types may have provided a selective advantage or susceptibility to disease. Although examples do exist of infectious disease transmission influenced by ABO type, there is no evidence showing a dramatic shift in the ABO types before or after the Black Plague.

Biochemistry

The A and B antigens are small, three- or four-sugar oligosaccharide epitopes decorating the termini of *N*-linked glycoproteins and glycolipids (see Figs 4-1 and 4-2). They are remarkably similar chemically, differing by a single immunodominant sugar: galactose (B antigen) and *N*-acetylgalactosamine (A antigen). In fact, the antigens differ only in the presence or absence of an *N*-acetyl group on



Mnemonic Device: To remember which immunodominant sugar residue corresponds to the A and B blood groups, think of the A in blood group A as standing for Acetyl and Amine, ie, A = *N*-AcetylgalactosAmine and B = just plain galactose.

Table 4-2. ABO Blood Group Frequencies in Some US Populations

Group	Frequencies (%)			
	White	Black	Asian	Native American
O	45	49	40	79
A	40	27	28	16
B	11	20	27	4
AB	4	4	5	<1

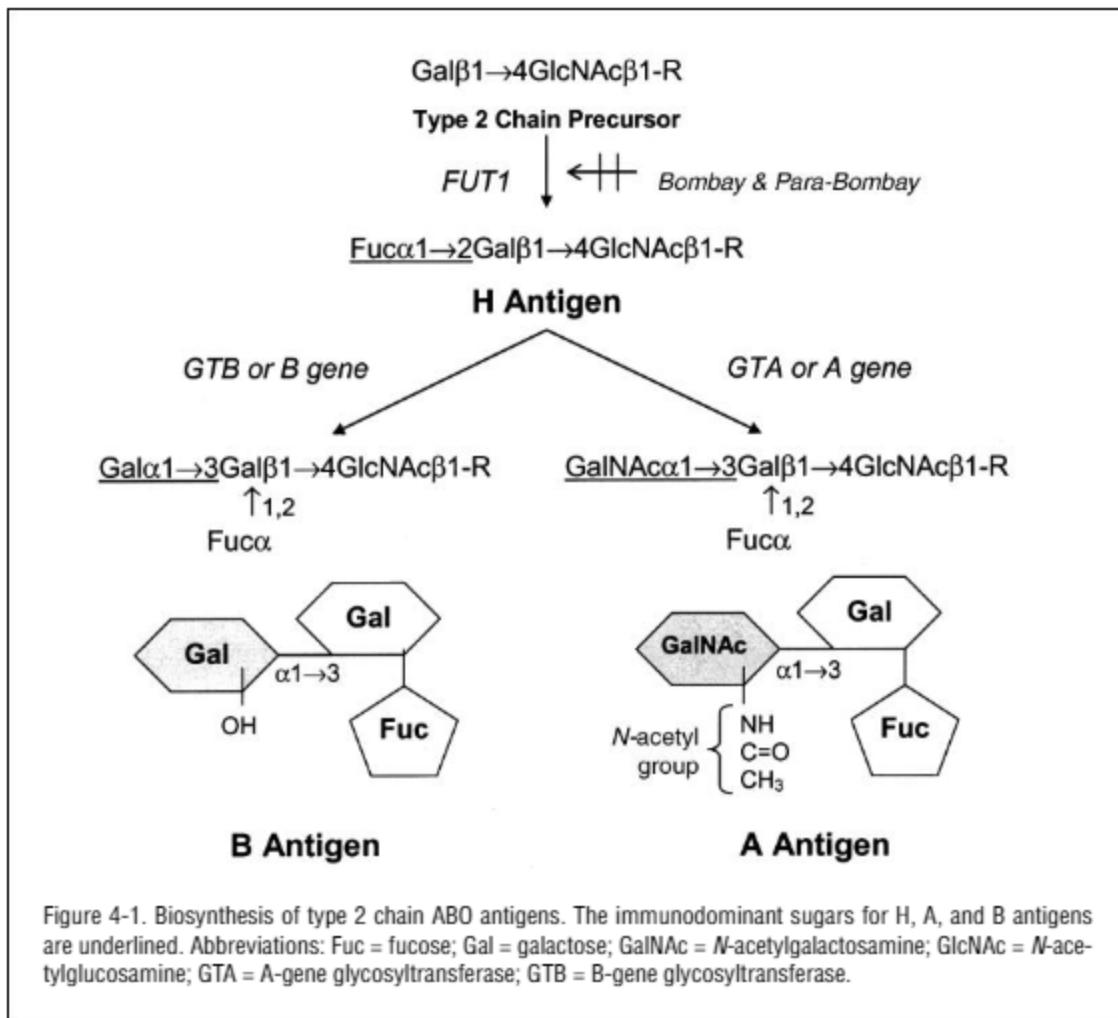


Figure 4-1. Biosynthesis of type 2 chain ABO antigens. The immunodominant sugars for H, A, and B antigens are underlined. Abbreviations: Fuc = fucose; Gal = galactose; GalNAc = *N*-acetylgalactosamine; GlcNAc = *N*-acetylglucosamine; GTA = A-gene glycosyltransferase; GTB = B-gene glycosyltransferase.

the galactose ring. An A antigen can be chemically converted to a B-like antigen by deacetylation. This change can occasionally occur in patients with gram-negative sepsis (acquired B phenotype).

The A and B antigens are also related to the H antigen, which serves as the immediate biosynthetic precursor for A and B antigen synthesis. As shown in Fig 4-1, the H antigen is defined by a terminal, immunodominant $\alpha1\rightarrow2$ fucose. In group A individuals, the H antigen is modified by the addition of an $\alpha1\rightarrow3$ *N*-acetylgalactosamine to the subterminal galactose to form the A antigen. In group B individuals, H is modified by the addition of an $\alpha1\rightarrow3$ galactose to form the B antigen. Group AB individuals, who have inherited both an A and a B gene (see Table 4-1), synthesize both A and B structures. In group O individ-

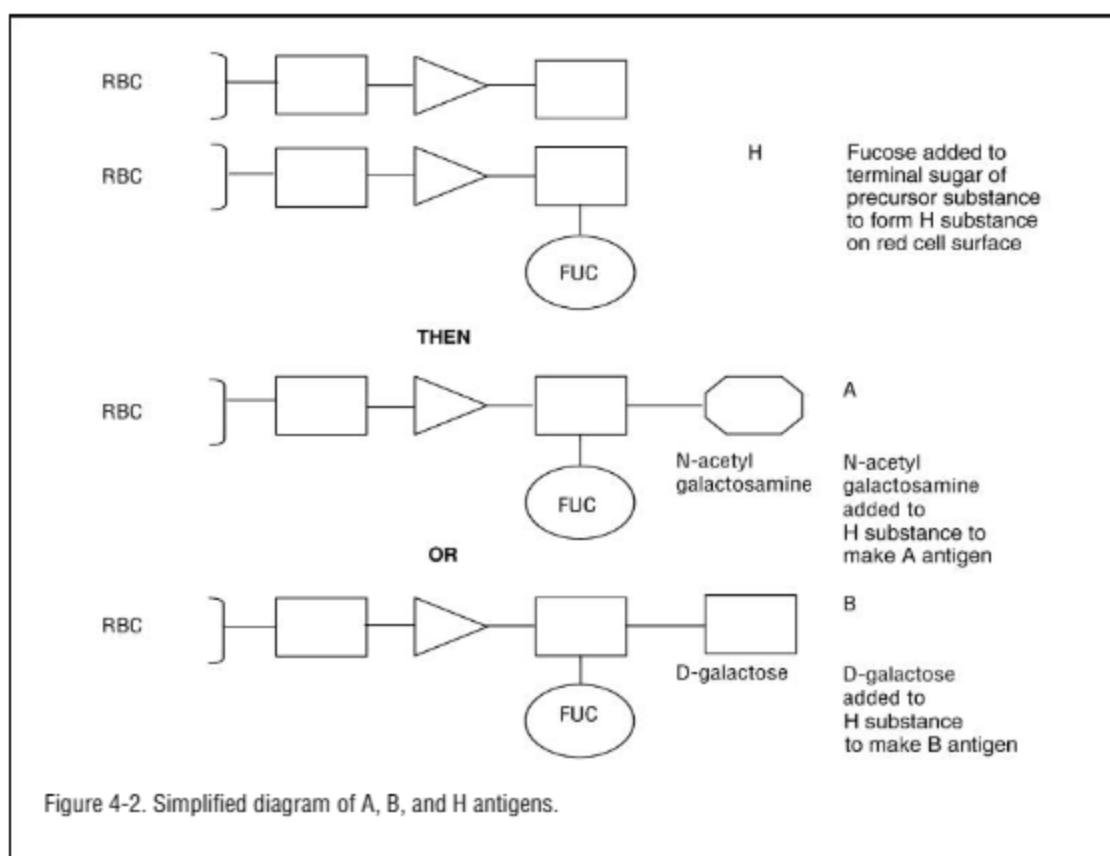


Figure 4-2. Simplified diagram of A, B, and H antigens.

uals, neither A nor B antigens are synthesized because of a mutation in the ABO gene. As a consequence, group O individuals express only the H antigen. An inverse relationship exists between A/B and H antigens on red cells and other tissues. That relationship is important to remember in regard to ABO subgroups, which are typically characterized by weak A/B expression and elevated H antigen. A and B antigens are also absent in the rare Bombay phenotype characterized by the absence of the H-antigen precursor (see the H blood group system below).

Several chemical and immunologic variants of the ABH antigens are attributed to heterogeneity in the oligosaccharide carrier, which can vary in size, complexity (eg, linear, branched), carbohydrate sequence, and anomeric (α or β) linkages. Because the synthesis and the ability to use different oligosaccharide substrates are genetically and transcriptionally regulated, many ABH variants display tissue-specific expression that contributes to antigenic differences between tissues and individuals. On human red cells, four basic ABH “chain” variants have been described



Type 2 precursors are converted to H substance by the addition of fucose to the terminal sugar. This is under the control of a transferase encoded by *FUT1*, also known as the H gene.

H substance forms the substrate onto which the immunodominant sugars for A and B antigens are added.



Type 2 chain ABH molecules are integral to the red cell membrane, whereas type 1 chain ABH molecules are synthesized by the gut and other secretory tissues but not by red cells.

on the basis of the carbohydrate sequence of the oligosaccharide scaffold immediately upstream of the terminal ABH motifs (see Fig 4-3). The major ABH variant on red cells is type 2 chain ABH (galactose $\beta 1 \rightarrow 4$ N-acetylgalactosamine), which is present on all *N*-linked glycoproteins and most ABH-active glycosphingolipids. Red cell glycolipids can also express type 3 (repetitive A) and type 4 ABH (globo-ABH), which contribute to some of the antigenic differences between A_1 , A_2 , and other weak A subgroups. In contrast, type 1 chain ABH antigens are not synthesized by red cells but originate in the gut and other secretory tissues. Those antigens are adsorbed onto red cells from a circulating pool of glycolipid antigen present in plasma. As will be discussed in the section of this chapter on the Lewis blood group system, synthesis and diversity of type 1 chain ABH-active antigens are determined by three genes: ABO, Lewis, and Secretor.

ABH Chain	Red Cell Origin	Structure
		A epitope
Type 1A	No	$\text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3\text{GlcNAc}\beta 1-(R)$ ↑ ₂ $\text{Fuc}\alpha 1$
Type 2A	Yes	$\text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1-(R)$ ↑ ₂ $\text{Fuc}\alpha 1$
Type 3A (repetitive A)	Yes (A_1 cells)	$\text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1-(R)$ ↑ ₂ ↑ ₂ $\text{Fuc}\alpha 1$ $\text{Fuc}\alpha 1$
Type 4A (globo-A)	Yes (A_1 cells)	$\text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}\beta 1 \rightarrow 3\text{Gal}\alpha 1 \rightarrow 4\text{Gal}\beta 1 \rightarrow 4\text{Glc-Cer}$ ↑ ₂ $\text{Fuc}\alpha 1$

Figure 4-3. Comparison of A antigen variants found on red cells. Type 3 and type 4 are relatively specific for A_1 red cells. Differences in the sequence and anomeric linkage of the upstream sequence, which define the four variants, are highlighted in bold. Abbreviations: Cer = ceramide; Gal = galactose; GalNAc = *N*-acetylgalactosamine; Fuc = fucose; GlcNAc = *N*-acetylglucosamine; R = additional carbohydrate sequences.

ABO Gene

The ABO gene is a large, 18-kb gene on chromosome 9q34. The gene contains seven exons that encode a 41-kD, 353-amino-acid enzyme. The ABO alleles responsible for the A₁ and B phenotype differ by a mere seven nucleotides and four amino acids (176, 235, 266, and 268). The two alleles appear to have arisen more than 13 million years ago, before the divergence of lineages leading to humans and old world primates. Seminal studies from the 1990s show that amino acids 266 and 268 in particular (see Table 4-3) play a major role in determining whether the enzyme can accept galactose (B antigen) or an *N*-acetylgalactosamine (A antigen). Variations at those two amino acids are associated with chimeric enzymes, *cis*-AB and B(A), which can synthesize both A and B antigens (see Table 4-3), or a loss of enzyme activity (O03 or O² O allele). Recent studies have linked the O03 allele as a common cause of ABO discrepancies in normal blood donors.

Since the cloning of the ABO gene, a large number of mutations have been identified. Weak ABO subtypes are often the result of point mutations in the enzyme (eg, B₃, B_x, B_{el}, B_w, A₃, A_x, A_w). In A₂ and A_{el} individuals, a nucleotide deletion and frameshift toward the end of the enzyme lead to the translation of additional amino acids that decrease enzyme activity. A specific ABO subgroup phenotype can arise from several different mutations (eg, B_w is associated with seven different mutant B alleles).



A antigen results from addition of *N*-acetylgalactosamine to the H antigen (H substance).

B antigen results from addition of galactose to the H substance.

Table 4-3. Molecular Differentiation of the A and B Genes

Phenotype	Nucleotide Sugar Donor	Amino Acid 235	Amino Acid 266	Amino Acid 268
A ₁	<i>N</i> -acetylgalactosamine (GalNAc)	Gly	Leu	Gly
B	Galactose (Gal)	Ser	Met	Ala
<i>cis</i> -AB	Gal and GalNAc	Gly	Leu	Ala
<i>cis</i> -AB	Gal and GalNAc	Gly	Met	Gly
<i>cis</i> -AB	Gal and GalNAc	Ser	Leu	Ala
B(A)	Gal >> GalNAc	Gly	Met	Ala
B(A)	Gal >> GalNAc	Ser	Met	Ala
O (O03)	—	Gly	Leu	Arg

Likewise, the same mutant allele can be implicated in more than one weak ABO subgroup.

Most of the mutations associated with the group O phenotype are frameshift and nonsense mutations. Of the more than 30 different O alleles identified, the two most common possess a nucleotide deletion and frameshift, leading to a truncated 117 amino acid mutant protein. Homozygous inheritance of two O alleles is responsible for the group O phenotype (see Table 4-1).

A and B Subgroups



Group A₂ individuals have an A antigen that is both quantitatively and qualitatively different from group A₁. The qualitative difference means that some A₂ patients are capable of making anti-A₁.



A₁ red cells are agglutinated by *Dolichos biflorus* lectin, enabling A₁ cells to be differentiated from A₂ cells (and red cells of other A subgroups).

The ABO system also contains several subgroups, usually identified by weakened expression of A and B antigens. Such subgroups are a common cause of ABO typing discrepancies and are more common in group A individuals. The two most common subgroups that are clinically encountered are A₁ and A₂, which account respectively for 80% and 20% of all group A donors. Both A₁ and A₂ red cells are strongly agglutinated by commercial group A monoclonal antibodies; however, only A₁ red cells are agglutinated by the anti-A lectin, *Dolichos biflorus*. Approximately 8% of A₂ and 22% to 25% of A₂B individuals may present with an ABO discrepancy resulting from an anti-A₁ in their sera (see Table 4-4). The anti-A₁ reacts with A₁ red cells in routine serum or reverse grouping but not with the patient's own red cells.

A comparison of A₁ and A₂ red cells shows both quantitative and qualitative differences in A antigen density and structure. Quantitatively, the number of A antigens on A₁ red cells is approximately four times that on A₂ red cells (1 million vs 220,000). That quantitative difference is the basis for A₁/A₂ subtyping by the lectin, *D. biflorus*. In addition, A₁ red cells express an array of glycans-bearing A antigen, including type 3 (repetitive A) and type 4 chain (globo-A) glycolipid (see Fig 4-2). The latter is related to the globoside blood group collection (see the section on P blood group) and was recently hypothesized to be an A₁-specific antigen. A₂ red cells, by contrast, express less A and more H antigen, reflecting inefficient conversion of H→A in affected individuals. The difference between A₁ and A₂ is even more dramatic on nonerythroid tissue such as platelets and endothelium. Platelets and organs from A₂ individuals essentially lack A antigen and can be considered group O compatible. Genetic analysis has shown that most A₂ individuals have a deletion late in the A transferase gene, leading to a frameshift and an additional 21

Table 4-4. Examples of ABO Discrepancies

Antibodies in Serum (Reverse Type)						
Red Cell Antigens (Forward Type)	A ₁ Red Cells	B Red Cells	0 Red Cells	Auto Control	Discrepancy	Possible Causes
A	-	+	-	-	None	Group A
AB	-	+	-	-	Extra B reactivity	Acquired B
AB	+	-	-	-	Extra A reactivity	B(A) phenotype
A _{weak}	(+/-)	+	-	-	Weak A expression	Weak A subgroup (with anti-A ₁)
					Infant	
					Massive transfusion (group O RBCs)	
A	-	-	-	-	Missing anti-B	Newborn
						Hypogammaglobulinemia
						Immunosuppression
						Transplantation
A	+	+	-	-	Extra serum reactions	Weak A subgroup (eg, A ₂)
						Out-of-group platelets, plasma
						IVIG
A	+	+	+	-	Extra serum reactions	IgM alloantibody (eg, anti-M, P ₁)
A	+	+	+	+	Extra serum reactions	IgM cold autoantibody
A	-	+	+	-	Extra serum reactions	Rouleaux
O	+	+	+	-	Extra serum reactions	A ₁ with anti-H, -H
						Bombay, para-Bombay

amino acids in the translated protein. The latter results in a 5- to 10-fold decrease in enzyme activity.

Additional subgroups are also known (eg, A₃, A_x, A_{el}, A_{firm}), but they are relatively rare (<0.1% of donors). A₃ and A_{end} red cells are often distinguished by weak, mixed-field agglutination with anti-A. Some phenotypes are agglutinated only by anti-A,B (A_x). In the A_{el} phenotype, no agglutination is observed. In those cells, A expression can be demonstrated only by adsorption and elution studies. Like the A₂ phenotype, those weak ABO subtypes have markedly increased H antigen expression and can be accompanied by anti-A₁. Weak group B phenotypes include B₃, B_x, B_{el}, and B_w.

Chimeric Phenotypes



Acquired B presents as red cells that (transiently) phenotype as AB on forward typing but demonstrate strong anti-B on reverse typing.

Acquired B is associated with bacterial sepsis, particularly infections by enteric organisms.

Chimeric phenotypes with both A and B or mixed-field expression are encountered. Usually, they are detected because of a discrepancy in ABO typing. Acquired B is a transient AB red cell phenotype in a genetically group A individual. Although the person may forward or red-cell-type as AB, his or her serum continues to react like group A serum (strong anti-B). (See Table 4-4.) Acquired B is most likely to occur in the setting of bacterial sepsis, particularly by gram-negative bacteria that can secrete a deacetylase enzyme capable of removing the N-acetyl group on the A antigen (see Fig 4-1). The resulting sugar can react with some monoclonal anti-B typing sera.

The B(A) phenotype is a genetic phenotype that is characterized by inheritance of an ABO enzyme capable of synthesizing B and A antigens (see Table 4-3). B(A) individuals will type as A_{weak}B on red cell typing but will reverse-type as group B (strong anti-A). Detection of the B(A) phenotype can depend on the monoclonal anti-A used for red cell typing.

Transient and stable chimeric phenotypes can also be encountered in ABO-mismatched allogeneic hematopoietic progenitor cell (HPC) transplants. Patients in that category have a circulating mixture of recipient and donor red cells, often accompanied by hemolysis. For example, a group A patient transplanted with HPCs from a group O donor will have a mixed-field typing because of circulating group A autologous red cells and group O red cells from both the donor and red cell transfusions. With full donor engraftment, the patient will eventually have only group O red cells. Even after engraftment, those patients may continue to have discrepancies on ABO typing as a

result of missing agglutinins. In the example above, the patient's red cells will eventually type as group O (donor), but the patient's serum may type as group A (anti-B only). That result is attributable to immune tolerance by group A antigen on endothelium and other tissues.

ABO Discrepancies

As discussed at the beginning of the chapter, identification of ABO group requires typing red cells for ABO antigens and screening plasma or sera for the appropriate antibodies. On occasion, there can be a discrepancy between red cell (forward type) and serum grouping (reverse type) results. ABO discrepancies fall into four basic categories: weak or missing antigens on red cells, weak or missing antibodies in sera, anomalous or extra reactions on red cell typing, and extra reactions during serum grouping. All ABO discrepancies must be investigated before an ABO type is concluded. An extensive discussion of ABO typing and potential causes of ABO discrepancies is found in Chapter 3. Several examples of ABO discrepancies are shown in Table 3-2 and Table 4-4.

Weak or missing antigens on red cell typing may reflect weak ABO subgroups, young age, and hematopoietic malignancies (eg, erythroleukemia). Massive transfusion with group O red cells will also give weak A or B reactions (or both) on red cell typing, with a mixed-field appearance. Anomalous red cell typing results can occur in acquired B, the B(A) phenotype, and in ABO-mismatched transplant patients. Anomalous red cell typing can also be the consequence of nonspecific agglutination caused by contaminants in the sample (eg, Wharton's jelly), sensitized red cells, or polyagglutination.

ABO discrepancies are most commonly caused by missing or extra serum reactivity. Extra reactivity with group A, B, and O red cells can be observed with cold autoantibodies, cold alloantibodies (eg, anti-M, P₁), anti-A₁, and rouleaux. The latter can occur in monoclonal gammopathies, such as Waldenstrom's macroglobulinemia, or after the infusion of a high-molecular-weight volume expander. On occasion, passively acquired isoagglutinins are encountered after an out-of-group platelet transfusion, after administration of intravenous immunoglobulin (IVIG), or after delivery when transplacental maternal antibody is present in a newborn. Missing isoagglutinins, however, are normal in newborns, who do not develop those antibodies until they are 3 to 6 months old. Weak or missing isoagglu-



An ABO typing discrepancy is said to exist when the patient's forward and reverse type do not agree, ie, when the patient's serum (reverse type) contains ABO antibodies that should not be present in a person whose RBCs contain the antigen(s) found on forward typing.

tinins are also encountered in some elderly patients, in patients receiving immunosuppressive chemotherapy, and in patients with congenital and acquired humoral immunodeficiencies, including those patients receiving intensive apheresis with albumin replacement and immunosuppression. As discussed earlier, ABO-mismatched transplant patients may have missing agglutinins because of immune tolerance.

Finally, ABO discrepancies can be the result of laboratory errors such as specimen mix-up, fibrin clots, failure to add reagents, reagent failures, a heavy red cell suspension, hemolysis, overcentrifugation of samples, and incorrect interpretation or recording of results.



Anti-A and anti-B are “naturally occurring” antibodies, meaning that they are produced in the absence of RBC exposure.



Anti-A and anti-B are both highly clinically significant antibodies, capable of causing both acute hemolytic transfusion reactions (acute HTRs) and hemolytic disease of the fetus and newborn (HDFN).

ABO Antibodies

The majority of anti-A and anti-B is immunoglobulin M (IgM), although some immunoglobulin G (IgG) and immunoglobulin A (IgA) can be present. Group O serum also contains a third antibody, anti-A,B, that recognizes an epitope common to both the A and B antigens. The antibodies are naturally occurring and do not require immune stimulation by transfusion or pregnancy. It is believed that the stimulus for anti-A and anti-B is exposure to environmental bacteria, particularly normal intestinal flora, which carry ABO-like structures on their lipopolysaccharide. Generally, ABO antibodies are not present in newborns until they are 3 to 6 months old, with most children expressing the appropriate isoagglutinins in their serum by the time they are 1 year old. Antibody titers achieve adult levels by the time children are 5 to 10 years old, and the titers may decrease with advanced age and illness, although most people have detectable isoagglutinins throughout adulthood.

Clinically, ABO antibodies are highly significant. They are capable of binding red cells at 37°C and fixing complement, which results in intravascular hemolysis. Transfusion of incompatible red cells (“major incompatible”) or plasma (“minor incompatible”) can lead to severe HTRs, with significant morbidity and mortality. Despite numerous redundant policies to prevent the transfusion of ABO-incompatible red cells and plasma, the transfusion of ABO-incompatible blood still occurs because of clerical errors in patient identification at the time of sample collection (wrong blood in tube) or at transfusion (wrong patient). In large transplant centers, ABO-mediated hemolysis is frequently observed in ABO-mismatched allogeneic

marrow and solid organ transplantation. In those cases, it usually follows transplantation from a group O donor to a group A or B recipient. Donor lymphocytes present in the marrow or organ continue to produce ABO antibodies that bind and hemolyze the patient's red cells.

ABO antibodies can also be associated with HDFN in the presence of a fetomaternal ABO incompatibility. That ABO incompatibility can stimulate production of IgG antibodies that are capable of crossing the placenta and affecting the fetus. ABO-associated HDFN is more likely to occur in group O mothers carrying group A infants because, in part, of the higher titer of anti-A IgG in group O serum. Although ABO-associated HDFN is usually mild, severe cases requiring phototherapy and exchange transfusion are not rare. It is believed that soluble ABO antigens that are present in fetal plasma, coupled with weak ABO expression on fetal red cells, may help neutralize maternal antibody and protect infants from severe hemolysis in most cases.



ABO-associated HDFN is usually mild, but severe cases that require phototherapy and exchange transfusion are not rare

Physiologic Role

The true function of the A and B antigens is unknown. Many epidemiologic studies have reported associations between ABO type and autoimmune, neoplastic, and infectious diseases. Because ABO antibodies are stimulated by environmental bacteria, it has long been speculated that the different ABO types are an evolutionary response to bacteria and other pathogens.

Changes in ABO expression can occur during normal development and neoplastic transformation. In colon cancer, A and B antigens appear to modify cell adhesion and signaling by integrins, which play a critical role in invasion and metastasis. Loss of A and B antigens, with increased expression of H and H-like antigens, is associated with a poor prognosis and increased metastatic potential in several epithelial cancers.

H Blood Group System

Summary Overview

The H antigen is the immediate precursor for A and B antigens and is defined by a terminal, immunodominant $\alpha 1 \rightarrow 2$ fucose. It is ubiquitously expressed on all red cells except for rare Bombay and para-Bombay phenotypes, which lack



The H antigen is the immediate precursor for A and B antigens.

H-gene or *FUT1* activity. The amount of H antigen is dependent on ABO type and is highest on group O red cells, followed by weak ABO subtypes. The least amount of H antigen is observed on A₁ and A₁B red cells because nearly all H antigen is converted to A or B antigen, or both (see Figs 4-1 and 4-2). The presence of H antigen can be detected with the anti-H lectin, *Ulex europaeus*. Antibodies against H and HI antigen are usually clinically benign; exceptions occur in the rare Bombay and para-Bombay phenotypes.

Biochemistry and Synthesis



Secretor status refers to the presence of A, B, and H antigens in glandular secretions. The "secretor gene" is necessary for synthesis of ABH antigens in secretions.



FUT1 = Type 2 H gene (red cells)

FUT2 = Type 1 H gene (secretor gene)

FUT3 = Lewis gene

The minimum structure for the H antigen is fucose $\alpha 1 \rightarrow 2$ galactose. In humans, two related genes are capable of synthesizing the H antigen. *FUT1*, also known as the H gene, specifically recognizes and fucosylates type 2 chain ($\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$) substrates. The product of *FUT1* is type 2 chain H (2H), which is the major H antigen synthesized on glycoproteins and glycolipids of red cells (see Figs 4-1 and 4-4). *FUT2*, or Secretor, is a second $\alpha 1 \rightarrow 2$ fucosyltransferase capable of synthesizing H antigen. Unlike *FUT1*, *FUT2* fucosylates type 1 chain substrates ($\text{Gal}\beta 1 \rightarrow 3\text{GlcNAc}$) to yield type 1 chain H (1H). Whereas nearly all individuals inherit and express *FUT1*, only 80% of Whites are positive for the Secretor gene (*Se*). Those individuals are considered ABH "secretors" because they express type 1 chain ABH antigens in saliva, plasma, and other secretions. Individuals who lack ABH substances in saliva are considered "nonsecretors" and are homozygous for a nonfunctional Secretor gene (*se/se*). In addition to type 1H, *FUT2/Secretor* may also be responsible for the synthesis of type 3 and type 4 H antigens, at least in epithelial tissues. For a discussion of *FUT2* in Lewis antigen synthesis, please see the section on the Lewis blood group system in this chapter.

Molecular Biology

FUT1 and *FUT2* genes reside on chromosome 19q13.3 and appear to be the consequence of gene duplication. They are approximately 70% homologous and encode a 361 and 365 amino acid glycoprotein, respectively. More than 20 different mutant *FUT1* alleles, which are associated with Bombay and para-Bombay phenotypes, have been identified to date. See the Lewis section for more on *FUT2*.

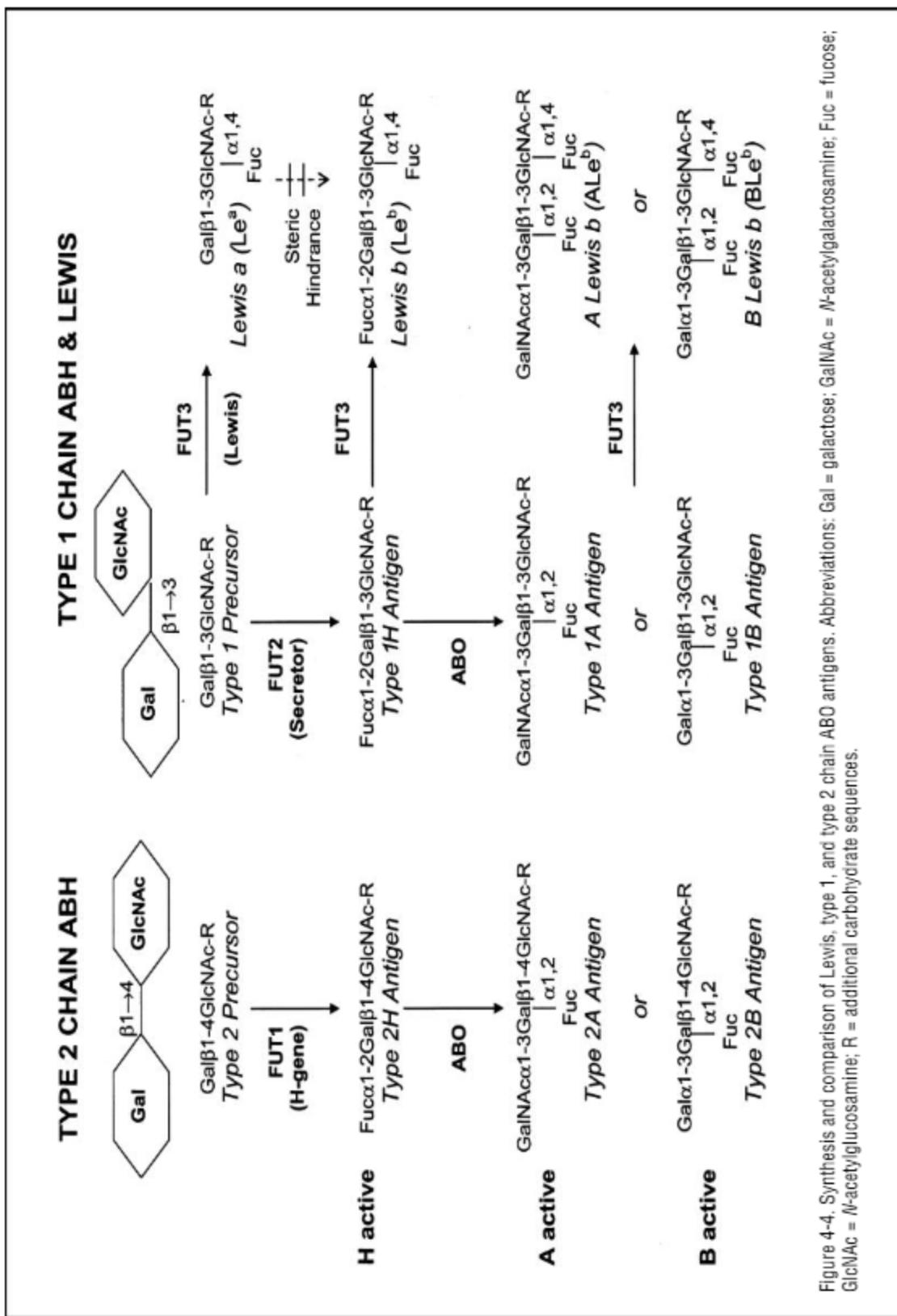


Figure 4-4. Synthesis and comparison of Lewis, type 1, and type 2 chain ABO antigens. Abbreviations: Gal = galactose; GalNAc = N-acetyl/galactosamine; Fuc = fucose; GlcNAc = N-acetyl/glucosamine; R = additional carbohydrate sequences.

Null Phenotypes



Bombay individuals have normal A and B genes but lack the ability to produce H substance, the precursor of both A and B antigens. As a result, Bombay RBCs lack A and B antigens and appear phenotypically to be group O.



Bombay patients have a potent, naturally occurring anti-H that reacts with all RBCs except those from another Bombay individual. They require transfusion with rare-donor (Bombay) RBCs.

Bombay (O_h) is a rare, autosomal, recessive phenotype characterized by the complete absence of H, A, and B antigens on red cells *and* in secretions. Individuals with the Bombay phenotype lack both *FUT1* (*hh* genotype) and *FUT2* (*se/se*). Bombay, therefore, is an H-deficient, “non-secretor” phenotype (see Table 4-5). Serologically, Bombay red cells fail to agglutinate with anti-A, anti-B, and the anti-H lectin, *Ulex europaeus*. On plasma or serum grouping, Bombay individuals possess anti-A, anti-B, and a strong allo-anti-H. On routine ABO typing, individuals with the Bombay phenotype initially type as group O. Usually, they are identified during antibody screening, which requires testing their sera against group O red cells (see Chapter 3). The phenotype is confirmed by typing red cells for H antigen with *Ulex europaeus*. In addition, the sera of individuals with the Bombay phenotype will react with all cells except autologous and O_h red cells. Because those individuals also lack Secretor/*FUT2*, they will phenotype as Le(b-) and will lack ABH substances in their saliva.

The para-Bombay phenotype is also an autosomal-recessive phenotype associated with an *hh*, or H-null, genotype. Unlike with the Bombay phenotype, most individuals with the para-Bombay phenotype are positive for Secretor/*FUT2* and, therefore, possess ABH substances in plasma, saliva, and other secretions (that is, they are H-deficient Secretors). In laboratory testing, para-Bombay red cells lack detectable H antigen but can carry small amounts of

Table 4-5. Bombay and Para-Bombay Phenotypes

	ABH Antigens on Red Cells			ABH in Secretions (Type 1)	Antibodies in Serum	Genotype	
	H	A	B			<i>FUT1</i>	<i>FUT2</i>
Bombay (O_h)	—	—	—	—	Anti-H,-A,-B	<i>hh</i>	<i>se/se</i>
Para-Bombay							
A_h	—	—/trace	—	A, H	Anti-H,-B	<i>hh</i>	<i>Se</i>
B_h	—	—	—/trace	B, H	Anti-H,-A	<i>hh</i>	<i>Se</i>

A antigen, B antigen, or both. This phenomenon is due to adsorption of type 1 chain ABH substance present in plasma (see the discussion of the Lewis blood group system). Individuals with para-Bombay also have a potent allo-anti-H in their sera. Para-Bombay red cells are designated A_h, B_h, and AB_h.

Antibodies

H antibodies are of IgM isotype and are naturally occurring. They are most commonly encountered as autoantibodies in the sera of groups A₁ and A₁B individuals, who have very little H antigen on their red cells. Auto-anti-H is usually of low titer and is clinically insignificant.

In contrast, the allo-anti-H present in individuals who have the Bombay or para-Bombay phenotype is a potent, clinically significant antibody capable of causing acute hemolytic transfusion reactions. Patients with Bombay or para-Bombay phenotype must receive O_h, crossmatch-compatible, red cells.

Physiologic Role

H antigen is expressed on early HPCs; it has been hypothesized to play a role in cell adhesion and development. In many cancers, loss of A/B antigens, accompanied by an increase in H and H-active antigens, is associated with resistance to apoptosis, increased metastatic potential, and decreased survival. H antigen is also a receptor for several pathogens, including Norwalk virus and some strains of *Candida*.

Lewis Blood Group System

Summary Overview

The Lewis blood group system consists of two major antigens, Le^a and Le^b, and three common phenotypes. The three phenotypes reflect the inheritance and interaction of two nonallelic fucosyltransferase genes: Lewis (*FUT3*) and Secretor (*FUT2*). Unlike most red cell antigens, the Lewis antigens are not of erythroid origin but are passively adsorbed onto red cell membranes from a soluble pool of secreted Lewis substance in plasma. Lewis antigens are widely expressed on tissues and in fluids, including plasma, saliva, urine, red cells, platelets, endothelium, and



The Lewis (*FUT3*) gene encodes for a fucosyltransferase that acts on type 1 ABH precursor oligosaccharides. Thus, Lewis antigens are not integral to the RBC membrane but, instead, are found in plasma, saliva, and other glandular secretions.

epithelial tissues. Like the I antigen, Lewis antigens are developmentally expressed, with most newborns typing as Le(a–b–). Lewis antigens are rarely a cause of HTRs, and they are not associated with HDFN. Lewis and Lewis-related antigens are oncofetal markers and serve as genetic, host-susceptibility factors for many infectious diseases.

Phenotypes

Three Lewis phenotypes are observed in adults: Le(a–b+), Le(a+b–), and Le(a–b–). As shown in Table 4-6, the Le(a–b–) phenotype is five times more common in Blacks than in Whites. The Le(a–b–) phenotype is also increased in neonates as a result of developmental delays in Lewis and Secretor expression. The Le(a+b+) is rarely encountered, but it can be transiently observed in some young children as they transition to an adult Le(a–b+) phenotype. An adult Le(a+b^{weak}) phenotype is present in Polynesian, Japanese, and Taiwanese populations because of inheritance of a weak or partial Secretor gene (*Se^w*). Because Lewis antigens are passively adsorbed onto red cell membranes, Lewis antigens can be eluted from red cells after transfusion or by increases in plasma volume and circulating lipoproteins. The literature contains several examples of transient changes in Lewis phenotype that are associated with pregnancy and ascites.



The Le^a antigen is found in nonsecretors who carry the Lewis gene, whereas the presence of both the Lewis gene and the secretor gene (*Se*) is required for formation of Le^b antigens.

Biochemistry and Synthesis

On red cells, platelets, and endothelium, which adsorb Lewis antigens from plasma, the Lewis antigens are present on glycosphingolipids. In tissues that actively synthesize Lewis antigens (eg, gut, salivary gland), Lewis antigens can be found on an array of glycans, including glycosphingolipids, glycoproteins, and mucins. Gastrointestinal epithelium is particularly rich in Lewis glycolipids and glycoproteins, and it is believed to be the source of Lewis antigens in plasma and, therefore, in red cells. This phenomenon was demonstrated *in vivo* in a Le(a–b–) patient who received a small bowel transplant. Seven months after transplantation, the patient's red cells typed as Le(a–b+).

Synthesis and expression of Le^a and Le^b antigens reflect inheritance and interaction of two distinct glycosyltransferases: fucosyltransferase type 2 (FUT2/Secretor) and fucosyltransferase type 3 (FUT3/Lewis). FUT3 is the product of the Lewis gene (*Le/FUT3*) and is an $\alpha 1 \rightarrow 3/4$ fucosyl-

Table 4-6. The Lewis Blood Group System

Phenotype	Frequency (%)		Genotype		Type 1 Chain Lewis and ABH in Plasma and Secretions		
	White	Black	Lewis	Secretor	Group O	Group A	Group B
Le(a+b-)	22	23	Le	Se/Se	Le ^a	Le ^a	Le ^a
Le(a-b+)	72	55	Le	Se	H, Le ^a , Le ^b	H, A, Le ^a , Le ^b	H, B, Le ^a , Le ^b
Le(a-b-)	6	22	le/le	Se or se/se	H	H, A	H, B
Le(a+b+)*	Rare	Rare	Le	Se or Se ^w	—	—	—
					H, Le ^a , Le ^b , Le ^{weak}	H, B, Le ^{weak}	H, B, Le ^{weak}

*Children can have a transient Le(a+b+) phenotype in early childhood. A Le(a+b^w) phenotype is present in some Asian populations as a result of a weak Secretor allele (Se^w).

transferase. FUT2, the product of the Secretor gene (*Se*/*FUT2*), is an H-like, $\alpha 1 \rightarrow 2$ fucosyltransferase. Both FUT2 and FUT3 preferentially recognize and fucosylate type 1 chain oligosaccharides ($\text{Gal}\beta 1 \rightarrow 3\text{GlcNAc}$). Figure 4-5 is a simplified diagram of Lewis antigens.

How Lewis, Secretor, and ABO genes interact to yield the different combinations of red cell and plasma phenotypes is shown in Table 4-6 and Fig 4-4. Individuals of the $\text{Le}(\text{a+b-})$ phenotype have inherited at least one functional *FUT3* (*Le*) gene but lack *Secretor* (*se/se* genotype). The FUT3, or Lewis, enzyme adds an $\alpha 1 \rightarrow 4$ fucose to the sub-terminal *N*-acetylgalactosamine of type 1 chain precursor (historically Le^e) to form the Le^a antigen. However, because those donors are negative for FUT2, or Secretor, no type 1 chain H (Le^d) or Le^b can be made. As a result, only Le^a is present in plasma, saliva, red cells, and other tissues.

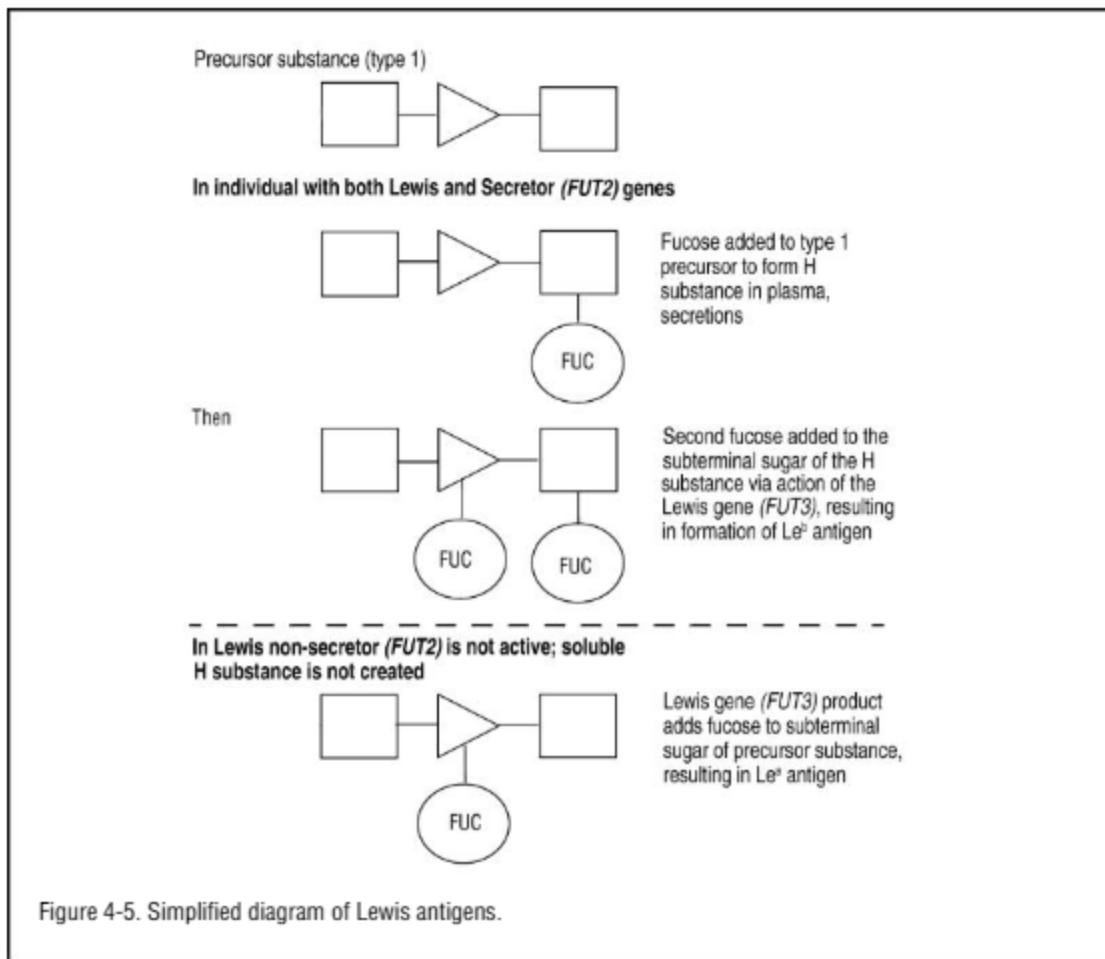


Figure 4-5. Simplified diagram of Lewis antigens.

The Le(a–b+) red cell phenotype reflects inheritance of a functional Lewis (*Le*) and Secretor (*Se*) gene. Although FUT3, or Lewis, still converts a small amount of type 1 chain precursor to Le^a antigen, most Le^c substance is converted to type 1 chain H (or Le^d) by FUT2 (see Fig 4-4), which adds a terminal, H-active $\alpha 1 \rightarrow 2$ fucose. FUT3 can subsequently add a second $\alpha 1 \rightarrow 4$ fucose to form Le^b antigen. Note that Le^b cannot be formed from Le^a: the presence of a subterminal fucose on Le^a inhibits binding by the FUT2/Secretor enzyme. Because Le(a–b+) synthesizes a small amount of Le^a antigen, it does not make an anti-Le^a.

In Le(a–b+) individuals, Le^b synthesis and immunogenicity are also influenced by ABO type. In group O individuals, only type 1H and Le^b are synthesized. In group A₁ and B individuals, type 1 chain H is further modified by the appropriate ABO glycosyltransferase to form type 1 chain A and B antigens, respectively. The latter can subsequently serve as substrates for FUT3/Lewis to form ALe^b and BLe^b (see Fig 4-4). In group A₁ donors, ALe^b is the major Le^b antigen found in plasma. The interaction of ABO with Lewis can influence antibody reactivity. Antibodies with both Le^b- and ABO-specificity are well known.

Unlike the Le(a+b–) and Le(a–b+) phenotypes, the Le(a–b–) red cell phenotype can arise from two genetic backgrounds, depending on the inheritance of Secretor/FUT2. By definition, all Le(a–b–) individuals lack a functional Lewis/FUT3 gene and are unable to synthesize either Le^a or Le^b antigens. In Le(a–b–) secretors (*le/le, Se* genotype), type 1 chain ABH antigens can still be synthesized by the action of FUT2/Secretor. Those individuals can be identified by testing saliva for the presence of ABH substances capable of inhibiting red cell agglutination. Le(a–b–) non-secretors (*le/le, se/se*), however, are unable to synthesize either Lewis or type 1 chain H antigens. As a result, only type 1 chain precursor (Le^c) is found in secretions and tissue.

Molecular Biology

FUT3, or Lewis, gene resides on chromosome 19p13.3 near two other $\alpha 1 \rightarrow 3$ fucosyltransferase genes, *FUT5* and *FUT6*. The gene encodes a 361-amino acid, type 2 glycoprotein with $\alpha 1 \rightarrow 4$ (Le^a) and very weak $\alpha 1 \rightarrow 3$ (Le^X) fucosyltransferase activity. Inactivating mutations in *FUT3* are responsible for the Le(a–b–), or null, phenotype. More than 10 missense mutations in the *FUT3* gene have been



FUT3 (the Lewis gene) codes for a transferase that adds fucose to the *subterminal* sugar of type 1 precursors.



FUT2 (Secretor gene) codes for a transferase that adds fucose to the terminal sugar of type 1 precursor chains, creating type 1 H substance.

identified, with most null alleles (*le*) possessing two or more mutations.

The *FUT2* (*Se*) gene resides on chromosome 19q13.3 as part of a 100-kb gene cluster that includes the H gene (*FUT1*) and *Sec1*, an inactive *FUT2*-like pseudogene. Multiple null alleles have been reported, with distinct geographic and ethnic distributions. Most null alleles are the result of nonsense mutations. The *Se^w* allele associated with the adult Le(a+b^{weak}) or partial secretor phenotype present in some Asian populations is characterized by a single missense mutation (Ile129>Phe).

Lewis Antibodies

Antibodies against Le^a and Le^b antigens are generally of IgM isotype and are naturally occurring. In laboratory testing, they are usually detected as room-temperature saline agglutinins, although rare examples are capable of hemolysis in vitro and in vivo. They are most often encountered in the sera of Le(a-b-) individuals, and they may contain a mixture of anti-Le^a, anti-Le^b, and anti-Le^{a+b}. Anti-Le^b is seen in Le(a+b-) individuals and occasionally in pregnancy. Because of an increase in plasma volume during pregnancy, Le(a-b+) females can develop a transient Le(b-) phenotype and a weak anti-Le^b. Le(a-b+) individuals do not make an anti-Le^a because those individuals synthesize a small amount of Le^a (see discussion on synthesis). Some examples of anti-Le^b can demonstrate ABH specificity (anti-Le^{bH}, anti-ALe^b, anti-BLe^b), reacting stronger with Le^{b+}-positive red cells of specific ABO types (Fig 4-4).

Lewis antibodies can be enhanced by treating red cells with enzymes to remove glycoproteins that are capable of masking Lewis antigens. Conversely, Lewis antibodies can be inhibited by adding soluble Lewis substance to plasma or serum samples. The latter can be a helpful trick when managing patient samples containing multiple alloantibodies. Soluble Lewis substance is available commercially or can be obtained by using plasma from a Lewis-typed donor [eg, Le(a-b+) donor], which should contain soluble Lewis substance of interest.

In general, Lewis antibodies are not clinically significant. Patients with Lewis antibodies may be safely transfused with crossmatch-compatible red cells. It is not necessary to type and provide Lewis-negative units. The one exception is a Lewis antibody that reacts at 37°C or shows in-vitro hemolysis (or both). In those cases, transfusion of Lewis antigen-negative, crossmatch-compatible, red cells



RBCs for transfusion to patients with Lewis antibodies should be crossmatch-compatible but need not be antigen-negative.

is recommended. If antigen-negative blood is not available, the infusion of plasma containing the soluble Lewis antigen may neutralize or inhibit circulating antibody before Red Blood Cell (RBC) transfusion.

Lewis antibodies are not associated with HDFN. As discussed, Lewis antigens are poorly expressed on neonatal red cells, with most newborns typing as Le(a–b–) with human Lewis antibodies. In addition, Lewis antibodies are of IgM isotype and will not cross the placenta.

Physiologic Role

Type 1H and Le^b are receptors for several strains of *Helicobacter pylori*, a gram-negative bacterium that is associated with chronic gastritis, gastric ulcers, and several types of gastric cancer. This finding appears to explain the known epidemiologic association between gastric ulcers, cancer, and a group O secretor phenotype. Le^b and type 1H antigen are also receptors for Norwalk virus, a virulent and common cause of gastroenteritis that has caused havoc for several ocean cruise lines. In contrast, the absence of Lewis or Secretor or both (Lewis null or nonsecretor phenotype) has been linked to an increased incidence of recurrent *Candida vaginitis*, urinary tract infections, and heart disease. Finally, several Lewis-related antigens (sialyl-Le^a, sialyl-Le^c, Le^X, and sialyl-Le^X) are markers for many epithelial cancers. CA19-9, a serologic marker used for monitoring patients with pancreatic cancer, recognizes the sialyl-Le^a antigen. Sialyl-Le^a and sialyl-Le^X are ligands for E-selectin, an endothelial adhesion molecule implicated in tumor-endothelial interactions and metastasis.



Individuals who are group O secretors have an association with gastric ulcers and gastric cancer. This association is believed to result from the fact that type 1 H and Le^b are receptors for several strains of *Helicobacter pylori*.

I Blood Group System

Summary Overview

The I blood group system consists of two related type 2 antigens, i and I, that differ only in the size and complexity of their structures. Those antigens are ubiquitously expressed on all cells and are the primary carriers of ABH antigens on red cells. The i antigen is a linear type 2 oligosaccharide and is highly expressed on cord and immature red cells. The i antigen is converted to a branched I antigen by the I gene glycosyltransferase, resulting in an adult I+ red cell phenotype. Low-titer, cold autoantibodies against i



i and I epitopes are found on the interior portions of the same oligosaccharide chains as A, B, and H antigens. The i antigen, which is unbranched, is found on neonatal RBCs, whereas the branched I antigen is present on almost all adult RBCs.

and I antigens are common in many sera and are clinically insignificant. High-titer antibodies with broad thermal amplitudes and hemolysis are associated with primary and secondary cold autoimmune hemolytic anemias.

Phenotypes



At birth, cord blood cells are I⁻, i⁺. These red cells convert to an adult I⁺ phenotype in early childhood.

Two major red cell phenotypes are recognized in the I system: I-i⁺ and I⁺. At birth, cord red cells express a primitive I-i⁺ phenotype but gradually convert to an adult I⁺ phenotype in early childhood. An increase in i antigen can also be observed in certain chronic hemolytic states—such as paroxysmal nocturnal hemoglobinuria and thalassemia—as a marker of stressed erythropoiesis. It has been hypothesized that increased i expression reflects early release of immature red cells. Strong i expression is also a feature of hereditary erythroblastic multinuclearity with positive acidified serum (HEMPAS). In HEMPAS disease, increased i is the direct result of enzyme defects in N-glycosylation.

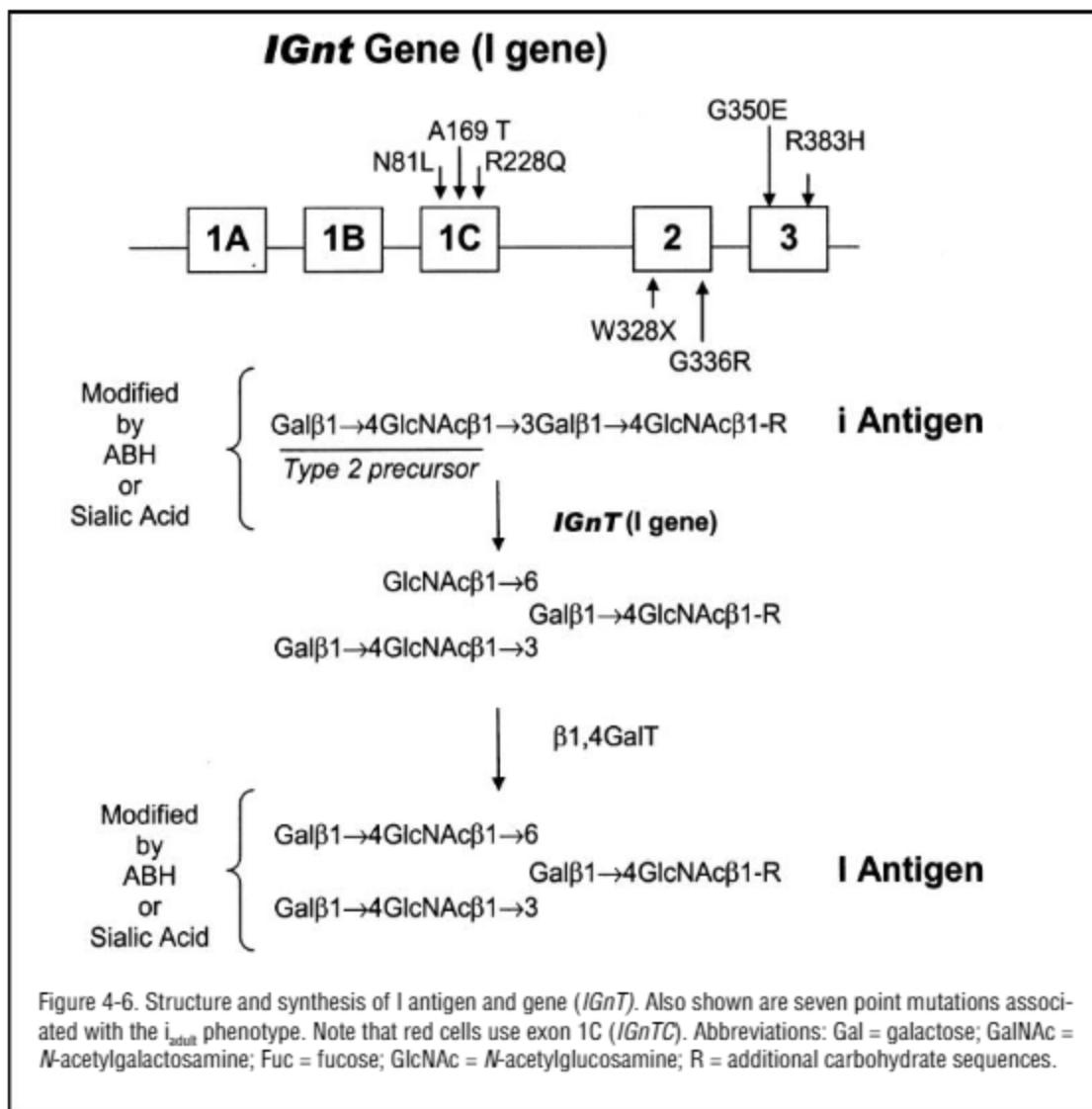
The I blood group system also contains a rare, autosomal-recessive, null phenotype (0.01-0.03%), characterized by an I-i⁺ phenotype in adults (*i_{adult}*). Two variants exist, *i_{adult}* with cataracts and *i_{adult}* without cataracts. The *i_{adult}* with cataracts is more frequently observed among Asians, whereas I-i⁺ without cataracts is common among Whites. Both variants reflect mutations in the I gene (*IGnT*).

Biochemistry and Synthesis

The i antigen is converted into the I antigen by the action of the I gene glycosyltransferase, $\beta 1 \rightarrow 6$ N-acetylgalactosaminyltransferase. That enzyme adds an N-acetylgalactosamine, in a $\beta 1 \rightarrow 6$ linkage, to an internally placed galactose (Fig 4-6). That action results in a branched structure that can be further modified and elongated to form large multivalent polylactosamines. On red cells, those oligosaccharide structures can reach 20 to 40 carbohydrates in size, with multiple branching sites. The structures can be further modified to express ABH and sialic acid. It is believed that the progressive increase in ABH expression during early childhood is the consequence of increased I antigen synthesis.

Molecular Biology

The I gene encodes an $\alpha 1,6$ glucosaminyltransferase and is located on chromosome 6p34. The gene contains five exons: three tissue-specific exons 1 (1A, 1B, 1C), exon 2,



and exon 3. Human red cells use exons 1C, 2, and 3 (*IGnTC* spliceform), whereas the human lens uses exons 1B, 2, and 3 (*IGnTB* spliceform). The i_{adult} red cell phenotype without cataracts usually involves single-point mutations in exon 1C. As a result, the I antigen is missing on red cells but is present in the lens and other tissues that use a different exon 1. In contrast, the i_{adult} phenotype with cataracts is linked to gene deletion or mutations in exons 2 and 3, which are shared by all I gene mRNA variants (*IGnTA*, *IGnTB*, *IGnTC*). The absence of I in the human lens is hypothesized to be responsible for the formation of cataracts.

I and i Antibodies



Auto-anti-I is associated with mycoplasma pneumonia and with cold autoimmune hemolytic anemia seen with lymphoid malignancy. Auto-anti-i is associated with infectious mononucleosis and with alcoholic cirrhosis.

Antibodies to i and I antigens are usually IgM and naturally occurring. Many sera contain a low-titer, cold-reactive auto-anti-I. Those autoantibodies are clinically benign and present few problems during laboratory testing. In contrast, hemolysis can be observed with high-titer autoantibodies with a broad thermal amplitude reactive above 30 to 32 C. Auto-anti-I is a common specificity in patients with cold autoimmune hemolytic anemia resulting from lymphoid malignancies or infection (mycoplasma pneumonia). Auto-anti-i is less common and is associated with infectious mononucleosis and alcoholic cirrhosis. An allo-anti-I is present in patients with the i_{adult} phenotype. Because i and I are structurally similar, anti-i and anti-I can exhibit considerable cross-reactivity in laboratory testing. In patients with high-titered antibodies, it may be necessary to titrate the antibody at several temperatures to discern the specificity of the antibody.

Finally, i and I structures are type 2 chain precursor molecules that can be modified to express other blood group determinants. Antibodies with "compound specificity" (anti-IA, anti-IBH, anti-IP₁, and anti-ILe^{B+}) can occur, and they preferentially bind with red cells of specific ABO, P₁, or Lewis types. Anti-HI is the compound specificity most frequently encountered, particularly in A₁ and AB individuals who express little H antigen. Anti-HI can be distinguished from anti-H and anti-I on the basis of its reactivity with group O, A₂, and A₁ red cells (see Table 4-7).

Table 4-7. Reactivity of Common Cold Antibodies

Antibody	Phenotype of Test Red Cells				
	O Adult	O Cord	O _b	A ₂	A ₁
Anti-I	+++	0	+++	+++	+++
Anti-i	0/+ ^w	+++	-/+ ^w	0/+ ^w	-/+ ^w
Anti-HI	+++	0	0	++	-/+ ^w
Anti-H	+++	++	0	++	-/+ ^w
Anti-A ₁	0	0	0	0	+++

P Blood Group and GLOB Collection

Summary Overview

The P blood group contains a single antigen, P_1 , which is red cell specific. The P , P^k , and Luke (LKE) antigens, which were considered part of the P system for years, are currently assigned to the GLOB collection. Unlike the P_1 antigen, P and P^k antigens are broadly distributed on a variety of blood cells (red cells, platelets, lymphocytes, monocytes) as well as on endothelial and epithelial tissues. P , P^k , LKE, and related globo-antigens play an important role in development, malignancy, cell signaling, and infectious disease, where they serve as receptors for several pathogens. Paroxysmal cold hemoglobinuria is associated with auto-anti-P.



P^k is the precursor of P and is part of the GLOB collection.

Phenotypes

The two phenotypes of the P_1 antigen are P_1 (P_1+) and P_2 (P_1-). Those two phenotypes account for nearly all donors (>99%). P_1 is the predominant phenotype in all populations, with some individuals having unusually strong expression. P_1 can be lost from red cells during prolonged in-vitro storage.

P^k and P antigens are high-incidence glycolipid antigens on all red cells, regardless of P_1 phenotype (Table 4-8). Three rare, autosomal-recessive, null phenotypes have been described (p , P_1^k , P_2^k) as well as two weak variants (P^k and P variants). Analogous to the ABO system, the rare p and P^k null phenotypes are associated with the presence of



P_1 is *not* part of the synthesis pathway for P^k and P . The same type 2 chain that forms the precursor for type 2 H (and, thus, for A and B antigens) is also the precursor for P_1 .



Paroxysmal cold hemoglobinuria is associated with auto-anti-P.

Table 4-8. Phenotype and Antibodies of the P Blood Group and GLOB Collection

Phenotype	Red Cell Antigens	Possible Antibodies	Frequency (%)		Comments
			White	Black	
P_1	P_1 , P^k , P	None	79	94	
P_2	P^k , P	Anti- P_1 (1-8%)	21	6	
P_1^k	P_1 , P^k	Anti- P	Rare	Rare	
P_2^k	P^k	Anti- P , anti- P_1	Rare	Rare	
p	—	Anti- P_1 , P , P^k	Rare	Rare	Spontaneous abortion and resistance to B19

naturally occurring antibodies against missing antigens (anti-P₁, anti-P, anti-PP₁P^k).

Biochemistry and Synthesis

The synthesis of the P^k, P, and P₁ antigens proceeds from the stepwise addition of sugars to lactosylceramide, a ceramide dihexose (CDH) (see Figs 4-7 and 4-8). The first step is the synthesis of the P^k antigen, which is the ultimate precursor of all globo-type glycosphingolipids. To make P^k antigen, α 1,4 galactosyltransferase 1 (α 4GalT1) adds a terminal galactose, in an α 1 \rightarrow 4 linkage, to CDH. The P^k antigen can then serve as a substrate for β 1,3 N-acetylgalactosaminyltransferase 1 (β 3GalNAcT1, or in older papers, β 3GalT3), which adds a β 1 \rightarrow 3 N-acetylgalactosamine to the terminal galactose of P^k/Gb₃. In red cells and other tissues, the P antigen is further elongated to form additional, globo-family antigens such as LKE and type 4 chain ABH antigens (globo-ABH or type 4 chain ABH).

Unlike the P^k and P antigens, the P₁ antigen is not a globo-glycosphingolipid; it is a member of the neolacto-family (type 2 chain glycosphingolipids). In P₁ individuals, α 4GalT1 or P^k synthase appears to be responsible for adding an α 1 \rightarrow 4 galactose to the terminus of paragloboside to form P₁. The P₁ antigen is not expressed on red cell glycoproteins.

Molecular Biology

Several inactivating mutations have been identified in both α 4GalT1 and β 3GalNAcT1 (see Fig 4-6). The p phenotype is the consequence of mutations in α 4GalT1. In the absence of α 4GalT1 activity, there is a loss of all globo-family antigens as well as P₁ antigen synthesis. Patients in that category have a compensatory increase in type 2 chain glycolipid synthesis as evidenced by increased sialoparagloboside and paragloboside on red cells. Mutations in β 3GalNAcT1 give rise to the P^k phenotype, which is characterized by a loss of P and LKE antigens and increased P^k expression. The absence of P₁ antigen in P₂ individuals may involve mutations in the α 4GalT1 promoter. Studies of P₂ donors have shown homozygosity for an allele containing an insertion (-551C-500) and a single nucleotide substitution (T-160 > C) in the promoter region. It is hypothesized that a decrease in α 4GalT1 transcription may be the basis for the P₂ phenotype.



P^k and P are related antigens, but P₁ is not a member of the globoside group.

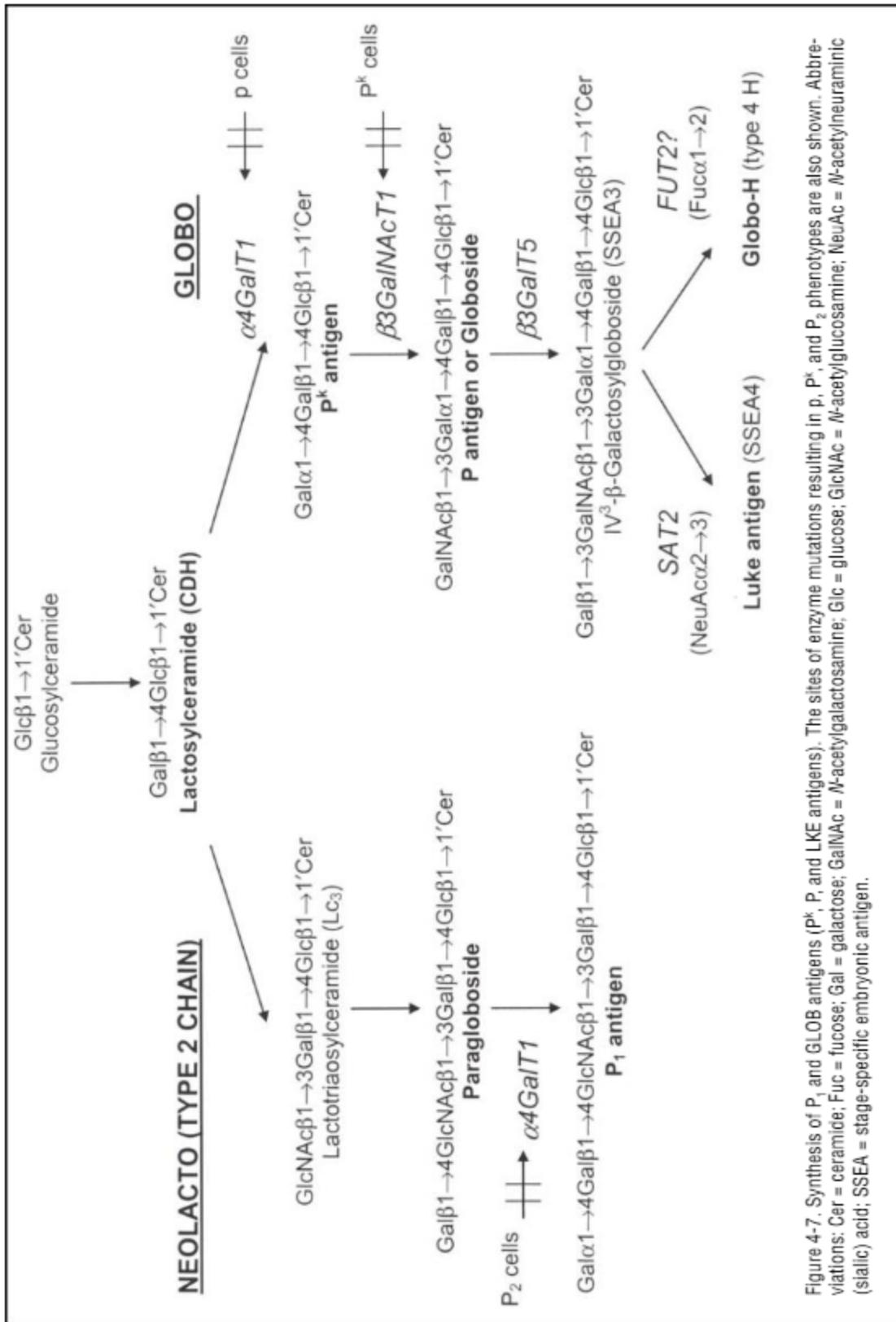
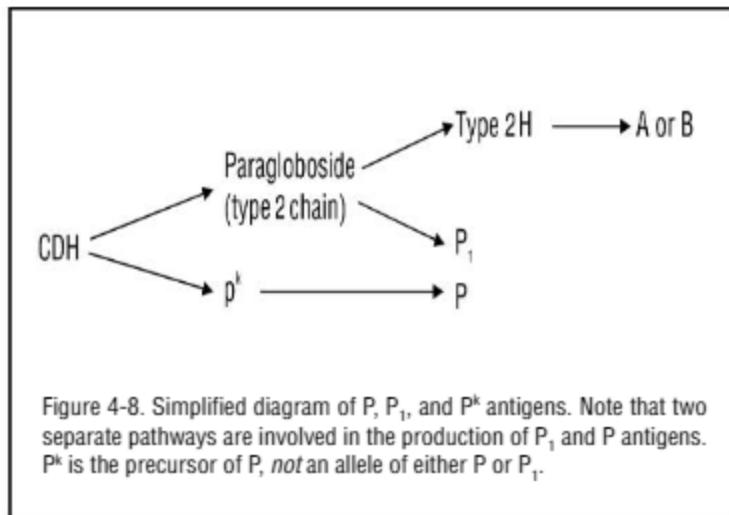


Figure 4-7. Synthesis of P_1 and GLOBO antigens (P^k , P , and LKE antigens). The sites of enzyme mutations resulting in P , P^k , and P_2 phenotypes are also shown. Abbreviations: Cer = ceramide; Fuc = fucose; Gal = galactose; Glc = glucose; GcNAc = N -acetylglucosamine; NeuAc = N -acetylneurameric (sialic) acid; SSEA = stage-specific embryonic antigen.



P Antibodies



Anti-P₁ can be neutralized by Echinococcus hydatid cyst fluid or by egg whites from pigeons and turtledoves.



Rare individuals who lack all three P antigens (P₁, P^k, and P) are said to have the p phenotype. Persons of this phenotype can produce a potent hemolytic antibody, anti-PP₁P^k (formerly known as anti-Tj^a), and, if they do, require rare-donor blood of the p phenotype for transfusion.



The Donath-Landsteiner antibody is an auto-anti-P that causes paroxysmal cold hemoglobinuria (PCH). This antibody binds to RBCs in the cold and causes complement-mediated RBC hemolysis when rewarmed to 37 C.

Anti-P₁ is a common, naturally occurring IgM antibody in the sera of P₂ donors. In general, anti-P₁ is clinically benign; it is not a cause of HDFN and is only rarely associated with decreased red cell survival or HTRs. Anti-P₁ titers can be elevated in patients with hydatid cyst disease or fascioliasis (liver fluke), as well as in bird fanciers, who are exposed to P₁-like substances present on parasites or in bird droppings. Because P₁ expression varies between red cell donors, anti-P₁ may not react with all P₁-positive cells tested. Anti-P₁ can be inhibited by hydatid cyst fluid and P₁ substance derived from pigeon eggs. Some examples of anti-P₁ have I blood group specificity (anti-IP₁).

Unlike anti-P₁, antibodies in the sera of p and P^k individuals are potent hemolysins that are capable of causing severe HTRs, HDFN, and spontaneous abortion. Anti-PP₁P^k (historically known as anti-Tj^a) is present in the sera of p individuals and is a separable mixture of anti-P, anti-P₁, and anti-P^k. An allo-anti-P is found in the sera of P₁^k and P₂^k individuals. Both p and P^k individuals require rare antigen-negative red cells for transfusion.

Finally, autoantibodies to P antigen are common in paroxysmal cold hemoglobinuria (PCH), a clinical syndrome most commonly observed in children after viral infection. In PCH, auto-anti-P is an IgG, biphasic hemolysin capable of binding red cells at colder temperatures, which is followed by intravascular hemolysis at body temperature. That characteristic can be demonstrated in vitro in the Donath-Landsteiner test.

Physiologic Role

The P^k antigen is implicated in cell signaling (via lipid rafts) and is a tumor marker in Burkitt's lymphoma (CD77). LKE or stage-specific antigen 4 is a marker of pluripotent embryonic stem cells and is a tumor marker in embryonic carcinoma and renal cell carcinoma. In renal and breast cancer, LKE expression has been linked to increased metastatic potential and immune suppression.

Several pathogens recognize P^k antigen, including shiga toxins, *Streptococcus suis*, and possibly human immunodeficiency virus. Shiga toxins are the causative agent of shigella dysentery, enterohemorrhagic *Escherichia coli*, and *E. coli*-associated hemolytic uremic syndrome. *S. suis* is a zoonotic illness capable of causing bacterial meningitis. The P antigen is a receptor for parvovirus B19, the etiologic agent of erythema infectiosum (fifth disease). In some patients, parvovirus B19 can cause a transient anemia, thrombocytopenia, and, occasionally, aplastic crisis. Patients particularly susceptible to the latter are patients with hemoglobinopathy (sickle cell, thalassemia), with underlying erythroid hyperplasia.

Finally, P₁, P^k, P, and LKE antigens can all serve as receptors for P-fimbriated uropathogenic *E. coli*, a common cause of chronic urinary tract infections, although LKE appears to be the preferred receptor on uroepithelium. There is a decreased risk of *E. coli* infections in patients of the Secretor phenotype. It is believed that Secretor/FUT2 competes with a sialyltransferase (SAT2) for galactosylgloboside (Gb₅, SSEA3), the immediate precursor for LKE synthesis (see Fig 4-7). In Secretors, there is preferential synthesis of globo-H, leading to a decrease in LKE synthesis and *E. coli* binding.



The P antigen is a receptor for parvovirus B19, which can cause transient aplastic crisis, particularly in sickle cell anemia patients.

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Appendix 4-1. Clinical or Disease Association of Blood Group Antibodies

Condition	Antibody
Cold agglutinin disease	Autoanti-I, rarely auto-i, -Pr
Paroxysmal cold hemoglobinuria	Autoanti-P
Pregnancy	Anti-Le ^b , less commonly anti-Le ^a (with loss of RBC Le antigens)
Infectious mononucleosis	Anti-i
Mycoplasma pneumonia	Anti-I
Dialysis	Anti-Nf (formaldehyde-altered RBCs)

5

Polypeptide Blood Group Systems

LAURA COOLING, MD, MS



CHAPTER 4 COVERED BLOOD GROUP ANTIGENS defined by carbohydrate epitopes. This chapter discusses the antigens and blood group systems defined by polypeptide epitopes. With one exception, polypeptide blood group antigens reside on endogenous, intrinsic red cell proteins that play a critical role in red cell maturation and physiology. Many blood groups reside on membrane transporters (Rh, Kidd, Colton, Diego, Gil) that facilitate solute and electrolyte transport across the red cell membrane. Several blood groups are associated with complement (Chido/Rodgers), complement regulation (Knops, Cromer), and adhesion molecules (Lutheran, LW, Indian, Scianna, and Ok). Unlike antibodies to carbohydrate antigens, which are usually naturally occurring, most antibodies against polypeptide blood group antigens are the result of immune stimulation.

Rh Blood Group System

Except for ABO, Rh is the most clinically significant and perhaps most complex blood group system in transfusion medicine. The most immunogenic and important antigen is the D antigen (Rh positive) present on the RhD protein.



The terms Rh positive and Rh negative refer respectively to the presence or absence of the D antigen. The D antigen is the most immunogenic of all non-ABO antigens. Anti-D can cause both clinically significant (extravascular) hemolysis of red cells and severe HDFN.

Alloantibodies against D are highly significant and are capable of causing severe hemolytic transfusion reactions (HTRs) and hemolytic disease of the fetus and newborn (HDFN). Patients lacking the D antigen are considered Rh negative and must be transfused with Rh-negative red cells to avoid Rh immunization. Additional Rh antigens reside on the RhCE protein, which is highly homologous. Like D, alloantibodies to other Rh antigens are the result of immune stimulation and are capable of causing HTRs and HDFN. For transfusion, antigen-negative, crossmatch-compatible red cells should be provided to patients alloimmunized to Rh family antigens.

Rh Phenotypes

The highly complex Rh system comprises more than 50 antigens. However, only a handful of Rh antibodies are commonly encountered in the transfusion service. To avoid immunizing Rh-negative patients, all blood donors and transfusion recipients are typed for the D antigen. Individuals positive for D antigen are Rh positive and individuals negative for D antigen are Rh negative (see Table 5-1). The C/c and E/e are two pairs of antithetical antigens found on the RhCE protein, which is a related but distinct Rh pro-

Table 5-1. Basis and Frequency of Rh+, Rh-, and Rare Rh Phenotypes

Rh D Phenotype	Whites	Blacks	Protein on Red Cells	Genotype
Rh positive	85%	92%	RhD present	<i>RHD</i> present
Rh negative	15%	8%	RhD absent	<i>RHD</i> deletion (Whites) <i>RHD</i> pseudogene (Blacks) <i>RHD</i> mutant (uncommon)
Rare Phenotypes				
Weak D	1%	—	RhD present	<i>RHD</i> missense mutant, or <i>Ce</i> or <i>CE</i> in <i>trans</i>
Partial D	Rare	Rare	RhD-CE protein	<i>RHD-RHCE</i> recombination
Rh _{null} amorph	Very rare	Very rare	RhD and RhCE absent	<i>RhD</i> deletion and <i>RHCE</i> mutation
Rh _{null} regulator	Very rare	Very rare	RhD, RhCE, and RhAG absent	<i>RHAG</i> mutation
Rh _{mod}	Very rare	Very rare	RhD, RhCE, and RhAG decreased	<i>RHAG</i> mutation

tein. Altogether, those five antigens that are present on two Rh proteins give rise to eight haplotypes (see Table 5-2).

Historically, three nomenclature systems have been used to describe the expression and inheritance of those five antigens: Rosenfield, Fisher-Race, and Wiener. The Fisher-Race and Wiener systems have somewhat complementary nomenclatures and are used interchangeably by current transfusion services (see Table 5-3). In the modified Wiener system, Rh positive, or inheritance of RhD (D) protein, is reflected by a capital "R." The absence of RhD (d or Rh negative) is reflected by a lowercase "r." The inheritance of C or E antigens is denoted by superscripts (numbers for Rh positive and primes for Rh negative). For example, the inheritance of C antigen is R^1 or r' and E is R^2 or r'' . Coinheritance of C and E is denoted by "z" or "y." Table 5-3 places the Wiener and Fisher-Race systems side by side, thereby revealing the similarities.

Because of their proximity on chromosome 1, the *RhD* and *RhCE* genes show genetic linkage. As a result, some haplotypes are much more common than others, particularly in certain ethnic groups (see Table 5-2). The inheritance and distribution of specific antigens and haplotypes in the population have a direct effect on the risk for alloimmunization. The distribution can be helpful in the selection of compatible blood. For example, R^1R^1 patients have



The term "Rh negative" does not mean the absence of all Rh antigens. Instead, the absence of all antigens is termed " Rh_{null} ".



Fisher and Race proposed that three genes encode the major Rh antigens (Cc, Ee, and Dd). Wiener proposed one gene for these antigens. Ironically, neither proposal was correct; there are two genes—one encodes the CeEe antigens (*RHCE* gene) and the other encodes the D antigen (*RHD* gene).

Table 5-2. Rh Haplotype Frequencies

	Rh Haplotype		Frequency (%) of American Donors			
	Fisher-Race	Wiener	White	Black	Asian	Native
Rh positive (D)	<i>Dce</i>	R^0	4	44	3	2
	<i>DCe</i>	R^1	42	17	70	44
	<i>DcE</i>	R^2	14	11	21	34
	<i>DCE</i>	R^z	0.2	0	0	0
Rh negative	<i>ce</i>	r	37	26	3	6
	<i>Ce</i>	r'	2	2	2	2
	<i>cE</i>	r''	1	0	0	6
	<i>CE</i>	r^y	0	0	0	0

Table 5-3. Comparison of Wiener and Fisher-Race Nomenclature for Rh Haplotypes

Wiener	Fisher-Race			Wiener	Fisher-Race			
Rh+	1	2	3	Rh-	1	2	3	Comments
R ⁰	D	c	e	r	d*	c	e	No C or E present
R ¹	D	C	e	r'	d*	C	e	Only C present (1 or ')
R ²	D	c	E	r''	d*	c	E	Only E present (2 or '')
R ^z	D	C	E	r ^y	d*	C	E	Both C and E present (Z or y)

*In this nomenclature, "d" denotes absence of the D gene. Today, the absence of D is indicated by omission of the letter D.



Anti-c and anti-E are often found together.



Patients with anti-c or anti-e should not receive Rh-negative red cells, because these are highly likely to be ce/ce.

a 30% to 50% risk of developing both anti-E and anti-c after exposure to E-positive blood. That risk is attributable to the increased frequency of the c antigen (98%) among E-positive donors (30% of donors). Because of the increased risk of developing anti-c and anti-E in those individuals, many transfusion services provide R¹R¹-matched red cells to R¹R¹ patients. Similarly, one would never transfuse Rh-negative blood to a patient with either anti-c, anti-e, or anti-f, which is an antibody with anti-c+e activity. As shown in Table 5-2, nearly all Rh-negative donors (98%) are genetically rr and therefore positive for c, e, and f antigens.

Racial differences in Rh haplotype frequency have the most significant implications for alloimmunization and transfusion support, however. For example, R⁰ (Dce) is the common haplotype in Blacks, whereas R¹ (DCe) is the predominant haplotype in Whites—and Whites constitute the majority of blood donors in most localities (see Table 5-2). R⁰R⁰ (Dce/Dce) and R⁰r (Dce/ce) Black transfusion recipients are therefore at significant risk for developing antibodies to either C or E antigens from a random Rh-positive red cell transfusion. For that reason, many centers that treat large numbers of patients with sickle cell anemia automatically type their patients for D, C, c, E, and e antigens to determine their probable Rh haplotype. Those centers will provide Rh-matched red cells for all their patients with hemoglobinopathy as part of a "hypertransfusion protocol" designed to prevent or delay Rh alloimmunization.

Biochemistry and Molecular Biology

Three related proteins constitute the Rh system: RhD, RhCE, and RhAG. RhD and RhCE are 30-kDa, 416-amino-acid-integral membrane, or “multipass,” proteins containing 12 transmembrane domains. RhAG is an evolutionarily and structurally related 45- to 70-kDa glycoprotein that is critical to the expression and assembly of Rh proteins on red cells. Those three proteins are physically associated in the red cell membrane as part of an Rh complex comprising two molecules of Rh (RhD and RhCE) and two molecules of RhAG. Several proteins are topologically associated with the Rh complex in the red cell membrane, including proteins with blood group activity (LW, Duffy, and glycoporphin B).

The genes for RhD and RhCE are on chromosome 1p34 and are separated by only 30 kb. RhAG resides on chromosome 6p11. It is believed that *RHAG* and *RH* genes arose from gene duplication nearly 300 million years ago. Approximately 10 million years ago, a second gene duplication gave rise to *RHD* and *ce* alleles (R^0 or *Dce* haplotype). The remaining haplotypes are the result of point mutations, recombination, and gene conversions.

Rh Positivity

Rh-positive individuals express the RhD protein and have inherited at least one copy of the *RHD* gene (see Table 5-1). Unlike the C/c and E/e antigens, the D antigen recognized by the immune system is complex, involving several spatially separated amino acids along the RhD protein. Computer modeling, combined with epitope mapping, suggests that three extracellular loops (3, 4, and 6) contribute to the tertiary structure of the D epitope.

Rh Negativity

In the United States, approximately 15% of Whites and 8% of Blacks type as Rh negative. The Rh-negative phenotype in Whites appears to have originated in the Basque region of eastern Spain, which has the highest incidence of Rh-negative donors in the world (20-25%). In Rh-negative individuals, there is a complete absence of RhD protein on red cells. As a result, Rh-negative individuals can develop a potent anti-D if transfused with Rh-positive red cells.

Rh negativity is an autosomal-recessive phenotype arising from a variety of genetic backgrounds (see Table 5-1). In Whites, Rh negativity is usually the result of gene dele-



There is speculation that the Rh-negative phenotype may have occurred first among the Basque people of Spain but was rare elsewhere. This might explain why the Rh-negative phenotype is more frequent among individuals of European ancestry than among other racial or ethnic groups.

tion. In Blacks, Rh negativity can arise from gene deletion or inheritance of an Rh pseudogene (*RHD Ψ*), which contains a 37-bp gene insertion. It is estimated that 60% of Rh-negative Blacks carry the *RHD Ψ* pseudogene. That racial difference must be remembered when molecular genotyping of prenatal samples is performed. Finally, rare *RHD* genes with nonsense mutations and nucleotide deletions have been reported.

Weak D and Partial D

Approximately 1% of Rh-positive individuals are considered weak D. Those individuals have reduced expression of D antigen on red cells. Historically, those individuals initially typed as Rh negative or very weak Rh positive with human polyclonal anti-D reagents in routine testing (see Chapter 3: Pretransfusion Compatibility Testing for a discussion of Rh typing). With antihuman globulin enhancement through an indirect antiglobulin test (IAT), those individuals would subsequently type as D positive. Current anti-D reagents are potent monoclonal and polyclonal-monoclonal blends that are capable of agglutinating most weak D samples on direct testing.

Several distinct backgrounds can give rise to a weak D phenotype (see Table 5-1). The most common is autosomal-recessive inheritance of two weak *RhD* alleles, which possess missense mutations in the transmembrane or the cytoplasmic domain (or both). Those mutations are believed to interfere with protein insertion or retention in the cell membrane. A second background is Ce in *trans*, in which C antigen is on one chromosome and D antigen is on the other chromosome. The presence of C on the opposite chromosome appears to suppress D expression, although the mechanism is unclear. D expression is also depressed in the *Rh_{mod}* phenotype because of mutations in the RhAG protein.

More important, weak D expression can reflect inheritance of partial D, a recombinant RhD-RhCE protein that is missing parts of the normal D protein. Partial D individuals can make an allo-anti-D to high-incidence RhD antigens that are missing on their own red cells. Partial D individuals also express novel low-incidence antigens that are generated by recombination events. In the blood bank, partial D individuals are often identified by an apparent "Rh discrepancy," which means an Rh-positive individual has made an allo-anti-D reactive with Rh-positive red cells but not with autologous red cells. There are reports that some



Weak D refers to red cells with reduced expression of the D antigen. In the partial D phenotype, however, some epitopes of the D antigen are missing. Thus, partial D individuals, although technically considered Rh positive, may nonetheless produce alloanti-D.

partial D phenotypes can be identified by using a panel of anti-D typing reagents, which recognize different epitopes on the RhD protein. Recent improvements in gene chip technology may permit rapid identification of partial D donors and recipients.

It is important to understand the consequences of weak D for blood collection and transfusion services. If one is to avoid missing a possible weak D individual, all Rh-negative blood donors are tested for weak D expression by using an enhancement method (in this case, antihuman globulin). Blood *donors* who are positive for D antigen, regardless of the strength of antigen expression, are considered Rh positive. That designation is to prevent transfusion of D-positive red cells to a true Rh-negative recipient. In contrast, female *recipients* who type as weak D are increasingly treated as Rh negative for transfusion purposes. That designation is to prevent possible anti-D alloimmunization in partial D individuals.



Blood donors who test D negative must be tested for weak D expression to be sure they are truly Rh negative, to prevent inadvertent transfusion of Rh-positive (weak D-positive) red cells to an Rh-negative recipient.

C/c, E/e, and Compound Antigens

The C/c and E/e antigens are the result of single amino acid polymorphisms on the RhCE protein. The C/c antigen resides at amino acid 103 on the second extracellular loop, where C is Ser103 and c is Pro103. The E/e antigen resides at amino acid 226 on the fourth extracellular loop, where E is Pro226 and e is Ala226. Several additional high- and low-incidence antigens (C^x , Mar, G, E^W , hr^s , V, Hr, VS, Hr^B , RH26, RH35) are also attributable to amino acid substitutions in the RhCE protein.

Because C/c and E/e antigens reside on the same protein, they can stimulate alloantibodies that require the presence of both C/c and E/e antigens on the same protein for antibody recognition. Four "compound antigen" specificities are recognized: ce, or f (RH6); Ce (RH7); cE (RH27); and CE (RH22). As shown in Table 5-4, those antigens are associated with specific Rh haplotypes. Anti-f is the most common compound alloantibody encountered clinically, particularly among R^1R^2 individuals (DCe/DcE).

G Antigen

G is an Rh antigen present on D-positive cells and C-positive cells. The G antigen epitope is a C-active antigen (Ser103) shared by RhD and the C antigen on the RhCE protein. After transfusion of C-positive blood, Rh-negative patients can make an anti-G that reacts like a mixture of anti-C and anti-D in laboratory testing. In fact, many ex-

Table 5-4. Compound Rh Antigens

Compound Antigen	Name	Rh Haplotypes		Frequency (%) in Whites
		Fisher-Race	Wiener	
ce	f or RH6	ce + Dce	r + R ⁰	41
Ce	RH7	Ce + DCe	r' + R ¹	44
cE	RH26	cE + DcE	r'' + R ²	15
CE	RH22	CE + DCE	r ^y + R ^z	0.2

amples of anti-G are accompanied by anti-C. Like other Rh antibodies, anti-G is clinically significant because it is capable of causing HTRs and HDFN. For transfusion purposes, it is not necessary to discern whether a patient has an anti-C with anti-D, or an anti-G. In both instances, the patient should receive Rh-negative blood (rr, or ce/ce), which lacks D, C, and G antigens.

In contrast, it is important to investigate whether either anti-G or an anti-D with anti-C is present in prenatal samples. A patient with an anti-G or an anti-G and anti-C mixture may still become immunized to D antigen. If one is to prevent sensitization to D antigen, patients with anti-G should receive Rh immunoprophylaxis. Patients with a true anti-D response, however, do not receive Rh immunoprophylaxis because they are already sensitized to D (see Chapter 11: Special Transfusion Situations). One clue to help discriminate between an anti-G vs an anti-D with anti-C is the antibody titer against D-positive and C-positive red cells. Anti-G has a stronger reaction with C-positive red cells in antibody titration studies, with anti-C titers at least four times higher than anti-D titers. That reaction is the opposite of an anti-D with anti-C, where anti-D should be significantly higher than anti-C.



Differentiating anti-G from anti-D plus anti-C is important in pregnancy because a pregnant female who has produced anti-G but not anti-D is still a candidate for Rh Immune Globulin prophylaxis.

Rh_{null} Phenotype

Rh_{null} is a very rare (<1:6,000,000) autosomal-recessive phenotype characterized by the complete absence of RhD and RhCE proteins on red cells. The absence of Rh proteins is accompanied by an absence or a decrease in other antigens (Fy⁵, LW, S/s/U). Rh_{null} red cells are abnormal; they possess a stomatocyte red cell morphology, phospho-

lipid asymmetry, increased osmotic fragility, and shortened red cell survival. As a result, Rh_{null} individuals have a chronic, mild hemolytic anemia (Rh-null syndrome). Rh_{null} individuals can make alloantibodies to several high-incidence antigens on RhD and RhCE proteins, which are reactive with all red cells except Rh_{null}.

The Rh_{null} phenotype arises from two genetic backgrounds—regulator or amorph (see Table 5-1). The Rh_{null} amorph type occurs in Rh-negative (D-negative) individuals who have inherited a nonfunctional *RHCE* gene. In the Rh_{null} amorph phenotype, RhAG protein is still present, although decreased relative to normal red cells. In contrast, Rh_{null} regulator is the result of mutations in the *RHAG* gene, thereby leading to a loss of RhD, RhCE, and RhAG proteins on red cells. Rh_{mod} is related to Rh_{null} regulator and is the result of nonlethal missense mutations in RhAG protein. Rh_{mod} red cells have markedly decreased RhD, RhCE, and RhAG proteins as well as a mild, chronic hemolytic anemia.

Rh Antibodies

Alloantibodies against Rh antigens are always clinically significant. They are capable of causing HTRs and severe HDFN. Unlike ABO antibodies, which fix complement with intravascular hemolysis, Rh antibodies clear incompatible red cells by extravascular hemolysis through the reticuloendothelial system. Antibodies against Rh antigens are almost always the result of immune stimulation by transfusion or pregnancy; exceptions are anti-E and anti-C^w, which can sometimes be naturally occurring. In laboratory testing, Rh antibodies are reactive at 37°C and in an IAT (see Chapter 3: Pretransfusion Compatibility Testing). Rh antibodies can also demonstrate dosage by reacting stronger with double dose, or homozygous, cells. Antibody reactivity is enhanced by the protease digestion of red cells, which unmasks the Rh antigens. In addition to alloantibodies, autoantibodies with apparent Rh specificity are not uncommon, particularly auto-anti-e.

Clinically, D is the most immunogenic Rh antigen, followed by c, E, C, and e. If one is to avoid sensitizing Rh-negative patients, all Rh-negative patients are provided Rh-negative red cells whenever possible. That practice is particularly important for females of childbearing potential to avoid the risk of Rh-associated HDFN. Rh-negative females are also at risk for developing anti-D during pregnancy as a result of Rh-positive fetal red cells. As a conse-



To avoid sensitizing Rh-negative patients, Rh-negative patients (particularly females of childbearing potential) are provided with Rh-negative cells whenever possible.

quence, unsensitized Rh-negative females are given Rh Immune Globulin prophylaxis during midpregnancy and immediately after delivery to prevent anti-D formation (see Chapter 11). As discussed earlier in the section on G antigen, Rh Immune Globulin is not given to females who have already formed anti-D.

Physiologic Role

RH homologues have been identified in early invertebrates, suggesting a critical physiologic role in early evolution. Current evidence suggests that Rh proteins are members of the Mep/Amt family of ammonium transporters.

Kell and Kx Blood Group Systems



Kell system antibodies are clinically significant. The Kell (K) antigen is a potent immunogen, second only to D in immunogenicity, but finding Kell-negative blood is not difficult because only 9% of donor red cells will carry K. On the other hand, the antithetical allele (k or cellano) is very high in frequency and patients with anti-k will require rare-donor, k-negative blood for transfusion.

Kell is a large single-pass glycoprotein on red cells and erythroid progenitors. It is covalently associated to a second red cell protein, XK, or to Kx antigen, which may help stabilize Kell protein. Kell antigens are highly immunogenic and are second only to RhD in their ability to stimulate alloantibodies. Antibodies against Kell antigens are immunoglobulin, gamma class (IgG), and are the result of immune stimulation. They are capable of causing HTRs and a reticulocytopenic fetal anemia resulting in marrow suppression. For transfusion, patients should receive antigen-negative, crossmatch-compatible red cells. Two important null phenotypes are known: K_0K_0 and McLeod, which arise from mutations in the *KEL* gene and *XK* gene, respectively. The McLeod phenotype can be associated with the McLeod syndrome and chronic granulomatous disease.

Kell Phenotypes

The Kell system has 25 antigens, including five sets of antithetical antigens and 14 high-incidence antigens. The most significant antigen pair is K (Kell, or K1) and k (cellano, or K2). Both are highly immunogenic. K is present in 10% of the population, whereas k is a high-incidence antigen on 99% of donor red cells. Kp^a/Kp^b and Js^a/Js^b are two additional antithetical antigen pairs included on antibody identification panels (see Table 5-5). Like k, Kp^b and Js^b are high-incidence antigens in the general population. It is important to note that Js^a is significantly more prevalent in Blacks (20%) than in Whites (<1%). Kell antigens can be targets for autoantibodies with suppression of Kell expression.

Table 5-5. Frequency of Antithetical Kell Antigens

Antithetical Kell Antigens	Phenotype	Frequency (%)		Comments
		Whites	Blacks	
K/k	K+k-	0.2	<0.1	
	K+k+	8.8	2	
	K-k+	91	98	k >99% of donors
	K-k-	Rare	Rare	K ₀ K ₀ or McLeod
Kp ^a /Kp ^b	Kp(a+b-)	<0.1	0	
	Kp(a+b+)	2	<0.01	
	Kp(a-b+)	98	>99.9	Kp ^b >99% of donors
	Kp(a-b-)	Rare	Rare	K ₀ K ₀ or McLeod
Js ^a /Js ^b	Js(a+b-)	<0.1	1.0	
	Js(a+b+)	<0.1	19	Js(a+) = 20% of Blacks
	Js(a-b+)	>99.9	80	Js ^b >99% of donors
	Js(a-b-)	Rare	Rare	K ₀ K ₀ or McLeod

The K₀ and McLeod phenotypes differ in serology and genetic background. K₀K₀ is characterized by a complete absence of Kell protein and of all Kell antigens on red cells. K₀K₀ red cells have increased expression of a Kx antigen, a Kell-associated protein (XK) that may help stabilize Kell protein in the cell membrane. K₀K₀ individuals can make a potent alloantibody (anti-Ku) reactive with all cells except rare K₀K₀ red cells.

The McLeod syndrome is an X-linked recessive phenotype that is characterized by an absence of XK protein on red cells and other tissues. McLeod red cells lack Kx antigen and have markedly reduced expression of all Kell antigens. McLeod red cells also display abnormal red cell morphology, which is sometimes referred to as "neuromuscular acanthocytosis" by neurology services. Clinically, the McLeod syndrome is associated with several rare genetic disorders, including muscular dystrophy, chronic granulomatous disease, and retinitis pigmentosa. McLeod individuals make alloantibodies against Kell and XK pro-



The McLeod syndrome is characterized by the absence of the Kx antigen on red cells and granulocytes, accompanied by significantly reduced Kell system antigen expression on red cells. The McLeod syndrome is associated with chronic granulomatous disease, muscular dystrophy, and retinitis pigmentosa.

teins and are incompatible with normal and K_0K_0 cells (which have enhanced expression of Kx antigen).

Biochemistry

Kell is a large, 732 amino acid glycoprotein on red cells and on early erythroid and megakaryocyte progenitors. The protein possesses 15 cysteine residues and multiple disulfide bonds, which lead to a highly folded protein that is exquisitely sensitive to sulfhydryl reagents such as dithiothreitol (DTT), 2-mercaptoethanol (2-ME), and ZZAP (2-ME and papain). Treatment of red cells with those reagents completely destroys Kell antigen, resulting in a red cell that types as K_0K_0 , or Kell null. That result can be useful in antibody identification, particularly when the transfusion service is dealing with an alloantibody against a high-incidence red cell antigen. A possible Kell specificity must be considered if an antibody fails to react with DTT- or 2-ME-treated red cells.

The Kell protein is covalently linked through a disulfide bond to the XK protein. The latter is a nonpolar multipass protein (10 transmembrane domains) that may help stabilize Kell protein in the red cell membrane. Unlike Kell, XK is not a red-cell-specific protein; XK is also found on the skeletal muscle, heart, brain, liver, and pancreas. The XK protein is resistant to sulfhydryl reagents.

Molecular Biology

The Kell gene (*KEL*) resides on chromosome 7q33 near the cystic fibrosis gene. With the exception of null phenotypes, all Kell antigens identified to date are the consequence of amino acid polymorphisms in the translated protein. The K and k antigens reside at amino acid 193, where K (K1) is Met193 and where k (cellano, or K2) is Thr193. The K, or K1, phenotype results in the loss of an *N*-glycosylation site. Three amino acid polymorphisms at amino acid 281 are responsible for Kp^a (Trp281; K3), Kp^b (Arg281; K4), and Kp^c (Gln281; K21) antigens. The Js^a/Js^b antigens are located at amino acid 597, where Js^a is Pro597 and Js^b is Leu597.

The K_0K_0 phenotype is autosomal recessive because of the homozygous inheritance of an amorph *KEL* gene. Multiple mutations have been identified to date, including nonsense and splice-site mutations. Missense mutations capable of depressing Kell expression have been identified in K_{mod} individuals.



Kell antigens are completely destroyed by treatment with DTT, resulting in a red cell that types as Kell null.

The *XK* gene is located on chromosome Xp21, close to cytochrome b and the gene for Huntington's disease. Several different mutations have been identified in the *XK* gene in McLeod individuals. The absence of *XK* on neuromuscular tissues and the brain is believed to be responsible for the clinical manifestations of McLeod syndrome. Those patients typically present with areflexia, muscular dystrophy, and choreiform movements late in life. Chromosomal deletions leading to loss of *XK* and cytochrome b occur in 7% of patients with X-linked chronic granulomatous disease.

Kell Antibodies

Alloantibodies against K1 and other Kell antigens are capable of causing immediate and delayed HTRs, making them clinically significant. The antibodies are usually of IgG isotype and are the result of immune stimulation by transfusion or pregnancy. Kell antibodies can cause severe HDFN. Unlike Rh-associated HDFN, Kell-associated HDFN is not the result of hemolysis. Kell antibodies have been shown to suppress erythroid precursors, leading to a reticulocytopenic anemia. There are also reports of neonatal thrombocytopenia caused by Kell antibodies. Autoantibodies with Kell-like specificity can occur in autoimmune hemolytic anemia, accompanied by transient suppression of Kell antigens. Because of antigen suppression, those autoantibodies can initially appear to be alloantibodies.



Kell antibodies can cause acute and delayed HTRs, as well as severe HDFN.



Anti-K is one of the most common and important causes of HDFN. The severity of HDFN associated with Kell antibodies is not the result of hemolysis, but rather is caused by suppression of erythroid precursors.

Physiologic Role

Kell belongs to the M13 family of zinc endopeptidases, which are associated with proteolytic cleavage of bioactive peptides. Kell is closest in homology to neutral endopeptidase 24.11, neprilysin, and two endothelin-converting enzymes. Kell appears to be a third endothelin-converting enzyme, with strong activity against endothelin-3. Kell may also play a role in red cell differentiation and maturation. Kell is phosphorylated in vivo, a common feature of cell-signaling molecules. In addition, Kell antibodies are able to suppress erythropoiesis in vivo and in vitro.

MNS Blood Group System

The M and N antigens reside on glycophorin A and the S, s, and U antigens are on glycophorin B. The M and N antigens are short, glycosylated polypeptide epitopes located on the extreme *N*-terminus of glycophorin A. S and s are



To remember which glycophorin bears the M and N antigens and which the S, s, and U antigens, use the mnemonic: BUS MAN. S, s, and U are present on glycophorin B, whereas M and N are present on glycophorin A.

antithetical antigens caused by a single amino acid polymorphism on glycophorin B. In general, antibodies against M and N are clinically insignificant, naturally occurring immunoglobulin M (IgM) antibodies. In contrast, antibodies to S, s, and U, on glycophorin B, are of IgG isotype, arising from immune stimulation. They are also associated with HTRs and HDFN. The MNS system contains several rare null phenotypes and low-incidence antigens resulting from recombination among glycophorins A, B, and E (Miltenberger system).

MNS Phenotypes

M and N are autosomal codominant antigens on glycophorin A that give rise to three phenotypes: M+N-, M+N+, and M-N+. As shown in Table 5-6, M and N are present in the population at approximately equal frequencies, with 50% of donors positive for both antigens. An N-like antigen ('N') is also present on the N-terminus of glycophorin B. The M-N- null phenotype is extremely rare. En(a-) is an M-N- null phenotype caused by loss of glycophorin A.

Table 5-6. Frequency of Major MNSs Antigens

Antithetical Antigens	Phenotype	Frequency (%) in US Donors		Comments
		White	Black	
MN	M+N-	28	26	
	M+N+	50	44	
	M-N+	22	30	
	M-N-	0	0	En(a-), M ^k
Ss	S+s-	11	3	
	S+s+	44	28	
	S-s+	45	69	
	S-s-	0	1	U-, M ^k , Henshaw*

*It is estimated that 23% of S-s-U- persons are U^{var} as a result of the Henshaw phenotype (B-A-B).

M^kM^k is a second M–N– null phenotype resulting from loss of glycophorin A and glycophorin B. As a result, M^kM^k cells are also negative for S, s, and U antigens.

S and s are autosomal codominant antigens present on glycophorin B. In most populations, s antigen is a high-incidence antigen (89–97%) regardless of race (see Table 5-6). The S antigen, however, is twice as prevalent in Whites (55%) as in Blacks (31%). A rare S–s– and S–s–U– phenotype is observed in 1% of Blacks. U is a high-incidence polypeptide antigen on glycophorin B that is located near the cell membrane. Three different genetic backgrounds can give rise to an S–s–U– phenotype (M^kM^k , Henshaw, and S–s–U–). Those individuals can develop a potent anti-U, making transfusion support extremely difficult.

Although the MN and SsU antigens reside on different proteins, the genes encoding their proteins reside as a gene cluster on chromosome 4q28. As a consequence, inheritance of MN and inheritance of Ss are not independent but are genetically linked. The relative haplotype frequencies for Whites and Blacks are: Ns>Ms>MS>NS (see Table 5-7).

Biochemistry

Glycophorin A is the major sialomucin on red cells, with sialic acid accounting for more than 50% of its total molecular weight. As a result, glycophorin A is a major contributor to the zeta potential, or net negative charge, of red cells. The M and N antigens reside at the extreme amino



Some S–s– individuals may also be U–. A U– patient who develops anti-U will require rare donor U– red cells for transfusion.



The rare S–s– and S–s–U– phenotypes are seen in 1% of Blacks but are rare in other populations.

Table 5-7. Estimated Frequency (%) of MNSsU Haplotypes

Haplotype	Frequency (%) in US Donors	
	White	Black
MS	25	10
Ms	30	34
NS	6	7
Ns	39	38
Mu (S–s–U–)	0	4
Nu (S–s–U–)	0	7

terminal end of the molecule (amino acids 1-5) and consist of both amino acids and carbohydrates. M and N antigens differ at two amino acid positions (Ser¹>Leu and Gly⁵>Glu) where M is Ser¹-Ser²-Thr³-Thr⁴-Gly⁵ and N is Leu¹-Ser²-Thr³-Thr⁴-Glu⁵. Three O-linked, sialylated tetrasaccharides reside on the intervening Ser and Thr residues (amino acids 2-4). Several low-incidence antigens (M⁸, M^c, Cad, Tm, and M₁) are associated with amino acid substitutions and alterations in O-glycosylation in that region. Glycophorin A also possesses several high-incidence antigens near the cell membrane [eg, En(a)] and interacts with band 3 to form the Wr^b antigen.



The high-incidence U antigen is found near the cell membrane in glycophorin B.

Glycophorin B is a related 72 amino acid glycoprotein that possesses 11 O-linked glycans. An N-like epitope ('N') is present at the amino terminus of the molecule and accounts for the rarity of anti-N. The S and s antigens are a single amino acid polymorphism, where s is Thr29 and S is Met29. The high-incidence antigen U is believed to be a polypeptide antigen stretching from amino acid 33-39 near the cell membrane.

Molecular Biology

The genes for glycophorin A (*GYPA*) and B (*GYPB*) are located on chromosome 4q28 as part of a 330-kb gene cluster that includes glycophorin E. Glycophorin B and E arose from glycophorin A through gene duplication and recombination. More than 20 hybrid glycophorin proteins have been described as a result of gene deletion and recombination among *GYPA*, *GYPB*, and *GYPE* genes. The M^kM^k null phenotype is the result of deletion of *GYPA* and *GYPB*, whereas En(a-) arises from recombination and deletion of *GYPA*. The Henshaw phenotype is a gene conversion mutant in which a portion of *GYPB* is replaced by *GYPA*, leading to a B-A-B glycophorin hybrid. It is estimated that 90% of Henshaw cells are S-s-U- or S-s-U^{var} and may account for almost 25% of all U- red cells. All of the antigens in the Miltenberger system are quite rare and are the result of recombination and gene conversion events.

MN Antibodies

Anti-M and anti-N are usually of IgM or IgM with IgG isotype and are naturally occurring. Anti-M is a relatively common alloantibody specificity. In contrast, anti-N is rare

because of the presence of 'N', an N-like antigen present on glycophorin B. When present, anti-N is usually an autoantibody. An anti-N_r was an antibody encountered in patients undergoing hemodialysis. Formaldehyde used to sterilize the dialysis machines was thought to modify red cells, leading to the neoantigen, N_r.

In general, anti-M and anti-N are clinically insignificant antibodies and are rarely a cause of either HTRs or HDFN. The one notable exception is rare M+N-, S-s-U- individuals who lack a normal glycophorin B. Those individuals can make a potent allo-anti-N that is capable of causing both HTRs and HDFN. For most patients, red cells that are crossmatch compatible in an IAT at 37 C are sufficient for transfusion.

SsU Antibodies

Antibodies against S, s, and U antigens are clinically significant IgG antibodies and are the result of immune stimulation. They are a cause of HTRs and, occasionally, of HDFN. Antibodies against S/s antigens can display dosage, thus reacting stronger with double dose, or homozygous, red cells. For transfusion, antigen-negative, crossmatch-compatible red cells should be provided.



Anti-S, anti-s, and anti-U are all clinically significant—capable of causing HTRs and occasionally HDFN.

Physiologic Role

Glycophorin A and glycophorin B can serve as receptors for *Plasmodium falciparum*. There are no clinical syndromes associated with the absence of glycophorin A and glycophorin B.

Kidd Blood Group System

The Kidd blood group system consists of two codominant antigens (Jk^a, Jk^b) on SLC14A1, a transmembrane protein that acts as a urea transporter. The Jk_{null} phenotype is very rare and is associated with an increased osmotic resistance to 2M urea, a lytic agent used in some automated hematology analyzers. Anti-Jk^a and anti-Jk^b are clinically significant, complement-fixing IgG antibodies that are capable of causing intravascular and extravascular hemolysis. They are the most common alloantibodies associated with delayed HTRs. For transfusion, antigen-negative, crossmatch-compatible red cells should be provided.



Anti-Jk^a and anti-Jk^b are clinically significant—capable of causing both intravascular and extravascular hemolysis.



The Jk(a–b–) phenotype is rare but, when present, it is most commonly seen in individuals of Polynesian or Finnish origin. Jk(a–b–) red cells are relatively resistant to lysis by 2M urea; this characteristic can be used as a screening test in populations in which this phenotype is prevalent.

Kidd Phenotypes

Three predominant serologic phenotypes are in the Kidd system. As shown in Table 5-8, nearly all individuals are positive for Jk^a or Jk^b, or for both. The Jk(a–b–), or null (Jk_{null}), phenotype is exceedingly rare in all populations except Polynesians and Finns. The Jk_{null} phenotype may be autosomal recessive or autosomal dominant [In(Jk)]. The latter is characterized by very weak Kidd antigen expression. Both Jk null and In(Jk) red cells show increased osmotic resistance to 2M urea.

Kidd Biochemistry and Genetics

Kidd is a urea transporter present on red cells and vasa recta cells of the kidney medulla. Like many solute transporters, the Kidd glycoprotein is a multipass transmembrane protein containing 10 transmembrane domains. The Jk^a/Jk^b antigens reside on the fourth extracellular loop at amino acid 280.

The *JK*, or *SLC14A*, gene is located on chromosome 18q12.21 and consists of 11 exons. A single base pair transition (G823A) is responsible for the Jk^a (Asp280) and Jk^b (Asn280) antigens. The autosomal-recessive Jk_{null} phenotype is the result of inactivating mutations (splice-site, nonsense, and missense), leading to the complete absence of

Table 5-8. Frequency of Kidd Phenotype

Phenotype	Frequency (%)		Comments
	White	Black	
Jk(a+b–)	28	57	
Jk(a+b+)	49	34	
Jk(a–b+)	23	9	
Jk(a–b–)	Rare	Rare	Jk _{null} – Autosomal-recessive – red-cell-resistant 2M urea – Polynesians and Finns
			In(Jk) – Autosomal-dominant

Jk protein on cell membranes. The molecular basis for the autosomal-dominant In(Jk) phenotype is unknown. It is hypothesized that the In(Jk) phenotype is the consequence of a suppressor gene at a distant unrelated locus.

Kidd Antibodies

Kidd antibodies are complement-fixing IgG antibodies that are a cause of intravascular and extravascular hemolysis. In addition, Kidd antibodies rapidly decline in the absence of immune stimulation, and they can be absent in later blood samples. If the transfusion service does not know that the patient has a Jk antibody history, the patient can accidentally receive Jk-positive, “crossmatch-compatible” units with devastating consequences. After transfusion of an antigen-positive unit, the patient will mount a brisk anamnestic response that can lead to severe delayed HTRs. Not surprisingly, Jk antibodies are the most common specificity (75%) associated with clinically significant delayed HTRs—thus, the old adage “Kidd kills.” For transfusion, patients must receive antigen-negative, crossmatch-compatible red cells.



Kidd antibodies are notorious for being evanescent, with antibody titers declining to undetectable levels after initial immunization. This explains why Kidd antibodies are among the most commonly implicated in delayed hemolytic transfusion reactions.

Kidd Physiologic Role

In the kidney, Kidd facilitates the transport of urea in the renal medulla during the concentration of urine. On red cells, Kidd may also preserve osmotic stability as the red cells pass through the kidney. Jk_{null} persons have only a minimal decrease in urine-concentrating ability. It is thought that other mechanisms may compensate for the absence of Kidd on renal vasa recta cells.

Duffy Blood Group System

Duffy antigens reside on a transmembrane glycoprotein known as Duffy-associated receptor for chemokines (DARC). The Duffy blood group system consists primarily of two codominant Duffy antigens, Fy^a and Fy^b, and two high-incidence antigens, Fy3 and Fy5. Alloantibodies against Duffy blood group antigens are considered clinically significant. Biologically, DARC, or Duffy, can serve as a receptor for malaria and a variety of chemokines. The Duffy glycoprotein also resides on other tissues, including the kidneys, where it may play a role in sickle cell nephropathy and renal transplant rejection. For transfusion, patients



The Duffy protein is a receptor for *Plasmodium vivax*; thus, Fy(a–b–) individuals are resistant to *P. vivax* malaria. This may explain why two-thirds of Blacks are Fy(a–b–).

should receive antigen-negative, crossmatch-compatible red cells.

Duffy Phenotypes



The Fy(a–b–) phenotype is rare in Whites but is the most common phenotype in Blacks.

The four major Duffy phenotypes are based on the presence or absence of the two codominant, antithetical antigens Fy^a and Fy^b (see Table 5-9). The high-incidence antigens Fy3 and Fy5 are found on all red cells except in the Fy(a–b–) phenotype. The Fy(a–b–) phenotype, although rare in Whites, is the most common phenotype in Blacks. An Fy^b-weak phenotype, known as Fy^x, is also observed among Blacks. Duffy antigens are found on several tissues besides red cells, including endothelium and epithelial cells of renal collecting ducts.

Duffy Biochemistry and Genetics

The DARC protein is a 338 amino acid glycoprotein with a long extracellular C-terminal domain that is anchored in the cell membrane by seven transmembrane domains. The extracellular domain is the home of Fy^a and Fy^b as well as the *Plasmodium vivax* binding site. Fy3 is a high-incidence epitope near the cell membrane that involves polypeptides on the third extracellular loop. The location of Fy5 is unknown, but Fy5 does require the presence of Rh proteins

Table 5-9. Frequency of Duffy Phenotypes

Phenotype	Frequency (%)		
	White	Black	Comments
Fy(a+b–)	17	9	
Fy(a+b+)	49	1	
Fy(a–b+)	34	22	
Fy(a–b–)	Rare	68	Blacks: GATA promoter mutation - absence on red cells only - <i>FY*B</i> or <i>FY*X</i> genotype - increased resistance to <i>P. falciparum</i>

for expression. Because Fy^a and Fy^b are located on the long extracellular domain, those antigens are destroyed by treating red cells with proteolytic enzymes; Fy3 and Fy5 are enzyme resistant. The chemokine binding site appears to be a pocket formed by the extracellular and transmembrane domains. Chemokines capable of binding DARC include interleukin-8, monocyte chemotactic protein-1, and RANTES.

The *DARC* gene resides on chromosome 1. The antithetical antigens Fy^a and Fy^b are the consequence of a single nucleotide polymorphism that results in either an Asp42 (Fy^a) or a Gly42 (Fy^b) in the translated protein. Two mechanisms are responsible for the Fy(a–b–) null phenotype. Among Blacks, the Fy(a–b–) phenotype is the result of a mutation in the *DARC* gene promoter region. Specifically, there is a mutation in an erythroid transcription-factor binding site (GATA-1) that causes an absence of DARC transcription in erythroid cells. It should be noted, however, that DARC, or Duffy, is still expressed in other tissues, including the kidneys.

On DNA sequencing, most Fy(a–b–) Blacks genotype as Fy(b+) because they carry at least one allele of *FYB*, and/or *FY*X*, a distinct Fy^b allele that is responsible for weak Fy^b expression. For that reason, anti-Fy^b (and anti-Fy3) is extremely rare in Blacks despite an absence of Fy^b and Fy3 on their red cells. In other ethnic populations, the Fy(a–b–) phenotype is usually the result of inactivating mutations in the *DARC* gene, thereby leading to a complete absence of Duffy glycoprotein on all tissues. As a consequence, those individuals can make alloantibodies to all Duffy antigens, including the high-incidence antigen Fy3.

Duffy Antibodies

Antibodies against Duffy antigens are clinically significant and have been associated with HTRs and, on rare occasions, with HDFN. The antibodies are generally of IgG isotype and are the result of immune stimulation. In laboratory testing, Duffy antibodies often display dosage, reacting stronger with homozygous, or double dose, cells [eg, Fy(a+b–) or Fy(a–b+)]. In general, anti-Fy^a is more common than anti-Fy^b. Antibody reactivity can be abolished by digesting red cells with proteolytic enzymes that cleave the long amino-terminal extracellular domain bearing Fy^a and Fy^b antigens. Antigen-negative, crossmatch-compatible red cells should be provided for transfusion.



Among Blacks whose red cells phenotype as Fy(a–b–), the lack of DARC protein may be confined to red cells, with normal expression of DARC, or Duffy, on other tissues including kidneys.

This explains why anti-Fy^b is rare in Fy(a–b–) Blacks—they often carry at least one allele of *FYB*, but it may be expressed only on nonerythroid cells.



Duffy antibodies are clinically significant, capable of both mediating hemolytic transfusion reactions and (rarely) causing HDFN. Duffy antigens are destroyed by proteolytic enzyme treatment.

Duffy Physiologic Role

Duffy is a receptor for *Plasmodium vivax*. Fy(a–b–) reticulocytes are resistant to *P. vivax*—a finding that likely accounts for the predominance of Fy(a–b–) among individuals of African descent. Recent clinical studies suggest that the Fy(a–b–) phenotype may be associated with increased renal proteinuria and decreased graft survival after renal transplantation.

Lutheran Blood Group System

The Lutheran blood group system resides on two glycoproteins, Lutheran (85kD) and basal cell adhesion molecule (B-CAM, 78 kD), that differ only in the size of their intracellular domain. Lutheran contains 19 antigens and three null phenotypes: autosomal-recessive, X-linked recessive, and In(Lu). Anti-Lu^a is the most common Lutheran antibody. Occasionally, Lutheran antibodies have been associated with cases of mild HTRs. Lutheran/B-CAM glycoprotein is a high-affinity receptor for laminin; it is upregulated on red cells of patients with sickle cell disease where it may contribute to circulatory stasis and vaso-occlusive crises.



Lutheran antibodies have occasionally been associated with mild HTRs.

Lutheran Phenotypes

The Lutheran blood group system is large. It has 19 antigens, including four pairs of antithetical antigens. Lu^a (LU1) and its antithetical partner, Lu^b (LU2), are the two major antigens that are encountered clinically, and they are included in standard antibody identification panels (see Table 5-10). The vast majority of donors are Lu(b+); the Lu(a+b–) and Lu(a–b–) phenotypes are quite rare.

The Lu(a–b–), or Lu_{null}, phenotype can be inherited as either an autosomal-recessive, X-linked recessive, or an autosomal-dominant [In(Lu)] phenotype. The autosomal-recessive Lu(a–b–) phenotype is a true null phenotype caused by homozygous inheritance of two amorph Lutheran genes. Individuals with that phenotype can make a potent anti-Lu^{ab} (LU3) that recognizes the Lutheran glycoprotein. In contrast, the In(Lu) and X-linked recessive Lu_{null} phenotypes are the result of distant unknown genes that are unrelated to Lutheran. It is hypothesized that those genes may suppress or downregulate Lutheran transcription. Both the In(Lu) and X-linked recessive Lu_{null} pheno-

Table 5-10. Frequency of Lutheran Phenotypes in Most Populations

Phenotype	Frequency (%)	Comments
Lu(a+b-)	0.2	
Lu(a+b+)	7.4	
Lu(a-b+)	92.4	
Lu(a-b-)	Rare	Autosomal-recessive – no Lutheran antigens Autosomal-dominant – In(Lu) phenotype – weak Lutheran expression X-linked, recessive – weak Lutheran expression

types have weak Lutheran expression and therefore do not make an anti-Lu^{ab}. The In(Lu) red cell phenotype is also associated with altered expression of other red cell antigens, including P₁, i, Indian, and CDw75. In(Lu) red cells can have increased poikilocytosis and increased hemolysis during in-vitro storage.

Lutheran Biochemistry and Molecular Biology

The Lutheran glycoprotein is a single-pass, Type 1 protein related to the immunoglobulin superfamily. It contains three immunoglobulin constant domains, two variable domains, and five N-glycosylation sites. Two isomeric forms (85 and 78 kD) of the Lutheran glycoprotein are caused by alternate splicing of the Lutheran mRNA transcript. The 85-kD isoform is the major Lutheran glycoprotein on red cells and differs from the 78 kD isoform only in the length of the cytoplasmic tail. Like Kell, the Lutheran glycoprotein contains several disulfide bonds and can be denatured by sulfhydryl reducing agents such as DTT and 2-ME. The Lutheran glycoprotein is widely distributed and can be found in the kidneys, spleen, heart, liver, pancreas, gut, lung, placenta, ovary, testis, prostate, brain, and marrow.

The Lutheran gene (*LU*) resides on chromosome 19q13.2 and contains 15 exons. Alternate splicing of exon 13 is re-



Lutheran antigens can be denatured by sulfhydryl reducing agents such as DTT and 2-ME.

sponsible for the 78-kD minor isoform. Most Lutheran antigens are the result of single amino acid polymorphisms. The Lu^a/Lu^b antigens are the result of a single nucleotide substitution, resulting in either His77 (Lu^a) or Arg77 (Lu^b).

Lutheran Antibodies

Alloantibodies to Lutheran antigens are relatively uncommon. Anti-Lu^a is the most common Lutheran alloantibody, and it can present as an IgG or an IgM, room-temperature agglutinin. Because Lutheran is heterogeneously expressed on red cells, anti-Lu^a can display mixed-field agglutination in laboratory testing, characterized by a mix of unagglutinated red cells and small red cell agglutinates. Anti-Lu^a, anti-Lu^b, and other Lutheran antibodies are inhibited by pretreatment of red cells with sulphydryl reducing agents (DTT, 2-ME, and 2-aminoethylisothiouronium bromide, or AET), which denature the Lutheran glycoprotein. In general, anti-Lu^a is clinically insignificant; it is only rarely associated with HTRs and HDFN. Because most donors are Lu(a-), it is not difficult to provide crossmatch-compatible blood for transfusion.

Anti-Lu^b is a rare IgG alloantibody that is usually identified in an IAT. There are rare reports of mild HTRs and HDFN after the transfusion of incompatible red cells. Because Lu^b is a high-incidence antigen present on 99% of donors, finding compatible blood can be a challenge. For elective procedures, the patient should be encouraged to donate autologous units. For patients with short- and long-term transfusion needs, it may be advisable to investigate family members as possible donors. For emergency transfusions, “least-incompatible” red cells can be selected.

Lutheran Physiologic Role

Lutheran and B-CAM (78-kD isoform) are high-affinity receptors for laminin. Lutheran and B-CAM are overexpressed on sickle red cells and are believed to contribute to vaso-occlusive crises by facilitating red cell adhesion to vascular subendothelium. Lutheran and B-CAM are also overexpressed in several cancers, including ovarian carcinoma and neuroblastoma.

Other Blood Group Antigens

Several additional blood group antigens and systems are not included in standard antibody identification panels.



Lu^b is a high-incidence antigen and is much more common than Lu^a. Anti-Lu^a is typically clinically benign, rarely causing mild hemolysis or mild HDFN. Anti-Lu^b is only slightly more likely to be clinically significant.

Antibodies against many of those antigens are encountered infrequently by hospital transfusion services.

Bg Antigens

Bennett Goodspeed, or Bg, antigens are residual HLA-class antigens on red cells. Although HLA Class I antigens are expressed by all nucleated cells, including early erythroid precursors, they are progressively lost with increasing erythroid maturation. As a consequence, red cells have historically been considered negative for HLA antigens on the basis of studies with human HLA antibodies. With the availability of potent monoclonal antibodies, it is now estimated that nearly 50% of donors may possess trace HLA Class I antigens on red cells. Some HLA types have higher residual red cell expression, particularly HLA-A2, -A8, -A9, -A10, -A28, -B7, -B12, -B15, and -B17. Three "Bg specificities" are recognized: Bg^a (HLA-B7), Bg^b (HLA-B17), and Bg^c (HLA-A28 and HLA-A2). There are rare reports of HLA antibodies causing shortened red cell survival and HTRs. HLA antibodies are not associated with HDFN, but there are anecdotal reports of maternal HLA antibodies causing neonatal alloimmune thrombocytopenia.



The carrier molecules for Bg (Bennett-Goodspeed) antigens are Class I HLA molecules. Antibodies to Bg antigens are clinically insignificant but their variable reactivity sometimes causes problems with antibody identification.

Cartwright Blood Group

The Cartwright blood group contains two autosomal codominant antigens: Yt^a (99.8%) and Yt^b (8.1%). The antigens reside on acetylcholinesterase, a glycophasphatidyl-inositol (GPI)-linked glycoprotein. Alloantibodies to Cartwright antigens are usually of IgG isotype, arising from immune stimulation. Although Cartwright antibodies are generally benign, examples of shortened red cell survival and delayed HTRs are known. Like other GPI-linked proteins, Cartwright antigens are absent on red cells of patients with paroxysmal nocturnal hemoglobinuria Type III because of defects in GPI synthesis.

Chido and Rodgers Blood Group

The Chido (Ch) and Rodgers (Rg) blood group system contains 10 high-incidence antigens (>90% of donors). Like Lewis, Chido and Rodgers antigens are of plasma, not red cell, origin. Chido and Rodgers antigens reside on complement C4, a product of two highly homologous genes (*C4A* and *C4B*). In general, Chido antigens are on C4B, and Rodgers antigens are on C4A. Antibodies against Chido and Rodgers antigens are not associated with HTRs or

HDFN. There are occasional reports of allergic reactions after the transfusion of platelets and plasma. Ch and Rg antibodies are inhibited by plasma.

Colton Blood Group

The Colton blood group system contains two autosomal codominant antigens: Co^a (99.7% of donors) and Co^b (0.3% of donors). The Co(a–b–) phenotype is extremely rare and has been described only in a handful of individuals. Colton antigens reside on aquaporin 1 (AQP1), a water-selective membrane channel on red cells, as well as kidney, choroid plexus, and other tissues. AQP1 is a major molecular water channel on red cells and the kidney, where it facilitates the concentration of urine. Despite the latter, few or no clinical adverse effects have been documented in Co(a–b–) individuals. Antibodies to Colton antigens are usually of IgG isotype, arising from immune stimulation. They are clinically significant and can be associated with shortened red cell survival, HTRs, and HDFN.

Cromer Blood Group

The Cromer blood group system is fairly large, containing 10 high-incidence (>99%) and four low-incidence antigens. The Cromer antigens reside on decay-accelerating factor (DAF), a widely distributed GPI-linked glycoprotein involved in complement regulation. Cromer antibodies are usually of IgG isotype, arising from immune stimulation. Historically, they were classified as high-titer, low-avidity antibodies because of their weak variable agglutination in titration experiments. Cromer antibodies can be associated with shortened red cell survival and HTRs. Cromer antibodies do not cause HDFN because of adsorption of antibody by DAF on trophoblast epithelium.



Diego blood group system antibodies can be clinically significant. However, these antibodies are rare in the United States because of the low incidence of Di^a (meaning exposure is unlikely) and the high frequency of Di^b, which means few recipients are capable of making anti-Di^b.

Diego Blood Group

The Diego blood group system consists of 21 antigens: two high-incidence (Di^b, Wr^b) and 19 low-incidence antigens. Diego antigens are expressed on a major red cell protein, band 3 or anion exchange protein 1 (AE1). AE1 is present in high-copy numbers on both red cells and renal collecting ducts, where it plays a critical role in gas transport and acid-base equilibrium, acid secretion, and bicarbonate readsorption. AE1 also helps anchor and stabilize the red cell membrane to the underlying cytoskeleton through interactions with ankyrin, protein 4.2, and protein 4.1. Mu-

tations in AE1 have been linked to abnormal red cell morphology, including spherocytosis and ovalocytosis. With the exception of Wr^b, all Diego antigens are the result of amino acid polymorphisms. The high-incidence antigen Wr^b is formed by an electrostatic interaction between band 3/AE1 and glycophorin A. No AE1 or Diego null phenotype has ever been identified, which is not surprising, given the importance of AE1 in acid-base metabolism.

Antibodies against Diego antigens can be naturally occurring or can be the result of immune stimulation. Most antibodies against Diego antigens are naturally occurring, room-temperature agglutinins. The most common are anti-Wr^a, anti-Wd^a, and anti-WARR (13-18% of donors). In contrast, antibodies against Di^a, Di^b, and Wr^b are usually of IgG isotype and immune stimulated. Wr^a, Wr^b, and Di antibodies have been associated with shortened red cell survival, HTRs, and HDFN. Di^a is normally a rare, low-incidence antigen, except in certain Asian and South American populations where the incidence can reach 50%.

Dombrock Blood Group

The Dombrock system contains five antigens: Do^a, Do^b, Gregory (Gy^a), Holly (Hy), and Joseph (Jo^a). The antigens reside on an adenosine diphosphate-ribosyltransferase, whose biologic function on red cells is unknown. Do^a and Do^b are present in 67% and 82% of White donors, respectively. Gy, Hy, and Jo^a antigens are very high-incidence antigens found on almost all donor red cells. Alloantibodies against Dombrock antigens are often clinically significant, resulting in shortened red cell survival as well as in acute and delayed HTRs. Because they often occur in highly alloimmunized individuals with multiple red cell antibodies (eg, sickle cell patients), antibodies against Dombrock antigens can be difficult to identify.

Gerbich Blood Group

The Gerbich blood group contains seven antigens and three distinct null phenotypes. Most Gerbich antigens are high-incidence antigens (Ge2, Ge3, Ge4, Ge5, Ge7, Ge8). Gerbich antigens reside on two related glycoproteins, glycophorin C and glycophorin D, which are the product of a single gene (*GYPC*). Glycophorin D differs from glycophorin C by only 22 amino acids as a result of leaky translation from an alternate methionine residue in exon 2 (Met22). The three Gerbich null phenotypes (Yus, Gerbich,

and Leach) are the result of deletion and recombination of the *GYPC* gene. Like band 3/Diego, glycophorins C and D bind several key cytoskeletal proteins (spectrin, protein 4.1, and p55). Therefore, Gerbich antigens can be markedly decreased in hereditary elliptocytosis caused by mutations or deficiencies in those cytoskeletal proteins. Elliptocytosis is also observed in the Leach phenotype, a Gerbich null phenotype characterized by the complete absence of both glycophorin C and glycophorin D.

Antibodies to Gerbich antigens may be IgM or IgG isotype and can lead to shortened red cell survival. Anti-Gerbich is rarely a cause of HTRs or HDFN. Gerbich can be a target for autoantibodies in warm autoimmune hemolytic anemia. Because they are rich in *O*-linked sialic acid, glycophorins C and D can bind influenza virus and *P. falciparum*.

Gil Blood Group

The Gil system contains a single high-incidence antigen, GIL (100% donors). It is present on aquaglyceroporin (AQP3), a membrane water channel capable of transporting urea and glycerol. Like Colton or AQP1, AQP3 is a multipass, hydrophobic protein composed of six transmembrane domains. It is expressed on red cells and on kidney, gastrointestinal, and respiratory tract tissue. Anti-GIL is a rare antibody of IgG isotype, capable of causing HTRs. There have been no reports of anti-GIL causing HDFN.

Indian Blood Group

The Indian blood group resides on CD44, a ubiquitous glycoprotein and adhesion molecule that recognizes a number of extracellular matrix proteins. Two antithetical antigens are recognized: In^a (Pro46) and In^b (Arg46). A third antigen, AnWj (>99% of donors), is also believed to be present on CD44. Nearly all donors express In^b (>99%). In^a is rare (<1%) in all populations, except some South American Indian and Arab populations, where the incidence ranges from 4% to 12%. Alloantibodies against Indian antigens are usually of IgG isotype, arising from immune stimulation. They can be clinically significant. Alloantibodies to Indian antigens can be inhibited by using plasma, which contains soluble CD44, or by treating red cells with proteases or sulphydryl reagents, which destroy the CD44 molecule. It is hypothesized that CD44 may participate in the adhesion of erythroid progenitors to stromal

fibroblasts. AnWj antigen is a potential receptor for *Haemophilus influenzae*.

JMH Blood Group

The JMH, or John Milton Hagen, system contains a single high-incidence antigen, JMH. JMH resides on a semaphorin-family, GPI-linked glycoprotein, SEMA-1. The molecule consists of a large, highly folded extracellular domain caused by the presence of 19 cysteine residues. Like Dombrock and other GPI-linked glycoproteins, JMH is missing on red cells from patients with paroxysmal nocturnal hemoglobinuria Type III. Antibodies against JMH can be naturally occurring or immune stimulated. They are usually of IgG isotype and are clinically insignificant. Antibody binding can be inhibited by pretreating red cells with proteases and sulphydryl reducing agents that destroy the JMH protein.



JMH and Knops antibodies are usually clinically insignificant.

Knops Blood Group

The Knops blood group contains 10 antigens, many of which are racially segregated. Mc^b, SI1, SI2(Vil), and SI4 antigens are observed predominantly in Blacks. A Knops weak, or Helgeson, phenotype is present in 1% of donors. The Knops antigens reside on complement receptor 1 (CR1, CD35), a 220-kD glycoprotein with a large extracellular domain. Antibodies against Knops antigens are usually clinically insignificant. When transfused, Knops-incompatible red cells have normal in-vivo survival. Historically, Knops antibodies were classified as high-titer, low-avidity antibodies.

LW Blood Group

The LW, or Landsteiner-Wiener, blood group system contains two autosomal codominant antigens, LW^a (99% of donors) and LW^b. The LW antigens reside on intracellular adhesion molecule type 4 (ICAM-4, CD242), a member of the immunoglobulin superfamily that acts as a counter receptor for β2-integrins. It is hypothesized that the LW glycoprotein may participate in adhesive interactions during early erythroid development. The LW antigens are the result of a single amino acid polymorphism, where LW^a is Gln70 and LW^b is Arg70. LW antigens are missing from Rh_{null} red cells and rare, autosomal-recessive LW(a-b-) red cells. Alloantibodies to LW antigens are usually clinically benign.

OK Blood Group

The OK system contains a single high-incidence antigen (Ok^a) that is present on CD147, a member of the immunoglobulin superfamily. It may participate in cell adhesion and wound healing. The OK(a-) phenotype is rare and has been described only in Japan. Anti- Ok^a is of IgG isotype and is associated with shortened red cell survival.

RAPH Blood Group

The RAPH system contains a single antigen, RAPH, or MER2, found on 92% of donors. MER2 is a 40-kD protein found on CD34 cells, stromal fibroblasts, and red cells. Anti-RAPH is usually of IgG isotype, arising from immune stimulation. The antibody has been shown to bind complement in some cases. There are no reports of HTRs or HDFN associated with anti-RAPH.

Scianna Blood Group

Scianna antigens reside on erythrocyte membrane-associated protein (ERMAP), a large glycoprotein belonging to the immunoglobulin superfamily. Four antigens are in the Scianna family: Sc1, Sc2, Sc3, and Rd. Alloantibodies to Scianna antigens are rare. They are not associated with HTRs, although cases of HDFN have been reported. Autoantibodies against Scianna antigens have been observed in warm autoimmune hemolytic anemia. It is hypothesized that Scianna antigens or ERMAP may participate in red cell adhesion, signaling, and immune recognition.

Xg^a Blood Group

The Xg^a system contains a single sex-linked antigen, Xg^a, encoded by a gene (*XG*) on the X chromosome. Not surprisingly, the Xg(a+) phenotype is more prevalent in females than in males. Antibodies against Xg^a are of IgG isotype, and they may be either immune stimulated or naturally occurring. They are not associated with HTRs or HDFN. Anti-Xg^a is more common in males because of gender differences in Xg^a expression.

Selection of Compatible Units

Most of the antigens discussed in this chapter are capable of stimulating alloantibodies. However, not all alloanti-

bodies are clinically significant. For transfusion purposes, a full antiglobulin crossmatch is required for all patients with a positive antibody screen, regardless of the antibody specificity (see Chapter 3: Pretransfusion Compatibility Testing). In many instances, units may require antigen typing in addition to a full crossmatch before blood can be released for transfusion. The following section discusses some of the issues surrounding blood selection in the presence of alloantibodies.

Antibody Significance and Blood Selection

The difficulty in identifying and selecting units for transfusion depends, in large part, on the clinical significance of the alloantibody (see Table 5-11). The most commonly encountered alloantibodies that are clinically significant are antibodies against Rh, Kell, Duffy, Kidd, and Ss antigens. Patients with clinically significant alloantibodies require units that are negative for the particular antigen of interest (ie, antigen-negative units). For the more common antigens, commercial antisera are available for

Table 5-11. Selection of Red Cells for Patients with Red Cell Alloantibodies

Antigen-Negative and Crossmatch-Compatible	Crossmatch-Compatible (IAT at 37 °C)	Crossmatch-Compatible or Least-Incompatible
ABO	A ₁	Gerbich
Rh	P ₁	Knops, Cost
Kell	Lewis	Chido and Rodgers
Kidd	I/i	Xg ^a
Duffy	MN	Bg
Co ^a	Lutheran	LW ^a , LW ^{ab}
Vel	Do ^a , Do ^b	Cromer
	Co ^b	Scianna
<i>Strong examples:</i>		Weak Yt ^a
Cartwright (Yt ^a)		JMH
Dombrock		Weak Gy ^a , Hy, Jo ^a
Cromer		Weak Lan
Lan		Weak Jr ^a
Jr ^a		

screening and identifying antigen-negative units. For many other antigens, no commercial antisera are available. In those instances, the blood bank may need help from the blood center to locate antigen-negative units by using unlicensed human antisera, historic donor typing, and, more recently, molecular phenotyping. Units labeled as antigen negative must be crossmatched against the patient's serum or plasma before blood is transfused.



For patients with antibodies against Lewis, M, P₁, H, I, i, and Lu^a that are reactive *only* at room temperature, it is usually not necessary to provide antigen-negative units. Crossmatch-compatible red cells suffice *unless* the antibody is reactive at 37 C or demonstrates in-vitro hemolysis.

(Note that a Bombay antibody, which has anti-H specificity, would be reactive at 37 C and cause in-vitro hemolysis.)

Some alloantibodies rarely cause in-vivo hemolysis and are considered clinically benign in most circumstances. In that category are many naturally occurring antibodies that usually react as room-temperature saline agglutinins (eg, Lewis, M, N, P₁, H, I, i, Lu^a). For the vast majority of those antibodies, crossmatch-compatible red cells are sufficient. It is usually not necessary to screen and provide antigen-negative units unless the antibody reacts at 37 C and in an antiglobulin test. In the rare instance when the antibody reacts in an antiglobulin test at 37 C or demonstrates in-vitro hemolysis, antigen-negative, crossmatch-compatible red cells may be required.

Crossmatch-compatible or least-incompatible red cells are provided for many other antibodies. Least-incompatible red cells are often the only choice for alloantibodies against high-incidence antigens such as Knops, LW^a, JMH, Cromer, and Yt^a. Antigen-negative units for high-incidence antigens can be difficult to locate. Furthermore, many of those antibodies only rarely cause HTRs. If the patient has an unusually strong antibody or if there is clinical evidence of shortened red cell survival, it may be necessary to perform specialized testing to assess the clinical significance of the antibody. Those tests are not available in all institutions, so the antibody may require testing by a licensed reference laboratory. The best test for predicting in-vivo survival requires chromium or biotin-labeled red cells and is very laborious. The monocyte-monolayer assay and chemiluminescent assay are two in-vitro assays that have proved useful in predicting the clinical significance of antibodies to high-incidence antigens. Not surprisingly, complex cases require extensive communication and coordination among the clinical service, transfusion service, and blood supplier.

Antigen Frequency and Blood Availability

The time, labor, and degree of ease required to identify and provide compatible red cells for alloimmunized pa-

tients are determined by the frequency of the antigen in the population. The frequency of "antigen-negative" donors will determine whether the hospital transfusion service will screen its own inventory to identify units or will contact the blood supplier. For instance, most transfusion services will screen their own inventories for K1-negative units because 90% of donors are K1 negative. In contrast, the blood bank will usually contact its blood supplier for help in locating units for patients with alloantibodies to high-incidence antigens (eg, anti-Kp^b, which occurs in <1% of donors) or for patients with multiple alloantibodies who require red cells that are negative for multiple red cell antigens. A rough guide to estimating antigen frequencies in the general donor population is shown in Table 5-12.

Table 5-12. Generalized Frequencies (%) of Major Blood Group Antigens in Whites

<1	2	10	25-30	50	70-80	85	98-100
Js ^a	C ^w	K	E	S	M, N	D	e
LW ^b	Kp ^a	Lu ^a	Le ^a		P ₁	G	k
In ^a		Yt ^b			C, c, f	Fy ^b	Kp ^b
Di ^a					Le ^b	s	Js ^b
Co ^b					Fy ^a	Sd ^a	U
Sc2					Jk ^a , Jk ^b		Lu ^b
					Xg ^a		LW ^a
					Do ^a , Do ^b		Yt ^a
							In ^b
							Di ^b
							Co ^a
							Sc1
							Ch/Rg
							Knops
							Vel

Courtesy Gary Stack, MD, PhD with modifications.

To determine how many units are required to find a compatible or antigen-negative unit, divide the number of units by the frequency of antigen-negative donors as shown in the following formula:

$$\text{Number of Units to Screen} = \frac{\text{Number of Units Desired for Transfusion}}{\text{Fraction of Donors Negative for the Antigen}}$$

For example, a patient with an anti-Fy^a requires a 2-unit transfusion. The frequency of Fy(a+) in White donors is 66%; therefore, 34% of donors are Fy(a-) and compatible with the patient. The approximate number of units that the blood bank must screen to find 2 Fy(a-) units is 6 units:

$$\text{Number of Units to Screen} = 2 \text{ units}/0.34 = 5.88 \text{ or } 6 \text{ units.}$$

In that instance, most hospital transfusion services would screen their own inventories to locate antigen-negative, crossmatch-compatible units.

The number of units that may have to be screened can increase dramatically in patients with more than one alloantibody. For example, a patient may have alloantibodies to Fy^a, E, and c antigens. Those are all clinically significant antibodies that require antigen-negative units. The approximate frequency of donors negative for all three antigens can be determined by multiplying the frequency of antigen-negative donors for each antigen of interest. For the patient in question, the fraction of donors or units negative for Fy^a, E, and c antigens is 0.45, or 4.76% of White donors. The calculation is:

$$\begin{aligned} \% \text{ Antigen Negative} &= \frac{[\text{Fraction Fy}(a-)] \times (\text{Fraction E-Negative}) \times}{\text{Donors}} \\ &\quad (\text{Fraction c-Negative}) \times \\ &\quad 100\% \text{ Fy}(a-), E-, c- \text{ Units} = (0.34) \times \\ &\quad (0.70) \times (0.20) = 4.76\% \text{ Donors} \end{aligned}$$

The number of units that must be screened to identify 2 units negative for Fy^a, E, and c is as follows:

$$\begin{aligned} \text{Number of Units to Screen} &= \frac{\text{Number of Units Desired (2 Units)}}{(\text{Fraction Units Negative for Antigen})} \\ &2 \text{ units}/(0.34)(0.70)(0.20) = 2/0.0476 = 42 \text{ units!} \end{aligned}$$

In that example, it may be more cost effective for the hospital to contact the blood supplier than to screen its own inventory. In addition, the clinician must be informed

of the increased time necessary to locate compatible units. Chapter 12 includes a discussion of transfusing incompatible red cells in transfusion emergencies.

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Appendix 5-1. Summary of Properties of Selected Blood Group Antigens and Antibodies

		Antigens		Antibodies					
		Antigen Name		Frequency (%)		Usual Immunoglobulin Class		Clinical Significance	
Blood Group	Antigen	Pronunciation	Whites	Blacks	IgM	IgG	HTR	HDFN	
MNS	M	M	78	70	X		Few	Few	
	N	N	72	74	X		No	Rare	
	S	Big S	55	31	Some	Some	Yes	Yes	
	s	Little s	89	97	X	Yes	Yes	Yes	
	U	U	100	99	X	Yes	Yes	Yes	
P	P1	P-one	79	94	X		Rare	No	
Rh	D	D	85	92	X	Yes	Yes	Yes	
	C	Big C	68	27	X	Yes	Yes	Yes	
	E	Big E	29	22	Rare	X	Yes	Yes	
	c	Little c	80	96	X	Yes	Yes	Yes	
	e	Little e	98	98	X	Yes	Yes	Yes	
	f	f	65	92	X	Yes	Yes	Yes	
C ^w	C-W (Willis)	C-W (Willis)	2	1	X	Yes	Rare		
G	G	G	84	92	X	Yes	Yes	Yes	

Lutheran	Lu ^a	Lutheran a	8	4	X		No
	Lu ^b	Lutheran b	99.9	99.9		X	Yes
Kell	K	Kell	9	2	Rare	X	Yes
		Cellano	99.8	100		X	Yes
	Kp ^a	K-p-a (Penny)	2	Rare		X	Yes
	Kp ^b	K-p-b	100	100		X	Yes
	(Rautenberg)						
	Js ^a	J-s-a (Sutter)	Rare	20		X	Yes
	Js ^b	J-s-b	100	99		X	Yes
	(Matthews)						
Lewis	Le ^a	Lewis a	22	23	X		Few
	Le ^b	Lewis b	72	55	X		No
Duffy	Fy ^a	Duffy a	66	10		X	Yes
	Fy ^b	Duffy b	83	23		X	Yes
Kidd	Jk ^a	J-k-a	77	91		X	Yes
	Jk ^b	J-k-b	72	43		X	Yes
Xg	Xg ^a	X-g-a	66(M) 89(F)	ND		X	No
							No

ND = no data available; HTR = hemolytic transfusion reaction; HDFN = hemolytic disease of the fetus and newborn; M = male; F = female.

Appendix 5-2. Blood Group Genes and Gene Products

Blood Group System Name	Abbrev.	No. of Antigens	Chromo-some	Gene Name	Gene Product	Property or Function
ABO	ABO	4*	9	<i>ABO</i>	N-acetylgalactosaminyl (A) and galactosyltransferases (B)	Glycocalyx
Hh	Hh	1	19	<i>FUT1</i>	Fucosyltransferase	Glycocalyx
Lewis	Le	3	19	<i>FUT3</i>	Fucosyltransferase	Glycocalyx
P	P	1	22	<i>P1</i>	Galactosyltransferase	Glycocalyx
MNS	MNS	43	4	<i>GYPA</i> <i>GYPB</i>	Glycoporphin A Glycoporphin B	Carrier of sialic acid; receptor for <i>P. falciparum</i> , bacteria, viruses
Rh	Rh	45	1	<i>RHD</i> <i>RHCE</i>	RhD protein RhCE protein	Possible transporter
Lutheran	Lu	18	19	<i>LU</i>	Lutheran glycoprotein	Adhesion; binds laminin
Kell	Kell	22	7	<i>KEL</i>	Kell glycoprotein	Endopeptidase
Duffy	Fy	6	1	<i>FY</i>	Fy glycoprotein	<i>P. vivax</i> and chemokine receptor
Kidd	Jk	3	18	<i>HUT11</i>	Urea transporter protein (JK)	Urea transporter
Diego	Di	19	17	<i>AE1 (SLC4A1)</i>	Band 3 (AE1)	Anion exchanger

Yt	Yt	2	7	ACHE	Acetylcholinesterase ?Acetylcholine clearance
Xg	Xg	2	X	XG	XG glycoprotein ?Adhesion
Scianna	Sc	3	1	SC	Scianna glycoprotein ?Adhesion
Dombrock	Do	5	12	DO	Dombrock glycoprotein ?
Colton	Co	3	7	AQP1	Channel-forming integral protein (CHIP); Aquaporin-1 Water transporter
Landsteiner-Wiener	LW	3	19	LW	Intercellular adhesion molecule-4 (ICAM-4) Adhesion; binds cell surface ligands (LFA-1, Mac-1)
Chido/Rodgers	Ch/Rg	9	6	C4A C4B	Complement component 4 (C4) Complement component
Kx	Kx	1	X	XK	Kx glycoprotein ?Neurotransmitter transporter
Gerbich	Ge	7	2	GYPC	Glycophorins C and D Carrier of SA; membrane stability
Cromer	Cr	10	1	DAF	DAF (CD55) Complement regulation; <i>E. coli</i> , virus receptor
Knops	Kn	7	1	CR1	CD35, CR1 complement receptor Complement receptor
Indian	In	2	11	CD44	CD44 Adhesion; hyaluronate receptor

*A₁, A₂, B, and AB are counted as antigens in the ABO group.
SA = sialic acid; DAF = decay-accelerating factor.

Appendix 5-3. Null Phenotypes Associated with Changes in RBC Morphology

Symbol	Phenotype	RBC Morphology	Other Clinical or Laboratory Associations
Rh _{null}	All Rh antigens missing	Stomatocytes	Chronic, often mild, hemolytic anemia
Jk _{null}	No Jk antigens	Discocytes	RBCs resistant to 2M urea Defective urine concentration
Lu _{null}	No Lu antigens	Acanthocytes	Clinically silent
McLeod	No Kx antigen Reduced Kell system antigens	Acanthocytes	Chronic granulomatous disease (X-linked only) Cardiomyopathy Progressive neuromuscular disorder with elevated creatine kinase and lactate dehydrogenase
K _o	No Kell system antigens Increased Kx	Discocytes	Clinically silent

6

Administration of Blood Components

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IDENTIFICATION OF THE PATIENT WHO IS to receive a unit of blood or a blood component is probably the most important part of the transfusion process. Errors in patient identification account for the vast majority of transfusion accidents, with most fatal hemolytic reactions resulting from the inadvertent administration of ABO-incompatible Red Blood Cells (RBCs). Identification errors can occur at one of three critical points: at the time a pretransfusion sample is drawn for compatibility testing, in the laboratory during testing, or when the unit is issued and transfused. Each of those critical points requires meticulous attention to detail in recipient identification to make sure that transfusion of the wrong unit does not occur.

The minimum requirements for proper identification of a sample submitted for pretransfusion compatibility testing, as specified in the 24th edition of the *AABB Standards for Blood Banks and Transfusion Services*, include the following: two independent identifiers, date of draw, and a means of identifying the person who drew the sample. The patient data must be taken from an armband attached to the patient, not from the chart or an addressograph plate or from a family member in the room, and the label must be affixed to the sample at the patient's bedside. Most transfusion services are inflexible in accepting improperly labeled samples, thereby enforcing a policy of discarding a mislabeled sample and requiring that a second, properly



Samples from potential blood product recipients must be labeled at the bedside, from an armband or other positive identification attached to the patient.



The label must contain:
Two independent identifiers
Date of draw
A way to identify the person who drew the sample



Even trivially mislabeled samples are markedly (40 times) more likely to be ABO-mismatched with the patient's real type.



Units are visually inspected before issue. Units that appear hemolyzed or discolored, or that contain clots or visible gas are not issued but rather are quarantined.

labeled sample be drawn. The rationale behind this inflexibility is based on studies that have shown that a mislabeled sample, even one with a trivial-appearing misspelling of the patient's name, is 40 times more likely to be an ABO mismatch when compared with the patient's true ABO type. Mislabeling a sample is regarded as a strong marker of carelessness in the process of obtaining and labeling the sample; thus, any mislabeled sample is destroyed. The mislabeled sample must be replaced by a properly drawn sample before type-specific, crossmatched blood is issued to the recipient.

At the time of issue of a blood unit from the transfusion service, the technologist releasing the unit verifies the identity of the intended recipient, including both unique identifiers, the ABO and Rh type of the unit and of the recipient, the results of compatibility testing, and the time of issue. The unit is also inspected before release. A unit that appears hemolyzed, that contains clots or visible gas, or that has a different color from the attached segments or from other units is not issued but is quarantined in the transfusion service pending investigation of possible contamination. Most transfusion services also require cross-check of the patient and unit identification by the person receiving the unit, although some facilities will permit this verification to be performed by two members of the transfusion service staff instead.

Likewise, before the unit is transfused, the patient's identity must be confirmed by inspection of his or her attached armband, and this information must be verified against the information on the unit. Many institutions require that the recipient identification be performed and documented by two individuals before the start of infusion.

Infusion Time



Transfusion must be completed within 4 hours of issue.

Units may be returned for reissue only if returned within 30 minutes of release from the blood bank.

Transfusion of blood components must be completed within 4 hours of issue from the transfusion service; thus, in most cases, only a single unit is issued at a time. A unit that has been out of the blood bank for less than 30 minutes may be returned and reissued to another patient. This interval is chosen because RBCs that have remained unrefrigerated for longer than 30 minutes are likely to have exceeded the maximum allowable transport temperature (10 C). In both cases (the 4-hour limit for transfusion and

the 30-minute interval for return), the period has been chosen because of concern about bacterial contamination of units stored out of the refrigerator.

Filters

All blood components, including Fresh Frozen Plasma (FFP) and Cryoprecipitated AHF, must be transfused through a filter capable of removing blood clots, fibrin strands, and other particulate matter that is potentially harmful to the patient receiving the unit. Standard blood infusion sets have an inline filter with a pore size of 170 to 260 microns, which will accomplish filtration. Leukocyte reduction filters act by affinity between the charged filter and leukocytes in the unit, but they also have a pore size of approximately 170 microns. Thus, a bedside leukocyte reduction filter is sufficient to remove particulate matter and need not be used in conjunction with a standard blood infusion filter. However, a unit that has undergone leukocyte reduction before storage is still at risk of developing fibrin strands or other cellular debris during storage and must be administered through a standard blood infusion set at the time of transfusion. Information about whether a filter is required for the infusion of a particular derivative, such as albumin or factor concentrate, is best determined by consulting the package insert accompanying the derivative because the requirement varies depending on the product in question.

Microaggregate filters with a pore size of 40 microns were advocated at one time for use in massive transfusion, in the hope that they would prevent development of acute lung injury reactions. However, microaggregate filters have fallen into disfavor because they have proven ineffective in preventing acute lung injury and because they significantly slow infusion rates in the very patients who require rapid transfusion of large volumes of blood.

The one exception to the rule that all blood components must be filtered at the time of transfusion is aliquots for very small pediatric patients, which are issued in syringes. Those aliquots are filtered in the transfusion service immediately before issue and, if transfused immediately, need not be filtered at the bedside. This situation permits the use of a syringe pump with a very slow infusion rate for transfusions to neonates and pediatric patients who could not



All blood components (even FFP and cryo) must be infused through a 170 to 260 micron (or smaller) filter.



Leukocyte reduction filters have a pore size of about 170 microns, as do the filters in standard blood infusion sets.

tolerate infusion through a standard intravenous line because of their tiny blood volumes.

Bedside leukocyte reduction filters are used considerably less frequently now that many donor centers have adopted a policy of universal leukocyte reduction of cellular components either during collection by apheresis or immediately after collection of a whole blood donation. However, there are occasions when units that have not been leukocyte reduced before storage must be transfused to a patient who requires leukocyte-reduced products. In such settings, leukocyte reduction is accomplished by leukocyte filtration, either in the transfusion service (a standard blood filter is still required for transfusion) or at the bedside (a leukocyte reduction filter suffices).

Several important caveats regarding leukocyte reduction filters should be observed. Although such filters have a pore size compatible with the removal of particulate matter, the mechanism of action in removing leukocytes is based on affinity between the charged filter surface and leukocytes in the unit being filtered. The filters are intended to be used in a strictly vertical position under gravity feed only. Their efficacy is reduced—and may be negated—if they are not maintained in the proper orientation or if pressure is used to force the component through the filter. The manufacturer's instructions with respect to priming of the filter must be strictly followed, and the filter should not be flushed after completion of the unit because such action will flush adherent, filtered leukocytes from the filter into the patient. Leukocyte reduction filters are designed to be used once and discarded. They should not be used to filter more than one unit of blood or apheresis platelets or one pool of pooled platelet concentrates.

Many institutions require the use of a different infusion set for each unit, although some will allow the same set to be used for up to 4 units administered within a 4-hour period. Leukocyte reduction filters are designed to be used once, then discarded, and should not be reused under any circumstances.



Leukocyte reduction filters must be strictly vertical during transfusion, must be fed by gravity only, and should not be flushed following infusion.

Needle Size and Choice of Intravenous Fluid

Although conventional wisdom specifies a minimum needle size of 18-gauge for blood transfusion, pediatric patients and adults with very small veins may require the use of smaller gauge needles. Thin-walled 23-gauge needles have

been used successfully in such settings, albeit with slower flow rates.

In general, the only intravenous fluid that should be used in conjunction with the transfusion of blood is normal saline (0.9% USP). It is permissible to use other fluids that are approved by the Food and Drug Administration (FDA) for use with blood, most notably 5% albumin, which is used for replacement in plasma exchange. However, in practical terms, regarding normal saline as the only acceptable fluid will avoid accidental use of other fluids that are clearly unacceptable.

Lactated Ringer's solutions, in particular, cannot be used because they contain calcium, which will neutralize the anticoagulant and precipitate clotting. If, for some reason, a "balanced salt" solution must be transfused along with red cells, several solutions do not contain calcium and thus are FDA-approved for use with blood; such solutions include Plasma-Lyte A Injection pH 7.4 (Baxter, Deerfield, IL) and Normosol-R pH 7.4 (Abbott Laboratories, Abbott Park, IL). Use of dextrose solutions and hypotonic saline will result in red cell hemolysis. In addition to causing swelling and hemolysis, dextrose solutions may also cause red cells to clump.

Medications must not be infused through the same line that is being used for the transfusion of blood. Multilumen central venous catheters may be an exception because they are designed to permit simultaneous infusion of fluids without allowing admixture in the lines themselves. In addition, high flow rates in the central vein are generally sufficient to prevent intermingling of fluids at the ports. However, simultaneous infusion of fluids, medications, or both even through a multilumen central catheter certainly should not be encouraged as a matter of routine. If the patient has an adverse reaction and if multiple fluids have been infused simultaneously, it may be difficult, if not impossible, to determine which fluid or drug caused the reaction.



Only normal saline (0.9% USP) should be used to transfuse blood.



Lactated Ringer's solution is contraindicated because calcium in the fluid will counteract citrate and may result in clot formation. Plasma-Lyte A injection pH 7.4 and Normosol-R pH 7.4 are safe alternative balanced salt solutions.



Dextrose and hypotonic saline will cause red cell hemolysis.

Blood Warmers

When large volumes of cold blood are transfused rapidly, there is a risk of arrhythmia, cardiac arrest, or both associated with chilling of the conduction system, particularly the sinoatrial node. Blood warmers may be indicated in the operating room and in trauma settings when patients

are receiving blood at a rate in excess of 100 mL a minute, but there is generally no need to warm blood for routine infusion, even if multiple units are to be transfused over a period of several hours. Blood warmers are also used for the transfusion of patients with potent cold agglutinins, although this use remains somewhat controversial.

Pretransfusion Preparation of the Patient



A full set of vital signs should be obtained immediately before transfusion of each unit as a baseline.



Although patients are often premedicated with acetaminophen and antihistamines, premedication is not absolutely necessary and may even mask signs of incipient transfusion reactions. Premedication with corticosteroids may be necessary in some patients who require repeated transfusions despite a history of frequent allergic reactions. Washed RBCs are an alternative to steroids in patients requiring RBC support, but washing is not feasible for FFP transfusion and may result in activation of platelets in a platelet unit with detrimental effects on their function.



Vital signs should be recorded 15 minutes after the start of transfusion and after completion of each unit.

A full set of vital signs (temperature and respiration as well as blood pressure and pulse) should be obtained immediately before transfusion to provide a baseline for comparison should the patient later develop signs of a possible transfusion reaction. Ideally, patients should be afebrile before transfusion because assessing a fever spike in a patient who was already febrile at the start of a transfusion is problematic. However, it is not always practical to withhold transfusion until a patient's fever can be brought under control.

Patients are often premedicated with acetaminophen and antihistamines before transfusion, but this practice is not essential, nor is it necessarily desirable. In particular, the use of antipyretics such as acetaminophen may mask fever, although, realistically speaking, the fever of a septic reaction will probably be evident despite premedication. Likewise, hemolytic reactions will have other hallmark manifestations, even in the absence of fever. Antihistamines are of specific benefit only in patients with allergic reactions, which involve mast cells, but antihistamines may also help by mildly sedating anxious transfusion recipients.

Monitoring the Patient during Infusion and after Transfusion

Patients undergoing transfusion should be closely observed during the first 15 minutes of transfusion because major hemolytic reactions often become apparent during this interval. If no reaction is noted, a second set of vital signs should be obtained at the end of this period, after which the patient should be observed intermittently until the unit is completed, at which time a posttransfusion set of vital signs should be recorded.

If, at any time during the transfusion of a blood component, the patient develops either symptoms or signs that might represent a transfusion reaction, the transfusion should be halted and the potential reaction evaluated. In many institutions, all possible transfusion reactions are reported to the transfusion service as well as to the patient's physician, although some facilities rely on the primary physician to make the decision about whether a patient is experiencing a possible reaction. All possible transfusion reactions must be reported to the transfusion service promptly. With the exception of mild, simple allergic reactions (isolated urticaria without dyspnea or oral edema), the report should be accompanied by the remainder of the unit with all associated tubing intact (needle removed) and by a sample of venous blood obtained after the onset of the possible reaction (the posttransfusion sample). Even in the case of a possible allergic reaction, the unit should not be restarted until clearance is received from the transfusion service.

Initial evaluation by the transfusion service of possible transfusion reactions should include a clerical check to verify that the blood was transfused to the intended recipient, examination of the posttransfusion venous blood for visible hemolysis, and performance of a direct antiglobulin test (DAT) on both posttransfusion and pretransfusion venous blood samples from the recipient. In addition, a STAT Gram's stain should be performed on a sample from the implicated unit if the reaction included significant fever (1 to 2 C rise in temperature). All of those procedures can be completed in less than 15 minutes, at which time a reasonable conclusion can be reached regarding the nature of the reaction and about what further measures are indicated. While the transfusion service is performing those tests, appropriate symptomatic treatment of the recipient should be instituted. For more information concerning the management of transfusion reactions, see Chapter 7: Adverse Effects of Transfusion.



If a possible transfusion reaction occurs, the unit should be stopped immediately. The unit and all attached tubing should be returned to the blood bank along with a posttransfusion sample from the patient.

Considerations in Neonatal Transfusion

There are a few key differences in the administration of blood components to neonates (less than 4 months old). Most of the differences arise because the neonate has an immature immune system. As a consequence, newborns are incapable of de novo production of antibody for about 4 months after birth, although rare instances of autoanti-



Newborn infants are incapable of de novo production of antibody for approximately 4 months.



As a matter of convenience, many hospitals choose to transfuse all newborns with group O-negative or group O Rh-compatible RBCs.

body production by 2-month-old infants have been reported. Selection of red cells for transfusion to newborns may be done according to ABO grouping and Rh typing of the infant's red cells and an antibody screen performed on either maternal or neonatal serum/plasma. Any antibodies present in the maternal serum should be honored with antigen-negative blood until such time as the infant's serum no longer shows evidence of antibody.

In terms of ABO selection of RBCs, many centers choose, as a matter of convenience, to transfuse all newborns with O-negative RBCs or with group O Rh-compatible units. If, however, a decision is made to use type-specific blood, the serum/plasma of a non-group-O newborn must be tested for the presence of maternal anti-A and/or anti-B unless it is known that the RBCs to be given are compatible with the maternal serum. If anti-A or anti-B is present in the newborn's serum/plasma, RBCs lacking the corresponding antigen(s) must be used. (See Table 6-1.)

In the past, there was some concern about the passive transfer of small amounts of anti-A or anti-B along with red

Table 6-1. Selection of RBCs for Neonatal Transfusion

	Acceptable ABO Groups for Transfusion to Neonate	Further Testing Required
Maternal or neonatal antibody screen negative	Group O	
Serum not examined for anti-A or anti-B		
Maternal or neonatal antibody screen negative	Group O or lacking antigen(s) corresponding to ABO antibody present	Repeat testing for anti-A and anti-B every 2 weeks until there is no antibody
Infant red cells not group O		
Infant's serum has been tested for anti-A and anti-B		
Maternal serum has non-ABO antibody that is clinically significant	ABO group as above either antigen-negative or crossmatch-compatible at Coombs	Repeat antibody screen as indicated—honor antibody until screen is negative
Maternal antibody screen negative or newborn's antibody screen negative	ABO group as above	No repeat needed until 4 months of age or new hospital admission if less than 4 months old

RBCs = red blood cells.

cells. For that reason and to reduce the amount of potassium passively infused, some centers chose to wash units for transfusion to newborns. However, if an appropriate amount of RBCs is transfused (10-15 mL per kg), the amount of passively transferred antibody is proportionately no greater than the amount given to any other patient receiving out-of-group but ABO-compatible RBCs. In such a case, the passive transfer of anti-A, anti-B, or both will not be clinically significant.

Both potassium levels and ABO antibody in the plasma accompanying red cell aliquots have recently been shown to be of less concern than was previously thought. Several studies have examined the safety of using multiple aliquots from a single unit of RBCs until its outdate in order to reduce donor exposures to low-birthweight infants. They concluded that, as long as transfusion is done slowly, potassium in the supernatant plasma does not present a clinical problem. Two of the studies used unwashed citrate-phosphate-dextrose-adenine (CPDA) RBCs (35-day outdate), whereas another used unwashed AS-1, or Adsol, RBCs. Two of the three studies used group O-negative RBCs for all patients. The latter two studies provide good evidence that just as potassium is not a problem with slow infusion rates, neither is the passive transfer of ABO antibodies. Finally, the safety demonstrated by the three studies also satisfactorily addresses earlier concerns about low levels of 2,3-diphosphoglycerate in red cells stored for 35-42 days. It should be noted that most, if not all, of those studies used RBC units with reduced plasma volume, either



Red cells for neonatal transfusion need not be washed to remove potassium or passive anti-A or anti-B.



Even 42-day-old AS-1 RBCs can safely be transfused to neonates without washing, provided the dose is appropriate (10-15 mL/kg) and the transfusion is given slowly.

Table 6-2. Doses and Expected Response to Transfusion in Infants and Small Children

Component	Dose	Expected Response
Red Blood Cells	10-15 mL/kg	3 g/dL in hemoglobin or 9% in hematocrit
Platelets	Per 10 kg of body weight, either: 1 whole-blood-derived platelet concentrate unit, or 50 mL apheresis platelets (1/6 of an apheresis unit)	30,000-50,000/ μ L
Fresh Frozen Plasma	10 mL/kg	Depends on starting level of coagulation factors



Many centers irradiate all cellular blood components before transfusion to newborn infants.



Neonates, especially low birthweight newborns, should receive CMV-reduced-risk blood components.

because they were centrifuged inverted before an aliquot was drawn from the down-facing ports or because they were stored inverted.

Cellular blood components transfused to newborns should be gamma irradiated to avoid the risk of transfusion-associated graft-vs-host disease, especially after intrauterine transfusion or when the unit is a directed-donor unit from a family member. Many centers irradiate all blood components for neonates on the premise that it may be several weeks to months before an infant with significantly compromised cell-mediated immunity (eg, severe combined immunodeficiency, Wiskott-Aldrich syndrome, or De George syndrome) may be identified.

In addition, because newborns are particularly sensitive to infection by cytomegalovirus (CMV), CMV-reduced-risk cellular blood components should be selected, particularly for very low-birthweight infants, as well as for recipients of intrauterine transfusion. (See Chapter 1: Blood Component Preparation and Chapter 7: Adverse Effects of Transfusion for further information about the prevention of CMV transmission by blood.)

Finally, it must be recognized that neonates require a higher hemoglobin concentration, with transfusion at hemoglobin levels of 13 g/dL being deemed justified on a prophylactic basis in newborns with severe pulmonary or cyanotic heart disease or heart failure (compared with the level of 10 g/dL for adults in the same settings). And, with respect to FFP, it is important to remember that reference range values for prothrombin time (PT) and activated partial thromboplastin time (aPTT) in newborns are considerably higher than they are in adults. Thus, a PT or aPTT that would justify prophylactic FFP in an adult might well be a normal value in the neonate. (See Table 6-2.)

Suggested Reading

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7

Adverse Effects of Transfusion

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THIS CHAPTER DISCUSSES GENERAL ASPECTS of adverse reactions to transfusion, including recognition, reporting, and evaluation of suspected transfusion reactions. Management of specific transfusion reactions is addressed—acute and delayed hemolytic transfusion reaction, febrile transfusion reaction, allergic reaction, anaphylactoid reaction, transfusion-related acute lung injury, transfusion-associated graft-vs-host disease, hypotension, and posttransfusion purpura. Transfusion-transmitted diseases, including bacterial sepsis and viral and parasitic infections, are also discussed. Alloimmunization to blood group antigens and HLA antigens is covered in Chapter 3: Pretransfusion Compatibility Testing and Chapter 11: Special Transfusion Situations.

Transfusion Reactions

Transfusion reactions may present with a plethora of different symptoms, and the same symptom(s) may be seen in a variety of reactions. Table 7-1 lists some of the more common manifestations of transfusion reactions.

The same symptom may be present in a relatively innocuous reaction (eg, febrile or allergic), but it also may be the harbinger of a life-threatening reaction such as acute hemol-



Whenever a transfusion reaction is possible, the transfusion must be stopped, pending assessment of the possible reaction.

Table 7-1. Signs and Symptoms Commonly Seen in Transfusion Reactions

- Fever
 - Febrile nonhemolytic transfusion reaction
 - Hemolytic transfusion reaction, acute or delayed
 - Septic transfusion reaction
 - Transfusion-related acute lung injury—variably seen
- Rash
 - Allergic and/or anaphylactic reactions
 - Hemolytic transfusion reaction
- Shock
 - Acute hemolytic transfusion reaction (intravascular hemolysis)
 - Anaphylactic reaction
 - Sepsis
 - Transfusion-related acute lung injury—variably seen
- Respiratory distress/dyspnea
 - Acute hemolytic transfusion reaction
 - Anaphylaxis
 - Fluid overload, cardiogenic pulmonary edema
 - Transfusion-related acute lung injury

ysis. Thus, the transfusion must be stopped immediately whenever the possibility of transfusion reaction exists, however remote.

Reporting Suspected Transfusion Reactions

Hospital procedures for managing suspected transfusion reactions will vary, but all procedures require minimum evaluation to exclude the possibility of a hemolytic reaction. After the transfusion is discontinued and the transfusion service has been notified, the following items should be sent to the transfusion service as soon as possible:

1. *The implicated unit with infusion set (minus the needle) attached.*

These items will be used to confirm that no clerical error has occurred, resulting in transfusion of the wrong unit to the patient. Further laboratory testing may be performed on the blood bag (eg, Gram's stain and culture of the unit if a septic reaction is suspected), on segments retained in the transfusion service before issue of the unit, or on both. For example, if a hemolytic reaction is suspected, the supplemental testing on the

unit itself might include repeat ABO typing of the unit, antibody screening of the donor unit, and repeat cross-match of the unit with the recipient's posttransfusion serum.

Inclusion of the infusion set will also allow detection of hemolysis caused by transfusion of blood components with inappropriate fluids such as dextrose solutions.

2. *A red top (clot tube) or a purple top (EDTA tube) containing a sample drawn from the recipient following discontinuation of the transfusion.*

These samples will be used to examine the posttransfusion plasma for evidence of free hemoglobin (indicating a possible hemolytic reaction) and to perform a direct antiglobulin test (DAT) to detect the presence of antibody bound *in vivo* to red cells in the recipient's circulation. In the case of a suspected hemolytic transfusion reaction, the positive DAT would represent recipient antibody coating of transfused red cells, whereas in an untransfused patient with warm autoimmune hemolytic anemia, the positive DAT would represent binding of the autoantibody to the recipient's own red cells. An EDTA-anticoagulated sample (purple-top tube) is preferred for the DAT because EDTA prevents *in-vitro* binding of complement, which might otherwise cause a false-positive test result. In addition, if the unit transfused was Red Blood Cells (RBCs), repeating ABO and Rh typing on pretransfusion and posttransfusion samples and on the transfused unit(s) is indicated whenever a hemolytic reaction is being considered.

If the preliminary evaluation suggests a hemolytic reaction, additional tests would also be performed, including repeat crossmatches of recipient pretransfusion and posttransfusion plasma with the affected unit and with any other units transfused immediately before the development of symptoms, antibody screening on pretransfusion and posttransfusion samples from the recipient, and repeat ABO and Rh typing on pretransfusion and posttransfusion samples from the recipient and on transfused RBC unit(s), if not already done.

3. *The first urine sample available after the suspected reaction (optional at some institutions).*

The urine sample will be used to detect the presence of free hemoglobin, which would suggest hemoglobin-



Actions to be taken immediately whenever a transfusion reaction is suspected include:

- Stop transfusion
- Report reaction to blood bank
- Return bag with all tubing attached
- Send posttransfusion blood sample
- Posttransfusion urine may be sent later

emia if it were detected in the absence of intact red cells (hematuria) in the same urine sample. A post-transfusion urine that is dipstick-positive for blood but that also contains intact red cells may result from a variety of causes unrelated to transfusion, including cystitis, bladder tumor, or renal calculus, and should be interpreted with caution.

On rare occasions, clearance of free hemoglobin from the plasma can be so rapid that hemoglobinemia may no longer be present. In such cases, hemoglobinuria—in the absence of hematuria—may provide a valuable indicator that hemoglobinemia was present.

NOTE: The blood bag and transfusion set and a post-transfusion blood-bank sample should be sent to the transfusion service without delay, along with the relevant paperwork. A urine sample should be sent whenever it becomes available.

Some institutions do not require a urine sample to be sent in all cases—reserving the request only for selected cases in which the urine is deemed crucial to the evaluation. Requiring a urine sample on all transfusion reaction workups can result in delay in receipt of samples if nurses wait until they can send the entire workup at once.

4. *The hospital's transfusion reaction report form on which the transfusionist, the physician caring for the patient, or both have noted the signs and symptoms that raised concern about a transfusion reaction.*

Evaluation and Management of Transfusion Reactions

Transfusion reactions fall into the following categories: immunologic, infectious, and other. (See Table 7-2.)

Acute Hemolytic Transfusion Reaction

By definition, an acute hemolytic transfusion reaction (AHTR) occurs within 4 hours of transfusion. AHTRs result from the reaction of preformed antibody (usually ABO antibody) with transfused red cells. The antibody coats the transfused red cells, activates the complement system to the membrane attack complex (C 5-9), and results in intravascular hemolysis. The resultant “cytokine storm” also activates the coagulation system, resulting in disseminated intravascular coagulation (DIC).

Table 7-2. Types of Transfusion Reactions

- Immunologic
 - Acute hemolytic transfusion reaction
 - Delayed hemolytic transfusion reaction
 - Febrile nonhemolytic transfusion reaction
 - Transfusion-related acute lung injury (granulocyte antibody model)
 - Allergic (urticarial) reaction
 - Anaphylactic reaction
 - Transfusion-related graft-vs-host disease
- Infectious*
 - Septic transfusion reaction (bacterial contamination)
 - Viral infection (HIV, HTLV, HBV, HCV, WNV)
- Other
 - Circulatory overload
 - Transfusion-related acute lung injury (lipid activators of neutrophils model)
 - Febrile nonhemolytic transfusion reaction (cytokine, other BRM mediated)

*See Table 7-7 for a more comprehensive list of transfusion-transmissible infectious diseases.

The result of the activation of the complement and coagulation systems is hypotension, often frank shock, and microcirculatory changes that result in decreased renal blood flow and in renal failure. The greater the volume of incompatible blood transfused, the more severe the reaction is likely to be.

The signs and symptoms of AHTR include the following:

- Dark urine (hemoglobinuria) and dark plasma (free hemoglobin)
- Persistent hypotension, often shock (mediated by cytokines, complement, or anaphylatoxin)
- Fever (usually greater than a 1 to 2 C rise over the pre-transfusion level)
- Severe flank pain (pain over the costovertebral angles)
- DIC, oozing of blood from IV sites and other orifices
- Pain at the infusion site
- Chest tightness, sense of impending doom
- Urticaria, hives, flushing
- Vomiting, diarrhea
- Bilirubin increase 5 to 6 hours after transfusion



Signs and symptoms of acute hemolytic reactions include:

- Hypotension
- Red urine (hemoglobinuria)
- Red plasma (hemoglobinemia)
- Flank pain/pain at infusion site
- Acute-onset of DIC

According to one report summarizing 355 transfusion-related deaths, the most common symptoms in AHTR were hemoglobinuria, DIC, hemolysis, renal failure, and hypotension. Other less common findings were the positive DAT, respiratory distress, chills, fever, and oliguria. Tachycardia, agitation or apprehension, hypertension, and seizures were relatively uncommon.

Treatment for suspected AHTR should be initiated immediately, including the following steps:

STOP TRANSFUSION.

Administer fluids and pressor agents to maintain blood pressure and renal blood flow.

Administer mannitol or furosemide (Lasix) to maintain urinary flow and to prevent acute tubular necrosis.

Table 7-3 lists suggested medications and starting doses for management of various reactions. They are intended as guidelines only. In all cases, management should be individualized to the specific patient and clinical presentation.

If a hemolytic transfusion reaction is confirmed by the transfusion service, some institutions advise consultation with the renal service for guidance in maintaining renal blood flow during the acute phase.

Delayed Hemolytic Transfusion Reaction

Delayed hemolytic transfusion reactions (DHTRs) involve anamnestic antibody production. DHTRs occur when preformed antibody is absent (or present in an amount insufficient for detection by routine methods) in the recipient's pretransfusion blood sample. In such cases, the recipient has been previously sensitized and, thus, has lymphocytes that are primed and ready to produce anamnestic antibodies upon rechallenge with the same antigen. Because the pretransfusion antibody level is undetectable, even the most diligent search for antibody before transfusion will not prevent the development of DHTRs.

Typically, DHTRs occur 5 to 14 days after transfusion, although they may occur as early as 2 or 3 days after rechallenge. Unlike AHTRs, DHTRs rarely involve activation of complement, meaning that the hemolysis seen in delayed HTRs is usually extravascular, occurring in the reticuloendothelial system. Thus, the presentation of a patient exhibiting a delayed HTR is considerably more subtle.

DHTRs may manifest as unexplained anemia (or failure to achieve expected posttransfusion hemoglobin increment) or as an unexplained increase in unconjugated



Management of acute HTRs includes:

- Stop transfusion
- Support blood pressure with pressors and fluids
- Support renal blood flow (mannitol or Lasix)
- Treat DIC, if present



Delayed HTRs typically manifest as:

- Unexplained decreases in hemoglobin/hematocrit
- Unexplained increase in (unconjugated) bilirubin with few, if any, associated symptoms.

Table 7-3. Suggested Drugs and Dosages

Reaction Type	Drug	Starting Dose	Route	Notes
Acute hemolytic reaction with shock	Dopamine or norepinephrine or epinephrine	2 µg/kg/minute Titrate to keep systolic BP at 90 mmHg or mean arterial pressure at 60 mmHg	Intravenous drip	Requires infusion pump capable of microdrop delivery Recommend central venous catheter If measurement of mean arterial pressure is available, this is the preferred monitor.
	Methylprednisolone or dexamethasone Intravenous fluid bolus	125 mg every 6 hours 4 mg every 6 hours Normal saline 1-2 liters	Intravenous (piggyback) infusion Intravenous bolus, may need frequent repetition	Use to treat rigors. Antihistamines are often used but have no specific effect because mast cells are not involved. (See allergic for dose.)
Febrile nonhemolytic	Meperidine	25 mg	Intravenous or intramuscular	Aspirin is contraindicated for thrombocytopenic/thrombocytopathic patients. (continued)
	Antipyretics (aspirin, acetaminophen)	Oral		

Table 7-3. Suggested Drugs and Dosages (continued)

Reaction Type	Drug	Starting Dose	Route	Notes
Allergic	Diphenhydramine and either ranitidine or famotidine	50 mg 50 mg 20 mg	Oral or intravenous or intramuscular	
Anaphylactic	Epinephrine	0.3-0.5 mL of 1:1000 solution (0.3-0.5 mg) Patients with shock and/or major airway compromise: 3-5 mL of 1:10,000 solution	Subcutaneous thorax Intravenous or instilled into endotracheal tube	May be repeated at 20-minute intervals if needed
	Methylprednisolone Dexamethasone Albuterol	125 mg every 6 hours 4 mg every 6 hours 2.5-5 mg in 3 mL saline	Intravenous (piggyback) infusion Nebulizer or endotracheal tube	For patients with anaphylactic shock For patients with bronchospasm
	Glucagon	1 mg; may repeat every minute up to 5 mg	Intravenous	For patients refractory to epinephrine—likely on beta blocker

BP = blood pressure.

bilirubin with few, if any, associated symptoms, occurring in the week or two after transfusion, or as both. However, many DHTRs go unrecognized clinically.

DHTRs may first be discovered by the transfusion service as a newly detected antibody in a recently transfused patient. The DAT is often positive but may be negative if all of the transfused red cells have already been removed from the circulation by the reticuloendothelial system. One important distinction in this context is between delayed serologic transfusion reactions (DSTRs) in which there is serologic evidence of incompatibility in the absence of evidence of hemolysis, and DHTRs which demonstrate both serologic incompatibility and clinical hemolysis.

Although DHTRs are usually not life threatening, on average two to four of the transfusion-related fatalities reported to the Food and Drug Administration (FDA) each year are caused by DHTR, about a tenth as many as those caused by AHTR. Although often only minimally symptomatic, DHTRs can nonetheless cause morbidity, particularly the need for additional transfusions. And, when DHTRs are not properly recognized, patients can be subjected to unnecessary testing for bleeding or for biliary stasis as part of a search for an explanation for anemia or hyperbilirubinemia that is actually the result of the DHTR.

A 1978 study from the Mayo Clinic found that anti-Jk^a accounted for more than one-third of DHTRs and that most of the remainder were caused by anti-D, -E, -c, -K, or -Fy^a, either singly or in combination with another antibody. Three deaths occurred among patients experiencing DHTRs but those deaths could not be attributed solely to the reaction because each of the three patients had serious underlying disease. A subsequent study, also from the Mayo Clinic, observed no DHTR-related fatalities.

Frequently, the only action that is required for a patient with a DHTR is determining the specificity of the antibody that caused the reaction. Obviously, subsequent transfusions must be antigen negative for the specificity defined as causing the reaction, as well as for any other red cell antibodies previously detected. However, when a DHTR is first detected in the laboratory rather than being reported by the patient's physician, it is crucial that the clinician be advised of the reaction and its significance, including the number of antigen-positive units at risk for premature removal from the circulation. Such action will forestall unnecessary evaluation of the patient for bleeding or jaundice because of a misinterpretation of anemia or hyperbilirubinemia.



DHTRs are rarely life threatening. However, on average two to four of the transfusion-related fatalities reported to the FDA each year are caused by DHTR.



The most common antigen specificities associated with DHTRs are: anti-Jk^a, -D, -E, -c, -K, and -Fy^a, either individually or in combination with another antibody.



Febrile nonhemolytic reactions present with fever, shaking chills (rigors), and possibly hypertension. Hypotension is not seen.

Febrile (Nonhemolytic) Transfusion Reaction

Febrile nonhemolytic transfusion reactions (FNHTRs) are usually characterized by rigors (shaking chills), fever (typically defined as a temperature above 38 C or a rise in temperature of more than 1 C in the 4 hours after transfusion), and diastolic hypertension. Those signs and symptoms may be accompanied by tachycardia, palpitations, or cough, but hypotension (shock) is not seen in FNHTRs.

FNHTRs are caused either by antibodies present in the recipient and directed against white cells, white cell stroma, and platelets in the transfused unit or by biologic response modifiers (BRMs) that accumulate in the plasma compartment of the unit during storage. Some of the cytokines that are found after storage appear to be leukocyte derived, which may explain why prestorage leukocyte reduction of cellular blood components reduces the incidence of FNHTRs. Others appear to be platelet derived. Furthermore, activation of complement by the alternative pathway has been described after the exposure of plasma to plastic surfaces, and it is postulated that, in turn, complement activation may in turn stimulate cytokine production by neutrophils, macrophages, and monocytes. A model for the pathogenesis of FNHTRs is shown in Fig 7-1.

The incidence of FNHTRs can be significantly reduced but not completely eliminated by leukocyte reduction of cellular blood components. It is not surprising that complete elimination of FNHTRs cannot be achieved by leukocyte reduction because leukocyte reduction will have no effect either on platelet-derived cytokines or on complement activation. Fresh Frozen Plasma (FFP), which is essentially acellular, need not be leukocyte reduced.

Leukocyte reduction is best performed before storage, before leukocytes can release BRMs into the unit, rather than at the bedside. Leukocytes can be removed before storage either by filtration or by the use of special apheresis machines that remove leukocytes from platelet and RBC components as they are collected. Universal prestorage leukocyte reduction is mandated in the United Kingdom and Canada, primarily to reduce the risk of variant Creutzfeldt-Jakob disease (vCJD), and many donor centers in the United States have also adopted a policy of universal leukocyte reduction of cellular blood components.

Antipyretics are used to treat the fever of patients with FNHTR, and meperidine (Demerol) may be administered for rigors, particularly if they are severe. Antihistamines are frequently ordered, but their effect is primarily as a seda-



Leukocyte reduction decreases the incidence of FNHTRs but does not prevent them entirely.



Merperidine (Demerol) is effective against the rigors of FNHTRs.

tive and not as specific therapy because mast cells are not involved in the pathogenesis of FNHTRs.

Allergic (Urticular) Reaction

Allergic (urticular) reactions result from hypersensitivity to allergens contained in the transfused unit. Because almost all blood components contain some plasma, urticarial reactions can be seen with virtually any blood component, with the exception of washed RBCs or frozen deglycerolized RBCs, neither of which contain plasma in sufficient volume to trigger an allergic reaction.

Some urticarial reactions appear to be rate dependent. Thus, it may be safe to restart the implicated unit at a decreased flow rate once the rash has subsided. Restarting the implicated unit should not be considered if the rash is extensive or if there is any evidence of perioral swelling or laryngospasm, which might suggest that the rash is the harbinger of an anaphylactoid reaction. Furthermore, a limited transfusion reaction workup should be performed to



Allergic reactions result from hypersensitivity to allergens in the donor plasma.

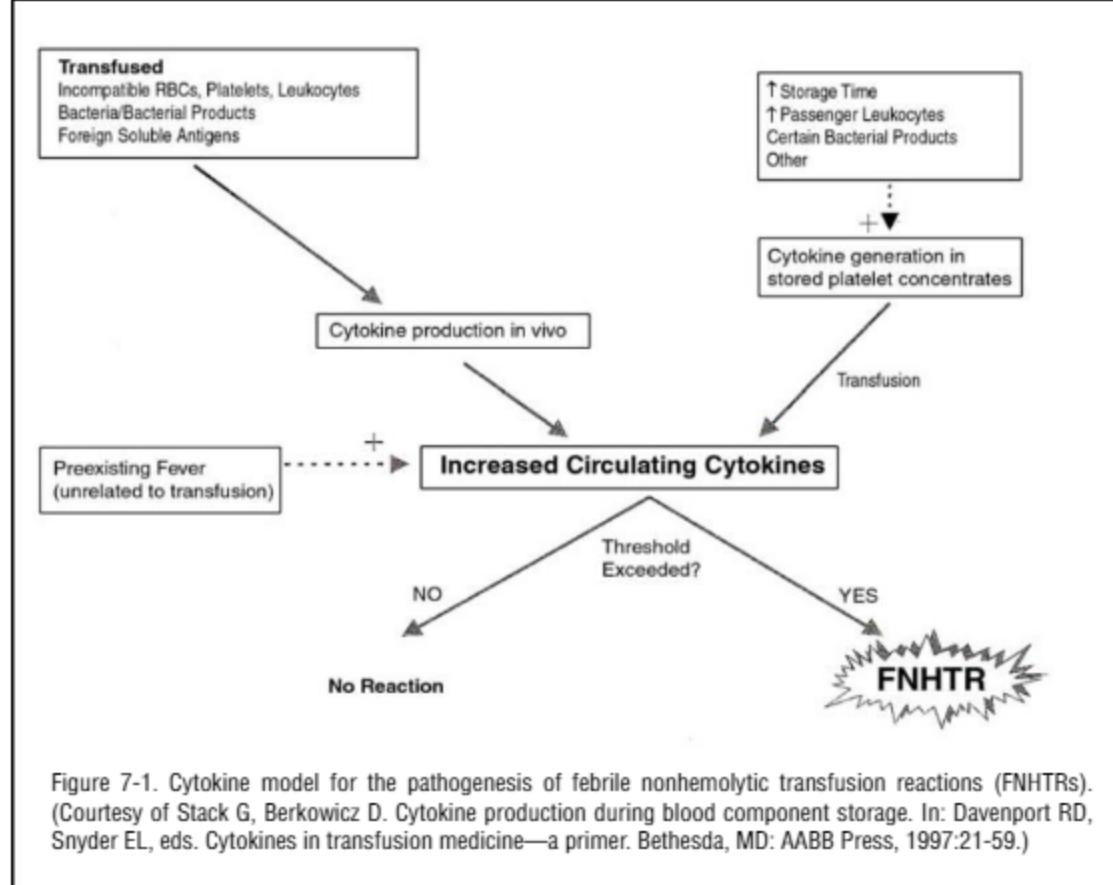


Figure 7-1. Cytokine model for the pathogenesis of febrile nonhemolytic transfusion reactions (FNHTRs). (Courtesy of Stack G, Berkowicz D. Cytokine production during blood component storage. In: Davenport RD, Snyder EL, eds. Cytokines in transfusion medicine—a primer. Bethesda, MD: AABB Press, 1997:21-59.)



Recurrent allergic reactions are uncommon because it is unlikely that another donor's plasma will contain the same allergen.

ensure that there is no evidence of hemolysis before administration of the unit is restarted.

Urticarial reactions are treated symptomatically with antihistamines. Ordinarily, no special precautions need to be taken with subsequent transfusions to the recipient because it is unlikely that a different donor will have the same allergen present in his or her circulation at the time of donation. Rarely, multitransfused patients, particularly those receiving large amounts of plasma on a frequent basis (eg, patients with thrombotic thrombocytopenic purpura undergoing daily therapeutic plasma exchange), will develop frequent allergic reactions. In such cases, it may be beneficial to premedicate the patient with steroids before transfusion or to consider combined H₁ and H₂ blockade.

Anaphylactoid/Anaphylactic Reaction



Anaphylactoid reactions to blood components can occur on the very first transfusion to a patient who is IgA-deficient. This is because anti-IgA is often a "naturally occurring" antibody.

Anaphylactic, or more correctly anaphylactoid, reactions occur when a plasma-containing component is transfused to an individual with preexisting antibody directed at an epitope contained in the donor plasma. The resulting antigen-antibody complex triggers mast cell degranulation and other mechanisms of anaphylaxis. Most commonly, this situation arises when an IgA-deficient recipient with preformed anti-IgA is transfused.

The distinction between labeling a reaction "anaphylactoid" rather than "anaphylactic" hinges on whether IgE antibody mediation can be demonstrated. True anaphylactic reactions are IgE-mediated, whereas the term anaphylactoid is used when IgE is not involved.

IgA-deficient recipients can develop "naturally occurring" antibody in response to exposure to IgA-like substances that are ubiquitous in the environment. Those preformed antibodies can react with IgA present in the plasma contained in a transfused component. As with anaphylactic reactions to drugs and other substances, the quantity required to precipitate an anaphylactoid response can be minuscule—in the nanogram range. Unlike most drug-related anaphylaxis, however, IgA-related anaphylactoid reactions to transfused blood components can occur with the first unit transfused because the antibody is "naturally occurring."

There have been episodes of anaphylaxis in response to other allergens (eg, penicillin) in the plasma compartment of the transfused unit. Anaphylaxis has also been precipi-

tated by donor IgE antibodies passively transfused into a recipient who has the corresponding allergen in his or her circulation (again, penicillin is an example). Both of those instances are rare, especially in light of the detailed questioning that donors undergo before donation—antibiotic usage in the period immediately before donation should result in deferral of the donor. Another, albeit rare, cause of anaphylactoid reactions is haptoglobin antibody in haptoglobin-deficient patients. Haptoglobin deficiency is seen almost exclusively in individuals of Asian heritage and is not seen in those of European and African heritage. In some cases, it may be impossible to precisely define the allergen, even by obtaining detailed medication and allergy histories from both the donor and the recipient.

The symptomatology of anaphylactoid transfusion reactions is the same as seen in anaphylaxis caused by other allergens, namely, respiratory distress, laryngeal edema, and hypotension. Anaphylactoid/anaphylactic transfusion reactions are treated in exactly the same manner as any other anaphylactic episode—with epinephrine, antihistamines, and, if severe, with intravenous steroids.

Prevention of anaphylactoid reactions with future transfusions involves verification that the recipient is IgA deficient with IgA antibody. Some institutions prefer to test first for the presence of IgA in the recipient and then to test individuals who lack IgA for the presence of anti-IgA. In testing for anti-IgA, it is important to look for both class-specific and limited-specificity anti-IgA. In particular, one must remember that it is possible for a patient to have normal total IgA levels, yet be subclass deficient, and that adverse reactions have been attributed to subclass-specific anti-IgA1 or -IgA2.

In cases where anti-IgA can be demonstrated, subsequent transfusions of plasma-containing components (RBCs, platelets, Cryoprecipitated AHF, and FFP) must be from IgA-deficient donors. Because only nanogram amounts of IgA are required to precipitate an anaphylactoid reaction, conventional washing of cellular blood components will not sufficiently reduce the associated plasma. However, frozen deglycerolized RBCs are acceptable, as are standard RBC units washed with 3 liters of normal saline. Several other washing protocols exist, including protocols that can be used to wash platelet units.

In patients with anaphylactoid/anaphylactic reactions for whom anti-IgA is not found, detailed medication and allergy histories may be obtained both from the recipient and from the donor of the implicated unit in an attempt to



Rarely, anaphylaxis can result from medications or other allergens in the donor plasma or even from the passive transfer of IgE antibodies from the donor to a recipient who has the target antigen (eg, penicillin) in his or her circulation.



Treatment of anaphylactoid reactions involves:
Epinephrine
Antihistamines
IV steroids, if severe



IgA-deficient patients with a history of anaphylactoid reactions require blood components from IgA-deficient donors.

define the allergen involved. In instances where it is critical to define the allergen, a history of the ingestion of foods known to precipitate anaphylaxis may be beneficial.

Transfusion-Related Acute Lung Injury



TRALI is noncardiogenic pulmonary edema caused by the passive transfer of either:

Granulocyte or HLA antibodies from the donor
Biologic response modifiers (such as lipids, CD40 ligand, or neutrophil antibodies) to a patient whose clinical condition has resulted in neutrophil sequestration in the pulmonary capillary bed.

Transfusion-related acute lung injury (TRALI) is noncardiogenic pulmonary edema associated with the passive transfer of donor granulocyte (neutrophil) or HLA Class I or II antibodies or both into the recipient of a plasma-containing blood component. Reaction of those donor antibodies with neutrophils in the recipient's lung results in neutrophil aggregation and activation in the lung microvasculature. The by-products of neutrophil activation result in altered vascular permeability and a pulmonary capillary leak syndrome, ending in noncardiogenic pulmonary edema and a clinical picture resembling acute respiratory distress syndrome (ARDS).

An alternative "two-event" hypothesis has been proposed in which the recipient's neutrophils are primed by a first event (eg, trauma, surgery, sepsis, receipt of exogenous cytokines) and then adhere to the pulmonary vascular endothelium, resulting in neutrophil sequestration in the pulmonary capillary bed. Overt TRALI ensues when lipid activators of neutrophils or other BRMs such as CD40 ligand found in donor plasma after storage are passively transfused into the susceptible recipient (ie, the second event), causing neutrophil activation with the release of proteases that damage the endothelium and result in capillary leak. This two-event hypothesis would explain the small percentage (approximately 10%) of cases in which no granulocyte or HLA antibodies can be detected.



Any plasma-containing blood component can potentially cause TRALI. Plasma derivatives, however, are very rarely associated with TRALI.

TRALI can occur with any plasma-containing blood component, including FFP, RBCs, whole blood, platelets, granulocytes, and even cryoprecipitate, which contains only 5 to 15 mL of plasma per unit. However, except in very rare case reports, TRALI has not been observed with plasma derivatives such as albumin, intravenous immune globulin (IVIG), Rh Immune Globulin, and coagulation factor concentrates. A probable explanation is that derivatives are made from pools of plasma obtained from thousands of different donors and contain only minuscule amounts of plasma from any one donor.

The signs or symptoms of TRALI include the acute onset of respiratory distress and hypoxemia within 6 hours after the end of transfusion of a plasma-containing blood com-

ponent, accompanied by clinical and radiographic evidence of acute pulmonary edema. Hypotension, fever, or both are often present as well. At the outset, TRALI is clinically indistinguishable from ARDS, but TRALI has a much more favorable prognosis. With prompt recognition and adequate ventilatory and pressor support, TRALI typically resolves within 3 to 7 days, without permanent sequelae. However, TRALI may be fatal in a small but significant proportion (5-10%) of cases.

TRALI is treated in the same manner as ARDS—with oxygen, ventilatory support, and pressors. Steroids are of limited, if any, value and diuretics have no place in the management of TRALI.

TRALI is largely a diagnosis of exclusion, meaning that other causes of acute pulmonary edema, including fluid overload and cardiac failure caused by myocardial infarction, should be excluded. It is important to note, however, that TRALI is still possible even if the patient has alternative risk factors for acute lung injury, such as underlying cardiac or respiratory disease. In light of recent work that suggests elevation of B-natriuretic peptide (BNP) may be a useful adjunct marker in confirming transfusion-related circulatory overload (TACO), research is ongoing into whether BNP can be used to help differentiate TACO from TRALI.

In addition to x-ray confirmation of the presence of pulmonary edema and documentation of hypoxemia by assessment of oxygen saturation or blood gases or both, laboratory evaluation of a possible episode of TRALI should include testing donors of plasma-containing units transfused within 6 hours of the development of symptoms for the presence of granulocyte and HLA Class I and Class II antibodies. When possible, a pretransfusion sample from the recipient should be similarly tested. The diagnosis is best established by demonstrating either a correspondence between donor antibody and antigenic determinants on the recipient's white cells or a positive lymphocyte cross-match (donor serum, recipient lymphocytes). However, such testing may not always be possible because of difficulties in obtaining appropriate samples from the recipient. This difficulty holds particularly true for the lymphocyte crossmatch because that testing requires a fresh (less than 24 hours old) sample from the recipient, who may have been discharged by the time samples can be obtained from donors.

The risk of TRALI has been estimated at 1:2000 to 1:5000 units, but it is likely that many cases go unrecognized. From 1992 to 2000, TRALI represented 13% of all



TRALI presents as acute onset respiratory distress and hypoxemia within 6 hours of transfusion, accompanied by physical and radiographic findings of acute pulmonary edema.



Treatment for TRALI includes:
Oxygen (with ventilatory support, if needed)
Pressors, if hypotension is present
Steroids and diuretics are of limited or no value.



TRALI represented 13% of all transfusion-related fatalities reported to the FDA from 1992 to 2000.



Donors implicated in an episode of TRALI or those associated with multiple episodes of TRALI need to be evaluated regarding their continued eligibility to donate plasma-containing components.

transfusion-related fatalities reported to the FDA, prompting the agency to issue a “Dear Colleague” letter encouraging heightened awareness of the possibility of TRALI in patients presenting with respiratory distress and hypoxemia within 6 hours after transfusion.

Aside from the need for prompt cessation of transfusion, the most important reason for identifying episodes of TRALI is to identify donors who are potentially at risk of precipitating TRALI in other recipients. If a donor with granulocyte or HLA antibodies can be identified as the cause, that donor should not be permitted to donate plasma-containing blood components in the future. Prompt notification of the donor center of suspected cases of TRALI is crucial so that any remaining products from suspect donors can be quarantined. Products from donors who are demonstrated to have antibodies corresponding to antigens present on the recipient’s white cells would then be destroyed. (See Table 7-4.)

A donor is considered to be *associated* with an episode of TRALI if a plasma-containing blood product from that donor was transfused within the 6 hours preceding the onset of symptoms. A donor associated with TRALI is considered to be *implicated* in an episode of TRALI only if he or she can be demonstrated to have granulocyte or HLA antibodies and if the antibody has specificity for an antigen present on the recipient’s white cells or if a positive

Table 7-4. Laboratory Evaluation of a Possible TRALI Episode

1. To detect possible antibodies associated with TRALI, perform the following: Test donors of the units transfused within 2 hours of the onset of symptoms for granulocyte (neutrophil) antibodies and for HLA Class I and Class II antibodies. If negative, extend the donor testing back (in 2-hour increments) to 6 hours before the onset of symptoms.
2. Test the recipient pretransfusion plasma for granulocyte and HLA (Class I and II) antibodies.
3. If antibody is detected in a donor, do one of the following:
 - a. Perform a lymphocyte crossmatch (recipient lymphocytes, donor plasma) for donors with HLA antibodies.
 - b. Determine whether the antibody detected has specificity for an HLA antigen or human neutrophil antigen (HNA) that is present on recipient white cells.

crossmatch exists between recipient white cells and donor plasma. Donors *implicated* in an episode of TRALI or *associated with multiple episodes* of TRALI shall be evaluated for their continued eligibility to donate plasma-containing components.

In addition to deferring donors implicated in TRALI, several donor strategies have been proposed to reduce the likelihood of first episodes of TRALI, but no one strategy has emerged as optimal. Proposed strategies include the collection of plasma only from male donors; deferral of multiparous women from donating plasma; and testing of multi-parous donors for antibodies, with donors found to be antibody positive deferred from donating plasma-containing components.

The relatively high per-unit risk and the fact that TRALI represented 13% of all transfusion-related fatalities reported to the FDA from 1992 to 2000 have resulted in heightened interest in methods to reduce the risk of TRALI. In November 2006, the ABBB published Association Bulletin #06-07, which recommended that donor centers implement interventions to limit the collection of high plasma-volume components from donors known to have leukocyte antibodies and those at increased risk of leukocyte alloimmunization. Noting that results from the Serious Hazards of Transfusion (SHOT) system showed a five- to sevenfold higher risk of TRALI with components having high volumes of plasma, the Bulletin primarily addressed plasma (including FFP, Plasma Cryoprecipitate Reduced, and Plasma Frozen within 24 hours After Phlebotomy), apheresis platelets, buffy-coat-derived platelets suspended in the plasma from a single donor in the pool, and whole blood. Not specifically addressed were lower risk products, such as RBCs, cryoprecipitate, and whole-blood-derived platelet concentrates. The Bulletin did not endorse any one strategy, but discussed a number of alternatives, including the ones listed in the preceding paragraph, as well as taking a lifetime transfusion history from all donors, male and female, and either excluding or testing those with a history of transfusion. Other alternatives mentioned were use of pooled solvent/detergent (SD)-treated plasma and storage of platelets in platelet additive solution rather than plasma. However, there are no FDA-approved SD plasma products or licensed platelet additive solutions available in the United States at the moment.

Typically, no special measures are required for further transfusions to the recipient experiencing an episode of TRALI. Repeat episodes of TRALI are rare and appear to be



Components with high volumes of plasma have a higher risk of TRALI.



Blood centers should implement interventions to eliminate the collection of high plasma-volume components from donors known to have leukocyte antibodies and those at increased risk of leukocyte alloimmunization.



Repeat episodes of TRALI are rare.

related to unusual instances in which the recipient has the antibody and the donor's white cells provide the antigenic target with which the antibody reacts. TRALI resulting from recipient antibody has always been an infrequent occurrence, and most cases have been associated with non-leukocyte-reduced units. Now that universal leukocyte reduction is practiced by many donor centers, the likelihood that recipient antibody will cause TRALI, much less a repeat episode of TRALI, is even lower.

As we learn more about the two-event hypothesis, we may some day be able to predict recipients who are more likely to experience TRALI. Some authorities have suggested that because RBCs less than 2 weeks old and platelets less than 3 days old may contain lipid levels that are insufficient to trigger a second event, fresher units, washed RBCs, or both might be considered for high-risk patients. However, given that risk factors for the development of TRALI still have to be definitively established, such a recommendation is clearly not the standard of care at present and is mentioned here merely as a portent of potential developments.

Transfusion-Associated Graft-vs-Host Disease



Transfusion-associated GVHD is caused by the engraftment of viable donor lymphocytes contained in cellular blood components. It has also been seen, albeit rarely, with fresh plasma but not with FFP.

Transfusion-associated graft-vs-host disease (TA-GVHD) results from the engraftment of viable lymphocytes contained in cellular blood components transfused to certain immunocompromised patients; it is almost always fatal. Once engrafted, the donor lymphocytes recognize the recipient-host as foreign and set about destroying the perceived interloper. Patients most at risk for TA-GVHD are marrow and peripheral blood progenitor cell transplant patients; premature neonates, especially recipients of intrauterine transfusions; patients receiving blood components from close relatives; and patients receiving fludarabine or other purine antimetabolites.

Theoretically, TA-GVHD can occur with the transfusion of any cellular blood component, but there have been no reported cases with frozen plasma. There have been rare cases reported with fresh plasma (the fresher the product, the more viable the lymphocytes), however.

The symptoms of TA-GVHD are seen primarily in organs with the highest turnover rate: skin, liver, marrow, and gastrointestinal tract. A generalized erythematous rash (often with desquamation), severe diarrhea, abnormalities of liver function, and pancytopenia are the hallmarks of TA-GVHD. There is no known treatment for TA-GVHD.

and, unlike the GVHD associated with marrow or peripheral blood progenitor cell transplantation, TA-GVHD is almost always fatal. In the transplantation setting, the marrow is derived from the engrafted donor and is not recognized as foreign, whereas in TA-GVHD, the engrafted donor lymphocytes see the recipient's marrow as foreign. Thus, TA-GVHD results in marrow aplasia, but the GVHD seen in marrow transplantation does not result in destruction of the marrow because it is donor derived.

TA-GVHD is prevented by gamma irradiation of cellular blood components before transfusion and patients at risk should receive irradiated blood components. Irradiation shortens the life span of RBC units to 28 days from the date of irradiation. Shortened outdate is usually not a problem for large transfusion services that have their own irradiator and can irradiate units immediately before release, but it does preclude routine irradiation of all blood components by the donor center at the time of collection. Leukocyte reduction is not effective in preventing TA-GVHD and should not be used for that purpose.

Marrow and peripheral blood progenitor cell transplants should never be irradiated because irradiation will prevent engraftment of the transplant. Granulocyte transfusions, on the other hand, should always be irradiated because they are given to profoundly immunosuppressed (severely neutropenic) patients and they obviously contain large numbers of viable lymphocytes.

Indications for irradiation of cellular blood components are discussed in more detail in Chapter 8: Indications for Transfusion.

Septic Transfusion Reaction (Bacterial Contamination)

Septic transfusion reactions are caused by the transfusion of bacterially contaminated blood components. Bacteria can enter a blood component as the result of unrecognized asymptomatic bacteremia in the donor, from the introduction of skin flora during phlebotomy, or by the entry of contaminants during processing. Bacterial contamination of blood components accounted for 16% of transfusion associated fatalities reported to FDA from 1986 to 1991. Nearly three-fourths of these fatalities were due to contaminated platelet units.

Platelet units are far more likely to be contaminated than are RBC units, probably because platelet units are stored at room temperature, whereas RBC units are refrigerated. Before the 2003 implementation of a requirement



Irradiation of blood components prevents GVHD by destroying the ability of lymphocytes to replicate and engraft. Leukocyte reduction *cannot* be used in substitution for irradiation in the prevention of GVHD.



Marrow and peripheral blood progenitor cell transplants cannot be irradiated because irradiation will prevent the desired engraftment of transplanted cells.



Bacterial contamination of blood components accounted for 16% of transfusion-associated fatalities reported to the FDA from 1986 to 1991. Nearly three-fourths of those fatalities were caused by contaminated platelet units.



Gram-negative bacterial contamination is often more severe than gram-positive because of production of endotoxin during storage.

RBC units tend to contain gram-negative bacteria, whereas platelets contain gram-positive bacteria. Either can cause fatal sepsis.



Autologous blood provides no protection against septic transfusion reactions.



When Gram's stain and culture are performed, the sample tested should come from the returned bag, not from a retained segment. Cultures taken from segments can be false negative as a result of sampling error.

that blood banks or transfusion services use methods to limit and detect bacterial contamination in all platelet components, it was estimated that between 1 in 1000 and 1 in 3000 platelet units were contaminated with bacteria. The effect of testing in reducing this figure is not yet known.

Contaminated RBC units are more likely to contain gram-negative organisms and, thus, are typically associated with more severe sepsis, possibly even with fatal gram-negative septic shock. Such reactions are caused by gram-negative endotoxin that is produced and stored in the unit and transfused intravenously into the recipient along with the blood.

Platelet units, in contrast, are more often contaminated with gram-positive organisms; thus, a smaller percentage of contaminated units result in overt sepsis. However, septic shock and death can occur with the transfusion of contaminated platelet units just as they can with contaminated RBC units. Mortality rates for platelet-associated sepsis are around 25%, whereas reported mortality rates for red-cell-associated sepsis are closer to 70%.

Thus, when one looks at fatality rates based on which component is contaminated, a smaller percentage of contaminated platelet transfusions are fatal because there are so many more contaminated platelet units than there are contaminated RBC units. However, if one looks instead at actual fatalities, a larger proportion of fatalities (72%) are caused by platelet sepsis.

Autologous blood affords no advantage over allogeneic transfusion with respect to septic reactions.

See Chapter 2: Blood Component Storage for information on new developments in contamination testing and storage of platelets.

As one might expect, the signs and symptoms of septic transfusion reactions include fever (often poorly responsive to antipyretics), chills, rigors, and shock. Any temperature rise of 1 to 2 C or more over the pretransfusion baseline should be evaluated for the possibility of a septic reaction.

One of the reasons the implicated unit must always be returned to the transfusion service in the case of a suspected transfusion reaction is that the bag itself must be cultured when a septic reaction is possible. Culture of retained segments from the unit may give false-negative results because of the small volume contained therein, which can result in sampling error.

In the case of a possible septic reaction, a Gram's stain should be performed at the same time the unit is cultured. A positive Gram's stain can help guide the choice of anti-

biotics for therapy, but a negative Gram's stain by no means rules out the possibility of a septic reaction. In cases of possible septic transfusion reactions, culture of the implicated unit(s) should always be performed, even if the Gram's stain is negative. In addition, it is advisable to culture the recipient at the same time in order to facilitate interpretation of a positive bag culture because, even in the best of hands, blood cultures may have a false-positivity (contamination) rate as high as 3%.

Possible septic transfusion reactions should be treated as one would treat any septic episode, with prompt initiation of appropriate antibiotic therapy guided by the results of the Gram's stain if positive, and with pressor support in the event of frank septic shock.

Just as crucial as prompt treatment for the recipient is immediate notification of the supplier of the implicated product when investigation of a febrile response suggests that a septic reaction is possible. Such notification enables the supplier to promptly recall and quarantine any additional outstanding components from that same collection. If contamination is subsequently confirmed, the quarantined components must be destroyed.

Hypotension Associated with Bedside Leukocyte Reduction Filters (Primary Hypotensive Reaction)

In addition to the well-established transfusion reactions delineated above, a small number of cases (less than 100 to date) of severe hypotension have been reported in conjunction with bedside leukocyte reduction by filtration. Initially, those episodes were attributed to the use of negatively charged filters in patients concomitantly receiving angiotensin-converting enzyme (ACE) inhibitors. Such a conclusion was based on the theory that negatively charged filters may result in the generation of bradykinin, the degradation of which is compromised by ACE inhibitors.

However, analysis of cases reported by the FDA has led to doubt about that conclusion because hypotensive episodes have also been reported in association with positively charged filters as well as in patients not receiving ACE inhibitors. Almost no cases have been reported in conjunction with prestorage leukocyte-reduced blood components, perhaps because bradykinin generated during filtration is degraded during subsequent storage.

In any event, all persons who order or administer blood should be aware of that potential complication. In addition



Possible septic reactions should be treated with prompt initiation of broad-spectrum antibiotic therapy, guided by the results of Gram's stain on the unit, if possible.



Rare episodes of isolated hypotension have been reported when bedside leukocyte reduction filters are used. These episodes usually respond rapidly to discontinuation of the transfusion.

to severe hypotension, a number of other symptoms may be seen, including respiratory distress, flushing, nausea, abdominal pain, and loss of consciousness. Whenever a severe hypotensive episode occurs within an hour of transfusion of a blood component through a bedside leukocyte reduction filter, such a reaction should be suspected; the transfusion should be stopped immediately and the transfusion service notified. Usually, symptoms resolve rapidly on discontinuation of the transfusion.

The use of prestorage leukocyte-reduced blood components instead of bedside filtration will likely reduce the risk of such hypotensive reactions, now termed "primary hypotensive reactions" by some. In certain cases, avoidance of ACE inhibitors in the 24 hours before transfusion or the use of washed blood components might be prudent.



Posttransfusion purpura (PTP) manifests as rapidly falling platelet counts and purpuric skin lesions occurring in the first 3 weeks after transfusion of red cells or platelets.

Posttransfusion Purpura

Posttransfusion purpura (PTP) is a rare disorder characterized by severe thrombocytopenia occurring in the first 3 weeks (range = 1-24 days; mean = 9 days) after transfusion in a patient with prior allogeneic exposure through transfusion or pregnancy. Sensitized by the earlier transfusion or pregnancy, the recipient mounts a brisk anamnestic response directed against the high-frequency platelet antigen HPA-1a (formerly known as PL^{A1}). Occasionally, platelet antigens other than HPA-1a have been implicated in PTP. Any platelet-containing blood component can be associated with the development of PTP in a sensitized recipient, and rare cases have been reported after transfusion of FFP.

Because the antibody is an alloantibody, one would expect the patient's own platelets to be spared, but this expectation appears to be false. Instead, the antibody paradoxically behaves as if it were an autoantibody, destroying both the transfused HPA-1a-positive platelets and the patient's own HPA-1a-negative platelets. In one recent case report, the anti-HPA-1a was detected in the patient's plasma—and it could be eluted off the patient's own antigen-negative platelets. After incubation of the patient's serum with antigen-negative and antigen-positive platelets, the same antibody was found in eluates from both the antigen-negative and the antigen-positive test platelets.

The mechanism by which destruction of the patient's antigen-negative platelets occurs is poorly understood, but one hypothesis is that the HPA-1a antigen is adsorbed onto the recipient's own platelets from plasma in the transfused unit. This hypothesis, as well as the theoretical risk of fur-



PTP is caused by anamnestic production of platelet-specific antibody, often directed against the high-incidence HPA-1 platelet antigen.

ther stimulating the patient's immune response, has led to the recommendation that plasma-containing transfusions should be avoided in patients with PTP and that, should red cells be required, they be washed to remove soluble platelet antigens and residual platelet membrane fragments.

Other suggested mechanisms for PTP include 1) adherence of immune complexes to antigen-negative platelets, resulting in accelerated clearance in the reticuloendothelial system or 2) production, early in the immune response, of autoantibodies or cross-reacting alloantibodies targeted against autologous platelet determinants.

Regardless of the pathogenesis of the phenomenon, PTP is associated with platelet counts below 10,000/ μ L in 80% of cases and, if untreated, may result in thrombocytopenia that persists for several weeks. Although PTP is self-limited, mortality rates in the range of 10% to 15% have been reported. IVIG, corticosteroids, and combined therapy with IVIG and corticosteroids have been successfully used in the treatment of PTP. Plasmapheresis to remove the offending antibody is generally reserved for those patients who fail to respond to other therapeutic interventions.

Because the patient is destroying autologous HPA-1a-negative platelets, one would predict that transfused HPA-1a-negative platelets would also be destroyed rapidly; this outcome is often the case. Although conventional wisdom holds that platelet transfusions are ineffective in PTP, at least one case report suggests that transfusion of antigen-negative platelets may be efficacious in emergent situations, such as intracranial hemorrhage. Furthermore, HPA-1a-negative platelets have been successfully transfused in conjunction with IVIG in severely thrombocytopenic PTP patients.

Table 7-5 contains a summary of the preceding transfusion reactions. Table 7-6 describes the risk estimates for noninfectious complications of transfusion.



The antibody involved in PTP is an alloantibody, but it behaves like an autoantibody, destroying autologous as well as allogeneic platelets.



Intravenous immunoglobulin followed by the transfusion of antigen-negative allogeneic platelets may be helpful in severely thrombocytopenic PTP patients.



Hepatitis A, B, and C are all transfusion-transmissible, but only hepatitis B and C cause chronic hepatitis.

Vaccines exist for HAV and HBV but not for HCV.

Transfusion-Transmitted Diseases

Several infectious agents—viruses, bacteria, and parasites—are transmissible by blood transfusion, at least in theory. However, most such transmissions result in transient, self-limited illnesses without chronic sequelae and will not be discussed in this chapter, except for enumerating them in Table 7-7. This chapter focuses primarily on

Table 7-5. Transfusion Reaction Summary

Type	Usual Cause	Signs/Symptoms	Action	Prevention
Hemolytic, intravascular (acute or delayed)	Typically, ABO or certain other blood group incompatibilities. Recipient's antibodies react with antigens on donor red cells, fully activate complement, and lyse RBCs. Other causes: use of improperly stored or warmed blood.	Hemoglobinuria and hemoglobinuria, fever, chills, chest pain, dyspnea, flank pain, facial flushing, anxiety, shock, oliguria, anuria, burning at site of infusion, DIC (signs/symptoms occur immediately or within several hours of transfusion for acute reactions or typically after 3-10 days for delayed reactions).	STOP TRANSFUSION, maintain IV, notify MD and blood bank, monitor VS, strict &O, support blood pressure and renal function. MD may order furosemide, mannitol, and/or renal-sparing doses of dopamine.	Ensure proper identification of patient, blood sample, and blood component. Monitor patient during transfusion and infuse blood slowly for first 25-50 mL if at all possible.
Hemolytic, extravascular (acute or delayed)	Blood group incompatibility, where antibodies to RBC antigens do not fully activate complement.	Fever, jaundice, elevated indirect bilirubin, falling hematocrit (usually 3-10 days after transfusion for delayed reactions or within several hours for acute reactions).	No acute treatment generally required. MD may order antipyretics. If severe, same action as above. Notify MD and blood bank of any reaction.	Patient must receive anti-gm-negative and cross-match-compatible RBCs in the future.

Febrile, nonhemolytic	Either 1) antibodies in recipient's circulation directed against leukocytes or platelets in donor blood components or 2) cytokines present in plasma/ supernatant portion of stored components.	Chills, fever ($\geq 1^\circ\text{C}$ increase in body temperature); rigors in severe reactions.	STOP TRANSFUSION, notify MD and blood bank, maintain IV, monitor VS. MD may order antipyretics. (Aspirin is contraindicated for thrombocytopenic/thrombocytopathic patients.) Exclude septic reaction by obtaining Gram's stain and culture on unit.	Take a careful history of any previous reaction. Premedicate with antipyretics. Consider leukocyte-reduced RBCs, especially if two previous reactions.
Allergic	Presumed allergy to a soluble substance in donor plasma.	Localized or generalized urticaria (hives). If severe, may have laryngeal or facial edema and hypotension (see anaphylaxis below).	HOLD TRANSFUSION, notify MD, monitor VS. MD may order oral antihistamines. MD may order restart of transfusion if mild urticaria clears and no other symptoms are present.	Premedicate with antihistamines (or corticosteroid for refractory cases). Consider washed RBCs for repeated or severe reactions.
Anaphylactoid	Recipient antibodies to a soluble substance in donor plasma, especially infusion of plasma with IgA into IgA-deficient recipient with anti-IgA.	Frank anaphylaxis (laryngospasm and severe respiratory distress with or without shock). Coughing, nausea, vomiting, abdominal pain, diarrhea, possible loss of consciousness variably present.	STOP TRANSFUSION, maintain IV. Notify MD and blood bank. Epinephrine STAT, antihistamines. Monitor VS, O ₂ , and IV corticosteroids may be required.	Sensitized IgA-deficient patients with a prior reaction must be transfused blood components that lack IgA.

(continued)

Table 7-5. Transfusion Reaction Summary (continued)

Type	Usual Cause	Signs/Symptoms	Action	Prevention
Septic	Bacterial contamination of blood component. Can occur with autologous units as well as allogeneic blood components.	Fever, chills, hypotension, shock, hemoglobinuria, DIC, renal failure.	STOP TRANSFUSION, maintain IV, notify MD and blood bank, monitor VS. MD may give antipyretics, treat shock, and give broad spectrum IV antibiotics until the organism is identified; collect blood cultures.	Infuse all blood components within 4 hours; inspect components for clots, clumps, discoloration, or hemolysis.
Circulatory overload	Blood volume too large or infusion too fast for compromised cardiovascular system.	Dyspnea, orthopnea, peripheral edema, systolic hypertension, cardiomegaly and pulmonary congestion on chest x-ray (compare with TRALI below).	Slow or stop transfusion, keep IV open. Notify MD, monitor VS and I&O. MD may order diuretics and oxygen. Consider comparing pretransfusion BNP with posttransfusion BNP (BNP >100 pg/ml with 1.5-fold increase suggests volume overload).	If due to recipient antibody, transfuse leukocyte-reduced blood components.
Transfusion-related acute lung injury (TRALI)	Either 1) donor leukocyte antibodies, 2) rarely recipient has leukocyte antibody, or 3) lipid activators of neutrophils in the donor plasma.	Dyspnea, pulmonary edema, normal cardiac pressures. Pulmonary vascular congestion and/or frank pulmonary edema on chest x-ray but without cardiomegaly.	STOP TRANSFUSION, notify MD and blood bank. Give oxygen. Support respiration (intubation may be necessary).	If donor antibody, donor should not donate plasma-containing products in the future.

Posttransfusion purpura	Antibody to platelet antigen in antigen-negative recipient. This alloantibody behaves like an autoantibody, destroying autologous platelets as well.	Severe thrombocytopenia about 1 week after transfusion.	Consult hematologist and clinical pathologist.	Avoid transfusion. If transfusion unavoidable, transfuse blood component from donors lacking implicated antigen or, alternatively, wash RBC unit to remove soluble antigen in plasma accompanying unit. Consider IVIg. Consider plasmapheresis, especially in refractory cases.
Graft-vs-host disease	Infusion of immunocompetent donor lymphocytes in an immunosuppressed recipient; an immunocompetent recipient shares HLA haplotype with an HLA-homozygous donor.	Fever, skin rash (often desquamative), hepatitis, diarrhea, marrow suppression, infection; high mortality.	Consult hematologist/oncologist and clinical pathologist.	Gamma-irradiate cellular blood components for immunosuppressed recipients including neonates. Irradiate all cellular components from HLA-matched or related (directed) donors.

RBCs = red cells; DIC = disseminated intravascular coagulation; VS = vital signs; I&O = input and output; BNP = B-type natriuretic peptide.
 (Courtesy Gary Stack, MD, PhD, with modifications.)

Table 7-6. Noninfectious Risks of Transfusion*

	Estimated Risk
Serious	
Mistransfusion	1:14,000 to 1:19,000
ABO-incompatible transfusion	1:38,000
Death due to ABO-incompatible transfusion	1:1.8 million
Acute hemolytic transfusion reaction	1:12,000
Delayed hemolytic transfusion reaction	1:4000 to 1:12,000
Transfusion-related acute lung injury	1:2000 to 1:5000 (5-10% fatal)
Anaphylaxis	1:20,000 to 1:47,000 1:150,000 1:1600 (platelets) 1:23,000 (RBCs)
Graft-vs-host disease	1:1 million (Canada)
Posttransfusion purpura	1:143,000 to 1:294,000
Fluid overload	1:708 to 1:3200 1:7000 to 1:15,000
Less Serious	
Febrile nonhemolytic transfusion reaction	1:500
Allergic (urticaria)	1:250

*Modified from Petrides M, AuBuchon JP. To transfuse or not to transfuse: An assessment of risks and benefits. In: Mintz PD, ed. *Transfusion therapy: Clinical principles and practice*. 2nd ed. Bethesda, MD: AABB Press, 2005:657-90.

those transfusion-transmitted diseases (TTDs) that result in chronic infection in the recipient of the contaminated blood or blood component. Bacterial contamination of blood components is discussed in the section on septic transfusion reactions earlier in this chapter.

Transfusion-Transmitted Hepatitis

Of the currently known hepatitis viruses, hepatitis A (HAV) and hepatitis E are primarily spread by the fecal-oral route, whereas hepatitis B (HBV), hepatitis C (HCV), and hepatitis D are primarily blood-borne and, thus, transfusion trans-

Table 7-7. Examples of Transfusion-Transmissible Diseases

Potentially Resulting in Chronic Disease	Resulting in Transient Illness without Long-Term Sequelae or in Asymptomatic Infection	Risk Exceedingly Low or Theoretical Only
Hepatitis B	Hepatitis A	Classical Creutzfeldt-Jakob disease (CJD)
Hepatitis C	Hepatitis G	Lyme disease
Human immunodeficiency virus	TT virus	Human herpesvirus-8
Human T-cell lymphotropic virus	Epstein-Barr virus	Parvovirus B19
Cytomegalovirus		Erlichiosis
Chagas' disease		Babesiosis
Syphilis		
vCJD (four reported transfusion-associated cases thus far)		

missible. Both HBV and HCV can also be transmitted by sexual contact, although that route of transmission appears to be less likely. Hepatitis D infection requires coinfection with HBV because the “delta agent” that causes hepatitis D infection cannot multiply in the absence of HBV. Vaccines are available for HAV and HBV but not for HCV.

Hepatitis A

Hepatitis A virus is transmitted primarily by fecal-oral contamination, but the virus is also transmissible by blood and blood components. HAV has no lipid envelope and thus is not inactivated by solvent/detergent treatment of pooled plasma derivatives (eg, coagulation factor concentrates). There have, in fact, been cases of HAV infection in hemophili A patients receiving human Factor VIII concentrates.

Typically, HAV infection presents with the classic symptoms of hepatitis (nausea, vomiting, jaundice, dark urine, and acholic stools) 2 to 6 weeks after infection, and those symptoms resolve without sequelae over the ensuing several weeks.

HAV infection alone is not associated with chronic hepatitis, making it far less of a concern to the transfusion recipient, than are HBV and HCV. Furthermore, seroconversion (production of measurable antibody against HAV) often coincides with—and may lag slightly behind—the



HAV infection alone is not associated with chronic hepatitis, making it far less of a concern to the transfusion recipient.

development of symptoms. As a consequence, donors are questioned about symptoms suggestive of hepatitis and about a history of hepatitis, but no serologic testing is performed for HAV in donors. Donors with a history of hepatitis before age 11 are most likely to have been infected with HAV and, therefore, are not excluded from donation, whereas donors with a history of viral hepatitis after the age of 11 are permanently excluded from donation of blood because HBV and HCV are more likely etiologies in older children and adults.

The most recent estimate of risk from HAV transmission through blood components is 1:10 million units.

Hepatitis B

Hepatitis B is a DNA virus of the hepadnavirus family. The classic symptoms of hepatitis, including nausea, vomiting, and jaundice, may be seen in HBV, but they are far more frequent in hepatitis A. In fact, many HBV infections (65%) are asymptomatic, and persons with subclinical HBV usually resolve the infection without long-term sequelae. Patients with subclinical HBV are relevant to transfusion medicine specialists only when their asymptomatic infection, either past or present, is detected on routine screening of blood donors. Although chronic hepatitis resulting in cirrhosis is possible with HBV infection, it is uncommon—unlike the situation with HCV infection.

When an individual is infected with HBV, there is an incubation period of approximately 8 weeks (range = 4–26 weeks) during which the infection will be asymptomatic and undetectable by serologic methods. Blood donation during this seronegative window period carries the potential of HBV infection in the recipient of blood or blood components from that donor. Transmission of HBV by pooled-plasma derivatives, such as coagulation factor concentrates and immunoglobulin preparations, is unlikely because those derivatives are subjected to virus inactivation either by heat or by solvent/detergent treatment.

A recombinant vaccine for hepatitis B is available and is commonly administered to health-care workers and other individuals at high risk of exposure to HBV. The vaccine results in the production of antibody against hepatitis B surface antigen (anti-HBs), which confers prolonged, although not necessarily lifelong, immunity to hepatitis B. Because a large number of potential donors have been vaccinated, anti-HBs is not used as a serologic marker for HBV infection in the donor population, although it may be



During the 8-week incubation period of HBV infection, patients may be asymptomatic and may lack any serologic evidence of infection. Nonetheless, blood donated during this “window” period may be capable of transmitting HBV.



Anti-HBs (surface antibody) cannot be used for screening because it is found in donors who have been vaccinated against HBV and would result in the unnecessary exclusion of a large number of safe blood donors.

of use in evaluating a recipient who develops clinical hepatitis following transfusion. The viral markers that are of use in screening donors for HBV are hepatitis B surface antigen (HBsAg) and antibody against hepatitis B core antigen (anti-HBc, also known as "anti-core").

Figure 7-2 illustrates the approximate timeline from infection with HBV to the development of symptoms, if any, and to the development of positive HBV marker studies. As is readily apparent from Fig 7-2, there is a seronegative window period of several weeks between infection with HBV and laboratory positivity during which an infected individual might still appear qualified to donate blood.

HBV risk in the setting of blood transfusion can arise in one of two ways: first, during the window-period donation by an infected donor before seroconversion and, second, through blood components collected from a chronic HBV carrier with undetectable levels of HBsAg.

The window period from infection to HBsAg positivity is 59 days, but chemiluminescence assays that are pending FDA approval would reduce this period by 9 days. Nucleic acid amplification testing (NAT) for HBV currently in investigational new drug (IND) evaluation would reduce this window period by an additional day, to 49 days when performed on minipools (MPs). However, if NAT were used to test donations individually, HBV infection could be detected 25 to 36 days earlier than currently licensed HBsAg tests allow, and NAT is more sensitive than HBsAg testing.

Unfortunately, NAT alone is no panacea. Because HBV levels may be very low late in the course of infection, sero-

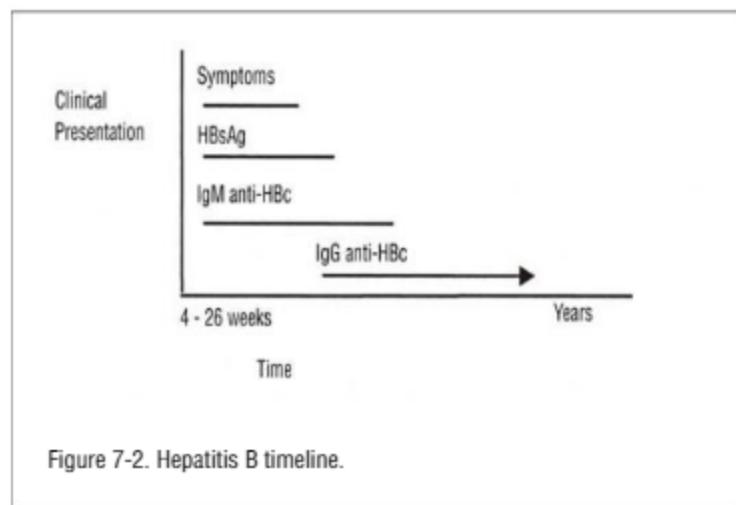


Donors are screened for HBs antigen and for anti-core (anti-HBc). The latter remains detectable for years after infection.



The window period from infection to HBsAg positivity is 59 days. This could be reduced considerably by using individual donation NAT testing.

However, HBV NAT might potentially miss some late-stage chronic carriers. Thus, implementation of HBV NAT remains optional and neither minipool nor individual donor NAT is yet regarded as an adequate substitute for either HBsAg or anti-HBc testing.



logic methods may be more effective than NAT for detecting chronic carriers. There is a 3% to 6% false negativity rate in individuals testing serologically positive (HBsAg positive, anti-HBc positive) when those samples are subjected to NAT, whether individual donor (ID) (3%) or MP (6%). As a consequence, implementation of HBV NAT remains optional, and the FDA has indicated that there are insufficient data for both MP and ID formats to allow replacement of either the HBsAg test or the anti-HBc test.

Despite the fact that anti-HBc is fraught with difficulty because of the lack of specificity of current screening tests (high false-positive rate) and the lack of a confirmatory test, "anti-core" testing remains a necessity, especially to detect chronic HBV carriers. Such testing is a necessity because chronic HBV carriers may be negative for HBsAg yet remain positive for anti-HBc.

Estimates of HBV residual risk range from 1:30,000 to 1:250,000 based on experience in the United States to 1:470,000 in France.

One intriguing question is why residual risk figures for HBV are so high compared to the one in one to two million figures for HIV and HCV, yet there have been few, if any, documented cases of transfusion-transmitted HBV. Such low numbers may be due in part to the fact that current estimates of residual risk are based on the full duration of the seronegative window period, but it is possible that a donor is infectious only during part of that window. Also, relatively few cases of HBV result in acute or chronic disease, meaning that subclinical cases may go unreported.

Hepatitis C

The causative organism of HCV is a genetically diverse RNA virus. The virus is spread primarily by the parenteral route (blood transfusion, intravenous drug use, occupational blood exposure from needlesticks) and occasionally by high-risk sexual exposure (multiple partners, history of sexually transmitted disease). In approximately 10% of cases, the route of infection cannot be determined. The vast majority (80-85%) of patients infected with HCV develop chronic hepatitis, and an estimated 20% of those infected develop cirrhosis over a period of two decades or longer. Furthermore, a percentage of those patients with cirrhosis caused by HCV subsequently develop hepatocellular carcinoma.

Until recently, pretransfusion detection of donors with HCV was problematic because most infected persons are



The majority (85%) of patients with HCV develop chronic hepatitis, but they may remain asymptomatic for years. A significant percentage will develop cirrhosis late in infection and some of them will also develop hepatocellular carcinoma.

asymptomatic until late in the disease when the stigmata of cirrhosis and liver failure develop. Disease progression in infected individuals is highly variable and transaminase levels [particularly alanine aminotransferase (ALT)] are notoriously unreliable at predicting which donors might be infected particularly because a third of patients with HCV infection will have persistently normal serum ALT levels. Although most patients with progressive disease will manifest some (albeit often mild) elevation of ALT, even this indicator is not invariably true. Thus, the only effective way of deciding whether an individual is infected with HCV is either by serology (detection of HCV antibody) or by NAT [detection of HCV by polymerase chain reaction (PCR)].

Anti-HCV testing of donors was introduced to the US blood supply in 1991, significantly reducing the risk of transmission. Serologic testing for HCV is far from perfect, however. First of all, there is a significant seronegative window period after infection with HCV (3-6 months), during which a donor can be infected without detectable anti-HCV. Furthermore, HCV infection is not always characterized by persistent antibody response, even in immunocompetent individuals, because HCV antibodies can disappear over time.

The application of NAT for HCV in the late 1990s was accompanied by a further decline in transmission risk, with current estimates of residual risk ranging between 1:1.9 million for repeat donors in the United States and 1:791,666 for first-time donors (2.4 times greater risk with first-time donors). The first NAT system to be licensed by the FDA for the detection of HCV was approved in 2001, and several others have subsequently achieved FDA licensure.

NAT can be performed for HCV and human immunodeficiency virus (HIV) individually or for both viruses simultaneously through the use of a multiplex HIV-1/HCV NAT. Testing is often performed on minipools of 16 to 24 donor samples. Pools that test positive are retested as single-donor samples to determine which of the multiple donors is positive. However, recent reports have shown that NAT is insufficiently sensitive to low viral loads, thus precluding the use of NAT to supplant antibody testing for either virus. This shortcoming is particularly true of MP NAT because of the dilution inherent in the pooling process. Even ID NAT will miss rare cases of HCV (or HIV-1), so, for the foreseeable future, it appears likely that antibody screening for those agents will continue to be required along with NAT.



ALT levels are unreliable for predicting which donors may be infected with HCV. A third of patients with HCV will have persistently normal serum ACT levels.



The seronegative window period for HCV is 3 to 6 months.



Screening of blood donors for anti-HCV has dramatically reduced the risk of transfusion-transmission of HCV. Estimates of current residual risk after screening range from 1:791,000 to 1:1.9 million in the United States.



The only reliable way to detect HCV infection is by either serologic testing or PCR testing (NAT). At the moment both are required, because even individual donation NAT, if used alone, would miss rare cases.

Initially, the only therapy for HCV was alpha-interferon, which resulted in early responses in 25% to 50% of treated patients but sustained responses in only 10% to 20%. Multidrug regimens using the combination of alpha-interferon and ribavirin have been tried with some success in patients who either failed to respond to interferon alone or who relapsed after an early response to interferon. In addition, a pegylated form of interferon has been developed. PEGylated interferon in combination with ribavirin has proved to be more effective than either standard interferon-ribavirin regimens or monotherapy with pegylated interferon alone. However, numerous unanswered questions remain concerning the type of regimen as well as the timing and duration of therapy.

Other Hepatitis Viruses

Hepatitis D and hepatitis G (HGV, also known as GBV-C) are both blood-borne viruses and potentially transmissible by transfusion. However, neither virus is regarded as having particular significance in transfusion medicine at this time.

Infection by hepatitis D requires coinfection with HBV because the causative "delta agent" cannot multiply in the absence of HBV. And HGV has not been convincingly associated with human disease and does not appear to cause non-A-E hepatitis. Although there is a high prevalence of HGV in patients with aplastic anemia, there is no evidence that the virus itself is the etiology of the disease.



Hepatitis G is transmissible by blood but is usually not associated with hepatitis, nor does it appear to worsen concurrent infection with HAV, HBV, or HCV.

Retroviral Infections



HTLV-I and -II are highly cell-associated, whereas HIV is transmissible not only by cellular blood components but also by plasma and by untreated plasma derivatives.

Transfusion-transmissible retroviral infections include HIV-1 and HIV-2 and human T-cell lymphotropic virus (HTLV-I and HTLV-II). HIV circulates as cell-free virions at some stages of infection; thus, HIV infection can be transmitted by plasma as well as by cellular components. Both HTLV-I and HTLV-II, in contrast, are highly cell associated, and there is evidence that viable lymphocytes may be required for the transmission of HTLV via transfusion.

Human Immunodeficiency Virus

HIV, the causative agent of AIDS, presents initially as a viremia of cell-free virus, which then infects CD4-positive T lymphocytes (T-helper cells). Eventually, the patient mounts an antibody response, and the infection enters a phase of clinical latency that can last for many years.

However, even during clinical latency, the virus continues to replicate and maintains a reservoir in tissue monocytes.

During the early viremic phase, infected individuals often develop nonspecific flulike symptoms—sore throat, cervical adenopathy, fever, and myalgias—that frequently go unrecognized. A period of asymptomatic latent infection lasting many years ensues, at the end of which viremia again rises, as does the proportion of infected lymphocytes. Eventually, a precipitous drop in CD4-positive T lymphocytes, resulting in the loss of T-helper cell function and impaired immunity, heralds the development of full-blown AIDS.

Death results either from opportunistic infection or from the complications of malignancies that arise in the setting of impaired immune surveillance, most notably Kaposi's sarcoma and central nervous system lymphoma.

Postinfection seroconversion produces detectable anti-HIV antibody at about 22 days. The window period during which a potential donor is infectious without detectable viral marker positivity has been reduced to about 10 days from the time of infection by NAT for HIV, using reverse transcriptase PCR (RT-PCR). The combination of laboratory testing for anti-HIV-1, anti-HIV-2, and HIV RNA by NAT and the questioning of donors about exposure and about potential high-risk behavior has reduced the residual risk of HIV infection to an estimated 1:2.13 million for repeat donors and 2.08 times that (1:1 million) for first-time donors in the United States.

The first NAT system to be licensed by the FDA for the detection of HIV-1 was approved in 2001; several others have subsequently achieved FDA licensure. It is also possible to perform multiplex NAT, which can simultaneously detect HIV-1 RNA and HCV RNA. In the United States, NAT, coupled with enzyme immunoassay (EIA) testing for anti-HIV antibody, has supplanted p24 antigen testing. Western blot is licensed as a confirmatory test for HIV detected serologically by EIA.

Initially, homosexuals and intravenous drug users were felt to be most at risk for developing HIV infection, although, in recent years, there has been a dramatic increase in the number of cases arising from heterosexual contact, from mother-to-child transmission at birth, and from breast milk. Before each donation, potential blood donors are questioned extensively about risk factors for HIV infection, even before a blood sample is screened for the presence of viral markers. Aggressive questioning of donors about risk factors, coupled with donor testing, has



Early in infection, HIV is present as cell-free virions. RT-PCR testing can detect the virus as early as 10 days after infection.



HIV antibody takes 3 weeks to reach detectable levels but remains detectable indefinitely. Thus, it can detect infection even after the initial viremic phase has passed.



Residual risk of HIV infection is estimated to be between 1:1 million and 1:2.13 million in the United States.



During the counseling of donors who screen positive for HIV, it must be borne in mind that only a small number of EIA repeat-reactive blood donors (10%) confirm positive on Western blot or other confirmatory HIV tests.



Blood donors with repeat-reactive EIA test results and indeterminate Western blot results are highly unlikely to exhibit true infection with HIV.



HTLV causes both adult T-cell leukemia/lymphoma and tropical spastic paraparesis, but the lifetime risk that an HTLV-positive person will develop either of them is very small (4% to 8%).

had a major effect in reducing the risk of viral transmission by transfusion not only for HIV but also for viral hepatitis.

In addition to being excluded from further donation (unless shown to be noninfected by subsequent testing), donors with repeat-reactive screening tests for HIV must be advised and counseled about the results. Likewise, it is mandatory that recipients who received potentially infectious units from prior donations by donors who later test positive for HIV be notified and counseled.

Given the requirements for donor counseling and look-back notification, it is worthwhile to review the significance of repeatedly reactive screening tests and the significance of indeterminate and positive confirmatory test results among low-risk donors. Among the donor population, only approximately 10% of those with EIA repeat-reactive test results are confirmed to be positive on Western blot or other confirmatory testing. Donors whose follow-up Western blot result is indeterminate are highly unlikely to exhibit true infection with HIV. In one study, follow-up testing (which included serology, HIV serum antigen testing, viral culture, and PCR) of 99 blood donors with repeat-reactive EIA tests and indeterminate Western blots failed to demonstrate any evidence of HIV-1 or HIV-2 infection. In a donor with no history of risk behavior, it is possible that a positive result on Western blot may be a false positive. Those statistics must be borne in mind by those who counsel repeat-reactive donors about the significance of their results and those who perform look-back notification of recipients.

Human T-Cell Lymphotropic Virus

HTLV-I causes adult T-cell leukemia/lymphoma (ATL) and HTLV-associated myelopathy (HAM), also known as tropical spastic paraparesis (TSP). HTLV-II has never consistently been associated with a human disease, but some studies have suggested that it may produce a myelopathy but not ATL. In addition to transmission by blood-borne contact, HTLV can be transmitted through sexual contact and from mother to child through breast milk. Risk factors for HTLV-I among blood donors are birth or sexual contact in areas endemic for the virus (Japan, the Caribbean, and sub-Saharan Africa); the risk factors for HTLV-II are IV drug use or sexual contact with an IV drug user.

The virus circulates as a provirus incorporated into the DNA of lymphocytes. No cases of transfusion-transmitted HTLV have been reported with noncellular components,

and it is possible that leukocyte reduction of cellular blood components may prevent HTLV transmission. Prolonged storage may also reduce the risk of transmission because no cases have been reported in blood products stored for more than 10 to 14 days. Exposure does not invariably result in infection. Recipients of HTLV-I/II-contaminated cellular blood components have been reported to become infected in only 20% to 63% of cases.

Infection with HTLV persists lifelong, but with extremely long latency periods (decades in the case of HTLV-I). The lifetime risk of an infected individual developing either ATL or HAM/TSP is extremely small, on the order of 4% to 8%, at least in regions where the virus is endemic, with the risk being less certain in other areas. Viral load may play a role in determining whether an individual infected with HTLV-I develops HAM because high levels of HTLV-I proviral DNA have been associated with the development of HAM. The mechanism remains uncertain but has been postulated to be either direct viral neurotoxicity or development of an autoimmune response that damages neural tissue. The association between high viral loads and development of ATL is less clear, but there have been a few studies suggesting such a relationship. The route of infection may affect viral load; one recent study found an association between high viral loads and a history of blood transfusion.

US donors are screened by EIA for both HTLV-I and HTLV-II. Just as the risk of developing symptomatic infection in those individuals truly infected is very low, the likelihood of a positive-screening EIA test confirming positive by Western blot or radioimmunoprecipitation assay (RIPA) is also low (13%) in the donor population, which is largely without risk factors for HTLV.

Residual risk of HTLV infection resulting from window-period donations by asymptomatic donors in the United States has been estimated to be 1:641,000. However, a recent analysis put the risk in Canada at a considerably lower level, one comparable to the residual risk of HIV infection, namely, 1:1.9 million. Reasons for this lower risk estimate include the implementation of universal leukocyte reduction in Canada, which can be expected to markedly decrease the viral load by removing infected leukocytes; lower prevalence figures for HTLV in Canada than in the United States; and the fact that evidence from look-back studies suggests that only one in three HTLV-contaminated cellular units transmits the virus.



Fewer than 13% of donors who are repeat-reactive on EIA screening for HTLV will confirm positive on confirmatory testing (Western blot or RIPA).



Influenza immunization can cause false-positive HTLV screening tests.

Other Viruses

Cytomegalovirus



CMV seropositivity rates in the United States range from 20% to 80%, depending on geographic locale.

Cytomegalovirus (CMV), a DNA virus of the herpesvirus family, is an obligate intracellular parasite that can be transmitted in the latent noninfectious state by carriage in leukocytes in cellular blood components. CMV is commonplace in nature and, not surprisingly, seropositivity rates for CMV in the general population range from 20% to 80%.

One area of ongoing debate over the past decade has been whether leukocyte reduction performed under quality-controlled conditions can produce cellular blood components that are equivalent to CMV-seronegative units in terms of safety. However, regardless of which method is used to render a cellular blood component "CMV-reduced-risk," there will nonetheless remain a small residual risk of transmission of the virus. Donors who test CMV-seronegative may be infected—in the window period before seroconversion. Even a properly leukocyte-reduced unit will contain some leukocytes that could conceivably harbor the virus.



CMV-seronegative cellular blood components and leukocyte-reduced components are both regarded as "CMV-reduced-risk" but neither is totally without risk.

A 2003 study showed that CMV DNA was not detectable in seronegative blood samples and was only rarely detectable in seropositive samples, suggesting that PCR testing for CMV will not improve the detection of potentially infectious blood products beyond the level already available through serologic testing. A 2005 study from Sweden suggested that some CMV-seronegative, CMV-DNA-positive individuals do produce antibodies against CMV, which can be detected in a modified enzyme-linked immunosorbent assay (ELISA) test—one using clinical isolate antigens rather than antigens from the laboratory strain AD169. In any event, such improved antibody testing, even if clinically feasible, is not commercially available at this time.

In 2001, the majority of a consensus panel discussing the prevention of transfusion-associated CMV in the era of universal leukocyte reduction in Canada recommended that patients at risk for CMV infection continue to receive both leukocyte-reduced and CMV-seronegative blood components. The rationale was that there might be an added benefit to serologic testing of leukocyte-reduced components in those patients at high risk and that there was no evidence to prove that abandoning serologic testing would not result in a clinically significant, albeit small, incremental increase in risk.

A literature review and meta-analysis of controlled trials on the subject published in 2005 came to similar conclusions. That review confirmed that both CMV-seronegative and leukocyte-reduced components markedly decreased the risk of CMV infection (more than a 90% reduction in risk for each intervention). However, in a head-to-head comparison, meta-analysis of the data found that CMV-seronegative components were associated with a 58% lower risk than those that were leukocyte reduced. That finding supports the conclusion that CMV-sero-negative components are more efficacious than leukocyte-reduced components at preventing CMV infection. On the basis of that analysis, the author concluded that in areas where universal leukocyte reduction is not practiced, CMV-seronegative components should be provided to patients at risk from CMV. In countries where universal leukocyte reduction has been implemented, CMV-seronegative components are preferable to CMV-untested components. Studies incorporated in that analysis were performed exclusively in the setting of marrow transplantation, so the generalizability of those conclusions to other settings has not been established.

Viral exposure obviously determines whether an individual becomes infected with CMV, but host factors play a crucial role in determining whether a particular recipient develops symptomatic CMV infection. Most immunocompetent hosts infected with CMV are asymptomatic, but newborn infants and profoundly immunocompromised patients are more likely to develop symptomatic CMV infection.

Congenital CMV infection may be associated with deafness and neurologic impairment of the neonate after birth, whereas newborns infected after birth may exhibit respiratory depression, hepatosplenomegaly, and lymphocytosis. Full-term neonates appear to be at less risk from postnatal CMV infection than are premature newborns.

Transplant recipients and HIV-positive patients are also particularly susceptible to developing symptomatic infection with CMV. In the case of transplant patients, particularly those undergoing marrow transplant, CMV pneumonitis is the primary concern, followed by hepatic and renal infection. Because reactivation of latent infection is a significant risk in such a setting, prevention of CMV infection is important not only in the posttransplant period (prevention of newly acquired infection) but also before transplant (reducing the risk of disease reactivation). Most HIV-infected patients are CMV seropositive but are nonetheless



A meta-analysis of controlled trials published in 2005 concluded that CMV seronegative cellular components are more efficacious than leukocyte-reduced components at preventing CMV infection.



Most immunocompetent persons infected with CMV are asymptomatic but neonates and profoundly immunocompromised patients are more likely to develop symptomatic CMV infection.



Patients at increased risk for CMV infection include:
Neonates, especially if premature
Profoundly immunocompromised patients (especially transplant recipients and HIV patients)

at risk for morbidity and mortality associated with CMV chorioretinitis, encephalitis, and enteritis.

West Nile Virus

West Nile virus (WNV) represents a relatively new concern in the field of transfusion medicine. The first documented cases of transmission of WNV by transfusion and by organ transplantation occurred in 2002. The causative organism is an arthropod-borne virus transmitted through mosquito bites in birds and appearing in humans as an incidental host. Infection with WNV manifests as encephalitis, meningitis, and, very rarely, poliomyelitis-like asymmetrical flaccid paralysis. The precise pathogenesis of WNV infection is still being studied, but recent work suggests that monocyte-macrophages may play a role in initial WNV replication and subsequent propagation in transfusion-transmitted WNV infection.

The vast majority (80%) of humans infected with WNV are asymptomatic, but approximately 20% develop non-specific flu-like symptoms such as fever, headache, myalgias, gastrointestinal complaints, eye pain, rash, and lymphadenopathy. A small number of infected individuals (1:150) develop more severe infection, and neuroinvasive disease occurs in 1:256 infected individuals. Infection proves fatal in a small percentage (4-14%) of individuals with severe disease. Immunocompromised patients, infants, and the elderly are particularly at risk for severe manifestations of WNV infection.

The incubation period between the time of infection and the development of symptoms lasts 2 days to 2 weeks, with viremia arising 1 to 3 days after the bite and lasting for an estimated 6.5 to 56 days. Unfortunately, routine serologic testing used for the diagnosis of WNV infection is of no use in screening blood donors because viremia has already receded or even disappeared by the time IgM antibodies against WNV become detectable by ELISA testing (Day 8 or later). Furthermore, the mere presence of IgM antibody, although indicating exposure at some time in the past, is indicative of acute infection only if accompanied by compatible symptomatology.

As a consequence, in June 2003, blood collection facilities in the United States began testing donated blood by NAT in minipools of between 6 and 16 donations, under the FDA's IND program. Reactive pools are resolved by ID NAT. In December 2005, the FDA approved the first NAT



West Nile viremia can last for up to 56 days following the infected mosquito bite. However, serologic testing is of no use in screening blood donors because by the time IgM WNV antibodies become detectable at day 8, viremia has already subsided.



Donor testing by NAT for WNV is mandated during endemic periods. In addition, donors with symptoms suggestive of WNV at the time of collection are deferred for 120 days.

for WNV, although several other tests are still being evaluated under IND protocols.

Initially, the FDA had recommended that donors be deferred if they reported a history of headache with fever in the week before donation, but this recommendation has been withdrawn. At present, in addition to deferral based on reactive NAT results, donors are also deferred for 120 days if they have symptoms suggestive of illness caused by WNV at the time of collection or within 2 weeks after donation. Serologic detection of IgM antibody is cause for deferral only in the presence of a WNV-compatible illness in the preceding 2 weeks.

The residual risk of transfusion-transmitted WNV after implementation of those measures (donor questioning and NAT) is unknown. However, it appears likely that MP testing will not completely eliminate the risk of WNV transmission. Indeed, during 2003, NAT detected more than 1000 cases of WNV in US blood donors who were deferred as a consequence, yet six cases of transfusion-transmitted WNV were nonetheless reported. A recent Canadian study that followed 14 asymptomatic WNV NAT-positive donors found two donors with prolonged, very low-level viremia resulting in false-negative follow-up NAT results. The follow-up tests on those two donors, who were seropositive for both IgG and IgM antibodies against WNV, demonstrated only the persistent presence of WNV by NAT when many replicate samples were tested. Thus, it appears that donors with low-level WNV viremia may have false-negative results sometimes when screened by NAT.

Although it is not feasible to cease collection of whole blood during WNV outbreaks, some collection facilities have chosen not to process frozen products during those intervals. Others have implemented voluntary withdrawal of frozen products from areas where the virus is endemic, substituting instead frozen products collected at other times or in other locations. Derivatives are most likely not at risk for carrying WNV because the virus is inactivated by heat and by solvent/detergent treatment.

Human Herpesvirus-8

Human herpesvirus-8 (HHV-8) is a transfusion-transmissible virus associated with Kaposi's sarcoma. HHV-8 infection is rarely a problem in immunocompetent individuals, who are unlikely to develop Kaposi's sarcoma unless they become immunosuppressed.



Donors with low-level WNV viremia may sometimes have false-negative results when screened by NAT.



Pooled-plasma derivatives are unlikely to present WNV risk because WNV is inactivated by heat and by solvent/detergent treatment.

Parasitic Infections

Malaria



Transfusion-transmission of malaria is rare in North America. Only 93 cases were reported in the United States from 1963 to 1999. However, 11% of those cases proved fatal.

Transfusion transmission of malaria is rare in North America. Only 93 cases were reported in the United States from 1963 through 1999, although 11% of those cases resulted in fatality. Most cases of transfusion-related malaria arose from red cell transfusions, but 6% of the 93 cases were caused by platelet units, presumably from parasite-infested red cells contained in the platelet unit.

Laboratory screening of US donors for malaria is not feasible at this time, but potential donors are questioned about travels or residence in endemic areas as well as their history of malaria. However, EIA tests for antibodies against *Plasmodium falciparum* and *Plasmodium vivax* and PCR tests are being investigated, primarily for use in endemic areas. In France, immunofluorescence antibody (IFA) assays have been used widely in donor screening. In a 2002 study, IFA detection was coupled with a dipstick antigen assay to improve sensitivity to 88%. Donors implicated in transfusion-transmitted malaria often exhibit very low levels of parasitemia (as few as 1 to 10 parasites in a unit of blood), thereby complicating the use of antigen-detection methods, even PCR.

It is unlikely that routine testing of all donors would be cost-effective in nonendemic areas, but some transfusion medicine specialists have proposed that targeted testing could be used to evaluate donors who would otherwise be deferred on the basis of their travel history.

The risk of acquiring malaria from blood transfusion in the United States and Canada is estimated at 1:4 million.



Laboratory screening of US donors for malaria is not feasible at this time. However, EIA and PCR tests are being investigated.



Blood donors in Mexico have a prevalence of Chagas' disease of 1 in 133.

Chagas' Disease

Chagas' disease is caused by *Trypanosoma cruzi*, a protozoan parasite that is transmitted by the bite of an infected reduviid bug. *T. cruzi* is endemic in Central and South America and in Mexico and large numbers of infected donors have been reported in these areas. The prevalence of Chagas' disease among blood donors in Mexico was recently shown to be 1 in 133, which is consistent with earlier figures from Brazil. Transfusion transmission of Chagas' disease is made possible by the fact that *T. cruzi* infection is a lifelong illness and most chronically infected individuals are asymptomatic. Transfusion transmission is

considered to be most likely with whole blood or platelets, because these components have the highest transmission efficiency.

The risk of transmission of Chagas' disease by blood transfusion in North America is considered to be extremely low, with only seven transfusion-transmitted cases having been reported since 1980—primarily in immunocompromised hosts and associated with platelet units. However, a 2006 study of the transmission of Chagas' disease by transfusion in Mexico may have implications for risk in areas north of the Mexican border. The study, which involved screening donors with Chagas IgG ELISA tests and confirming positive results by RIPA, found a prevalence rate of 1 in 133 donors tested. Because earlier studies reported a 13% to 26% risk of transmission per unit of contaminated blood transfused, the authors concluded that this 0.75% prevalence rate indicated a significant risk to transfusion recipients. Indeed, four of nine recipients of blood products from RIPA-positive donors were in turn infected with Chagas' disease. This corresponds to an estimated 1800 new cases of Chagas' disease in Mexico that are attributable to transfusion annually.

In the United States, donors are questioned about a history of Chagas' disease, but donor testing has not been performed routinely until recently because there was no FDA-approved screening test for *T. cruzi*. Despite the low overall risk in the United States and Canada, some donor centers located in areas with large numbers of immigrants from Central and South America have been voluntarily questioning donors and testing those identified to be at high risk for specific antibodies to *T. cruzi*. In Los Angeles from June 1993 to October 1995, 1131 of 3320 (39.5%) allogeneic donors were defined as being at risk for Chagas' disease based on questionnaire responses. When these donors were tested serologically, seven demonstrated EIA positivity and six of these were also positive by confirmatory RIPA testing. Thus, while the risk of Chagas' disease is low in the United States overall, the risk is probably higher in certain geographic regions.

In mid-December 2006, FDA approved a whole cell lysate ELISA test for *T. cruzi* and following this approval, a number of additional donor centers began testing all donations for Chagas' disease. Such testing is not yet mandated by the FDA, which has indicated its intention to mandate donor testing once a licensed test kit becomes available.



Whole blood and platelets appear to have the highest transmission efficiency for Chagas' disease.



Donors are questioned about history of Chagas' disease but donor testing has not been performed routinely because no FDA-licensed screening test existed until December 2006.



In December 2006, the FDA approved a whole cell lysate ELISA screening test for Chagas' disease. Screening for Chagas' disease is not yet mandated but probably will be in the near future. Many blood centers have indicated that their institution will begin Chagas' testing on a voluntary basis.

Other Organisms

Syphilis

Treponema pallidum, the causative organism of syphilis, does not survive refrigerated storage for more than 96 hours and survives only a few days to a few weeks at -10°C to -20°C, the temperature range at which most frozen blood products are stored. Therefore, although it is possible for a donor with a negative screening serologic test for syphilis to be harboring live spirochetes at the time of donation, there have been only two cases of transfusion-transmitted syphilis in the English literature since 1950. The risk of transfusion transmission of syphilis would be very low if no testing were performed and is virtually nonexistent with the use of serologic screening tests.

Creutzfeldt-Jakob Disease



Classic CJD is a rare fatal disease associated with progressive dementia. It can be sporadic, familial, or, rarely, transmitted by an apparently infectious route.



The causative agent of CJD appears to be an abnormal isoform of a cellular protein involved in neural synaptic transmission. This abnormal protein, termed a prion, is resistant to protease degradation.

Cases of apparent infectious transmission of classic CJD have been reported with growth hormone, dura mater, or corneal transplants and with intracerebral electrodes. No blood-borne transmission has been reported to date.

Creutzfeldt-Jakob disease (CJD) is a rare, fatal, degenerative neurologic disease characterized symptomatically by progressive dementia and pathologically by spongiform degeneration with neuronal loss in the absence of inflammation. CJD occurs in one of four forms: sporadic, transmitted, familial, and the recently described variant form (vCJD). CJD is characterized by the presence of the prion, an abnormal isoform (PrP^{SC}) of a normal cellular protein (PrP^{C}). The normal prion protein (PrP^{C}) is readily degraded by cellular proteases, whereas PrP^{SC} resists protease degradation. PrP^{C} (the normal form) appears to play a role in synaptic transmission.

The vast majority of classic CJD is the sporadic form, the mechanism for which remains unknown. Familial cases are caused by mutations in the *PRNP* gene that result in conformational changes, which in turn favor production of the abnormal prion form (PrP^{SC}) in middle age to later life.

In rare instances, transmission of classic CJD has arisen through an apparently infectious route by direct inoculation of tissue derived from the central nervous system (CNS) and associated with exposure to pituitary-derived human growth hormone, to cornea or dura mater transplants, or to reused intracerebral electrodes. Latency periods of greater than 30 years have been reported in such cases of iatrogenic CJD. The hypothesized basis for the transmission of CJD is that the normal protein (PrP^{C}) assumes an abnormal conformation that causes normal prion proteins to convert to abnormal forms. Experimental data with PrP^{C} -null mice suggest that the normal PrP^{C} form

must be present for signs or symptoms to develop and for abnormal prions to propagate; PrP^C-null mice do not develop disease even when inoculated with mouse-specific PrP^{SC}.

Experimental rodent models of infectivity have suggested that cellular blood components and even, to a lesser degree, plasma and plasma fractions (derivatives) might present a risk, albeit minimal, for transfusion transmission of classic CJD. However, to date, there have been no cases in which transfusion has been implicated in the transmission of classic CJD. Indeed, a number of case-control, look-back, and autopsy studies have failed to find a link between receipt of blood components and classic CJD. As a consequence, there is a growing consensus that the transmission of classic CJD by blood transfusion is unlikely.

Variant CJD appears to be a different matter. vCJD was first reported in the United Kingdom in 1996 and appears to be caused by the same agent (likely a prion) that causes bovine spongiform encephalopathy (BSE). vCJD differs from classic CJD in a number of ways, including the fact that it occurs in younger patients, is more likely to be associated with behavioral changes and ataxia, and tends to have a more acute course, progressing rapidly to death within 2 years. vCJD is distinguishable pathologically from classic CJD by the presence of large aggregates of prion protein surrounded by spongiform vacuoles, which are aggregates not seen in classic CJD.

More important from the perspective of transfusion medicine is the fact that vCJD is known to have spread from cattle to humans, presumably by ingestion. Furthermore, prion protein can be demonstrated in high concentration in the lymphoid tissue of vCJD patients, although data concerning the distribution of vCJD in human peripheral blood are still lacking. Those observations have raised concern that vCJD may be transmissible by blood transfusion and, thus, have resulted in a mandate for universal leukocyte reduction in the United Kingdom and Canada. This mandate has been controversial elsewhere, however. In the United States, for example, the vast majority of the members of the FDA's Transmissible Spongiform Encephalopathy Advisory Committee felt that leukocyte reduction was not indicated for reducing the risk of vCJD transmission, citing a lack of sufficient evidence that leukocyte reduction would significantly reduce the infectivity of blood, given that plasma-based prions can pass through current technology filters.



Transmission of classic CJD by blood transfusion is unlikely.



Variant CJD occurs in younger persons than does classic CJD and has a more acute course, progressing to death within 2 years.



Detection of prion protein in lymphoid tissues of patients with vCJD has raised the possibility of transmissibility by blood, especially circulating lymphocytes.



Four cases of transfusion-associated vCJD have been reported in the United Kingdom to date.

Until 2003, there were no reported cases of transfusion-transmitted vCJD but since then there have been three cases discovered in the United Kingdom and reported in the medical literature, all of them involving non-leukocyte-reduced RBCs. A fourth case was reported in the lay press in the UK in January 2007. Twenty-three additional recipients of blood products from donors who subsequently were diagnosed with vCJD remain alive and at risk. To date (early 2007), no vCJD cases have been associated with plasma products, but a draft assessment compiled by the FDA Transmissible Spongiform Encephalopathies Advisory Committee in December 2006 of the risk of vCJD transmission by human-source Factor VIII concentrates concluded that "while the risk is estimated to be very low, it may not be zero." Furthermore, in the United States, which had been BSE-free until 2003, at least one BSE-infected cow has been discovered in Washington state.

Several steps have been taken to reduce the risk of transmission of CJD and vCJD in the United States, primarily in the form of more restrictive donor deferral criteria. Donors at particular risk of exposure because of travel or residency in areas with BSE epidemics are deferred, as are donors who have received pituitary-derived human growth hormone, bovine insulin since 1980, or dura mater transplants. Also deferred are donors with blood relatives diagnosed with CJD unless the donor can be demonstrated by genetic testing to lack abnormal prion genes. In the United Kingdom, donors who have received blood transfusions in the United Kingdom since 1980 are deferred. In addition, plasma collected in the United States is used in the production of plasma derivatives in the United Kingdom, and patients in the United Kingdom who were born after January 1, 1996 are treated with US plasma as a precautionary measure.

In addition to the steps taken to defer donors with potential risk of CJD/vCJD infectivity, efforts are under way to develop screening tests capable of detecting CJD in blood components before issue, as well as methods to remove CJD infectivity from blood units by filtration. The former is complicated by the fact that, compared with levels of viruses like HIV or hepatitis, the concentration of PrP^{SC} in infected asymptomatic donors is likely to be very low, yet this low-level infectivity may still be sufficient to transmit disease because of the relatively large quantities of blood involved in transfusion. Another challenge will be differentiating normal from abnormal prion forms, given the similarities between the two.



Because no reliable screening test for vCJD exists at present, risk reduction measures in the United States hinge on donor deferral. In the United Kingdom and Canada, universal leukocyte reduction of cellular blood components is mandated.

Table 7-8. Infectious Risks of Transfusion*

Infectious Agent	Source of Estimate	
	Kleinman et al [†]	Other [‡]
HIV (with NAT)	1:4.7 million	1:2.1 million US—repeat donors 1:1 million US—first-time donors
HCV (with NAT)	1:3.1 million	1:1.9 million US—repeat donors 1:791,000 US—first-time donors
HTLV	1:1.9 million	1:641,000
HBV	1:31,000	1:30,000 to 250,000 US 1:470,000 France
Syphilis	Virtually nonexistent	
HAV	1:10 million	
Malaria	1:4 million	
Chagas' disease	Extremely low	1:133 donors tested in Mexico
CMV	Unknown	
WNV	Unknown	
Bacterial contamination		
Platelets [§]		1:1000 to 1:3000 US 1:14,000 to 1:38,000 France
Platelet fatality [§]		1:140,000
RBCs	1:5 million US 1:66,000 NZ	1:172,000 France
RBC fatality		1:1 million France 1:8 million US

*Modified from Petrides M, AuBuchon JP. To transfuse or not to transfuse: An assessment of risks and benefits. In: Mintz PD, ed. *Transfusion therapy: Clinical principles and practice*. 2nd ed. Bethesda, MD: AABB Press, 2005:657-90.

[†]Kleinman S, Chan P, Robillard P. Risks associated with transfusion of cellular blood components in Canada. *Transfus Med Rev* 2003;17:120-62.

[‡]For other data sources, see Petrides M, AuBuchon JP reference (above).

[§]Without pretransfusion culture of the component.

In terms of removal of infectivity from blood components, filtration appears to have more promise at the moment than pathogen inactivation. Current pathogen inactivation technologies target nucleic acids, which are obviously not present in prion proteins. Two companies are working on developing leukocyte reduction filters with the added capability to remove prions. However, it is too early to assess their efficacy, particularly in the absence of a test for CJD infectivity in human blood components.

Table 7-8 provides a summary of the estimated infectious disease risks associated with transfusion.

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8

Indications for Transfusion

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HEIGHTENED CONCERN ABOUT TRANSMISSION of infectious diseases has generated considerable interest in the appropriate use of blood components. Continued concern about emerging pathogens, the role of HLA alloimmunization in the development of platelet refractoriness, and the possibility that transfusion-related immunomodulation could result in recipient immunosuppression all highlight the importance of avoiding unnecessary exposure to allogeneic blood. The most reasonable guideline of all is to avoid arbitrary transfusion "triggers" and, instead, to regard blood and blood components the same as any other medication: to be used only when necessary and then at the lowest effective dose and frequency.

However, many physicians rely on consensus guidelines and "practice parameters" when making transfusion decisions. As with all of medicine, blood banking is in a state of constant flux with new information continually challenging established practice. Thus, this chapter begins with a review of current consensus guidelines then turns to a discussion of data collected since publication of those guidelines. Indications for special components, such as leukocyte-reduced and irradiated cellular blood components, follow.

One must remember, however, that those guidelines—and much of the data on which they are based—all address the *prophylactic* use of blood components, that is,



Blood components should be treated like any other medication:

Used only when truly needed AND
In the minimal effective dose.

transfusion in an attempt to forestall morbidity. In the case of *therapeutic* use, patients who do not meet the criteria set forth below may nonetheless require transfusion. Likewise, those guidelines are neither absolute indications nor absolute contraindications for blood component usage. In all cases, physician decision-making should be based on the patient's entire clinical presentation, not on a particular consensus document.

Red Blood Cells

In general, Red Blood Cells (RBCs) should be used only when there is a need for improved oxygen-carrying capacity and should not be transfused to increase colloid osmotic pressure, to be a volume expander, to be a substitute for iron or B12 supplements, to improve wound healing, or to enhance a patient's subjective sense of well-being. The one exception to this rule involves patients with sickle cell anemia. In such cases, RBC transfusions are indicated to reduce the concentration of hemoglobin S in the circulation as a means of decreasing viscosity and preventing the consequences of sickle cell occlusive episodes. Chapter 11: Special Transfusion Situations discusses transfusion in sickle cell disease at length.

Despite general agreement with the concept that RBC transfusions are indicated only for improved oxygenation, definition of hemoglobin (or hematocrit) levels that represent thresholds for RBC transfusion remains elusive. For many years, physicians relied on the so-called 10/30 rule, meaning that they would transfuse whenever the hemoglobin level decreased below 10 g/dL or the hematocrit below 30%. However, those figures, which are derived from combat experience with otherwise healthy soldiers, are not well supported by scientific evidence. In fact, a 1988 National Institutes of Health (NIH) consensus panel failed to find good evidence to support the 10/30 rule, noting in particular the experimental data that showed that cardiac output does not increase dramatically until the hemoglobin level decreases below 7 g/dL in healthy humans.

Both the NIH consensus document and a guideline published by the American College of Physicians in 1992 stress that RBC transfusion should be based on clinical assessment of each patient as an individual and should take into account that individual's response to the level of



Red cell transfusion is indicated only when there is a need for increased oxygen-carrying capacity or to decrease percent hemoglobin S in sickle cell disease patients.



Cardiac output does not increase dramatically in healthy humans until the hemoglobin decreases below 7 g/dL.

anemia present. Thus, patients with coexisting cardiac disease, pulmonary disease, or both may require transfusion at a higher hemoglobin level than would an otherwise healthy 18-year-old trauma victim. Acutely anemic patients with concomitant volume depletion, such as those with anemia caused by acute hemorrhage, may well respond to volume expansion with crystalloid alone. Duration and speed of onset of anemia are also factors; it is well documented that chronically anemic patients tolerate lower hemoglobin levels than do patients who have not yet had time to adjust to their anemia.

Consensus guidelines are generally in agreement that transfusion to a hemoglobin level above 10 g/dL is rarely indicated but that even healthy patients may require transfusion if the hemoglobin level decreases much below 7 g/dL. Likewise, patients with symptomatic anemia who do not respond to volume replacement require transfusion, especially if they have comorbid conditions such as coronary artery disease, cerebrovascular disease, peripheral vascular disease, or underlying pulmonary disease (such as chronic obstructive pulmonary disease). However, prophylactic transfusion of asymptomatic patients whose hemoglobin levels fall between 7 and 10 g/dL remains an area of controversy, particularly in the preoperative and postoperative periods.

What are the scientific data to support those consensus guidelines? Experimental data in healthy animals and human volunteers all point to 7 g/dL as a reasonable hemoglobin threshold in most cases. Data from studies in dogs and baboons have shown that normal animals can tolerate hemoglobin levels as low as 3 to 5 g/dL without adverse effects. In dogs with experimentally induced critical coronary artery stenosis, isovolemic reduction in hemoglobin was well tolerated until the hemoglobin level decreased below 7 to 7.5 g/dL. However, the dogs showed both ischemic EKG changes and diminution of cardiac function below that level. Likewise, healthy human volunteers subjected to isovolemic reduction in hemoglobin levels to as low as 5 g/dL showed no significant changes in oxygen consumption, although two of the 32 subjects did show transient ischemic changes on Holter monitoring. In another study involving human subjects, normal cognitive function was preserved until the hemoglobin level decreased below 7 g/dL. Subtle changes in cognition were observed at hemoglobin levels between 5 and 6 g/dL, and that dysfunction responded to autologous transfusion to levels above 7 g/dL.



Patients with acute anemia due to hemorrhage may respond to volume replacement alone and may not need red cell transfusion.



Transfusion to a hemoglobin above 10 g/dL is rarely indicated, but even healthy patients may require transfusion if the hemoglobin falls acutely to below 7 g/dL.

Data in human patients, however, yield a murkier picture. On the one hand, a retrospective cohort study of patients 60 years or older who were undergoing surgical procedures for hip fractures found that perioperative transfusion in patients with hemoglobin levels of 8 g/dL or higher did not influence the risk of mortality at 30 days or 60 days postoperatively. And, similarly, a prospective study of 2202 patients undergoing coronary artery bypass graft (CABG) surgery found that immediate postoperative hematocrit levels below 24% correlated with decreased risk of myocardial infarction (MI), whereas high hematocrits (34% or higher) were associated with increased MI risk.

On the other hand, several studies have reported a trend toward increased mortality in cardiac patients with hemoglobin levels below 9 to 9.5 g/dL. One of those, a large ($n = 6980$) study reported by DeFoe et al found increased risk of post-CABG death when nadir hematocrits decreased below 23%. A very large retrospective review by Wu et al of charts from nearly 80,000 Medicare patients who were admitted for acute MI found that transfusion at hematocrits below 30% was associated with a decreased odds ratio of death in the 30 days after admission. The latter study, however, suffers from a number of limitations, most notably the fact that it is based solely on a retrospective chart review.

To date, there has been only one large-scale, prospective randomized controlled trial comparing the effect of restrictive vs liberal transfusion regimens on morbidity and mortality—the Transfusion Requirements in Critical Care (TRICC) trial. In that study, 838 critical care patients were randomly assigned to either a restrictive transfusion group in which hemoglobin levels were maintained between 7 g/dL and 9 g/dL or a liberal transfusion arm in which hemoglobin levels were maintained between 10 g/dL and 12 g/dL. Patients in the restrictive arm did as well as those in the liberal arm with respect to 30-day survival and multiorgan dysfunction score (MODS). Indeed, in younger patients and less severely ill patients, mortality and MODS were significantly lower in the restrictive transfusion group. A subsequent subset analysis of 357 patients with cardiovascular disease in the TRICC study yielded similar findings: the restrictive group demonstrated no increase in mortality, no increase in complication rates (including new MI), and no difference in measures of multisystem dysfunction. Finally, a similarly styled secondary analysis of mechanically ventilated patients showed no difference between the



One size does not fit all:
Healthy patients can tolerate hemoglobin levels of 7 to 8 g/dL.
Patients at risk for silent ischemia, or those with underlying vascular or pulmonary disease, may require hemoglobin levels as high as 10 g/dL.



The TRICC trial found that critical care patients maintained at a hemoglobin between 7 and 9 g/dL did equally well as patients maintained between 10 and 12 g/dL hemoglobin. This was true even in a subset analysis of patients with cardiovascular disease.

two groups either in total ventilator time or in difficulty weaning.

Although the TRICC trial would appear to have resolved the issue, the sheer number of patients in the study by Wu et al makes it impossible to disregard their findings, especially in light of the experimental data in dogs with critical coronary stenosis and the findings reported by DeFoe et al in CABG patients.

The conclusion from those data is that “one size” clearly does not “fit all.” An RBC transfusion threshold of 7 to 8 g/dL is likely preferable in patients without ischemic heart disease, whereas those at risk for ischemic cardiac complications, including patients with diabetes mellitus having an increased likelihood of silent ischemia, might well benefit from a more liberal regimen targeted at maintaining hemoglobin levels in the vicinity of 10 g/dL.

Platelets

Until the early 1990s, the most widely quoted trigger for prophylactic platelet transfusion was a platelet count of 20,000/ μ L. That figure dates back to a paper from 1962 by Gaydos et al that noted that gross hemorrhage rarely occurred at platelet counts above an arbitrarily selected level of 20,000/ μ L. Although that paper is often cited as supporting the 20,000/ μ L trigger, the authors specifically noted that, although it would have been desirable to be able to establish a threshold level above which hemorrhage was unlikely to occur, in their study “no ‘threshold’ platelet level was observed.” Furthermore, the Gaydos study was performed at a time when the deleterious effects of aspirin on platelet function had not yet been elucidated, meaning that thrombocytopenic patients were often treated with aspirin-containing medications. That fact alone suggests that a lower setpoint for prophylactic platelet transfusion might be feasible.

Indeed, several recent studies have reexamined the 20,000/ μ L trigger and have arrived at the conclusion that a prophylactic platelet threshold of 10,000/ μ L is safe in many settings. As with RBC transfusion, it is equally apparent that any threshold for prophylactic platelet transfusion must take into account comorbid conditions that might necessitate transfusion at higher platelet counts.

Theoretical support for a 10,000/ μ L threshold can be found in a 1985 study by Hanson and Schlichter that



The 20,000/ μ L platelet transfusion trigger is based on old data and may be invalid.



A platelet count of 10,000/ μ L may be sufficient prophylaxis in patients who are not bleeding and who do not have fever, sepsis, hypersplenism, coincident soluble coagulopathy, or an anatomic lesion predisposing to bleeding.

Patients with any of these comorbid conditions, however, may require higher platelet counts for prophylaxis.



A small fixed number of platelets (approximately 7100/ μ L) are removed from the circulation each day. This may explain why 10,000/ μ L counts suffice for routine prophylaxis.



Several studies in leukemia patients have demonstrated the safety of a platelet threshold of 10,000/ μ L in the absence of comorbidity such as coagulopathy, fever, or active bleeding. In two studies, transfusing at a lower threshold resulted in significant reduction in the number of platelet units transfused.

looked at the posttransfusion clearance of ^{51}Cr -labeled autologous platelets. They found that a small fixed number of platelets (approximately 7100/ μ L) are removed from the circulation each day, presumably as a result of consumption in the process of endothelial repair.

The first of the studies to reassess prophylactic platelet thresholds, published in 1991 by Gmur et al, examined bleeding in acute leukemia patients. In the study population, the authors found a platelet count of 5000/ μ L to be safe in the absence of bleeding or fever—the former because of consumptive loss and the latter because fever results in a hypermetabolic state even in the absence of consumption caused by sepsis. In the presence of bleeding or fever, a platelet count of 10,000/ μ L appeared sufficient, whereas maintaining a platelet count of 20,000/ μ L was justified in the face of coincident coagulopathy, including heparin therapy, and in patients with anatomic lesions predisposing to bleeding.

Since the Gmur study, several other studies have confirmed the safety of a 10,000/ μ L-platelet transfusion threshold in patients with acute myelogenous leukemia (AML). The earliest of those was the Platelet Transfusion Trigger Trial (PTTT) conducted by the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto in 255 adult patients with AML. In that study, the liberally transfused group of patients received transfusions at a threshold of 20,000/ μ L, whereas the restrictive group received transfusions at a level of 10,000/ μ L except in the presence of fever, active bleeding, or invasive procedures, in which case the 20,000/ μ L trigger was applied. There was no significant difference between the two groups either in survival or in number of RBC units transfused, but the restrictive group received 21.5% fewer platelet transfusions ($p = 0.001$). However, the authors of that report cautioned against generalizing their findings to other clinical settings without further study.

Wandt et al came to similar conclusions when they compared eight centers using a 10,000/ μ L transfusion trigger with nine centers that transfused prophylactically at 20,000/ μ L. As in the PTTT, adjustments were made in the restrictive group for comorbid conditions such as coagulopathy or fever with rapid decline in platelet count, and the platelet count was maintained above 20,000/ μ L for biopsies and major bleeding episodes. Bleeding complications and number of RBC units transfused were comparable in the two groups, but, once again, the restrictive arm received significantly fewer platelet transfusions. Heckman

et al, looking at 78 patients undergoing induction chemotherapy for AML, found no statistically significant difference in number of bleeding episodes and number of RBC units transfused between patients who received a unit of platelets daily as prophylaxis at a platelet count of 10,000/ μL and those who received platelets at a more liberal level of 20,000/ μL .

A retrospective analysis at one institution compared 87 marrow transplant patients transfused at a time when the institutional platelet trigger was 20,000/ μL with 103 transplant patients transfused at a time when the trigger had been decreased to 10,000/ μL ; the study found no statistically significant difference between the two groups in relative risk of bleeding or of death from hemorrhage. Finally, a prospective but nonrandomized study looked at bleeding events, morbidity, and mortality in 98 chemotherapy patients transfused after implementation of a stringent prophylactic platelet transfusion policy at a hospital in the United Kingdom (10,000/ μL threshold for stable patients and 20,000/ μL in the presence of major bleeding or additional risk factors). The authors of that study concluded that a 10,000/ μL transfusion threshold was safe in the absence of fresh bleeding or other risk factors such as fever, sepsis, or disseminated intravascular coagulation (DIC). They did, however, caution that the presence of such additional risk factors was associated with significantly increased hemorrhagic risk.

So, although some physicians remain uncomfortable with stable patients having platelet counts below 20,000/ μL , there is a growing trend toward lowering the trigger for strictly prophylactic transfusion to 10,000/ μL . Higher levels may be required, however, when the use is not strictly prophylactic. For example, patients undergoing surgery or invasive procedures may require platelet counts as high as 50,000/ μL . In the setting of massive hemorrhage, extensive vascular injury, or both—particularly when platelet counts are rapidly falling—thresholds as high as 100,000/ μL may be appropriate.

Because the potential consequences of hemorrhage into the eye, the central nervous system, and the lung are so devastating, platelet counts of 100,000/ μL may be necessary in ophthalmic and neurosurgical patients, as well as in patients at risk for pulmonary hemorrhage.

One final indication for platelet transfusion is bleeding in patients with a known or suspected defect of platelet function, regardless of the (quantitative) platelet count. Congenital platelet function defects manifest in conditions



Prophylactic use of platelets is not the same as therapeutic use.



Patients with acute hemorrhage may require transfusion at platelet counts of 50,000/ μL . In massive hemorrhage or in patients at risk of bleeding into critical spaces (eye, lung, CNS), platelet counts as high as 100,000/ μL may be needed.



Patients with platelet dysfunction may require transfusion at platelet counts within the normal range.



If platelets are to be given to a patient with platelet dysfunction, the underlying cause of the dysfunction should be removed first—if possible (eg, platelet-toxic drugs such as aspirin should be discontinued and patients should be off cardiac bypass circuitry before platelets are transfused).

such as Glanzmann's thrombasthenia, whereas acquired platelet defects can be seen in cardiopulmonary bypass and in patients on extracorporeal membrane oxygenation therapy. A number of drugs, most notably aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), cause platelet dysfunction. The effect of NSAIDs is reversible, meaning that once the drug is removed, affected platelets return to normal function.

Aspirin, in contrast, irreversibly poisons the platelet's cyclooxygenase system, making it imperative to discontinue the drug before transfusing platelets. Although acetylsalicylic acid (ASA) has an irreversible effect on platelets, ASA is rapidly metabolized to salicylic acid, which is only a weak inhibitor of cyclooxygenase. ASA itself has a plasma half-life of only 15 to 20 minutes. Thus, if needed, platelet transfusion can be considered within a matter of hours after discontinuance of aspirin therapy—with the expectation that the transfused platelets will not suffer irreversible damage from the salicylate in the circulation at the time of transfusion. Indeed, aspirin-treated patients regain normal platelet function when as few as 20% of circulating platelets have not been exposed to aspirin.

Cardiothoracic surgery represents a special case because pump-induced platelet dysfunction can result in postoperative bleeding, even at relatively high platelet counts if the platelets in question were present in the circulation while the patient was on the bypass pump. Whether to routinely transfuse platelets after removal of a patient from the bypass pump or to transfuse only those patients with ongoing bleeding remains controversial. Clearly, however, if significant chest tube drainage continues after heparin reversal, platelet transfusion should be considered, even in the face of relatively normal (quantitative) platelet counts. Failure to respond to platelet transfusion in a cardiac surgery patient with normal soluble-coagulation system parameters [prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen level] should prompt investigation into surgical sources for the ongoing hemorrhage.

Management of bleeding during the perioperative period for cardiopulmonary bypass is in a nearly constant state of flux. First, there is the evolution of our understanding of the contribution of aspirin to bleeding in cardiac surgery—with some studies suggesting that aspirin is associated with increased blood loss and increased transfusion requirements and other studies failing to demonstrate this effect, even in repeat CABG procedures. Second, antifi-

brinolytic agents, particularly aprotinin, are being used more commonly in cardiac surgery. Those agents significantly decrease postoperative bleeding and transfusion requirements, and some cardiac bypass specialists have suggested that aprotinin may be more effective than platelets in facilitating postbypass hemostasis.

On the other side of the cardiac bypass equation is the increasing use of platelet inhibitors, such as clopidogrel, as well as GPIIb/IIIa inhibitors, to reduce thrombotic risk in patients with coronary artery disease. When patients taking those agents require emergent cardiac surgery, they are at increased risk of bleeding and may require prolonged (several days) platelet support. Optimal management of patients in the setting of cardiac surgery is under study by several large clinical trials networks.

It is important to note that uremic platelet dysfunction is not amenable to correction by platelet transfusion because transfused platelets become “uremic platelets” almost immediately after infusion. Uremic platelet dysfunction is discussed in more detail in Chapter 11: Special Transfusion Situations.



Patients receiving platelet inhibitors (such as clopidogrel) or GPIIb/IIIa inhibitors at the time of cardiothoracic surgery may require platelet transfusions for several days.



Uremic platelet dysfunction is not amenable to correction by platelet transfusion.

Fresh Frozen Plasma

Fresh Frozen Plasma (FFP) contains significant amounts of all of the coagulation proteins, both procoagulant and anticoagulant. Thus, FFP is indicated to correct deficiencies of coagulation factors for which no specific factor concentrates are available. Whenever possible, alternative therapies, such as vitamin K for reversal of warfarin effect, should be tried first. Factor concentrates are preferable to FFP in the case of single-factor deficiencies because such concentrates are all virus inactivated in some way and, thus, carry far less infectious disease risk than does FFP.

Published guidelines for the prophylactic use of FFP in patients scheduled for surgery or other invasive procedure generally focus on PT and/or aPTT values 1.5 times or more above the midpoint of the reference range for the test in question. Studies dating back to the 1950s and 1960s have demonstrated that certain surgical procedures (eg, cholecystectomy) could be safely performed on patients taking warfarin with PT values in the therapeutic range. Later studies likewise demonstrated no increase in bleeding after paracentesis and thoracentesis in patients with a PT or aPTT up to 1.5 to 2.0 times the midpoint of normal



FFP is generally indicated for prophylactic use only when the PT or PTT is 1.5 times the midpoint of normal or higher.



PT and aPTT are, at best, only crude prognosticators of bleeding risk and some studies even question whether they have any utility at all as predictors.

range. Another study found PT and aPTT to be good predictors of surgical bleeding in massively transfused trauma patients only when the PT or aPTT exceeded 1.5 times the control value.

Although the figure of 1.5 times midnormal is often quoted and appears in published guidelines for FFP use, one must remember that PT and aPTT are, at best, crude prognosticators of surgical bleeding. Some studies question whether they have utility at all as predictors. For example, in 1980, Ewe studied patients undergoing open liver biopsy, looking for parameters that correlated with prolonged liver bleeding time. The author found that none of the parameters examined, including PT, correlated with bleeding. A systematic literature review by Segal and Dzik published in 2005 addressed the issue of whether a prolonged PT/international normalized ratio (INR) correlates with the risk of bleeding at the time of invasive procedures; the study concluded that there are insufficient data to support the use of PT/INR to predict bleeding risk.



A PT of 1.5 times midnormal corresponds to an INR of 2.0, not an INR of 1.5.

Burns et al provide a possible explanation for the lack of correlation between mild to moderately prolonged PT/aPTT and increased risk of bleeding. When the authors measured PT or aPTT on two plasma samples, each 50% deficient in a single (different) coagulation factor, the test results were within the reference range. However, when the two 50%-deficient plasma samples were mixed, resulting in 75% levels of each factor, the PT and aPTT of the resulting sample were prolonged—likely reflecting the fact that the PT and aPTT are designed to be particularly sensitive to simultaneous deficiency of multiple factors.

One important caveat to remember with respect to PT values is that a PT of 1.5 times midnormal is *not* the same as an INR of 1.5. In actuality, an INR of 2.0 typically corresponds to a PT 1.5 times midnormal. This correspondence makes practical sense if one considers that target ranges for PT and INR in therapeutic warfarin use are 1.5 to 2.5 times normal for PT, which corresponds to a range of 2.0 to 3.0 for INR.

It is important to note that even when those guidelines are met, the clinical efficacy of prophylactic FFP remains in question. A recent systematic review of 57 randomized controlled trials (RCTs) involving the use of FFP for a variety of indications concluded that for most clinical situations there was insufficient RCT evidence to support or refute the efficacy of treatment with FFP. In response to the paucity of RCT studies examining the efficacy of FFP in patients with mildly prolonged PT/INR, the NIH Transfu-

sion Medicine/Hemostasis Clinical Trials Network has embarked on a large multicenter RCT designed to compare transfusion of FFP with no treatment in patients undergoing invasive hepatic procedures with a preprocedure INR of 1.3 to 1.9. That study is ongoing.

Two final points merit mention. First, FFP is not effective at correcting INRs that are only minimally elevated, largely because the correlation between PT or aPTT and coagulation factor levels is nonlinear, particularly at factor levels below 30% (which corresponds to mild-to-moderate prolongation of PT). Second, when the INR of FFP units was measured, INRs as high as 1.3 were noted—not surprising given that FFP is a biological product collected from clinically healthy donors, some of whom will have lower levels of coagulation factors than others. Thus, one cannot expect FFP to “normalize” only minimally prolonged INR values because the product itself might have a comparably “prolonged” INR, if one were measured on the contents of the bag. Indeed, a study published in 2006 found that when FFP was transfused to patients with INR measurements between 1.1 and 1.85, fewer than 1% of patients exhibited complete correction of INR and only 15% corrected halfway to normal.

In any event, if FFP is to be used, the timing and dose should be carefully considered. If correction of a markedly abnormal PT or aPTT is required before surgery, FFP should be given immediately before the patient is called to the operating room, not the night before. Several coagulation factors have very short half-lives, and if FFP is given 8 hours preoperatively, those factors will be largely gone from the circulation by the time surgery begins. In particular, Factor VII has a biologic half-life of 3 to 5 hours, meaning that very little Factor VII will remain after 8 hours.

Likewise, the dose should be based on the patient’s size, with a common rule of thumb being to begin by infusing 10 mL/kg of recipient body weight and then measuring posttransfusion PT, aPTT, or both 15 to 30 minutes after infusion. If substantial correction of the coagulopathy has not taken place, further FFP can be given. Because a unit of FFP has a volume of approximately 200 mL, an appropriate starting dose for a 70-kg patient would be 3 to 4 units. Patients with liver failure may require substantially higher starting doses because they are incapable of producing any coagulation proteins, most of which are produced in the liver. Some authorities recommend a starting dose of 20 mL/kg for liver failure patients.



FFP is not effective in correcting INRs that are only minimally elevated.



FFP should be transfused immediately before it will be needed because some factors (especially Factor VII) have very short in-vivo half-lives (3-5 hours).



An appropriate initial dose of FFP is 10 mL/kg. This dose should raise the factor levels by about 25% unless the patient is bleeding or consuming factor in some other way (eg, DIC).



Plasma exchange using FFP is the mainstay of treatment for TTP.

Beyond the correction of markedly prolonged PT, aPTT, or both in patients scheduled for surgery or invasive procedures and in bleeding patients with prolonged PT or aPTT, other indications for FFP are emergent reversal of warfarin therapy when time does not allow use of vitamin K; correction of dilutional coagulopathy in massively transfused patients; and replacement of coagulation regulatory proteins such as antithrombin, protein C, or protein S when specific concentrates are unavailable. Finally, therapeutic plasma exchange, with replacement of the patient's entire plasma volume by allogeneic plasma, is the mainstay of treatment for thrombotic thrombocytopenic purpura (TTP), which is discussed at length in Chapter 14: Therapeutic Apheresis.

Solvent/Detergent-Treated Plasma

At one time, solvent/detergent-treated plasma, a pooled plasma product that was virus inactivated with a solvent/detergent treatment method similar to that used in the manufacture of certain clotting factor concentrates, was available in the United States. However, the product has been voluntarily withdrawn from the market because of concerns that decreased concentrations of protein S and alpha₂-antiplasmin might result in adverse effects. An additional consideration was that the solvent/detergent method is not effective against viruses that lack a lipid envelope, meaning that the treatment is ineffective against viruses such as parvovirus B19 and hepatitis A.

Cryoprecipitated AHF



Cryo contains fibrinogen, vWF, Factor VIII, Factor XIII, and fibronectin but is most commonly used as a source of fibrinogen. Virus-inactivated alternatives exist for vWF and Factor VIII.

Cryoprecipitated antihemophilic factor (AHF) (commonly called cryo) is used primarily for replacement of fibrinogen. It may rarely be used, however, in treating von Willebrand disease when von Willebrand factor-containing concentrates such as Humate-P (ZLB Behring, King of Prussia, PA) and Alphanate (Grifols, Inc., Los Angeles, CA) are unavailable and when desmopressin (DDAVP) is either contraindicated (as in Type IIb von Willebrand disease or concomitant renal failure with creatinine clearance below 50 mL/minute) or ineffective (as in Type III von Willebrand disease).

When used for hypofibrinogenemia, cryo is indicated when the fibrinogen level decreases below 80 to 100 mg/dL, especially in the face of ongoing hemorrhage. That threshold comes from both practical laboratory considerations

and from clinical observations in massively transfused patients. For one thing, many PT and aPTT assays may exhibit prolongation when fibrinogen levels decrease below that level. More clinically relevant is a study from 1987 of bleeding risk in trauma patients that found the most sensitive laboratory predictors of microvascular bleeding were a fibrinogen level of less than 50 mg/dL and a platelet count of less than 50,000/ μ L.

Each unit of cryo contains 150 to 250 mg of fibrinogen in 5 to 15 mL of plasma. Generally, multiple units of cryo are thawed and pooled before issue by the transfusion service.

Although it is possible to calculate the required dose more precisely, a good rule of thumb is that it takes 14 units of cryo or its equivalent to raise the fibrinogen of a 70-kg patient by 100 mg/dL. Expressed slightly differently, each unit of cryo given in the absence of any other products such as FFP can be expected to raise the fibrinogen level of a 70-kg patient by somewhere between 7 and 8 mg/dL.

It must be recalled, however, that FFP also contains significant amounts of fibrinogen (2-4 mg/mL of FFP or the equivalent of 2 units of cryo/unit of FFP). The advantage to cryo over FFP in massive hemorrhage is that cryo allows infusion of large amounts of fibrinogen very rapidly. Ten units of pooled cryo contain about half the volume of a single unit of FFP. In the previous example, the 14 units of cryo required to produce a 100 mg/dL-increment in fibrinogen would have a volume of only 100 to 200 mL, whereas the equivalent amount (7 units) of FFP would have a volume of nearly 1.5 liters—and would take considerably longer to infuse.

In the past, cryo was used to make topical fibrin sealant for cardiothoracic surgery and neurosurgery, but commercially available, virus-inactivated fibrin sealants are now on the market and have largely obviated the need for cryo in such settings.

Table 8-1 contains a summary of the contents of common blood components, as well as the indications for their use and the expected posttransfusion response.



One unit of FFP contains the equivalent of 2 units of cryo—but in a much larger volume.



It takes approximately 14 cryo-equivalents to raise the fibrinogen of a 70-kg adult by 100 mg/dL. One unit of cryo increases fibrinogen by about 7 mg/dL in the same size individual.

Granulocytes

The granulocyte fraction of whole blood can be collected by apheresis for use in severely neutropenic patients (absolute neutrophil counts <500/ μ L) with bacterial sepsis that

Table 8-1. Transfusion Threshold

Component	Indications for Use	Suggested Transfusion Threshold	Notes
Red Blood Cells	<p>Restoration of oxygen-carrying capacity</p> <p>Reduction of percentage sicklable hemoglobin in SCD patients</p>	<p>Hb <7 g/dL or Hct <21% in healthy asymptomatic patients</p> <p>Range of 7-9 g/dL in asymptomatic patients with cardiopulmonary or cerebrovascular risk factors</p> <p>Transfusion to levels above 10 g/dL rarely indicated</p> <p>HbS <30% for stroke prevention See Chapter 11 for other SCD indications</p>	<p>Levels at upper end of range (9-10 g/dL) suggested by some for elderly patients at risk of silent ischemia, eg, those with diabetes mellitus.</p> <p>Infuse within 4 hours after removal from monitored platelet agitator.</p> <p>Administer slowly or in divided amounts in patients at risk of volume overload.</p>
Platelets	<p>Bleeding caused by thrombocytopenia or platelet dysfunction</p> <p>Prophylactic use in severe thrombocytopenia (usually <10,000/μL)</p>	<p>Prophylaxis in absence of any bleeding: <10,000/μL (range: <5000-20,000/μL)</p>	<p>In setting of significant hemorrhage <50,000/μL</p> <p>In patient bleeding or at risk of bleeding into closed space (CNS including around spinal cord, eye, lung) <100,000/μL</p>

Fresh Frozen Plasma (FFP)	Deficit of plasma coagulation factors with bleeding or risk of bleeding when specific factor concentrates are unavailable	Prophylactic use: PT or aPTT >1.5 times midnormal range* Therapeutic use: Bleeding patient with documented factor deficiency or prolonged PT or aPTT	Transfuse as close to the time FFP will be needed as possible because several factors have very short half-lives. (Factor VII half-life 3-5 hours)
Cryoprecipitated AHF (cryo)	Fibrinogen replacement in massive hemorrhage	Fibrinogen level <80-100 mg/dL in patient with ongoing bleeding Consider cryo at fibrinogen <125 mg/dL if patient has ongoing massive hemorrhage and hemostasis has not yet been achieved	Each unit of FFP contains the equivalent of 2 units of cryo.

*INR = 2.0 corresponds to PT 1.5 times midnormal.
SCD = sickle-cell disease; CNS = central nervous system; PT = prothrombin time; aPTT = partial thromboplastin time; Hb = hemoglobin; Hct = hematocrit.



Granulocyte transfusion may be indicated in patients with absolute neutrophil counts (ANC) <500/ μ L and bacterial or fungal sepsis unresponsive to appropriate antibiotic therapy.



Traditionally, increment in ANC has not been used to monitor granulocyte transfusions because, until recently, granulocyte infusions rarely resulted in any *measurable* rise in neutrophil count.

is unresponsive to appropriate combination antibiotic therapy or in severely neutropenic patients with disseminated fungal or yeast infections. The use of granulocyte transfusions in those settings, especially in fungal or yeast infections, remains controversial, however. In one literature review from the early 1990s, seven studies were examined. Only three of those studies showed overall benefit in infected neutropenic patients, whereas two studies demonstrated benefit only in specific subgroups; another two studies failed to demonstrate any benefit. However, the latter pair of studies used doses less than the currently mandated minimum of 1×10^{10} granulocytes per infusion.

One potential problem with studies of the efficacy of granulocyte transfusion may stem from inadequate dosing. Until recently, it was rare to be able to demonstrate any measurable increment in neutrophil count after granulocyte transfusion. However, in the past decade, improved donor mobilization techniques that combine granulocyte colony-stimulating factor (G-CSF) along with the more conventional corticosteroid mobilization regimen have resulted in products containing far higher granulocyte concentrations (between 4 and 8×10^{10} per bag). Those high-concentration products have, in turn, resulted in demonstrable posttransfusion neutrophil increments of 3000 to 4000/ μ L. Furthermore, in another study, doses at the high end of the range of granulocyte concentration resulted in posttransfusion increments in excess of 2500/ μ L, and patient neutrophil counts above 2500/ μ L were sustained until the next morning.

Given those encouraging developments, one might anticipate that higher concentration granulocyte preparations would be more clearly efficacious with respect to resolution of infection and patient survival, but, to date, the efficacy of even those high-dose granulocyte transfusions remains unclear. Indeed, one trial being considered for study by the recently established Transfusion Medicine/Hemostasis Clinical Trials Network of the National Heart, Lung, and Blood Institute (NHLBI) would address that topic.

In any event, once begun, granulocyte transfusions should be continued on a daily basis until the infection resolves or the absolute neutrophil count remains above 500/ μ L for 48 hours. Because granulocytes must be collected by apheresis on the day they are to be transfused and because the decision to begin granulocyte transfusions implies a commitment to continue them until the patient either improves or dies, most authorities would

not consider granulocyte therapy unless the patient is expected eventually to recover marrow function.

Because neutrophils undergo apoptosis rapidly after collection, granulocytes for transfusion should be infused as soon as possible, definitely within 24 hours of collection. Care must be taken to allow the greatest possible time interval between granulocyte infusion and doses of amphotericin-B because there have been reports of severe acute pulmonary reactions when the two were given concurrently.

Granulocyte components contain significant numbers of red cells and viable lymphocytes. Thus, granulocytes for transfusion must be ABO compatible (and, ideally, Rh compatible as well) with the recipient, and they should be irradiated to prevent development of graft-vs-host disease (GVHD). Irradiation is particularly important when HLA-matched products are used.

Obviously, leukocyte reduction filters must not be used for the transfusion of granulocytes.



Granulocyte transfusions must be ABO-compatible with the recipient, should be irradiated before use, and should not be infused through a leukocyte reduction filter.

Leukocyte-Reduced Components

Leukocytes in cellular blood components can be removed by a number of methods, most notably leukocyte reduction systems incorporated in apheresis collection devices or leukocyte reduction filters. Leukocyte reduction filters are charged (usually negatively charged) in a way that causes leukocytes to adhere to the filter while allowing desired blood components to flow freely. Cellular blood components can be filtered immediately after collection (prestorage leukocyte reduction) or at the time of transfusion (bedside filtration). Universal leukocyte reduction is mandated in the United Kingdom and Canada, largely in response to the perceived risk of variant Creutzfeldt-Jakob disease (vCJD), which is discussed in more detail in Chapter 7: Adverse Effects of Transfusion. However, that mandate is controversial; in the United States, most members of the Transmissible Spongiform Encephalopathy Advisory Committee of the Food and Drug Administration (FDA) have maintained that there is insufficient evidence that leukocyte reduction would reduce the infectivity of blood because plasma-based prions are able to pass through currently available filters.

Research is ongoing to develop filters that are effective at reducing vCJD infectivity, but proving efficacy will be



Universal leukocyte reductions of cellular blood components is mandated in the United Kingdom and Canada, but not in the United States. However, in many areas of the United States virtually all cellular components are leukocyte reduced before storage on a voluntary basis.



Indications for leukocyte reduction include:

- Reducing incidence of HLA alloimmunization and platelet refractoriness
- Reducing risk of CMV transmission
- Reducing incidence of febrile nonhemolytic reactions
- Possible reduction in TRIM (transfusion-related immunomodulation) effect



Leukocyte reduction before storage is more effective than bedside filtration.

problematic until reliable tests for vCJD infectivity in blood donors become available. Despite the controversy surrounding universal leukocyte reduction, in many areas of the United States, virtually all cellular blood components are leukocyte reduced before storage, with the primary exception being whole-blood-derived platelet concentrates, which are too costly to filter individually before pooling. However, late in 2005, the FDA approved a pre-storage pooling bag and test system combination that permits prestorage pooling of whole-blood-derived platelet concentrates, enabling a single test for bacterial contamination to be performed on the pool instead of six tests on individual concentrates.

Leukocyte reduction is used to prevent febrile transfusion reactions; to decrease the rate of alloimmunization to HLA antigens, along with the platelet refractoriness that HLA alloimmunization may engender in multitransfused recipients; to reduce the risk of transfusion-transmission of cytomegalovirus (CMV); and to reduce the immunomodulatory effects of blood transfusion.

Leukocytes and white cell fragments, as well as cytokines released by leukocytes during storage, are felt to be the cause of febrile nonhemolytic transfusion reactions. Prestorage leukocyte reduction has the advantage of removing leukocytes before they can release cytokines and, thus, is more effective than bedside filtration. Additionally, prestorage leukocyte reduction ensures better quality control over the filtration process because nursing personnel vary considerably in their degree of familiarity with the use of leukocyte reduction filters.

Leukocyte reduction can result in cellular blood components with reduced risk of transmitting CMV infection. In most normal individuals, CMV infection is self-limited and mild. However, in certain populations, most notably those with profound immunosuppression and those with HIV infection, CMV infection can cause significant morbidity and even mortality. CMV is an obligate intracellular pathogen; thus, removing leukocytes containing CMV is effective in reducing the risk of CMV infection. However, the relative efficacy of leukocyte reduction as compared with use of units from seronegative donors has been controversial. Even a properly leukocyte-reduced unit will contain some white cells that can harbor the virus. Moreover, some CMV-seronegative donors may be infected—but in the seronegative “window period”—and therefore CMV-negative components are not without risk either. Further complicating the matter is the possibility, at least theoret-

cally, for CMV infection to occur because of plasma viremia in “window-period” donors.

A recent meta-analysis by Vamvakas of controlled trials on the subject may have resolved the controversy. That study concluded that CMV-seronegative components are more efficacious at preventing CMV infection than are leukocyte-reduced components. CMV is discussed in more detail in Chapter 7: Adverse Effects of Transfusion.

Even more controversial is the role of leukocyte reduction in preventing transfusion-related immunomodulation (TRIM). The concern is that transfusion may result in immunosuppression, thereby increasing the risk of postoperative bacterial infection and perhaps even cancer recurrence. Although it is well known that transfusion improves the survival of renal allografts, thus lending some credence to the existence of a TRIM effect, the importance of TRIM remains uncertain and, thus, so does the efficacy of leukocyte reduction in reducing such an effect.

Finally, the generally held dogma is that hard-spun plasma should contain essentially no leukocytes, red cells, or platelets and that FFP, as a result, does not need to be leukocyte reduced; there have been some reports demonstrating significant leukocyte content in FFP units, however. In the future, more definitive data may result in rethinking the necessity of leukocyte reduction of FFP.



CMV-seronegative components are more efficacious at preventing CMV infection than are leukocyte-reduced components.

Irradiated Cellular Blood Components

Unlike the GVHD seen in marrow transplant recipients, transfusion-associated graft-vs-host disease (TA-GVHD) is almost always fatal. TA-GVHD is caused by engraftment of viable lymphocytes contained in donated blood when that blood is transfused into a recipient who is incapable of rejecting the donor cells. Persons who are especially at risk for TA-GVHD are profoundly immunocompromised patients (especially marrow or peripheral blood progenitor cell transplant recipients), premature neonates, and recipients of blood from first-degree relatives or from HLA-matched donors. Also at risk are patients receiving nucleoside analog drugs such as fludarabine. TA-GVHD is discussed in more detail in Chapter 7: Adverse Effects of Transfusion.

Gamma irradiation of cellular blood components with 2500 rads (2500 cGy) will prevent GVHD by rendering any lymphocytes incapable of mitosis and, thus, incapable



Irradiation renders lymphocytes incapable of mitosis and, thus, prevents engraftment and GVHD.

of engrafting. The only disadvantages to irradiating cells are a slightly shortened red cell outdate (28 days from irradiation) and the time and cost of the irradiator. Because irradiation is usually performed immediately before issue of the unit from the transfusion service, the shortened shelf life is generally of little practical concern. FFP has never been associated with TA-GVHD and need not be irradiated. Although most institutions irradiate cellular blood components only for patients considered to be at risk for TA-GVHD, some transfusion medicine specialists advocate universal irradiation of cellular components—arguing that some patients may not be recognized as being at risk until after nonirradiated components have been transfused.

One important caveat is that none of the current techniques used for leukocyte reduction (washing, leukocyte filtration, and leukocyte reduction during apheresis collection of blood components) are reliable at removing all potentially engraftable lymphocytes. Thus, leukocyte reduction is not an acceptable substitute for irradiation.



Washing and leukocyte reduction do not prevent GVHD. They are *not* acceptable substitutes for irradiation.

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9

Autologous Blood

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THIS CHAPTER COVERS AUTOLOGOUS BLOOD collection and transfusion—ie, the use of an individual's own blood or blood components for his or her transfusion needs. The goal of autologous transfusion is to eliminate the need for allogeneic transfusion as much as possible. The role of the transfusion specialist is to continually reassess the benefits, risks, and costs of autologous blood techniques as part of an overall strategy of blood conservation and management.

Most autologous blood programs are designed specifically to address surgical blood loss. Autologous blood can be collected at one or more stages in the perioperative period and by various techniques, including the following: 1) preoperative collection and storage of single units of Whole Blood (WB) or Red Blood Cells (RBCs) in the days to weeks before surgery, 2) collection of units of WB in the operating room immediately before surgery (acute normovolemic hemodilution) or during surgery (intraoperative recovery), and 3) postoperative collection of shed blood from wound drainage.

The decision to use autologous blood is best made after a thorough discussion between the patient and his or her physician about the following:

- Whether autologous blood use is indicated (Table 9-1).
- Which techniques might be considered.



Autologous blood use can be an important aspect of a facility's multidisciplinary, patient-centered approach to blood management.



It is important to remember that perioperative blood techniques should be implemented in conjunction with each other because few of them can achieve the desired results in isolation.

For each applicable technique, the benefits to the patient. For each technique, the risks to the patient.

Patients for whom autologous blood use elicits serious consideration are those patients for whom crossmatch-compatible blood is not easily obtainable—eg, patients with rare blood types and those with multiple red cell allo-antibodies.

Each facility considering offering autologous blood options must first analyze its institutional and regional resources, including the available technology and the trained personnel required, before determining the types of services that can be offered to patients. A strategy for autologous blood use is best managed with a team approach because multiple disciplines, hospital clinics, and often regional sites or facilities may be involved. Established lines of communication with all concerned are key to a successful program.

Preoperative Autologous Blood Donation

In preoperative autologous blood donation (PAD) programs, the patient donates his or her own blood, typically as single WB units, on multiple occasions at a hospital blood bank or blood center over a period of up to 5 to 6 weeks before surgery. This period corresponds to the allowable storage times of WB (up to 35 days) and Additive Solution RBCs (up to 42 days). The collection period may be longer if autologous RBC units are stored as glycerolized frozen units.



Many procedures likely to require transfusion (major orthopedic, vascular, cardiac, and thoracic surgeries) are nonemergent. The procedure can be scheduled far enough in advance to allow the patient sufficient time to donate the requested number of units.

Benefits

Theoretically, patients should achieve a net gain in total red cell mass available for surgery (circulating red cells plus donated red cells) by donating autologous blood. The overall gain in red cell mass is possible because the blood typically is collected over a period of weeks before surgery, allowing the patient's marrow time to at least partially replace the donated red cells. The amount of red cell replenishment constitutes the net gain in red cell mass.

A second and unintended benefit of autologous blood donation, ironically, is that the patient's hematocrit usually does not return to predonation levels before surgery, leading to normovolemic hemodilution. At the time of surgery, autologous patients generally have hematocrit levels that are below those of patients not making autologous dona-

Table 9-1. Autologous Blood Use for Specific Surgical Procedures*

Surgical Procedure	Preoperative Autologous Donation	Intraoperative Blood Recovery	Postoperative Blood Recovery	
Coronary artery bypass graft	+	+	+	+
Major vascular surgery	+	+	+	-
Primary hip replacement	+	+	+	+
Revision hip replacement	+	+	+	+
Total knee replacement	+	-	-	+
Major spine surgery with instrumentation	+	+	+	+
Selected neurologic procedures (eg, resection of arteriovenous formation)	+	+	+	-
Hepatic resection	+	+	+	-
Radical prostatectomy	+	+	+	-

*Adapted from National Heart, Lung, and Blood Institute Expert Panel on the Use of Autologous Blood. Transfusion alert: Use of autologous blood. Transfusion 1995;35:703-11.

+ indicates appropriate indication; - indicates inappropriate indication.

tions. Although plasma volume is fully restored within about 3 days of donation of a unit of blood, red cell replenishment takes several weeks. A patient who undergoes surgery before replenishment takes place will be hemodiluted at the time of surgery and will lose fewer red cells for any given volume of surgical blood loss. The reduction in loss of red cells becomes more significant when the operative blood loss is greater.

Through the combination of a modest gain in overall red cell mass and the modest reduction in red cell loss caused by hemodilution, the patient may achieve some decreased dependence on allogeneic RBC transfusion.

Limitations and Risks



Preoperative autologous donations typically result in anemia.

Also, the preoperative collection of autologous blood does not eliminate all risks of transfusion.

The perception on the part of the public and many physicians appears to be that PAD programs are more effective in avoiding allogeneic blood transfusion than they are. Perhaps a false sense of security is created by having several units of autologous blood available for a patient for surgery. What may be overlooked is that the autologous donations typically result in an anemia that increases the likelihood that the patient will require transfusion.

PAD programs are generally limited to supporting elective surgery, when at least several weeks are available to collect multiple units of blood. For more urgently needed procedures, too little time may be available to collect enough units to be of benefit. Accordingly, PAD is available to only a limited number of surgical patients.

Donating multiple units of autologous blood over the course of several weeks is time-consuming and inconvenient for patients and resource-intensive for the collecting facility. Thus, collecting autologous blood adds expense to medical care compared with the use of allogeneic blood. The added cost of autologous blood is exacerbated by the wastage of about 30% to 50% of all autologous units collected before surgery.

Also, the preoperative collection of autologous blood does not eliminate all risks of transfusion. Several transfusion reactions occur in recipients of autologous blood. One hospital survey revealed that the rate of febrile, non-hemolytic transfusion reactions per autologous unit transfused was 0.12% and the rate of allergic reactions was 0.01%. Although the rates are approximately 5 to 10 times lower than those observed with allogeneic transfusions, they indicate that febrile and allergic reactions are still possible. Septic transfusion reactions (see Chapter 7: Ad-

verse Effects of Transfusion) have also been reported with autologous blood. *Yersinia enterocolitica* appears to be the most common contaminant. Bacterial contamination may occur because of the following: 1) an undetected bacteremia in the patient, 2) inadequate skin sterilization before venipuncture, or 3) a break in the integrity of the plastic storage containers during handling or storage that allows bacterial entry. There is no reason to think that those routes of bacterial contamination are any less common in autologous blood than in allogeneic blood.

Autologous blood transfusion, like allogeneic transfusion, carries a risk of patient or component misidentification and the possibility of infusion of the wrong unit of blood to a patient. Therefore, hemolytic reactions caused by ABO mismatch are still possible with autologous blood. Autologous transfusions also may contribute to volume overload in patients at risk for congestive heart failure. Even transfusion-related acute lung injury has been reported.

Paradoxically, some autologous donors have received more units of allogeneic blood during and after surgery than patients who did not participate in an autologous program. The reason for that unexpected result is not clear. One hypothesis is that hemostasis is impaired as hematocrits decrease. This phenomenon is evidenced by the longer bleeding times in uremic patients when their hematocrits are below 28% to 30%. Because autologous donors already have lower hematocrits at the start of surgery, perhaps further operative blood loss can cause their hematocrits to decrease to the point where hemostasis is impaired.

The actual gain in red cell mass (circulating plus collected) during autologous blood collection is relatively modest in many cases because the degree of anemia induced by most autologous blood collection schedules is not a strong erythropoietic stimulus. Red cell production usually does not attain a rate greater than the upper limit of normal. In addition, the time available before surgery is a limiting factor to the amount of red cell replenishment that can be achieved. Furthermore, many patients' starting hematocrits are too low to allow the collection of the desired number of autologous units. All of these factors can cause the amount of autologous blood collected to be insufficient to meet transfusion requirements.

The administration of recombinant human erythropoietin (rhEPO) to autologous donors can further stimulate erythropoiesis. The data, however, are not convincing that this practice decreases allogeneic transfusion requirements



Autologous donation does not eliminate the risk of bacterial contamination. Clerical errors can occur, resulting in hemolytic transfusion reactions. Improper handling or storage can lead to hemolysis or other compromise of the quality of the product.



The yield of preoperative autologous donation can be increased by the use of recombinant human erythropoietin (rhEPO), but this remains an off-label use.

for most patients. The “off-label” unlicensed use of rhEPO adds considerable expense to autologous blood collection. Nevertheless, some centers will consider use of rhEPO to improve the yields of autologous collection in patients who are at particular risk from allogeneic transfusion, such as patients with multiple red cell alloantibodies for whom compatible RBC units are rare.

Alternatively, rhEPO is licensed for the purpose of increasing the circulating red cell mass and decreasing allogeneic transfusions in anemic patients with hemoglobin levels >10 g/dL, but <13 g/dL, who are scheduled to undergo elective noncardiac and nonvascular surgery. (Note: rhEPO use creates a thrombotic risk.) The Food and Drug Administration approval specifically excludes rhEPO use in these patients if they are willing to donate autologous blood. For some patients, preoperative administration of rhEPO lessens the need for transfusion during surgery and also appears to increase the ability of the marrow to replace operative blood loss more rapidly during the immediate postoperative period. Thus, rhEPO administration is a possible alternative to autologous blood donation in selected patients.



Patients who would not ordinarily be eligible for allogeneic donation may be considered for autologous donation.



Patients with certain health conditions such as severe cardiac and/or pulmonary disease or an uncontrolled seizure disorder are considered poor candidates for preoperative autologous donation. Obstetric patients and those with bacteremia, malignancy, hematologic disorders, or cardiac abnormalities should not donate or should donate only after careful evaluation by the physician overseeing the program.

Patient Eligibility

Although the criteria for autologous donor selection are not as rigid as those for allogeneic blood donation, some standards and guidelines are necessary to ensure patient safety (see Table 9-2). The planned surgical procedure should have a reasonable expectation of transfusion, and the number of units requested by the patient’s surgeon should correspond approximately to the typical transfusion requirements for that procedure. A schedule of expected transfusion requirements for specific surgical procedures, commonly known as a maximum surgical blood order schedule (MSBOS) or standard blood order schedule, should be prepared jointly by the transfusion service and surgery department (see Chapter 15: Managing a Hospital Transfusion Service). The MSBOS is used to determine the number of RBC units that the blood bank should type and screen or crossmatch for specific surgical procedures. The MSBOS also can be used as a guide for determining patient eligibility for autologous transfusion and for determining a reasonable number of autologous units to collect.

A patient should be considered eligible to donate autologous blood only if the MSBOS calls for the availability of

Table 9-2. Suggested Contraindications for Preoperative Autologous Donation*

- Patient not medically cleared for surgery yet
- Hematocrit <33% or hemoglobin <11 g/dL at time of donation
- Surgery <72 hours from time of donation (unless intravenous volume replacement will be given)
- Evidence or risk of bacteremia at time of donation
 - Oral temperature >37.5 C (99.5 F)
 - Indwelling devices, eg, urinary catheters, some orthopedic devices
 - Recent (within 24 hours of donation) invasive procedure, such as dental work, minor surgery
 - Recent episode of diarrhea
 - Open unhealed skin or other wounds
 - Patient receiving antibiotics
- Significant cardiac abnormalities
 - Unstable angina
 - Symptomatic aortic stenosis
 - Significant atrioventricular block
 - Cyanotic heart disease
 - Recent myocardial infarction (within 6 months)
 - High-grade left main coronary artery disease
- Uncontrolled hypertension
- Active seizure disorder
- Severe occlusive cerebrovascular disease
- Acute viral illness at time of donation
- Conditions that require further evaluation
 - Irregular heart rhythm
 - Rate <50 or >100 per minute

*The physician overseeing autologous collections should discuss the listed conditions with the referring surgeon. Further medical evaluation of the conditions may be necessary.

crossmatched RBC units for the planned surgical procedure. Patients undergoing surgical procedures that ordinarily do not require transfusion or routine crossmatching of RBC units in anticipation of transfusion, such as tonsillectomy and cholecystectomy, should not be considered



Blood transfusion during routine uncomplicated obstetric delivery is usually unnecessary. Thus, most pregnant women should not donate autologous blood.

candidates for autologous donation. Autologous blood donation in those settings would subject the patient to a potentially inconvenient and uncomfortable procedure, the risk of iatrogenic anemia, and a possible donor reaction for little or no gain. Moreover, autologous donation in such settings would add additional, unnecessary medical costs.

The majority of pregnant women should not donate autologous blood. The estimated blood loss in uncomplicated vaginal delivery is about 600 mL, slightly more than a unit of autologous blood. Generally, this blood loss is tolerated without symptoms, especially because there is an expansion of blood volume in pregnancy. For that reason, blood transfusion of any kind during routine, uncomplicated obstetric delivery is usually unnecessary. Similarly, routine collection of autologous blood for anticipated cesarean section is not justified by blood usage data. However, some centers permit pregnant women with persistent, late placenta previa to donate autologous blood. The eligibility of patients with placenta previa for autologous donation, as with any other medical condition or procedure, should be considered in light of how often patients with that condition typically require any transfusion and should take into account each patient's unique clinical circumstances.

Sufficient time must be available before surgery for collecting the desired number of autologous units. The risk, if any, of delaying surgery must be weighed against the advantages of using autologous blood instead of allogeneic blood. Thus, patients requiring urgent surgery are not eligible. Patients also must have transportation and must be able and available to make multiple trips to a blood collection facility for the blood donations.



There are no minimum or maximum age limits for autologous blood transfusion.

No minimum or maximum age limit exists for autologous blood donation. In fact, older donors have fewer reported vasovagal reactions and often tolerate the donation of a unit of blood better than younger donors. At the other extreme, children as young as 7 years old have donated autologous blood without adverse effect. The only restrictions relevant to pediatric donations are that the patient's vein(s) be able to accommodate the phlebotomy needle and that patients weighing <50 kg donate a volume of blood proportional to their weight. That restriction applies to small adults as well.

Patients must have good venous access in order to make repeated autologous blood donations. In addition, autologous donors should be emotionally prepared to donate blood. Patients who are intimidated by the thought of multiple donations may prefer not to participate in an autolo-

gous program. Sensitivity to this issue is especially relevant when pediatric patients are involved. Concerned parents may insist that their child enroll in an autologous blood program despite their child's reluctance. The blood bank medical director should be prepared to advise the patient's physician and family about the current safety of allogeneic blood, the availability of directed donations, and the risks vs the benefits of autologous blood so that properly informed choices can be made.

Patients with potential bacteremia should not donate autologous blood. Once bacteria gain entry into a unit of blood, they can proliferate during storage, even at 4°C. This concern is particularly the case for psychrophilic organisms, such as *Y. enterocolitica* and *Pseudomonas* species. Contaminated units can cause septic transfusion reactions that can be severe or even fatal. Because of the risk of bacteremia, donors who have open skin lesions, suspected abscesses, or indwelling devices (such as bladder catheters or percutaneous orthopedic devices), or who have undergone recent minor invasive procedures, including dental work, should be excluded. Autologous donors should be questioned regarding gastrointestinal symptoms in the days preceding donation in order to screen for possible *Y. enterocolitica*-related gastroenteritis.

Patients with some cardiac abnormalities, such as mild valvular lesions or chronic arrhythmias, have had few reported adverse reactions to autologous blood donation and may be acceptable as autologous donors. However, patients with unstable angina, symptomatic aortic stenosis, and recent myocardial infarction or stroke should be excluded. Other medical conditions that should exclude patients from participation in an autologous program or that should be investigated further are shown in Table 9-2.

Some patients, such as those with known carcinoma and a variety of other medical disorders, are eligible for autologous donation, whereas they are unacceptable for allogeneic blood donation. Patients with sickle cell trait are eligible for autologous donation, as they are for allogeneic blood donation. However, it should be noted that their red cells should not be frozen because gelation and excessive hemolysis may occur after deglycerolization.

In general, any medical condition that the physician overseeing the autologous blood program believes may place a patient at risk during blood donation should be discussed with the requesting physician and should be weighed against the potential advantages of autologous blood. In the absence of specific guidelines that cover all



Because of the risk of bacteremia, autologous donors should be questioned carefully about:

- Gastrointestinal symptoms in the days preceding donation
- Open skin lesions
- Suspected abscesses
- Indwelling devices
- Recent minor invasive procedures, including dental work

patient conditions and circumstances, sound medical judgment must be exercised in determining donor eligibility.

Procedure

Patients who qualify for PAD should be identified and counseled by their surgeon. The surgeon must write a medical order for the collection of autologous blood that specifies the number of units to collect and on what schedule. The number of autologous units to be collected should be based on the MSBOS and the surgeon's own usual blood usage for the procedure being planned. It is common and usually convenient for patients to donate at 1-week intervals, although twice-a-week donations (ie, separated by a minimum of 3 days) are possible for some donors should circumstances call for a more condensed schedule. The last donation should be at least 3 days before surgery, although donations made in the week or two before surgery achieve little more than hemodilution because not enough time is available for significant new red cell production. For that reason, an optimal collection should begin as far in advance of surgery as possible, depending on whether the autologous blood will be stored as WB or RBCs in citrate-phosphate-dextrose-adenine-1 (CPDA-1) with a 5-week shelf life, or as Additive Solution RBCs with a 6-week shelf life.

Oral iron supplementation may be prescribed for patients before or at the start of the autologous blood collection. Female donors with low body mass and limited total body iron stores may benefit most from iron supplementation, particularly when donating multiple units. For patients giving fewer donations, especially males with starting hematocrits in the reference range, iron supplementation may be less important. However, all autologous donors can be encouraged to increase their dietary iron intake to help compensate for the iron deficit. Each unit of donated blood contains about 200 mL of red cells, which corresponds to a loss of about 240 mg of iron. Tables that list the iron content of various foods are readily available and can be given to patients to guide them in enriching the iron in their diet. It is useful to keep in mind that iron from animal hemoglobin (ie, heme iron), such as that found in red meat and liver, is absorbed better than iron from grain and vegetable sources (ie, nonheme iron). The absorption of nonheme iron is improved if the meal contains meat, fish, or vitamin C but is reduced by coffee, tea, egg yolks, soy protein, wheat bran, and other dietary fiber as well as by food high in oxalates such as spinach.



Females in particular should be considered for iron supplementation because they often have lower iron stores than males.

One commonly prescribed oral iron supplement provides 65 mg of ferrous iron per dose (325 mg of ferrous sulfate). Patients donating multiple autologous units typically may be prescribed that preparation three times a day for a total daily dose of 195 mg. However, that figure does not represent the actual bioavailable dose because the percentage of oral iron absorbed varies from about 10% in individuals with normal iron stores to about 20% to 30% in those whose iron reserves are reduced or depleted. Therefore, a dose of 195 mg is more likely to provide about 20 to 40 mg of bioavailable iron daily. Over the course of a week, that amount is approximately enough to replenish the iron lost with a once-a-week autologous donation schedule. The iron supplement should be taken between meals (ie, at least 1 hour before a meal or 2 hours after the last meal) because certain foods interfere with iron absorption, as noted above. Gastrointestinal side effects of oral iron supplementation, such as abdominal discomfort, nausea, vomiting, constipation, or diarrhea, may be seen in up to 25% of patients. Those side effects can often be controlled by decreasing the dose or instructing the patient to take the iron with meals. It may also help to switch the patient to a different iron preparation, such as ferrous gluconate or ferrous fumarate.

Before beginning autologous blood collection, the patient should be asked to provide pertinent medical history. The history does not need to be as extensive as for allogeneic blood donors because risk factors for transfusion-transmitted viral infections are not a cause for autologous donor disqualification. The purpose of obtaining the medical history is predominantly to determine if the patient can safely donate blood and if the patient is at risk of bacteremia. Sample topics for an autologous donor medical history questionnaire are shown in Table 9-3.

The volume of blood collected at any one time should not exceed 15% of the donor's blood volume. That limit is unlikely to be exceeded during collection of units of blood with a volume of 450 ± 45 mL when the donor's body mass is 50 kg or greater. However, for patients weighing less than 50 kg, the following formula may be used for calculating the amount of blood that can be withdrawn safely:

$$\text{Volume to collect} = (\text{donor's weight in kg}/50) \times 450 \text{ mL}$$

The volume of anticoagulant/preservative solution in the storage bag when using CPDA-1 is 63 mL for collections of 450 ± 45 mL (70 mL for collections of 500 ± 50 mL). If less



The maximum allowable volume of blood withdrawn at each donation, including samples for testing, must not exceed 15% of the patient's estimated blood volume.

Table 9-3. Sample Topics for Autologous Donor Medical History Screening

- History and nature of any cardiac disease or dysfunction, seizure disorders, other major illnesses, and organ dysfunction
- Prior blood donations, if any, and description of any donor reactions or difficulties
- Prior fainting episodes
- Current medications (in particular to see if patient is on antibiotics, cardiac medications, or anticonvulsants)
- Recent infections or fevers
- Recent gastrointestinal illness or diarrhea (a risk factor for *Yersinia enterocolitica*, psychrophilic bacteria that can grow in cold-stored RBC units)
- Recent or planned minor invasive procedures other than the procedure for which the patient is donating blood
- History of hepatitis or human immunodeficiency virus infection, particularly if the autologous blood will be shipped to another facility



When surgery is postponed, every effort should be made to prevent autologous units from expiring and being discarded.

than 300 mL of blood will be collected, a proportionately reduced amount of anticoagulant must be used. This amount is calculated as: (volume of blood collected/450) × 63 mL. Reducing the amount of anticoagulant/preservative solution requires transferring excess volume (ie, 63 mL minus this calculated appropriate amount) out of the primary collection bag, while still maintaining the integrity and sterility of the closed blood bag system. If a satellite bag is not already attached to the primary bag into which the excess anticoagulant/preservative solution can be transferred, a sterile connection device may be used to attach an additional sterile bag or pouch.

If surgery must be postponed, RBC units may be glycerolized, frozen, and stored for a longer period (up to 10 years). A reasonable effort should be made to prevent autologous units from expiring and being discarded; otherwise, the patient is at risk of going into the rescheduled surgery with a lowered hematocrit and an increased probability of requiring allogeneic transfusion—the opposite of the desired outcome. RBC units should be frozen within 14 days of collection; if not, they may be “rejuvenated” up to 3 days after expiration to increase 2,3-diphosphoglycerate and adenosine triphosphate levels before the blood is glycerolized and frozen.

Another option for avoiding the expiration of autologous blood is known as "leap-frogging." This technique involves reinfusion of an autologous unit just before its expiration, coupled with collection of a new unit. However, it has the disadvantage of exposing the patient to the risk of both donor and transfusion reactions for each leap-frogged unit. It also adds expense, is time-consuming, and is inconvenient for the patient.

Adverse Donor Reactions

As with any blood donation, patients donating autologous blood may experience a variety of adverse reactions. Conflicting data exist regarding whether autologous donors overall are more likely to suffer adverse reactions than are allogeneic donors. However, more severe reactions that require hospitalization appear to be more common in autologous donors, perhaps related to the fact that they are patients rather than healthy volunteers.

One of the most common reactions is the vasovagal response. Studies on vasovagal reactions have revealed that they occur in about 2% to 8% of donations. They are more common in 18- to 40-year-old patients than in patients older than 40 years. In addition, they are more commonly seen in first-time donors and in individuals who already have a lower baseline heart rate and diastolic blood pressure. Patients with a history of fainting are at greater risk of syncopal episodes. Although younger patients suffer vasovagal reactions more often, older patients appear somewhat more likely to have serious sequelae that require hospitalization.

In vasovagal reactions, the patient has a sudden onset of weakness, sweating, pallor, hypotension, and possibly nausea or vomiting, in anticipation of the needle insertion or at the time of needle insertion. The patient may report dimmed vision, may feel faint, and may suffer syncope. The vasovagal reaction is a parasympathetic response caused by vagus nerve stimulation that results in a bradycardia-mediated decrease in cardiac output and peripheral vasodilation. If the vasovagal response sufficiently reduces blood flow to the brain, the patient will transiently lose consciousness. The syncope is usually brief, often lasting only a few seconds up to 1 to 2 minutes.

One way to distinguish vasovagal syncope from other causes is to take the patient's pulse and to measure the blood pressure. Vasovagal reactions are often characterized by a slowed pulse and lowered blood pressure. By



Because autologous donors are patients rather than healthy volunteers, they may experience more severe reactions to donation than healthy volunteers.



On rare occasions, vasovagal reactions to donation have led to cardiac arrest. It may be advisable for emergency room personnel to evaluate the autologous donor more fully for cardiac or other abnormalities.

contrast, in a hypovolemic reaction (see below), the patient will have an increased heart rate. In the treatment of vasovagal reactions, it is helpful to make sure the patient is placed in a supine position with legs elevated to improve venous return and to improve blood flow to the head. Sometimes, it is sufficient to have the patient bend forward at the waist while sitting down in order to lower the head to or below the level of the heart. If the risk of vomiting appears, the patient should be positioned on his or her side and the airway should be kept clear. An ammonia inhalant may be used if the patient's syncope lasts more than a few seconds. Despite the usually rapid return of consciousness, patients may need an hour or more to recover. Vasovagal reactions are uncomfortable and frightening, but usually have no serious sequelae. However, on rare occasions, they have led to cardiac arrest. During the time the patient is recovering, it may be appropriate for the emergency department to evaluate the patient more fully for possible cardiac or other abnormalities.

Patients who have just donated blood may also suffer hypovolemic reactions because of the acute loss of intravascular volume. Usually, such reactions are characterized by postural dizziness. Patients may have an increased heart rate, as opposed to the decreased rate seen in vasovagal reactions. To avoid postural dizziness, all patients should remain in a recumbent position for about 10 minutes after donation and may be given oral liquids to begin to replenish the lost blood volume. Patients should be allowed to rest in a supine position until they have recovered.

On very rare occasions, blood donors may suffer seizures when donating blood. If that happens, it is important not to restrain the patient or to force anything into his or her mouth. Such efforts may lead to bone or soft tissue trauma and broken teeth. Objects should be cleared away from the patient's area, and attempts may be made to cushion violent movements in order to prevent injury. Most seizures stop on their own in a short time. Patients should be evaluated by the hospital emergency department, and they will need time to recover from possible postictal fatigue, amnesia, or confusion.

After blood donation, hematomas in the antecubital fossa at the site of venipuncture are common. These hematomas are usually more unsightly than serious. To prevent hematomas, pressure should be placed over the venipuncture site as the needle is removed by the phlebotomist. Often, the patient will be asked to assist in continuing to apply pressure to the site while the phlebotomist

attends to the collected blood. A pressure bandage consisting of tape placed over a folded gauze pad should be placed over the puncture site. The patient should be advised to leave the bandage in place for about 15 to 30 minutes to allow clotting to take place. Usually, donors are advised to avoid heavy lifting with the affected arm for at least several hours.

In an estimated 1 in 20,000 to 25,000 donations, the needle may strike a nerve and cause nerve injury, resulting in numbness, tingling, pain, or even loss of strength in the hand or arm. In a small percentage of cases, paresthesias in the venipuncture area have been reported to persist for 6 months or longer. In addition, given that skin integrity is broken at the venipuncture site, the opportunity for skin infection exists. Because such problems are difficult to anticipate or prevent, using sterile or aseptic technique throughout the procedure and minimizing soft tissue trauma during venipuncture are imperative.

On rare occasions, patients will suffer angina, myocardial infarction, or cerebrovascular accidents that may or may not be directly related to the autologous donation. Therefore, adequate space and access for emergency personnel must be available in the autologous blood donation area. Enough space should be available to accommodate an additional stretcher in case the patient needs to be moved to another area and to accommodate several health-care providers attending to the patient. Yet, at the same time, the area needs to provide sufficient privacy to the patient for the collection itself.

Testing

The autologous donor's ABO group and Rh type must be determined by the collecting facility. If the transfusing facility is different from the collecting facility, the transfusion service or blood bank of the transfusing facility must confirm the ABO and Rh type of the autologous unit. Screening for unexpected red cell alloantibodies or crossmatching is optional. However, an immediate-spin crossmatch that confirms ABO compatibility is an appropriate safety check to confirm that the appropriate unit was chosen for the patient.

In addition, because the patient may require allogeneic units in addition to the autologous blood, an antibody screen is often appropriate so that the blood bank can be prepared for that contingency. Tests for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-



Access for emergency personnel must be available in the autologous donation area.



If a preoperative autologous donation tests positive for infectious disease or if any other medically significant abnormalities are identified, the patient and his or her physician must be notified of the findings.

HBc), antibody to hepatitis C virus (anti-HCV), HCV RNA, antibody to human immunodeficiency virus types 1 and 2 (anti-HIV-1/2), HIV-1 RNA, antibody to human T-cell lymphotropic virus types I and II (anti-HTLV-I/II) as well as a serologic test for syphilis must be performed by the collecting facility on at least the first unit collected in every 30-day period, if the collection facility is not the transfusing facility. However, those tests do not have to be performed if the units will be transfused in the same facility where they are collected. If any of the infectious disease test results are positive, the patient's physician and the patient must be notified, and the collecting facility must notify the receiving facility of the results.

Labeling and Storage



Proper labeling and storage of autologous units are particularly important, because such blood may be collected from donors with viral or other infectious illnesses.

A biohazard label should be placed on units from donors who test positive for infectious disease markers.

Units of autologous blood or components should be labeled and stored in the blood bank in a fashion that clearly distinguishes them from the rest of the blood inventory. That precaution is necessary to decrease the risk of transfusing autologous blood to an unintended recipient. Because autologous blood, unlike allogeneic blood, may be collected from donors with viral or other infectious illnesses, proper labeling and storage are particularly important. Moreover, transfusion to the wrong recipient would deny the autologous donor his or her own blood and would increase the likelihood of allogeneic transfusion, which would negate the autologous collection effort.

Some facilities use a different color for the main bag label on autologous units (fluorescent green instead of white); all facilities must have a label or tag that states "Autologous donor." If the autologous unit does not meet all the requirements of an allogeneic unit in terms of donor eligibility or testing, the phrase "For autologous use only" should be placed on the bag label in place of the standard ABO label. Either the label or the tag should have unique patient identifying information, including the patient's name, an identification number, and, if available, the name of the facility where the patient will be transfused and a patient identification number assigned by the transfusing facility. If the transfusing facility's identification number for the patient is unavailable at the time of blood donation, the patient's social security number or birthdate may be substituted. A biohazard label should be placed on units collected from donors testing positive for infectious markers, if such testing is performed.

A segregated space in a refrigerator, such as a separate shelf, or a dedicated refrigerator should be set aside in the hospital blood bank for autologous units only to further reduce the risk of mistaken use of autologous units for unintended recipients. Units from donors testing positive for infectious markers may be further segregated for improved safety. Some facilities put such units in an extra plastic bag or container (such as a cafeteria-style clear plastic salad container) on a separate shelf to call attention to the fact that those units are different. The purpose of the segregation, packaging, and labeling is to have redundant mechanisms in place to prevent those units from being transfused to the wrong recipient, especially because they could be infectious.

For patients who have made multiple donations, the autologous units should be labeled by the hospital blood bank in a way that identifies the correct order of transfusion, which should be the same as the order of collection; in other words, the oldest unit should be transfused first. In addition, if both autologous and allogeneic units are provided to the operating room for the same patient, the autologous units must be labeled in a way that ensures they will be transfused before any allogeneic unit. For example, a tag could indicate by number or letter (in alphabetical or numerical sequence) the order of transfusion, with autologous units first, starting with the oldest unit, followed by any allogeneic units. A sticker or tag should be placed on the allogeneic units indicating that autologous units are available. Members of the operating room staff need to be trained to be compliant with the chosen labeling system. The transfusion of allogeneic units when autologous units are still available is unacceptable practice and unnecessarily exposes the patient to other donors' blood. Such action would violate the patient's trust and would represent a performance error or accident that requires investigation and corrective action.



Autologous units must be labeled and stored using methods that clearly distinguish them from the rest of the blood inventory. The units should be labeled in a manner that identifies the correct order of transfusion.

Transfusion Trigger

The transfusion trigger refers to the factor or factors that "trigger" the decision to transfuse a patient. The trigger for RBC transfusion usually includes a threshold level of the patient's hematocrit, the signs and symptoms of anemia, or both. No clear consensus exists on whether the trigger for autologous transfusion should be the same as that for allogeneic transfusion. Some transfusion specialists argue that because autologous blood does not pose a risk of transfusion-transmitted infection and because it is the patient's

own blood, a lower threshold should be used. From that perspective, failure to transfuse autologous blood represents a waste of a valuable resource because it usually is discarded otherwise. Furthermore, some argue that even autologous blood transfusions have risks, so why subject the patient to unnecessary risk by transfusing blood unless it is medically necessary? That argument presumes that medical necessity does not change merely because autologous units are used instead of allogeneic units. Because the risk-to-benefit ratio of receiving autologous blood transfusion is not the same for all recipients, in the final analysis, the decision to transfuse depends on the unique medical circumstances of each patient. Nevertheless, each medical center should establish its own transfusion guidelines for autologous blood and should specify whether those guidelines differ from the transfusion criteria for allogeneic blood components.



About 30% to 50% of all autologous units are not transfused.

Quality Control

Autologous units may be monitored for bacterial sterility as a means to determine if arm preparation, blood collection, and blood storage are being carried out in a fashion that minimizes bacterial contamination. Because about 30% to 50% of all autologous units are not transfused, the opportunity exists to perform bacterial cultures on outdated units to determine whether sterile conditions were maintained from collection through storage. In addition, the quality (ie, efficacy) and safety of autologous units can be monitored by determining whether transfused autologous units cause an appropriate and expected increase in posttransfusion hematocrit and whether the patient experiences any adverse reaction associated with the autologous transfusion.



Acute normovolemic hemodilution refers to the collection of whole blood with the concurrent restoration of the blood volume with an acellular solution. The whole blood is reinfused later during the surgery or in the immediate postoperative period.

Intraoperative Blood Collection and Administration

Acute Normovolemic Hemodilution

Acute normovolemic hemodilution (ANH) refers to the collection of WB in the operating room just before or just after the patient has been placed under anesthesia and before the surgical procedure begins. The volume of WB removed is simultaneously replaced with crystalloid or colloid solutions, thereby maintaining blood volume and permit-

ting more than 1 WB unit to be collected without significantly affecting the patient's vital signs. The collected blood is kept as WB and usually stays in the operating room, where it is available for transfusion during or at the close of surgery. The intention of ANH is to reduce the patient's hematocrit so that less red cell mass will be lost for a given volume of surgical bleeding. The number of units of blood removed depends on the patient's expected tolerance of anemia and the anticipated surgical blood loss.

Hemodilution can be tolerated in many patients because oxygen delivery is normally in excess of that required by tissues. Moreover, hemodilution results in some compensatory effects that allow individuals to tolerate a greater degree of anemia than otherwise would be possible. First, hemodilution lowers the viscosity of blood, resulting in higher flow rates through the microvasculature and, hence, some compensatory gain in oxygen delivery. Second, hemodilution may cause a modest increase in cardiac output. This is due to an increase in stroke volume caused by decreased resistance to left ventricular ejection and increased venous return. The goal of ANH is to take advantage of those effects in order to hemodilute the patient as much as possible safely. The greater the hemodilution, the greater will be the potential benefit of ANH.

Benefits

ANH provides several advantages. Preoperative hemodilution may decrease the amount of red cells lost during surgery. Patients participating in an ANH program do not have to make multiple inconvenient trips to a collection facility weeks in advance of surgery. That makes ANH suitable for both urgent and elective procedures. Also, donor discomfort is largely eliminated because the collection usually takes place while the patient is anesthetized.

Unlike blood collected in anticipation of surgery, blood collected for ANH usually does not have to be tested, stored, or tracked administratively because it remains in the operating room with the patient until it is transfused. This method represents a monetary and logistic advantage over preoperative collections. Also, the wastage of blood, which is common with PAD, is avoided because, typically, all the blood collected by ANH is reinfused.

Autologous blood collected immediately before surgery and used within several hours provides a source of functional platelets and coagulation factors in addition to red cells. Such a source of autologous platelets can be an ad-



The rationale behind ANH is that intraoperative bleeding occurs at a lower hematocrit, and, therefore, the red cell loss is minimized.

vantage in surgical cases where there is significant, surgery-related platelet consumption or thrombocytopenia. For example, in open-heart surgery, platelets often become dysfunctional as a result of cardiopulmonary bypass (CPB), presumably because of partial activation after contact with synthetic materials in the extracorporeal circuit. Some patients develop a hemorrhagic syndrome at least partially related to this thrombocytopenia and require platelet transfusion. The reinfusion of fresh autologous WB after CPB theoretically could decrease the need for allogeneic platelet transfusion, thereby decreasing allogeneic donor exposures. It is not clear whether this benefit is evident in practice—in part because most patients do not require platelet transfusion in this setting. However, logic dictates that this source of autologous platelets should benefit some patients.

In a similar fashion, a unit of autologous blood stored for no more than 8 hours will provide coagulation factors equivalent to those in a unit of Fresh Frozen Plasma. Transfusion of this blood theoretically could improve hemostasis and decrease the need for plasma transfusion in patients who develop intraoperative coagulation factor deficits.

Limitations and Risks

ANH has been criticized for yielding limited savings in red cell mass, except when large blood loss occurs in the setting of extreme hemodilution. Moreover, many patients do not tolerate hemodilution to the extent necessary to achieve a significant reduction in red cell loss or allogeneic transfusion requirements. Even moderate hemodilution may increase the risk of organ ischemia in some patients. Hypervolemia is a concern for patients who receive large volumes of crystalloid solution when blood is collected or who are normovolemic when multiple units of autologous blood are beginning to be reinfused.

Patient Eligibility

As with PAD, the surgical procedure should have an expected requirement for transfusion (in other words, cross-matched units are normally provided for the procedure). ANH should be considered only when the patient's blood loss is expected to be significant (ie, 1 liter or 20% of blood volume) because only such patients will benefit from the hemodilution effect. ANH has been used with success in cardiothoracic, vascular, urologic, and orthopedic surgery.



ANH is of greatest benefit when the patient's blood loss is expected to be more than 2 or 3 units—only then will the red cell volume saved be of clinical significance.

Medical restrictions for ANH are similar to those for PAD described previously. Patients with significant cardiac, pulmonary, and liver disease are ineligible for ANH because of their increased susceptibility to organ ischemia. Patients with impaired renal function are also ineligible because adequate kidney function is required for the patient to handle the large fluid volume administered during the blood collection and hemodilution phase as well as during the reinfusion of the collected blood. Patients with bacteremia or risk of bacteremia should be excluded because of the potential for bacterial growth in the collected blood during its holding period of up to 8 hours at room temperature in the operating room.

The patient's preoperative hemoglobin should be at least 12 g/dL so that several units of WB can be removed without adversely affecting oxygen delivery to the tissues. No age restrictions exist for ANH, which has been used for both small children and the elderly.

Procedure

Blood is collected from the patient in the operating room just before surgery. The patient's vital signs are monitored carefully, and hypotension and tachycardia that is unresponsive to fluid replacement are avoided. Normovolemia is achieved by the administration of crystalloid in a ratio of about 3:1 compared to the volume of blood collected, colloid solution in a ratio of 1:1, or both. The blood is collected, as with other blood donations, into a standard blood bag with anticoagulant/preservative solution, and the bag is agitated during blood collection. The blood is stored in the operating room for up to 8 hours at room temperature. If the surgical procedures last longer than 8 hours or if the transfusion is deferred, the blood may be stored in a monitored blood bank refrigerator at 1 to 6 C for up to 24 hours as long as it is refrigerated within 8 hours of collection and is properly labeled.

The number of units of blood that should be collected depends on the estimated blood loss as well as on the patient's clinical status, hematocrit, and blood volume. Those parameters depend on each patient's unique clinical status. In limited hemodilution, the patient's hematocrit is reduced to about 28%, whereas in extreme hemodilution, the hematocrit is reduced to 21% or less. Extreme hemodilution is reserved for selected patients because many patients will not tolerate such aggressive hemoreduction.



ANH units should be reinfused in the reverse order of collection. In that way, units with the highest red cell volume are reinfused when they will be of maximal benefit to the patient.

The amount of blood that is removed during hemodilution can be calculated with the equation:

$$\text{Volume} = \frac{\text{EBV} \times (\text{Hct}_{\text{initial}} - \text{Hct}_{\text{final}})}{\text{Hct}_{\text{average}}}$$

where EBV is the estimated blood volume (body weight in kg \times 70 mL/kg), and Hct_{initial}, Hct_{final}, and Hct_{average} are the patient's initial, final, and average [(Hct_{initial} + Hct_{final}) \div 2] hematocrits.

The units collected should be numbered in order of collection. If blood replacement is needed during or after surgery while the patient is hemorrhaging, the last unit collected, which has the lowest hematocrit, is usually transfused first. Thereafter, the units are transfused in the reverse order of collection. The first unit collected, which is expected to have the highest hematocrit, can be saved for near the end of surgery when it should be most beneficial. The most hemodiluted units are more appropriately transfused during ongoing blood loss. In that way, the patient will continue to benefit from the decreased loss of red cell mass achieved with hemodilution. For open-heart surgery cases, the first unit collected will also have the highest levels of platelets and coagulation factors. The first unit collected will provide the most benefit after CPB because platelets may be rendered at least partially dysfunctional during CPB.



With ANH, testing is not usually performed because the blood is reinfused without being stored or removed from the operating room.

Testing

One of the advantages of ANH is that the blood remains in the operating room with the patient and is reinfused usually without need of blood bank storage. Thus, typically, testing is not performed.

Quality Oversight of ANH

Blood banks and transfusion services usually do not directly manage ANH, which is typically carried out by anesthesiologists. However, it is essential that the blood bank/transfusion service medical director and technical supervisory staff serve in at least an advisory capacity. That role includes ensuring that written procedures for ANH exist and comply with all regulatory requirements and voluntary standards. Both AABB and the College of American Pathologists (CAP) have accreditation programs for perioperative activities. Both organizations have requirements that delineate a role for the blood bank or transfusion ser-

vice medical director in helping to design and implement a perioperative blood program.

The blood bank or transfusion service director should ensure that personnel are appropriately trained and their competency assessed periodically; equipment is properly maintained; supplies and materials are appropriate and available in adequate quantity; and that blood collection, documentation, and record retention are subjected to quality control.

Intraoperative Blood Recovery

Intraoperative blood recovery is the collection of the patient's own blood shed during surgery. After collection, the blood can be reinfused immediately without further processing, or it can be washed with isotonic saline and concentrated by centrifugation. Open-heart surgery is a common setting for the use of intraoperative recovery, where reinfusion of up to one-half of the operative red cell loss has been reported. Accordingly, routine use of intraoperative blood recovery in cardiac surgery has been associated with a decrease in allogeneic RBC transfusion by about half. In that setting, typically, an automated cell recovery instrument is used in which the blood is centrifuged, washed, and filtered.



Intraoperative blood recovery is the collection, washing (optional), and reinfusion of blood shed during surgery.

Benefits

Intraoperative blood recovery has the advantage of being available to many patients who are ineligible or unable to donate autologous blood before surgery, either because of medical restrictions or because their surgery cannot be delayed. In addition, intraoperative blood recovery may be used as an adjunct to PAD for patients who have not donated sufficient blood to cover their total transfusion needs. Because the blood does not leave the patient's side usually, the risk of administrative errors and patient misidentification may be reduced. The blood is not stored or tested, which reduces laboratory costs and labor.



Blood recovery should not be attempted from any operative site that is contaminated with bacteria, such as can occur with bowel surgery, penetrating abdominal wounds, or infected wounds.

Limitations and Risks

Intraoperative blood recovery alone rarely collects enough blood to meet total RBC transfusion needs. On occasion, intraoperatively recovered blood may be excessively hemolyzed, which could result from overly vigorous aspiration with high vacuum settings. Air emboli, a serious and potentially fatal complication, have been reported during

the reinfusion of intraoperatively recovered blood with automated blood cell processors. Techniques have been described to decrease the risk of air emboli by transferring the blood from the initial holding bag into a transfer bag, then burping the air out of the transfer bag back into the holding bag. Various pharmacologic agents used during surgery, biologic contaminants, and other factors are possible contraindications to the use of intraoperative blood recovery (see Table 9-4).

Table 9-4. Possible Contraindications to Intraoperative Blood Recovery*

Pharmacologic Agents

- Clotting agents (Avitene, Surgicel, Gelfoam, etc)
- Irrigating solutions (Betadine, antibiotics meant for topical use)
- Methylmethacrylate
- Anticoagulants

Contaminants

- Urine
- Bone chips
- Fat
- Bowel contents
- Infection
- Amniotic fluid
- Cellular stroma
- Activated leukocytes, platelets, complement, plasmin

Hematologic Disorders

- Sickle cell disease (if blood will be refrigerated)
- Thalassemia

Miscellaneous

- Carbon monoxide (electrocautery smoke)
- Catecholamines (pheochromocytoma)
- Oxymetazoline (Afrin)

*Modified with permission from Waters J, Cheng D, Shander A, et al, eds. Perioperative blood management: A physician's handbook. Bethesda, MD: AABB/SABM, 2006:64.

Patient Eligibility

Intraoperative blood recovery is useful for patients undergoing surgical procedures with a large anticipated blood loss—ie, greater than 2 to 3 units of blood. Intraoperative blood recovery has been used most often in cardiovascular and vascular surgery. There are no age restrictions, and blood recovery techniques are used in pediatric surgery.

Blood recovery should not be attempted from any operative site that is potentially contaminated with bacteria. This caution applies to abdominal surgery in which the surgical field could be contaminated with intestinal contents or to any procedures involving preexisting infections at the surgical site. Furthermore, many centers do not perform blood recovery from surgical sites involving malignancies because cancer cells may also be collected. To do otherwise creates the theoretical risk of promoting metastasis by the parenteral infusion of malignant cells. However, no evidence that metastasis occurs has been reported, and consensus on this issue has not been achieved.



Intraoperative blood recovery is typically most successful when combined with other techniques, such as PAD or use of hemostatic agents.

Testing and Storage

Recovered blood is usually not tested because it expires 4 hours from the end of collection and typically does not leave the patient's bedside (see Table 9-5). However, blood that has been collected during surgery and that has been saline-washed and processed can be stored for 24 hours from the start of collection, but only if it has been refrigerated within 4 hours of the completion of collection. Whenever any recovered blood is stored in the blood bank, it should be appropriately labeled as if it were PAD units. Positive identification of the recipient and the recovered blood is particularly critical at the time of reinfusion in this setting because other tests and checks of compatibility are not performed.

Transfusion Trigger

Typically, recovered blood is all reinfused into the patient without consideration of a transfusion trigger. However, the patient's cardiac status and intravascular volume should still be considered in order to avoid hypervolemia.

Quality Oversight of Intraoperative Blood Recovery

Usually, intraoperative blood recovery is managed by surgeons and operating room nurses. However, the transfusion service director and supervisor should play a role in

Table 9-5. Handling, Storage, and Expiration of Perioperative Autologous Red Cell Blood Products*

Collection Type	Storage Temperature	Expiration	Special Conditions
Acute normovolemic hemodilution (whole blood)	Room temperature	8 hours from start of collection	None
Acute normovolemic hemodilution (whole blood)	1-6 C	24 hours from start of collection	Storage at 1-6 C shall begin within 8 hours of start of collection
Intraoperative blood recovery with processing	Room temperature	4 hours from completion of processing	None
Intraoperative blood recovery with processing	1-6 C (NA if bacterial contamination is suspected)	24 hours from start of collection	Storage at 1-6 C shall begin within 4 hours of completion of processing
Intraoperative blood recovery without processing	Room temperature	6 hours from start of collection	None
Shed blood under postoperative or posttraumatic conditions with or without processing	NA	6 hours from start of collection	None
Combined intraoperative and postoperative blood recovery with processing	Room temperature	Intraoperatively processed units: 4 hours from the completion of processing Postoperatively processed units: 6 hours from the start of post-operative collection	None
Red Blood Cells prepared by apheresis and intended for transfusion	Room temperature	8 hours from the start of collection	None
Red Blood Cells prepared by apheresis and intended for transfusion	1-6 C	24 hours from the start of collection	Storage at 1-6 C shall begin within 8 hours of collection

*Used with permission from Ilstrup SJ, ed. Standards for perioperative autologous blood collection and administration. 3rd ed. Bethesda, MD: AABB, 2007:21-5.

NA = not applicable.

1) designing and implementing written procedures for collecting and processing (if applicable) recovered blood, 2) ensuring the proper training and competency of personnel, 3) managing the quality control of equipment function and procurement of supplies, and 4) ensuring adequate documentation of activities and retention of records. Helpful in this effort is the AABB *Standards for Perioperative Autologous Blood Collection and Administration*, which identifies the elements of a well-designed program and forms the basis for AABB accreditation of perioperative activities. The CAP inspection checklist and laboratory accreditation program also address intraoperative blood recovery.

Postoperative Blood Collection and Reinfusion

Postoperative blood recovery is the collection of the patient's blood from surgical site drainage tubes after surgery. The technique is commonly used after cardiac and orthopedic procedures.

Benefits

As with intraoperative techniques, postoperative blood recovery has the advantage of being available to many patients who are unable to donate autologous blood preoperatively or who have not donated sufficient blood to cover their total transfusion needs. The blood does not usually leave the patient's bedside, so the risk of administrative errors and patient misidentification may be reduced. The blood is not stored or tested, which reduces laboratory costs and labor.

Limitations and Risks

Blood recovery after surgery, as with recovery during surgery, rarely collects enough blood to meet total RBC transfusion needs. Blood collected postoperatively, except in cases of brisk hemorrhage, usually has undergone extensive coagulation factor activation and fibrinolysis and is substantially hemodiluted. Frequently, coagulation factor levels and platelet numbers are greatly reduced. As a result, if large volumes are collected and reinfused, dilutional thrombocytopenia and coagulopathy are possible. Shed blood that is not saline-washed contains fibrin degradation products, pro-inflammatory cytokines, tissue debris, and bacteria. Infusion of those and other cellular products



Processing of shed blood before reinfusion will reduce the frequency and severity of adverse events experienced by the patient.

is likely related to the hypotensive, febrile, and septic reactions (as well as nephrotoxicity and disseminated intravascular coagulation) that have been reported after postoperative blood recovery. Because of concern that these reactions could be related to the amount of shed blood reinfused, some facilities place volume restrictions on the reinfusion of unprocessed, postoperatively shed blood.

Patient Eligibility



Significant reductions in allogeneic transfusion are best achieved when postoperative blood losses exceed 500 to 1000 mL, or an ongoing postoperative blood loss of >100 mL/hour.

For postoperative recovery of shed blood, patients must exhibit significant ongoing postoperative blood loss (eg, >100 mL/hour) for the effort to be worthwhile. Postoperative recovery has been used most commonly after orthopedic and cardiovascular surgery. Postoperative blood recovery without cell washing is contraindicated when the operative field has been contaminated with irrigating solutions such as povidone-iodine, certain antibiotics not intended for intravenous use, and topical hemostatic agents.

Testing and Storage

Generally, blood shed and recovered after surgery is not tested because it expires 6 hours from the start of collection and typically does not leave the patient's bedside. Whenever any recovered blood is stored in the blood bank, it should be appropriately labeled and segregated. Positive identification of the recipient and the recovered blood is particularly critical.

Transfusion Trigger

Typically, all the blood recovered after surgery is reinfused into the patient without consideration of a transfusion trigger. However, the patient's cardiac status and intravascular volume should be considered in order to avoid hypervolemia.

Quality Oversight of Postoperative Blood Recovery



The management of postoperative blood recovery is typically a focus of the nursing staff.

Blood banks and transfusion services usually do not directly manage postoperative blood recovery programs. Typically, postoperative blood recovery is a focus of the nursing staff. However, it is essential that the blood bank or transfusion service medical director and the technical supervisory staff maintain at least an advisory role. Their role should involve giving input and ensuring that written procedures exist for all techniques as well as reviewing

and approving procedures with regard to their compliance with regulatory and accreditation standards. The blood bank or transfusion service director should ensure that postoperative blood recovery is subject to quality management, including appropriate training of personnel, competency assessment, proper maintenance of equipment, procurement of adequate and appropriate supplies, quality control of collected blood, documentation, and retention of records. The AABB has created *Standards for Perioperative Autologous Blood Collection and Administration*, which addresses aspects of postoperative blood recovery. The AABB has a voluntary accreditation program for compliance with those standards. The College of American Pathologists' laboratory accreditation program also has a checklist of items that apply to the role of the blood bank medical director.

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Erythropoietin, Blood Substitutes, and Enzymatically Converted Red Cells

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NO TRANSFUSION IS RISK FREE. MANY INDIVIDUALS will not accept blood transfusions for religious reasons or because of concern about transmission of infectious diseases. Thus, recipient consent for blood transfusion must include discussion not only of the risks and benefits of transfusion, but also the alternatives a patient may choose to consider. Alternatives to transfusion of Red Blood Cells (RBCs) include pharmacologic agents designed to stimulate marrow erythropoiesis, hemoglobin solutions and hemoglobin substitutes, and enzymatically converted red cells that can be used in lieu of group O RBCs in emergent transfusion situations. One must bear in mind, however, that neither red cell substitutes nor enzymatically converted red cells are commercially available at this time. Thus, any potential benefits mentioned remain theoretical.



Recipient consent for blood transfusion must include risks, benefits, and alternatives.

Recombinant Erythropoietin

Human erythropoietin (EPO) is a heavily glycosylated protein that fosters maturation of marrow erythroid progenitors in response to hypoxic stimuli and, thus, indirectly in response to anemia. The glycosylation of EPO stabilizes



Despite the fact that recombinant erythropoietin (rhEPO) is not of human origin, most preparations are stabilized in a small amount of virus-inactivated human-source albumin, which may be important to some Jehovah's Witnesses. Aranesp (darbepoetin alfa) comes in two formulations, one of which contains polysorbate but no human albumin.



rhEPO takes 3 to 4 days to begin working and results in the production of the equivalent of 1 g/dL of hemoglobin in a week.

Thus, rhEPO is of no value in the acute treatment of anemia resulting from hemorrhage.

the tertiary structure of the protein and is required for proper functioning in humans. Sialic acid residues in the carbohydrate moiety of glycosylated EPO retard clearance of EPO by the liver but are not critical to EPO's function per se.

In fetal life, EPO is produced in the liver until late in gestation when there is a gradual shift to the kidneys as the primary site of synthesis, where it remains in adults. Once formed, EPO circulates and binds to the EPO receptor on the surface of erythroid progenitor cells in the marrow. Plasma levels of EPO are inversely proportional to the level of circulating hemoglobin and do not increase consistently outside the normal range until the hemoglobin concentration decreases below 10.5 g/dL.

Recombinant human erythropoietin (rhEPO) has been available for therapeutic use in humans for nearly two decades. As a pharmacologic preparation, rhEPO is dispensed in vials of 2000 U, 3000 U, 5000 U, and 10,000 U; it is available for either subcutaneous or intravenous injection. Because the thrice-weekly dosing regimens that were typical with first-generation rhEPO preparations can be inconvenient for some patients, a modified rhEPO (darbepoetin) with a longer half-life and weekly dosing is now available; typical dosing for darbepoetin begins at 2.25 µg/kg given subcutaneously once a week. The rhEPO must be stabilized in albumin or polysorbate to prevent adherence to glass surfaces, plastic tubing, or both because of the hydrophobic nature of the molecule. Thus, despite the fact that rhEPO itself is not of human origin, the presence of human-source albumin in most rhEPO preparations, albeit in minuscule amounts, may be of importance to some Jehovah's Witness patients. Darbepoetin alfa (Aranesp, Amgen, Thousand Oaks, CA) is available in two formulations, one of which contains polysorbate but no human albumin.

Adequate iron stores are required if rhEPO is to effect an increase in hemoglobin concentration. Therefore, rhEPO is often given in conjunction with supplemental iron. When administered parenterally, rhEPO takes 3 to 4 days to elicit a response and results in the production of the equivalent of 1 g/dL of hemoglobin in approximately 1 week. Thus, rhEPO is of no value in the acute treatment of anemia caused by hemorrhage and can be expected to effect only modest increases in hemoglobin over a period of weeks, not hours or minutes.

Over the past decade, it has become apparent that chronic use of rhEPO, particularly in renal failure patients, can be accompanied by the development of pure red cell

aplasia (PRCA), which manifests as profound anemia associated with reticulocytopenia and the virtual absence of erythroid precursors in the marrow. The causative relationship between PRCA and rhEPO administration is confirmed by demonstrating the presence of erythropoietin-neutralizing antibodies in the patient's circulation. Patients with PRCA caused by rhEPO may require immunosuppression and even renal transplant with immunosuppression because simple cessation of rhEPO therapy does not suffice to reverse the process. The number of cases of PRCA varies with the nature of the product—92% of the cases in one study were associated with Eprex (Janssen-Ortho, Toronto, ON, Canada), a product marketed outside the United States. That figure suggests that unique aspects of the manufacture or delivery of the product may play a role in the risk of PRCA. In addition, the cause of the patient's underlying anemia and the route of administration are also factors; the vast majority of patients who developed PRCA had chronic renal disease and were receiving rhEPO by the subcutaneous route. It has been postulated that immunosuppression resulting from cancer chemotherapy may account for the absence of cases in such a setting.



Treatment of chronic renal failure patients with rhEPO may cause pure red cell aplasia, probably the result of induction of EPO-neutralizing antibodies. Certain rhEPO preparations (Eprex) are more likely than others to be associated with this complication.

rhEPO in Renal Disease

Despite effective dialysis, severe anemia remains a major impediment to the rehabilitation of renal dialysis patients, a quarter of whom require RBC transfusions. The diseased kidneys of patients with end-stage renal disease are unable to produce adequate EPO, resulting in a hypoproliferative anemia characterized by very low levels of endogenous EPO.

The first clinical trials of rhEPO were in renal failure patients. The rhEPO was shown to be highly efficacious in raising hemoglobin levels and reducing transfusion requirements in those patients, with acute response rates as high as 97% and with similar results with maintenance therapy. Immunologic resistance to rhEPO was not encountered, nor was there evidence of the formation of antibodies directed against EPO. After approval by the Food and Drug Administration (FDA) in 1989, rhEPO again proved effective. Response rates were lower, though, than had been anticipated on the basis of the results of the clinical trials, both in terms of the proportion of patients achieving hematocrits above 30% and in the percentage of patients achieving transfusion independence. Subsequent studies have suggested that, in dialysis-dependent patients, this



rhEPO has proven efficacious in chronic renal failure, but target hemoglobin levels should be approximately 11 g/dL and should not exceed 12 g/dL.



Inadequate dialysis may result in poor response to rhEPO. Other causes of poor response or loss of earlier responsiveness include:

- Bleeding
- Relative iron deficiency



Side effects of rhEPO in patients with chronic renal failure include hypertension, seizures, and thrombotic events. Maintaining high hemoglobin levels (>12 g/dL) has been associated with increased risk of stroke and myocardial infarction and appears to be no more beneficial than correcting hemoglobin levels to around 11 g/dL.

disparity may reflect inadequate dialysis, which has been shown to be associated with a poor response to rhEPO. Although still speculative, the poor response in that setting may result from inadequate removal of uremic inhibitors of erythropoiesis.

Patients undergoing dialysis can receive rhEPO intravenously after hemodialysis; those not undergoing dialysis receive rhEPO subcutaneously. When renal patients fail to respond to rhEPO or lose responsiveness after an initial improvement, it is likely that another source of anemia is present. Failure of the rhEPO response should trigger a search for other causes such as blood loss, relative iron deficiency, inadequate dialysis, or a combination of these.

No discussion of the use of rhEPO in patients with chronic renal failure would be complete without a discussion of what constitutes an appropriate target hemoglobin in this setting. Current practice guidelines recommend partial correction to a hemoglobin level around 11 g/dL rather than complete correction into the normal range, noting that there is insufficient evidence to support maintaining hemoglobin levels at 13 g/dL or higher. This recommendation is borne out by two trials published in the *New England Journal of Medicine* in November 2006, both of which found no benefit to maintaining hemoglobin in the normal range in chronic renal failure patients. Indeed, one of these studies found an increased risk of stroke, myocardial infarction, hospitalization for congestive heart failure, and even death in the group whose target hemoglobin was 13.5 g/dL (actual mean hemoglobin achieved 12.6 g/dL) rather than a more conservative 11.3 g/dL. In addition, the higher hemoglobin group did not show an incremental improvement in quality of life.

Reported side effects of rhEPO treatment in renal failure patients include hypertension, seizures (sometimes in the setting of hypertensive encephalopathy), clotting of venous access, myalgias, and a flulike syndrome. Several reasons have been proposed to explain the occurrence of hypertension in rhEPO-treated renal patients, most notably, changes in blood viscosity and increased peripheral vascular resistance resulting from reversal of the compensatory vasodilatation seen in anemia. Seizures and vascular access thrombosis, although seen in rhEPO-treated renal patients, also occur in renal patients who are not treated with rhEPO. Relative or absolute iron deficiency is seen in a significant proportion (43%) of treated patients, most likely reflecting mobilization of iron stores to support increased

erythropoiesis. Treatment with rhEPO often requires concomitant supplemental iron.



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rhEPO in the Anemia of Chronic Disease

The anemia of chronic disease (ACD) is a normocytic anemia characterized by diminished circulating iron despite adequate iron stores. Thus, the measured serum iron level in ACD is low, transferrin levels are normal to decreased (unlike the increased levels seen in iron deficiency anemia), and serum ferritin is increased. Marrow iron is increased, thus reflecting poor mobilization of stored iron. ACD is seen in chronic inflammatory states such as rheumatoid arthritis and in a variety of infections, both acute and chronic.

Decreased red cell survival and impaired mobilization of iron from the reticuloendothelial system play a role in the pathogenesis of ACD, as does impaired production of, and impaired response to, EPO. Inflammatory cytokines are felt to be central players in that they appear to be responsible for both the submaximal production of EPO in response to anemia and the impaired response of erythroid progenitors to EPO that is produced. Furthermore, although not strictly included under the heading of ACD, other disease states such as cancer and human immunodeficiency virus (HIV) infection are accompanied by some degree of chronic inflammation and may also present with intercurrent infection. The same processes that cause poor EPO production and blunted response to EPO in ACD are probably also at work in cancer and HIV infection.

Most patients with ACD are only moderately anemic and, thus, may not benefit from rhEPO therapy. However, in the 20% to 30% of ACD patients whose anemia is severe enough to require RBC transfusion, rhEPO may be beneficial. rhEPO has been used effectively in patients with rheumatoid arthritis and, according to an anecdotal report, in a patient who required preoperative autologous blood donation because of the presence of multiple allo-antibodies but who would otherwise have been unable to donate because of ACD.



The anemia of chronic disease (ACD) is characterized by poor mobilization of stored iron, but suboptimal production of EPO and a poor response to the EPO that is produced also play a role.



rhEPO may be beneficial in ACD patients who develop anemia severe enough to require transfusion support, but these are the minority of patients with ACD.

rhEPO in Malignant Disease

The anemia seen in cancer patients is multifactorial in origin. The anemia of chronic disease; marrow invasion by tumor; and the effect of chemotherapy, radiation therapy, or both on marrow may play a role, as may subclinical



rhEPO is effective in cancer patients receiving chemotherapy but may be detrimental to those patients whose anemia is caused by the underlying disease rather than by chemotherapy.



New evidence suggests that rhEPO may be detrimental in patients receiving radiation therapy for cancer.

hemolysis. Current evidence suggests that blunted endogenous EPO production mediates a number of these causes of anemia. For example, levels of endogenous EPO that are lower than anticipated given the level of anemia have been demonstrated in anemic patients with solid tumors, and this effect is exacerbated by chemotherapy.

In view of the relative deficiency of endogenous EPO, one might imagine that anemic cancer patients would benefit from rhEPO therapy. Indeed, rhEPO has proven efficacious in patients with chemotherapy-related anemia. Current guidelines from the American Society of Clinical Oncology/American Society of Hematology recommend the use of rhEPO for chemotherapy-related anemia when the hemoglobin falls below 10 g/dL and occasionally in patients with declining hemoglobin levels below 12 g/dL. Continued treatment beyond 6 to 8 weeks in the absence of response does not appear to be beneficial. On the other hand, one must recognize that rhEPO may well be ineffective in anemia caused by underlying malignancy rather than anemia resulting from chemotherapy.

In the past, some workers advocated that hemoglobin levels greater than 12 g/dL might improve relapse-free intervals and overall survival in certain cancers, based on the theory that improved oxygenation may render tumor cells more sensitive to both radiation and cytotoxic drugs. However, others have observed that raising the hemoglobin beyond levels at which anemia is corrected might be detrimental because doing so is associated with a higher risk of thrombotic and cardiovascular events and might have a negative effect on tumor control.

Indeed, recent data submitted to the FDA have resulted in the issuance of an alert covering the use of erythropoietin stimulating agents (ESAs) to achieve target hemoglobin levels of 12 g/dL or higher in cancer patients receiving radiotherapy and in cancer patients whose anemia was not the result of chemotherapy.

With respect to the theoretical benefit of higher hemoglobin levels in patients receiving radiation therapy, the FDA reported on receipt of interim results from Danish Head and Neck Cancer Study Group trial (DAHANCA 10) in December 2006. In this study, the group treated with rhEPO (Aranesp) to maintain hemoglobin concentration during radiotherapy did significantly worse in terms of "loco-regional disease control." Overall survival figures showed a trend, albeit not statistically significant, toward worse outcomes in the EPO-treated group. These findings were consistent with observations made in a 2004 study

that found increase tumor progression and increase mortality in patients treated with rhEPO.

With respect to patients not receiving chemotherapy, in January 2007 the FDA was notified of the results of a large ($n = 989$) multicenter randomized controlled trial of Aranesp in anemic cancer patients not receiving chemotherapy. In this study, the EPO-treated group showed not only increased mortality compared to those receiving placebo but also no reduction in the need for RBC transfusions. In February 2007, the FDA was notified of final results of a placebo-controlled trial of rhEPO (epoetin alfa) in non-small-cell lung cancer patients not receiving chemotherapy that yielded similar results--shorter median survival in the rhEPO-treated arm, with the majority of deaths resulting from disease progression, and no significant reduction in RBC transfusion needs nor any improvement in quality of life. In addition, in February 2007, the FDA halted the study of an investigational new ESA due to safety concerns, namely an "imbalance in the number of deaths across the four arms of the study."



Treatment with rhEPO has been associated with poorer outcomes in two studies of anemic cancer patients who were not receiving chemotherapy.

rhEPO in the Perioperative Period

Because rhEPO administered intravenously takes 3 to 4 days to elicit a response and results in the production of only modest amounts of hemoglobin (1 g/dL in a week), rhEPO has no role in the management of acute hemorrhage, except perhaps in Jehovah's Witness patients for whom there is no alternative therapy. Two more realistic potential roles for rhEPO in surgical patients are the following: 1) improving the yield of preoperative autologous blood donation (PAD) and 2) accelerating the postoperative recovery of hemoglobin, thus reducing the need for transfusion.

However, data on the role of rhEPO in reducing allogeic blood exposure by improving the yield of PAD have been mixed, data on the efficacy of rhEPO in facilitating recovery from surgical anemia are at best sparse, and the FDA has recently reemphasized the lack of indications for perioperative use of rhEPO in orthopedic surgery patients. Furthermore, one must keep in mind that rhEPO is not without risk and concerns about the thrombotic risk are rising, particularly after the recent publication of data on chronic renal failure patients. Specifically, the FDA recently reported on preliminary results from a large multicenter randomized controlled trial of epoetin alfa in adults under-



Data supporting the use of rhEPO to reduce perioperative allogeneic blood exposure are weak, at best. More important, the safety of rhEPO in this setting has been questioned because of an apparent increase in thrombotic events, especially deep venous thrombosis, in rhEPO-treated patients.

going elective spinal surgery. In this study, the frequency of deep vein thrombosis in patients treated with rhEPO was more than twice that of patients treated with more conventional blood conservation techniques.



Endogenous EPO production has been found to be inappropriately low in:

- chronic renal failure
- anemia of chronic disease
- cancer



rhEPO is the standard of care in chronic renal disease (partial correction of anemia), is effective in cancer patients receiving chemotherapy, and possibly effective in patients with anemia of chronic disease.

However, rhEPO has been associated with increased thrombotic risk in several settings:

Perioperative use in patients with renal failure. Patients with hypertension.

It has been associated with poorer outcomes in:

- Cancer patients receiving radiation therapy.
- Cancer patients whose anemia is not the result of chemotherapy.

Summary of EPO and rhEPO

Erythropoietin is produced by the kidneys in response to increased transcription of the EPO gene induced by hypoxia and, thus, indirectly by anemia. It serves the vital function of stimulating erythropoiesis, thereby restoring oxygen delivery. Endogenous EPO production has been found to be inappropriately low in a number of anemic states, most notably, in chronic renal failure, ACD, and cancer.

rhEPO has long been the standard of care in patients with chronic renal failure but recent data in this population emphasize the fact that the aim should be partial, not complete, correction of anemia. In particular, the target hemoglobin should not exceed 12 g/dL and dosing should be aimed at achieving the lowest possible hemoglobin level that suffices to avoid RBC transfusion. rhEPO is widely used, albeit with variable results, in the management of chemotherapy-induced anemia in patients with malignancies, but may actually be detrimental in patients when used to raise the hemoglobin level to a target of 12 g/dL or higher in cancer patients not receiving chemotherapy and in patients receiving radiation therapy. rhEPO may be of benefit in anemia associated with other chronic diseases (ACD).

While initially appearing to have potential in the perioperative setting, rhEPO has since been associated with increased risk of thromboembolic complications in orthopedic patients. As a consequence, the FDA recently reminded practitioners that Aranesp (darbepoetin alfa), in particular, is not approved for use in this setting. In addition, rhEPO should not be used in patients with uncontrolled hypertension. Patients with hypertension and/or a history of cardiovascular disease require close monitoring, with particular attention to blood pressure control.

Finally, in a small number of cases, chronic use of certain rhEPO preparations (most notably Eprex, which is not sold in the US) has been linked to development of PRCA caused by erythropoietin antibodies, although reformulation of the products in question may have alleviated the problem.

Blood Substitutes

Any discussion of strategies for use of blood substitutes (also called artificial oxygen carriers or oxygen therapeutics) must begin with clarification of the expected benefit. The two obvious goals are 1) limiting exposure to allogeneic red cells and 2) rapidly providing compatible oxygen transport even outside the hospital setting. Less obvious is the fact that some blood substitutes have lower viscosity than red cells, enabling their use in situations where viscosity may be a limiting consideration. Other considerations include extending shelf life, thus ensuring availability when conventional blood supplies cannot meet demand, and, perhaps, avoiding the immunosuppressive effects of blood transfusion.

Given those goals, the settings in which blood substitutes, all of which have a shorter in-vivo survival rate than do human red cells, are most likely to be beneficial include the following:

Trauma and emergent surgical hemorrhage

Elective surgery when significant blood loss is anticipated

Situations in which reducing blood viscosity is desirable (eg, in patients with sickle cell disease and those at risk for cerebral vasospasm)

Cardiopulmonary bypass as a substitute for red cell pump prime

Situations in which it is desirable to improve blood flow to poorly vascularized tumors to increase their sensitivity to radiation therapy

Several of those potential uses will be discussed later, but first let us summarize the current state of development of the various blood substitutes and their unique characteristics.

Perfluorocarbons

Perfluorocarbons (PFCs) are fluorine-substituted hydrocarbons that dissolve oxygen and other gases. The amount of oxygen dissolved is directly proportional to the partial pressure of oxygen. In other words, PFCs do not have the sigmoidal dissociation curve that hemoglobin-based oxygen transport does. PFC-dissolved oxygen is not affected by temperature, pH, and 2,3-diphosphoglycerate levels.

PFCs are insoluble in water and must be emulsified for intravenous use. Although there were concerns about complement activation associated with the emulsifiers used with early perfluorocarbons (Fluosol-DA, Green Cross Corp., Osaka, Japan), the second-generation PFC prepara-



The search for blood substitutes stems from the desire to:

Improve availability of oxygen-carrying capacity in emergencies:

- in the field before arrival at a hospital
- in times of critical red cell shortages

Limit allogeneic blood exposure



Other potential uses for blood substitutes may include:

Reducing blood viscosity in sickle cell crises

As a substitute for allogeneic red cell prime in cardiopulmonary bypass

As replacement fluid for use in acute normovolemic hemodilution (ANH).

tions pose less of a concern. High viscosity limited the concentration of Fluosol that could be infused and, thus, its capacity to carry oxygen, and concerns about prolonged tissue retention limited the volume of Fluosol used in clinical trials. It is not surprising, then, that infusion of that first-generation PFC did not improve outcomes in Jehovah's Witness patients with acute anemia.

Second-generation perfluorocarbons do not exhibit the same viscosity problems, and they can be infused at much higher concentrations. However, they are still limited by short half-lives (2-4 hours) and require very high fractional-inspired oxygen (FIO_2). That limitation confines their use to settings in which supplemental oxygen is available.

At present, the only FDA-approved blood substitute is a first-generation PFC (Fluosol), and the only approved indication for its use is as a supplemental oxygen carrier in percutaneous transluminal coronary angioplasty. Its efficacy in that setting is based on the ability of Fluosol to diffuse into poorly vascularized tissue, thereby improving oxygenation in ischemia. Fluosol, however, is no longer marketed, in part because its use was associated with a fullike syndrome resulting from the release of cytokines and the activation of complement. In addition, its frozen storage, which necessitated thawing before use, made it difficult to use in emergent angioplasty.

A newer perflubron emulsion, Oxygent (Alliance Pharmaceutical, San Diego, CA), has undergone some Phase III testing in Europe, but further testing has been impeded by toxicities observed in early studies (stroke and increased postoperative bleeding).



Perfluorocarbons are limited by short half-lives (2-4 hours) and by the fact that they require access to supplemental oxygen because they are useful only in the presence of high FIO_2 .



Extravasation of hemoglobin results in the binding of nitric oxide (NO), which makes NO unavailable as a vasodilator. This effect on NO is the primary cause of the toxicity (renal, especially) seen with early free hemoglobin solutions.

Hemoglobin-Based Oxygen Carriers

The source of hemoglobin used in hemoglobin-based oxygen carriers (HBOCs) can be human, recombinant, or bovine. Most of the toxicity (renal dysfunction, vasoconstriction) of cell-free hemoglobin in early studies was attributable to extravasation of unmodified hemoglobin tetramers that bind nitric oxide (endothelium-derived relaxing factor), making it unavailable to act as a vasodilator. As a consequence, HBOCs being evaluated are modified in some way to prevent extravasation. Those modifications include conjugation with a macromolecule such as polyethylene glycol, intramolecular crosslinking (eg, diaspisin-crosslinked hemoglobin preparations: 3,5-bis-dibromosalicyl-fumarate), and intermolecular crosslinking (eg, polymerization using glutaraldehyde).

Crosslinking and polymerization of hemoglobin decrease renal filtration and extend its intravascular half-life. The half-life in animals may be as long as 48 hours, but human half-life appears to be considerably shorter, in the range of 3 to 24 hours.

In addition to modifying hemoglobin to prevent extravasation, pyridoxyl phosphate is used to improve oxygen delivery by raising the P50 and right-shifting the oxyhemoglobin dissociation curve. HBOC preparations are formulated so that 1 unit delivers a mass of hemoglobin comparable to 1 RBC unit.

Liposome encapsulation to produce "artificial red cells" has thus far proven elusive, as have attempts at producing recombinant human hemoglobin. Tests of first-generation recombinant hemoglobin preparations were abandoned because of adverse effects caused by scavenging of nitric oxide, with resultant vasoconstriction. Second-generation recombinant hemoglobin appeared less toxic in that regard, at least in animal studies. Nonetheless, Baxter Healthcare (Deerfield, IL) discontinued development in 2003 because Phase I clinical results did not meet the company's expectations.

Emergency Uses: Trauma, Emergent Surgical Hemorrhage, and Sickle Crisis

Both the prolonged shelf life (1 year for hemoglobin solutions, 4 years for perfluorocarbons) of red cell substitutes and the fact that they do not require compatibility testing before use make them ideal candidates for use in emergent situations such as trauma or emergent surgical blood loss. Outside the hospital setting, it is conceivable that infusion of HBOCs could safely be instituted by paramedics instead of, or in addition to, fluid resuscitation. Use of PFCs in such a setting would be limited by the availability of supplemental oxygen and a delivery system capable of attaining an FIO₂ approaching 100%. Even in the hospital emergency room, red cell substitutes could potentially be of benefit in stabilizing acutely hemorrhaging patients in substitution for group O Rh-negative red cells, especially when O-negative red cells are in short supply.

Human-Source Polymerized Hemoglobin

Initial reports of human trials using polymerized human stroma-free hemoglobin (PolyHeme or Poly SFH-P, Northfield Laboratories, Evanston, IL) in trauma and urgent sur-



More recent hemoglobin preparations use polymerization or macromolecule crosslinking to prevent hemoglobin extravasation.



Early recombinant hemoglobin preparations had toxicity caused by nitric oxide scavenging, resulting in vasoconstriction. Work on a second-generation recombinant hemoglobin was suspended in 2003.



Red cell substitutes have a prolonged *shelf-life* (1 year or more) and do not require pretransfusion compatibility testing.



Early trials showed polymerized stroma-free hemoglobin to be effective in maintaining total hemoglobin levels and reducing allogeneic blood exposure in trauma patients. Subsequent studies have been less encouraging.



Postinfusion half-life of PolyHeme was 24 hours, with none of the preparation remaining in the circulation 3 days after infusion.



In a comparison of patients receiving PolyHeme for urgent trauma or surgical blood loss, PolyHeme was associated with lowered morbidity than historical control patients who received red cell transfusion (both with nadir hemoglobin levels <3 g/dL). However, questions have been raised about whether adverse effects were appropriately reported.

gery were encouraging. In preliminary trials, 59% of 39 patients treated were able to avoid allogeneic transfusion during the acute bleeding episode. Similarly, the first prospective randomized trial of the same preparation in 44 trauma patients demonstrated that PolyHeme was effective in maintaining total hemoglobin levels without significant adverse effects and that patients receiving PolyHeme received significantly less allogeneic blood during each of the first 3 days after trauma. The half-life of PolyHeme was 24 hours, with all of the preparation being cleared from circulation within 3 days. Equally important was the statistically significant reduction in allogeneic blood transfusion in the study group during each of the first 3 days after trauma.

In a subsequent trial, 171 patients who received PolyHeme in lieu of RBC transfusions for urgent traumatic or surgical blood loss were compared with historical controls. In the study, red cells and plasma were separated, allowing quantification of the contribution of red cell hemoglobin (RBC Hb) to the measured total hemoglobin (total Hb). Total Hb minus the RBC Hb represented the amount of hemoglobin provided by PolyHeme. Of the 171 patients who received PolyHeme, 18 (10.5%) died, compared with 48 deaths (16%) among the 300 bleeding patients who refused blood transfusion in the control group. Of the 18 fatalities in the PolyHeme group, 10 occurred early because of exsanguination, 3 occurred within the first week (cause unspecified), and 4 occurred late (3 of those attributed to multisystem organ failure). More striking was the comparative mortality among patients whose RBC Hb decreased below 3 g/dL. Mortality in the PolyHeme group with nadir RBC Hb below 3 g/dL was 25.0% (10/40), compared with 64.5% (20/31) mortality in control patients with comparable nadir RBC Hb. Of 12 patients taking PolyHeme whose RBC Hb decreased below 1 g/dL, nine survived. Although the study appears encouraging, significant problems remain, most notably the use of historical controls and the lack of reported data on the incidence of specific adverse effects.

The issue of appropriate reporting of adverse effects has plagued PolyHeme lately. A *Wall Street Journal* article published in February 2006 alleged that the manufacturer had abruptly discontinued a Phase III clinical trial of PolyHeme in elective abdominal aortic aneurysm surgery because 10 of the 72 patients in the study developed myocardial infarctions (MIs). The article further alleged that the PolyHeme trial was discontinued without public disclosure.

sure of significant possible side effects. In March 2006, Northfield published a summary of the trial on the company's website; the company maintained that the cardiac events were due to "complex fluid management issues in these patients" rather than to "direct pharmacologic effect of PolyHeme." Details of the press release can be found at www.northfieldlaboratories.com. Those details will not be covered further in this chapter because they have not been published in a peer-reviewed journal.

Proper disclosure of "known adverse effects" has also become an issue in a trial of PolyHeme in trauma patients, particularly insofar as such disclosure is required in order to obtain truly informed consent from study subjects. As a consequence, there is an ongoing probe by the US Senate into how the FDA and Northfield are handling informed consent in a current large trauma trial. At issue is the fact that the prehospital portion of the trauma trial compares PolyHeme to crystalloid resuscitation, but patients randomly assigned to receive PolyHeme in the field are continued on the product once they arrive at a hospital, rather than receiving blood transfusions. Of particular concern is the requirement that individuals living in any of the 18 states involved in the study who wish to opt out of the study need to wear an armband indicating this desire—a requirement that is highly dependent on the efficacy of educational outreach and on full public disclosure of potential adverse effects.

In December 2006, Northfield reported on the website preliminary results from this Phase III trauma trial. A total of 712 patients were randomly assigned to two groups—349 received PolyHeme and 368 received standard therapy of saline in the field followed by red cells when available. Patients in the PolyHeme arm received up to 6 units of PolyHeme during the first 12 hours before receiving RBC units. These preliminary results failed to demonstrate superiority of PolyHeme over standard treatment: 30-day mortality for the PolyHeme group was 13.2%, compared with 9.6% in the control arm (saline plus red cells). Indeed, when compared on a modified intention-to-treat basis, these figures failed to demonstrate noninferiority. Final analysis of data will need to be completed before any decision can be made regarding submission to the FDA.

Finally, in a nonsurgical emergency use of PolyHeme, a single case report details the successful use of PolyHeme on a compassionate basis in a patient who was at high risk of respiratory failure because of acute chest syndrome (ACS) resulting from sickle cell disease (SCD). The patient,



In a trial of PolyHeme in elective aortic aneurysm repairs, 10 of 72 patients developed myocardial infarctions.



A trial comparing PolyHeme to crystalloid resuscitation in prehospital trauma has come under fire recently because of ethical concerns.



Preliminary data from a trial of PolyHeme in trauma patients failed to demonstrate noninferiority of PolyHeme compared to standard therapy with saline and red cells.

a 26-year-old woman who refused blood transfusions on religious grounds, received a total of 12 units of PolyHeme over the course of 13 days, with improvement in her respiratory status and eventual discharge from the hospital.

Bovine-Source Polymerized Hemoglobin

PolyHeme is made from outdated human red cells and requires 2 units of (outdated) RBCs to produce 1 unit of PolyHeme—meaning that the availability of substrate from which this product can be made is limited. However, bovine red cells provide an alternative source of hemoglobin. That approach has been used by Biopure (Cambridge, MA), which is conducting Phase III clinical trials in the United States of the company's bovine-source polymerized hemoglobin, known as HBOC-201 (Hemopure). This product has shown early promise in a Phase I/II US study in adult patients with SCD who were not in crisis and subsequently in Phase III clinical trials involving orthopedic, vascular, and cardiac surgery. However, there was evidence of vasoconstriction manifested as slight but statistically significant increases in blood pressure in the treated arm; methemoglobinemia and oliguria (without significant change in renal function) were also reported. Another study reportedly detected immunoglobulin G (IgG) antibodies directed against HBOC-201 at follow-up. Those studies, which involved elective surgery, are discussed in more detail in the following section.

Use in Elective Surgery When Significant Blood Loss Is Anticipated

In acute normovolemic hemodilution (ANH), whole blood is removed shortly before surgery and is replaced with crystalloid or colloid. The blood, which is collected into blood bags, is stored and returned to the patient either at the end of surgery after cessation of blood loss or intraoperatively, if needed. ANH has the advantage of allowing surgical blood loss to occur at a lower hematocrit, thus minimizing red cell loss; in fact, ANH has been proposed as a cost-effective alternative to preoperative autologous blood donation.

Infusion of blood substitutes during the period of ongoing blood loss can enable delayed reinfusion of allogeneic blood until bleeding has abated, further minimizing red cell loss. A canine model of surgical blood loss examined dogs hemodiluted to a hemoglobin of 7 g/dL and then given



Bovine source polymerized hemoglobin (Hemopure) has been associated with elevated blood pressure, methemoglobinemia, and IgG antibodies directed against Hemopure.

a single dose of 60% perflubron, a second-generation perfluorocarbon. After those measures, further normovolemic hemodilution was performed to a hemoglobin of 3 g/dL. Perflubron-treated dogs sustained the second episode of hemodilution without impairment of tissue oxygenation and without significant differences from a group of dogs who were hemodiluted to 7 g/dL hemoglobin and maintained at that level.

Results in Phase III human normovolemic hemodilution trials with HBOCs have yielded mixed results. The controversial study of PolyHeme in elective abdominal aortic aneurysm involved its use to increase the volume of autologous blood collected by ANH. According to the company's press release, even though a greater volume of autologous blood was collected, the study failed to achieve the objective of avoiding transfusion of allogeneic blood. As previously noted, 10 of the 71 patients in the study developed MIs, although whether there is any relationship between the use of PolyHeme and MI remains uncertain.

Results of studies involving the bovine hemoglobin substitute HBOC-201 in elective surgical blood replacement have been more encouraging thus far. In one study involving nearly 700 orthopedic surgery patients, 350 of whom received HBOC-201, patients were randomly assigned to receive either RBCs or HBOC-201 at the first perioperative allogeneic transfusion decision. More than 95% of the patients receiving HBOC-201 avoided RBC transfusion for at least 24 hours, and nearly 60% avoided RBC transfusion throughout the 6-week study period. Overall medical risk to patients treated with HBOC-201 was said to be "not inferior" to that of patients who received allogeneic RBCs.

Likewise, in a randomized, single-blind study of 72 patients undergoing elective infrarenal aortic reconstruction, 27% (13/48) of patients receiving HBOC-201 did not require allogeneic RBC transfusions. The number of perioperative deaths was not significantly different between the two groups [3 of 48 (6%) in the HBOC-201 arm as opposed to 2 of 24 (8%) in the RBC arm], and there was no significant difference between the groups with respect to a wide variety of complications, including postoperative bleeding, stroke, heart failure, ventricular arrhythmia, and renal insufficiency. However, HBOC-21 was associated with transient small (15%) increases in systemic blood pressure and transient jaundice.

A randomized, double-blind study in 98 patients undergoing cardiac surgery likewise found small but statistically significant increases in mean arterial pressure in the



Phase III normovolemic hemodilution trials with HBOCs have yielded mixed results.



HBOC-201 resulted in small but statistically significant increases in blood pressure in cardiac surgery and in elective infrarenal aortic reconstruction patients.

HBOC-201 group but no statistically significant differences in heart rate, pulmonary artery wedge pressure, oxygen delivery index, and oxygen consumption index. Treatment with HBOC-201 eliminated the need for postoperative RBC transfusion in 34% of cases. Despite that figure, however, use of HBOC-201 resulted in only a modest degree of blood conservation—only about half a unit. One explanation is that HBOC-201 was observed to oxidize to methemoglobin, thereby limiting the efficacy of the product. In the limited subset of patients for whom methemoglobin levels were available, 15% of circulating HBOC-201 was in the form of methemoglobin on postoperative day (POD) #1, 40% on POD #2.

In 2002, the FDA denied an application for licensure of HBOC-201 (Hemopure), evidently because of the limited benefit observed in sparing RBC transfusions. In July 2005, a clinical trial designed to test the use of HBOC-201 in prehospital trauma was placed on hold by the FDA because of concerns about the blood pressure effects and the risk-benefit ratio in the setting described in the test protocol. In December 2006, the Blood Products Advisory Committee (BPAC) once again recommended that the FDA reject a proposal put forth by the US Navy for testing Hemopure in civilian trauma patients, under waiver of consent. However, although testing is at a standstill in the United States, Biopure has obtained licensure for this product in South Africa, and the company began selling it there in January 2006. A similar product (HBOC-301) is approved for the treatment of canine anemia both in the United States and Europe.

Data on the longer-term use of HBOC-201 are limited to anecdotal reports. In one case, compassionate use of bovine hemoglobin enabled successful support of a 21-year-old patient with severe autoimmune hemolytic anemia refractory to conventional therapy (pulse steroids, intravenous immunoglobulin, and cyclophosphamide) until the autoimmune process could be brought into control with cyclosporine. HBOC-201 was also used on a compassionate basis for the relatively long-term (18 days) postchemotherapy support of a Jehovah's Witness patient who eventually succumbed to her acute myelogenous leukemia. Although her death was attributed to the underlying disease, it is worth noting that this patient developed renal failure 15 to 16 days after transfusion with HBOC-201. Although her kidney failure was most likely the result of multisystem organ failure, toxicity caused by nitric oxide scavenging could not be completely excluded.



Testing of the bovine source hemoglobin preparation Hemopure has been placed on hold by the FDA.



Bovine-source hemoglobin preparations have been used on a compassionate use basis to treat a patient with severe autoimmune hemolytic anemia refractory to other therapies.

Potential Uses in Situations Where Reducing Blood Viscosity Is Desirable

Recombinant hemoglobin solutions have lower viscosity than either albumin or normal human hemoglobin at the same concentration. Mixing recombinant hemoglobin with RBC suspensions results in dose-dependent decrease in whole blood viscosity. In fact, use of recombinant hemoglobin as a replacement solution in an in-vitro simulation of blood loss resulted in blood viscosity comparable to that of electrolyte solution—lower than either plasma or hydroxyethyl starch, which are often used in hemodilution. Likewise, neither red cell aggregability nor red cell deformability, as measured by transit time through small cylindrical pores, was adversely affected by the addition of recombinant hemoglobin.

An HBOC solution might, therefore, conceivably be beneficial in the setting of acute sickle cell crises. By reducing viscosity and, thus, improving blood flow while simultaneously raising hemoglobin concentration and decreasing relative percentage of hemoglobin S, simple infusion of an acellular hemoglobin solution could potentially obviate the need for red cell exchange in sickle crises such as stroke, priapism, and ACS. Simple infusion would have the benefit of being quicker and less personnel-intensive than manual or automated red cell exchange. Furthermore, use of a hemoglobin-based red cell substitute could reduce recipient exposure to allogeneic donor blood and, thus, could decrease the potential for development of red cell alloantibodies—which is always a risk in multitransfused SCD patients. Because none of the hemoglobin solutions are long-lived, the patient would eventually require RBC transfusion; if performed after the crisis is past, the transfusion could be with the minimum volume of allogeneic RBCs required to raise the hemoglobin and to reduce the percentage of sicklable hemoglobin to acceptable levels.

Reducing viscosity while increasing oxygen-carrying capacity is also of potential benefit in myocardial or cerebral ischemia, particularly in the period immediately after subarachnoid hemorrhage when the patient is at risk for vasospasm. In such a case, cerebral vasodilators cannot be used because they dilate normal blood vessels and result in shunting blood away from ischemic areas. Infusing an HBOC to reduce viscosity while increasing oxygen-carrying capacity, if possible, would represent a better alternative than the current standard: hypervolemic hypertensive



Recombinant hemoglobin, when mixed with red cells, lowers whole blood viscosity without affecting red cell deformability or aggregability.



Because they are less viscous than red cells and do not contain red cell membrane antigens that can cause alloimmunization, red cell substitutes hold promise in the acute management of sickle cell crises.



Development of a second-generation recombinant hemoglobin was discontinued in 2003 because of disappointing results in early clinical trials.

Likewise, neither human-source nor bovine hemoglobin solutions appear close to FDA approval at this time.



Red cell substitutes may be useful as replacement for allogeneic red cells as pump prime for small children who cannot tolerate the extracorporeal red cell volume required for apheresis or cardiac bypass.



Although in theory blood substitutes have immense potential, results of clinical trials have thus far been disappointing.

therapy (increasing intravascular volume with blood or colloid and inducing hyper-tension with dopamine or dobutamine).

However, at the moment, recombinant hemoglobin has failed to meet expectations, and development of the second-generation recombinant product, rHb 2.0 (Baxter Hemoglobin Therapeutics, Boulder, CO), was discontinued in 2003 when it did not meet the company's expectations in early clinical trials. Likewise, neither human-source nor bovine hemoglobin solution appear close to FDA approval at this time (see above).

For information about a case report detailing the use of human-source polymerized hemoglobin (PolyHeme) in a patient with SCD with ACS, also at risk of respiratory failure, see the earlier section on emergency uses.

Potential Use in Cardiopulmonary Bypass as a Substitute for Red Cell Pump Prime

When children with small intravascular blood volumes undergo cardiopulmonary bypass or apheresis procedures, the extracorporeal volume in the pump circuit represents a significant percentage of the patient's blood volume, necessitating that the pump tubing be primed with blood. Theoretically, if the pump circuit could be primed with an acellular solution that maintains oxygen delivery as well as vascular volume, an allogeneic donor exposure could be eliminated. Again, one must bear in mind the necessity of 100% FIO₂ if PFCs were to be used rather than an HBOC.

Final Caveats About Blood Substitutes

Although, in theory, blood substitutes have immense potential in the situations described above, practical experience with a variety of those products has fallen short of expectations. For example, clinical trials of a diaspirin-linked hemoglobin solution were suspended because there were disproportionately large numbers of deaths in the group receiving the blood substitute. That suspension was followed several months later by suspension, for unspecified reasons, of trials of the same product in elective surgery cases. More recently, there are possible adverse cardiac effects with Poly SFH-P (PolyHeme) in the elective aortic aneurysm trial, concerns about elevation of blood pressure in HBOC-201 (Hemopure) trials, and the discouraging preliminary results just reported in trials of PolyHeme in trauma patients. Thus, it is not surprising that

considerable work remains to be done to ensure the safety and efficacy of the blood substitutes in development. The unknowns are particularly evident when those products are tested in the trauma setting where confounding variables frequently need to be taken into account.

Finally, with respect to ensuring availability in times of blood shortage, PFCs, bovine-source hemoglobin-based solutions, and rhEPO (for nonemergent use) all have promise, but the benefits of human-derived hemoglobin solutions remain to be seen. If rising demand for blood results in increasingly short supplies of RBC units, will there be sufficient outdated or otherwise unused human RBC units for processing into human-source hemoglobin solutions? Will the ability to store hemoglobin solutions in times of relative abundance for use at times of shortage normalize those fluctuations in supply, or is the benefit largely illusory? Finally, can a recombinant hemoglobin solution with an acceptable side effect profile be developed and in large enough quantities to meet demands? Even if hemoglobin solutions eventually prove to be safe and efficacious, those supply questions must be answered.

Enzymatic Conversion of Group B or A RBCs to Group O

In an attempt to ensure adequate supplies of compatible RBC units for use in emergent situations in which the patient's ABO group may not be known, research is ongoing into enzymatic conversion of non-group O red cells into group O cells and into the creation of "stealth" red cells by masking antigenic determinants with polyethylene glycol (PEG). If the latter approach could be perfected, it would have the advantage of masking antigenic sites other than ABO, resulting in truly universal red cells that are compatible with all patients, even those with known alloantibodies.

Research into enzymatic conversion of A or B red cells into O red cells has yielded more progress in the conversion of B cells to O cells than in the conversion of A cells to O cells. Group A antigens are structurally more complex, potentially contain more than one antigenic site, and, more important, may have antigenic sites that are not terminally located, making them relatively inaccessible to the converting enzyme.



Enzymatic conversion of group B red cells to group O is possible. Phase I and II clinical trials have shown that transfusion of these converted red cells into persons with circulating anti-B produces both the expected increase in hemoglobin and normal red cell survival.

Enzymatic conversion of group A red cells is more complex but work is progressing nonetheless.

Current techniques for converting B cells to O cells involve washing the red cells, adding alpha-galactosidase, and incubating the cells for 2 to 3 hours in the presence of the enzyme. Originally, the enzyme used was derived from green Santos coffee beans, but it is now also available in a recombinant form. That technique results in red cells that, aside from loss of B antigenic determinants, show no weakening of other major antigens, with the exception of P1 and p^k. Treated red cells are nonreactive with both human polyclonal and murine monoclonal B antisera.

In Phase I and II clinical trials, transfusion of enzymatically converted group B RBCs has been shown to produce the expected rise of 1 g/dL in hemoglobin per unit and to demonstrate normal survival *in vivo*. Multiple-unit transfusions in both healthy volunteers and patients were well tolerated, as were units transfused in more than one episode separated by months. No evidence of hemolytic reaction was seen, although a recent study did report posttransfusion increase in anti-B titer in five of 19 recipients of converted RBCs. Studies into the conversion of group A cells to group O cells remain in Phase I trials.

The second approach, creation of "stealth" RBCs, has proven more problematic. Because PEG attracts water molecules, it has been theorized that PEGylated red cell membranes would become coated with a shell of water, preventing antibodies from reaching antigens on the red cell surface. In theory, PEG treatment could result not only in "stealth" RBCs that do not react with preformed red cell antibodies, but also in the prevention of alloimmunization caused by red cell exposure. The reality, however, is not nearly as rosy because PEGylated RBCs have caused a number of problems.

PEG-treated RBCs present several problems during *in vitro* compatibility testing. First of all, they tend to adhere to the surface of test tubes. They also adsorb proteins nonspecifically onto their surface, causing significant positivity in monocyte monolayer assay testing—the very testing that is used to predict the clinical significance of a circulating antibody.

More important, it appears that, although PEGylation masks the majority of antigenic sites, sufficient unmasked sites may remain to permit antigen binding by antibody. Indeed, lysis of PEGylated group A RBCs by anti-A present in some group O sera has been noted. Little animal data and no human data yet address the efficacy of PEG treatment in preventing alloimmunization. Likewise, no human red cell survival studies have been reported.



PEGylation of red cell membranes has been tried in an attempt to create "stealth" red cells capable of escaping immune surveillance. However, like the hemoglobin-based red cell substitutes, PEG-treated red cells have not met their promise to date.

Finally, despite the fact that PEG is reputed to be nonimmunogenic, that conclusion may not be the case. Many healthy humans appear to have "naturally occurring" antibodies evidently directed against PEG itself—which is not surprising when one realizes that PEG is present in cosmetics, drugs, and food additives. Furthermore, in a rabbit model, when PEGylated RBCs are transfused, not only do the rabbits make antibody against those RBCs but also the antibody results in shortened survival of the PEG-RBCs. Clearly, there is much work to be done before PEGylated RBCs can live up to their potential, if, in fact, they are capable of doing so at all.

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11

Special Transfusion Situations

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THERE ARE A NUMBER OF CONDITIONS IN which transfusion of blood or blood components is especially important. This chapter focuses on the following such settings: hemolytic disease of the fetus and newborn (HDFN); neonatal alloimmune thrombocytopenia (NAIT); sickle cell disease (SCD); massive transfusions; platelet refractoriness; post-transfusion purpura (PTP); and the role of platelet transfusion in immune thrombocytopenic purpura (ITP), heparin-induced thrombocytopenia (HIT), and platelet dysfunction.

Hemolytic Disease of the Fetus and Newborn

Red cell hemolysis in fetal life, in the immediate postnatal period, or in both has a number of possible etiologies, including those unrelated to blood group alloimmunization, such as congenital and acquired red cell defects. Typically, however, the term is reserved for hemolysis of fetal or neonatal red cells caused by the transfer of maternal alloantibody across the placenta.

The problems associated with HDFN fall into two categories: those directly related to hemolysis (anemia) and those arising from the hyperbilirubinemia that occurs as a consequence of red cell hemolysis. Hemolysis of fetal red cells in utero results in extramedullary hematopoiesis,



Problems in hemolytic disease of the fetus and newborn are either the direct result of hemolysis (anemia) or secondary to hyperbilirubinemia.



Fetal hemolysis, if severe, can result in profound distortion of liver architecture by extramedullary hematopoiesis and in hypoxic cardiac injury, culminating in hydrops fetalis and eventual fetal demise.



Hyperbilirubinemia is not a risk during intrauterine life because bilirubin is processed via transplacental passage to the maternal circulation.



Kernicterus is a risk if hemolysis continues after birth because:

Low levels of glucuronyl transferase decrease the neonate's ability to conjugate bilirubin.
Bilirubin that is unconjugated and unbound to albumin can cross the newborn blood-brain barrier.

which may be sufficient to compensate for the anemia of mild hemolysis. More severe hemolysis with more pronounced extramedullary hematopoietic response can cause distortion of the architecture of the liver and can result in portal hypertension. In addition, severe hemolytic anemia in utero leads to hypoxic injury to the heart and liver, with resultant circulatory failure and loss of colloid osmotic pressure caused by defective protein synthesis by the liver. The ultimate result of severe in-utero hemolysis is hydrops fetalis and intrauterine fetal demise.

If the fetus survives to delivery, anemia in the immediate postnatal period may be severe enough to require support with either simple or exchange transfusion of red cells.

Because bilirubin is processed in utero by transplacental passage to the maternal circulation, the consequences of hyperbilirubinemia are confined solely to the newborn period. In the immediate postnatal period, newborns have low levels of glucuronyl transferase and thus diminished capacity to conjugate bilirubin. The neonate also has a blood-brain barrier that is more permeable to bilirubin than it is in older infants and in adults. Bilirubin that is neither bound to albumin nor conjugated is capable of crossing the newborn's blood-brain barrier and depositing in the brain, resulting in kernicterus.

Alloimmunization to Red Cell Antigens

The fetus (and neonate) is immune incompetent and cannot make red cell alloantibodies. However, a mother whose red cells lack a particular antigen can become sensitized and can produce alloantibody directed against that antigen. Sensitization can arise either as the result of transfusion of antigen-positive blood or by exposure to fetal red cells containing the antigen (inherited from the father of the fetus). If the antibody produced is immunoglobulin G (IgG), it can cross the placenta and bind to antigen-positive fetal red cells, resulting in hemolysis.

For example, if an Rh-negative mother carries an Rh-positive fetus, hemorrhage of fetal blood into the maternal circulation, referred to as fetomaternal hemorrhage, or FMH, can elicit maternal alloantibody directed against the D antigen. The IgG anti-D thus formed can cross the placenta, enter the fetal circulation, and bind to D-positive fetal red cells. Sensitized fetal red cells can then bind to Fc receptors on phagocytes, which triggers either phagocytosis or extravascular lysis of the coated red cells.

Some degree of FMH takes place at delivery in the vast majority of pregnancies, but FMH can also occur with abdominal trauma, abortion (even if unrecognized by the mother), placental abruption, amniocentesis, or other invasive procedures. Even minuscule amounts of FMH can present a sensitizing stimulus, depending on the antigen involved.

Although FMH takes place from the fetal to the maternal circulation, the transfer of antibodies occurs exclusively from the mother to the fetus, who is unable to produce antibody on his or her own. Only IgG antibodies are transported across the placenta. Immunoglobulin M (IgM) antibodies, even if present in the maternal circulation, are incapable of causing HDFN because they do not cross to the fetal circulation. Transplacental transport of antibody is slow until about the 24th week of gestation; that timing explains why HDFN is rare, although not unheard of, earlier in pregnancy.

Even though Rh antibodies and antibodies directed against the Kell antigen are typically thought of as being most important in inciting HDFN, other antibodies are also capable of resulting in severe HDFN. Antibodies in the Kidd, Kell, Duffy, MNS, and Diego systems have all been known to cause severe HDFN, as have ABO antibodies. ABO antibodies, however, are usually associated with only mild HDFN. Most other antibodies, even if capable of crossing the placenta, typically cause only mild HDFN and can be managed expectantly.

Aside from the antigen specificity of the antibody involved, other factors that influence the development and severity of HDFN are the strength of expression of the antigen on fetal red cells, the quantity of maternal antibody produced, and the amount of antibody transported across the placenta. The IgG subclass of the antibody may also be a factor in that IgG1 and IgG3 subclass antibodies appear to be of most importance in HDFN. However, the significance of IgG subclass remains controversial.

One final consideration in determining the likelihood of HDFN is antigen frequency, which determines the likelihood of an antigen-negative mother being exposed to a fetus who is antigen positive. For example, a Kell-negative mother is relatively unlikely to be exposed to a Kell-positive fetus because the antigen frequency of Kell is approximately 9%. Of course, if one can determine the phenotype of the father, one would have a much better idea of whether the fetus is at risk, even before delivery.



An antigen-negative mother bearing an antigen-positive fetus can be sensitized to the antigen by way of fetomaternal hemorrhage.

If the antibody the mother produces is IgG in class, it can cross the placenta and bind to antigen-positive red cells in the fetal circulation.



HDFN is rare, but not unheard of, before the 24th week of gestation because transplacental transport of antibody is slow during this period.

HDFN Caused by Anti-D

HDFN caused by sensitization to the D antigen is the most significant form of HDFN for several reasons. First, before the advent of Rh immune globulin (RhIG) prophylaxis, anti-D was the most common cause of HDFN. Second, anti-D can—and often does—cause severe HDFN, which can often result in intrauterine fetal demise caused by hydrops fetalis. Third and most important, Rh HDFN is preventable with the use of RhIG. Since RhIG was approved by the Food and Drug Administration in 1968, the incidence of HDFN caused by sensitization to the D antigen has fallen to approximately 1:1000 (or 0.1%).

The clinical significance of Rh HDFN primarily reflects the fact that the D antigen is a very potent immunogen, as well as the fact that there is a reasonably high likelihood that an Rh-negative mother will bear an Rh-positive fetus because about 85% of the population is Rh positive (D positive).

RhIG is a preparation of human-source IgG anti-D. An intramuscular preparation is intended for immunoprophylaxis during pregnancy. Intravenous preparations of RhIG are also available, but they are used primarily for treatment of immune thrombocytopenic purpura (ITP) in Rh-positive patients.

RhIG is prepared by pooling human plasma from individuals sensitized to the D antigen, subjecting the pooled plasma to cold alcohol (Cohn) fractionation, and purifying it so that it contains almost exclusively IgG. In the process of manufacturing, RhIG is virus inactivated and, despite more than 30 years of use, has only rarely been implicated in transmission of infectious disease.

RhIG suppresses the primary response to Rh in most women in whom it would otherwise occur, but it is of no value in a woman who has already been sensitized to the D antigen. It is typically dispensed in 300- μ g vials, each of which suppresses the immune response to 15 mL of Rh-positive red cells or 30 mL of Rh-positive whole blood.

The exact mechanism of action of RhIG is not known. The most simplistic explanation is that the anti-D in RhIG functions by masking antigen sites on fetal Rh-positive red cells in the maternal circulation, thus preventing recognition of those cells by the mother's immune system. A similar explanation is that RhIG facilitates clearance of antibody-coated Rh-positive red cells before they can elicit immune recognition. A final postulate involves central feedback inhibition of antibody formation by the IgG immune complexes formed by RhIG and its antigenic target.



Rh Immune Globulin (RhIG) can be given to an Rh-negative mother for prophylaxis against production of anti-D.

RhIG is a preparation containing polyclonal, human-source IgG anti-D designed for intramuscular or intravenous injection.



1 vial (300 μ g) of RhIG covers FMH of up to:
15 mL of red cells or
30 mL of whole blood

The half-life of RhIG in the circulation is approximately 24 days. Thus, RhIG must be given during each pregnancy of an Rh-negative mother carrying an Rh-positive fetus at 28 weeks of gestation and again within 72 hours after delivery. Two injections are required because a single 300- μ g dose at 28 weeks will not be sufficient to cover FMH at delivery, and a small percentage (1% to 2%) of women given RhIG only at delivery will nonetheless develop anti-D. In contrast to the single 300- μ g dose given at 28 weeks, the amount given at delivery must be adjusted according to quantitation of FMH. Even if more than 72 hours have passed, it may still be worth considering RhIG, although the studies demonstrating efficacy were all performed using a 72-hour interval, making the efficacy of delayed doses uncertain.

Every Rh-negative mother delivering an Rh-positive infant should receive at least one vial (300 μ g) of RhIG at delivery. Before administration of the postnatal dose, the Rh type of the neonate should be determined, usually from a sample of cord blood; an Rh-negative neonate would obviate the need for RhIG at delivery.

If the newborn is Rh positive, assessment of the quantity of RhIG required is accomplished through the use of two laboratory procedures performed on a maternal blood sample collected after delivery. The first procedure, the fetal cell screen (rosette test), is capable of screening for FMH greater than 30 mL of whole blood, that is, amounts of FMH in excess of what is covered by a single dose of RhIG. A negative fetal cell screen indicates that a single 300- μ g dose is sufficient, whereas a positive fetal cell screen requires follow-up quantitation of FMH either by a Kleihauer-Betke test or by flow cytometric measurement of cells containing fetal hemoglobin. Appendix 11-1 describes the fetal cell screen and Kleihauer-Betke test in more detail.

HDFN Caused by Antibodies Other Than Anti-D

HDFN caused by ABO antibodies occurs most often with mothers who are group O because they are more likely to have IgG isoantibodies, including IgG anti-A as well as anti-A,B. ABO HDFN is usually mild and is rarely severe enough to require exchange transfusion. The reasons are that A and B antigens are not fully developed at birth and that significant amounts of maternal antibody are neutralized by A and B substance present in body fluids and tissues. If exchange transfusion is required in such a setting, it is usually for hyperbilirubinemia and not for anemia.



RhIG should be given at 28 weeks of gestation and within 72 hours after delivery, if the newborn is Rh positive.



Rh-negative mothers delivering Rh-positive infants should be screened for evidence of FMH greater than 30 mL of whole blood (fetal cell screen).

If the screen is positive, the amount of FMH should be measured (Kleihauer-Betke test or flow cytometric measurement of cells containing fetal hemoglobin).



The fetal cell screen detects D-positive red cells and is of no use in evaluating FMH when the mother is Rh-positive or if both mother and infant are Rh-negative.

Paradoxically, the simultaneous appearance of ABO and Rh incompatibility appears to have a "protective" effect in Rh-negative mothers who are not already sensitized to the D antigen, thereby resulting in a lower risk of Rh sensitization than if the fetus did not carry A or B antigens. One postulate to explain this observation is that A or B antigen-containing Rh-positive fetal cells that enter the maternal circulation are destroyed before Rh sensitization can occur. In any event, once a mother has been sensitized to the D antigen, the protective effect of ABO incompatibility is lost.

A small percentage (2%-5%) of cases of HDFN result from antibodies other than ABO or anti-D. Those cases are caused by antibodies to the other Rh antigens and antibodies to antigens in the Kell, Kidd, Duffy, MNS, and Diego systems. Of those, the two most important are anti-Kell (anti-K) and anti-c. Sensitization to the former usually results from prior transfusion exposure, whereas c-antigen sensitization most often arises from FMH. RhIG is not protective against formation of any of those antibodies. By the same token, the presence of an antibody other than anti-D in an Rh-negative mother does not obviate the need for RhIG prophylaxis whenever she bears an Rh-positive fetus. All Rh-negative mothers with Rh-positive fetuses who have not already formed anti-D should receive RhIG, regardless of whether other significant alloantibodies are present.

Management of the Sensitized Pregnancy

All obstetric patients should have a type and screen at the outset of pregnancy, and the procedure should include testing for the presence of weak D if preliminary results suggest that the mother is Rh negative. In the absence of other risk factors such as abdominal trauma or amniocentesis, and if the initial antibody screen is negative in an Rh-positive mother, no further evaluation is required before delivery. Rh-negative mothers with negative initial antibody screens, however, should be retested immediately before receiving RhIG at 28 weeks.

If a maternal antibody is detected at any stage, it should be identified and, if significant, followed with periodic titers. Although there are no hard and fast rules, an anti-D titer in the range of 1:8 to 1:32 or greater or a twofold increase in titer is often cited as the "critical titer," the point at which aggressive management should be initiated. Because anti-K results in marrow suppression as well as sequestration of red cells in the spleen, the critical titer of this antibody is said to be lower (ie, 1:8). Critical titers for



After anti-D and ABO antibodies, the next most important antibodies in HDFN are anti-c and anti-K.



All obstetric patients should have a type and screen at the outset of pregnancy, including testing for weak D.

If the mother is Rh negative, she should be retested for anti-D before receiving RhIG at 28 weeks.

other antibodies vary depending on the antigen specificity of the antibody.

There have been a number of advances in the past decade in the way that fetal antigen status is determined. Although it is still possible to determine fetal phenotype from blood drawn by cordocentesis [the so-called percutaneous umbilical blood sampling (PUBS) procedure described below], fetal antigen status for D and several other antigens can also be ascertained through the use of polymerase chain reaction (PCR) techniques on amniocytes from amniotic fluid or chorionic villus sampling (CVS). If the intention is to continue the pregnancy even if the fetus is antigen positive, it is better to perform amniocentesis (taking care to avoid crossing the placenta during passage of the needle, rather than CVS, because disruption of chorionic villi can result in FMH. An even more exciting development was the discovery in 1997 that free fetal DNA (ffDNA) can be detected in maternal plasma during pregnancy. ffDNA is demonstrable as early as 32 days of gestation, rising to levels of up to 6% in the late third trimester, and disappearing within 2 hours of delivery. In the years since this phenomenon was first reported, assays have been developed for D typing on ffDNA. Such assays are used extensively in the United Kingdom as an alternative to performing amniocentesis for D typing, but those assays are not yet commercially available in the United States. A recent meta-analysis of studies on ffDNA concluded that Rh genotyping of ffDNA in maternal serum or plasma had a greater than 96% accuracy in determining fetal RhD type. However, concerns remain about the accuracy of testing in different ethnic groups and in the presence of Rh antibodies. There is also the issue of establishing a contingency plan to deal with the small percentage of cases that are misdiagnosed by this technique.

If incompatibility exists between the antibody-positive mother and the fetus, ultrasound testing should be performed early to establish the correct gestational age. Knowing gestational age is crucial to proper interpretation of amniotic fluid bilirubin levels. The presence of fetal hydrops on ultrasound is particularly ominous because fetal hemoglobin levels in the presence of hydrops are often one-third of normal or less.

When a high or rising antibody titer is detected, serial testing either with amniocentesis for measurement of amniotic fluid bilirubin level or with Doppler ultrasound measurement of peak flow in the fetal middle cerebral artery (MCA) is initiated. Measurement of amniotic fluid



It is possible to phenotype the fetus *in utero* either by cordocentesis (percutaneous umbilical blood sampling) or by PCR on amniocytes obtained by amniocentesis or chorionic villus sampling.



D phenotyping can be performed on free fetal DNA in the maternal circulation. Although this test is available in the United Kingdom, it is not commercially available in the United States at present.



Whenever a clinically significant antibody is detected in a pregnant woman, it should be titered.

High titers or rising titers may indicate that further evaluation is necessary.



Further evaluation and treatment may include:

- Amniocentesis and measurement of amniotic fluid bilirubin level
- Doppler flow velocity study of the fetal MCA
- PUBS to measure fetal hemoglobin and to phenotype the fetus
- Intrauterine transfusion
- Molecular phenotyping performed on amniocytes
- D phenotyping performed on free fetal DNA in the mother's circulation

bilirubin, referred to as ΔOD_{450} , is based on the change in optical density at 450 nm, which is the absorbance spectrum of bilirubin. That value is plotted on a curve known as the Liley curve, which stratifies risk into three zones on the basis of measured bilirubin level and gestational age. It is important to note that the Liley curve is useful only after 27 weeks and cannot be extrapolated to earlier dates. However, a modified version of the Liley curve, devised by Queenan et al, can be used to assess sensitized pregnancies before 28 weeks.

Bilirubin levels that fall into the lowest zone (Zone 1) on the Liley curve indicate that the pregnancy can be managed expectantly, whereas intermediate measurements (Zone 2) suggest that intrauterine transfusion, delivery, or both may be necessary. Values in the highest range (Zone 3) are more critical, and such cases usually require intrauterine transfusion, early delivery, or both. Similarly, a value falling within the intrauterine transfusion zone on the Queenan curve requires aggressive management. If the fetus is older than 34 weeks and has mature lungs, delivery is generally appropriate, whereas less mature fetuses or those with immature lungs are treated with intrauterine transfusion.

A noninvasive method of detecting fetal anemia uses the performance of Doppler flow velocity studies of the fetal MCA. Increased flow rates suggest the presence of anemia. In one study, a value of 1.5 multiples of the median (MOM) for gestational age was found to detect all cases of fetal anemia, with only a 12% false-positive rate. Because of the demonstrated 100% sensitivity and the noninvasive nature of the procedure, MCA Doppler flow studies are rapidly supplanting amniotic fluid bilirubin in the assessment of fetal anemia.

Both amniotic fluid bilirubin and MCA Doppler flow rates are indirect measures of fetal hemoglobin. It is also possible to obtain a sample of fetal blood and to measure fetal hemoglobin through the use of a technique called PUBS. In the PUBS technique, the umbilical vein is cannulated under ultrasound guidance and a sample of fetal blood is removed. The fetal blood sample can be used to measure fetal hemoglobin concentration and to phenotype the fetal red cells, if desired.

The PUBS technique provides direct intravascular access to the fetal circulation, permitting intravenous transfusion to occur in utero. Whenever PUBS is performed, it is advisable to have Red Blood Cells (RBCs) available for intrauterine transfusion should the measured hematocrit be less than 30%. Serial PUBS procedures can result in a



MCA Doppler flow studies are rapidly supplanting amniotic fluid bilirubin in the assessment of fetal anemia.

1% to 2% rate of fetal loss, so PUBS is usually reserved for patients with increased amniotic bilirubin, increased MCA Doppler flow rate, or both.

Although it is also possible to perform intrauterine transfusion through the intraperitoneal route, intravenous transfusion is a more effective method. Alternatively, some institutions prefer a combined approach, using both intravenous and intraperitoneal transfusion in an attempt to mitigate the sawtooth pattern of rising then falling hematocrit seen with intravenous fetal transfusion. Intrauterine transfusions can be started as early as the 18th week of gestation. (No in-utero deaths from HDFN have been reported before 17 weeks.) The results of intrauterine transfusion can be dramatic, even lifesaving, but the procedure is not without risk. Perinatal loss rates in the range of 1% to 3% have been reported with fetal transfusion.

The RBCs provided for intrauterine transfusion are usually O negative unless the antibody is anti-c (Rh-negative blood is very likely to be c positive), and the RBCs should be compatible with any maternal antibodies. Some would advocate the prophylactic use of RBCs that are also Kell negative if the mother is Kell negative. Usually, the hematocrit is adjusted up to 85% to 90% by removal of excess plasma. Experts recommend that RBCs for intrauterine transfusions be CMV negative, leukocyte reduced, and irradiated to prevent development of graft-vs-host disease (GVHD).

Several other therapies have been tried—with inconsistent success—to modulate the maternal immune response. Attempts have been made to decrease phagocytosis by the fetal reticuloendothelial system through the use of promethazine. Plasmapheresis alone is unreliable in keeping antibody levels down, and rebound has been described after cessation of plasmapheresis. Maternal intravenous immune globulin (IVIG) therapy has also been tried with some success (mechanism unknown). An acute reduction in antibody level with plasmapheresis is often more effective when coupled with some form of immunomodulatory therapy to keep the antibody level low (see Chapter 14: Therapeutic Apheresis). Thus, some centers are experimenting with daily plasmapheresis for 5 days during week 10 of gestation, followed by two loading doses of IVIG, then weekly IVIG maintenance until week 20.



Unless the antibody is anti-c, RBC units for intrauterine transfusion are usually Rh-negative and should be compatible with any other maternal antibodies. Irradiated CMV-negative units should be provided.

Management of the Newborn with HDFN

On admission to labor and delivery, every mother should have a type and screen, even if one was performed earlier;

any antibody present on the antibody screen should be identified. After delivery, a sample of cord blood from every newborn should be tested for ABO (forward typing only) and Rh, and a direct antiglobulin test (DAT) should be performed. Testing for weak D should be performed on every newborn who appears on initial testing to be Rh negative to ensure that all Rh-negative mothers of Rh-positive (even if only weak D) newborns do receive immunoprophylaxis with RhIG.



A positive cord blood DAT does not establish a diagnosis of HDFN—there must also be evidence of hemolysis (anemia or rising bilirubin).



If the newborn has a positive cord blood DAT, an antibody screen should be performed on either a maternal sample or a (venous) sample from the infant.



Causes of a positive cord blood DAT with negative maternal antibody screen include:

- ABO incompatibility between fetus and mother
- Antenatal RhIG coating neonatal red cells
- Maternal antibody directed against low-incidence red cell antigen present on neonatal red cells but not on panel red cells

For all positive cord blood DAT results, information regarding the presence or absence of symptomatic anemia should be sought. It is important to recognize that a positive DAT result alone does not establish the diagnosis of HDFN. The positive DAT must be accompanied by signs or symptoms of premature destruction of fetal red cells, such as hemolytic anemia or hyperbilirubinemia, before HDFN is diagnosed.

Evaluation of an initially positive neonatal DAT, whether on cord blood or on a heelstick sample, should begin with a check of the mother's antibody screen, her antibody identification panel, or both, if available. If the positive DAT is from a cord blood sample, a heelstick sample should be obtained from the neonate for confirmation of the DAT, ABO, and Rh type. (It is important to note that one of the causes of a false-positive DAT is Wharton's jelly.) If a maternal blood sample is unavailable, an antibody screen should be performed on a blood sample, preferably venous, from the newborn. Although identification of antibodies detected on the screen can be performed on the neonatal sample, often the volume of serum is insufficient, and it may be preferable to attempt to obtain a maternal sample when antibody identification is required. Finally, an eluate should be performed on the neonate's red cells.

The possible causes of a positive DAT with a negative maternal antibody screen are ABO incompatibility, antenatal RhIG (passive anti-D coating neonatal red cells), and a maternal antibody to a low-incidence antigen that happens to be present on neonatal cells but not on any of the antibody panel cells. If the mother is Rh negative and the infant is Rh positive, a history of antenatal RhIG should be sought. If the mother and infant are ABO incompatible, a simple Lui freeze-thaw elution of antibody from the newborn's red cells may be performed and the resultant eluate tested against group A and B cells to confirm that the causative antibody is anti-A or anti-B.

If the causative antibody remains unidentified after the evaluation, it may be necessary to test the maternal or neo-

natal serum with a panel of red cells selected to facilitate identification of antibodies to low-incidence antigens. Alternatively, obtaining a blood sample from the father and testing his red cells against the maternal serum, neonatal serum, or both may yield useful information.

For treatment of the newborn with HDFN, it is crucial to assess how high the infant's bilirubin is and how fast the bilirubin is rising. If the bilirubin is less than 20 mg/dL with a rate of rise less than 0.5 mg/dL per hour (1 mg/dL per hour if ABO HDFN), phototherapy usually suffices. Phototherapy uses light at a wavelength of 420 to 480 nm to convert unconjugated bilirubin, which is insoluble in water, to the water-soluble biliverdin, which can be excreted through the kidneys. High levels of bilirubin or steeper rates of rise in bilirubin may necessitate exchange transfusion.

Exchange transfusion, although more invasive than phototherapy, has the advantage of removing both maternal antibody and sensitized red cells. Exchange is usually performed manually through the umbilical vein and carries a mortality rate of less than 1%. A double volume exchange will replace approximately 85% of the newborn's red cells. RBC units for exchange transfusion should be antigen negative for any antibodies present in the maternal serum, and they should be irradiated. Reconstitution of RBCs with Fresh Frozen Plasma (FFP) is used when replacement of coagulation factors is desired. Although the AABB *Standards for Blood Banks and Transfusion Services* mandates only that blood for neonatal transfusion be either antigen negative or crossmatch compatible with maternal or neonatal serum, some transfusion services continue to prefer crossmatch-compatible, antigen-negative units for exchange transfusion.

It is important to remember that the risk of anemia and hyperbilirubinemia in the neonate does not end at hospital discharge. Neonates who receive multiple intrauterine transfusions may exhibit significant suppression of erythropoiesis and will likely require a "top-up" transfusion in the 1- to 3-month period after delivery.



Treatment of a newborn with HDFN will depend on severity of anemia, as well as level and rate of rise of neonatal bilirubin. Treatment options include:

- Phototherapy ("bili lights")
- Exchange transfusion



RBCs for exchange transfusion should be antigen negative for any maternal antibodies and should be irradiated.



Neonatal Alloimmune Thrombocytopenia/ Fetal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia is most easily understood as being analogous to "HDFN for platelets." Because the resultant thrombocytopenia is present not only

Neonatal alloimmune thrombocytopenia (NAIT) is basically "HDFN for platelets."



In NAIT/FAIT, an antigen-negative mother, exposed to a platelet-specific antigen via FMH from an antigen-positive fetus, makes IgG antibody that crosses the placenta to find its target on fetal platelets.

The most common antibody specificity is anti-HPA-1 (anti-PI^{A1}).



Intracranial hemorrhage (ICH) can occur either in utero or after birth in a fetus or neonate with NAIT/FAIT.



A woman who delivers an infant with NAIT/FAIT is likely to have affected offspring with subsequent pregnancies. IVIG and/or intrauterine transfusions of antigen-negative platelets may be required if the mother has delivered a seriously affected infant in the past.

after delivery but also in utero, the term fetal alloimmune thrombocytopenia (FAIT) is also used to describe this condition.

Like HDFN, NAIT/FAIT results from alloimmunization of an antigen-negative mother to a platelet-specific antigen. If the corresponding antigen is present on fetal platelets, transplacental passage of alloantibody can result in destruction of fetal platelets, neonatal platelets, or both.

The most common antigenic specificity in NAIT/FAIT in the United States is human platelet antigen-1a (HPA-1a), which is a high-incidence antigen present on 98% of all platelets. A mother who lacks HPA-1a (formerly known as PI^{A1}) is very likely to bear an HPA-1a-positive fetus, who can present antigenic stimulation to the mother through FMH. Improved diagnostic techniques have increased the likelihood of identifying the antigenic target of the maternal antibody involved, but identification is still not possible in all cases—most likely because the target is one of the rare “private” alloantigens. It remains unclear whether HLA antibodies or ABO incompatibility ever cause NAIT.

NAIT/FAIT occurs in approximately 1:1000 live births, and the first-born infant is affected in about half of the cases. A mother who delivers an infant with NAIT/FAIT is likely to have affected fetuses or neonates with subsequent pregnancies. Although thrombocytopenia is often more severe in subsequent fetuses born to an affected mother, the only consistently useful predictor of severity is antenatal intracranial hemorrhage (ICH) in the older affected sibling.

Thrombocytopenia in the newborn may be severe and can last as long as several weeks if untreated. The diagnosis of NAIT is often made after birth when bruising, petechiae, or purpura are noted in the newborn. More severe bleeding occurs in 10% to 20% of symptomatic cases, particularly ICH, which often occurs in utero (25% to 50% of the time). ICH can also occur after birth, with the greatest risk of postnatal ICH occurring in the first 24 to 36 hours of life.

A woman with a history of a previously affected infant with either severe thrombocytopenia or ICH can be treated with IVIG, with or without corticosteroids, in an attempt to reduce the risk of antenatal ICH. Unfortunately, 15% to 38% of fetuses do not respond to maternal IVIG therapy. Furthermore, the lag between initiation of IVIG therapy and response is typically 1 to 2 days, making it unsuitable as a solitary therapy in emergent situations. The alternative is intrauterine transfusion of antigen-negative platelets, although it must be remembered that both simple PUBS

and intrauterine transfusions carry some risk in HDFN and that those risks, particularly the risk of exsanguination, are magnified when the fetus is profoundly thrombocytopenic. In addition, it has been recommended that when fetal blood sampling is performed, antigen-negative platelets should be available during the procedure and they should be transfused before removal of the sampling needle if the platelet count is found to be less than 50,000/ μ L. Obviously, this scenario requires the ability to obtain a platelet count within a minute or two of obtaining the fetal blood sample.

Obtaining allogeneic platelets for transfusion to affected infants is difficult because of the high incidence of antigen positivity (assuming the antibody is anti-HPA-1a) and because of the remote possibility that the antibody might be directed against a different high-incidence platelet antigen. The situation is particularly difficult when the neonate is the first affected infant, meaning that there is no warning that antigen-negative platelets might be needed and there is no time to identify the antigenic target of the maternal antibody. However, as long as the mother is an acceptable blood donor, maternal platelets can be collected by apheresis or from a whole blood donation for transfusion either to the fetus or the newborn infant. If maternal platelets are to be used, however, it is important to consider, whenever possible, plasma reducing the platelet product before transfusion. Such action reduces the amount of maternal antibody passively transfused along with the platelets.

If maternal platelets and antigen-negative allogeneic platelets are unavailable, a trial of IVIG accompanied by transfusion of random-donor platelets may be attempted. The premise behind this approach is that the antibody is passive rather than being actively produced by the neonate and, thus, will be present only in limited quantities.

Transfused platelets, regardless of origin, should be gamma irradiated to avoid the risk of transfusion-associated GVHD.



Serial intrauterine platelet transfusion is usually restricted to situations where treatment of the mother with IVIG and/or steroids has failed to increase the fetal platelet count.



Maternal platelets can be used to transfuse an affected fetus or newborn, assuming the mother qualifies as a blood donor. IVIG may also help but takes 1 to 2 days to work. When maternal platelets are transfused, they should be plasma reduced to minimize the amount of antibody passively transfused to the neonate.



Sickle cell disease patients may require red cell transfusion to reduce the percent hemoglobin S in their circulation because intraerythrocyte concentration of hemoglobin S (or hemoglobin C) is the critical determinant for initiation of sickling.

Sickle Cell Disease

Special Indications for Transfusion in Sickle Cell Disease

Although improved oxygen-carrying capacity is often considered as the only real indication for RBC transfusion (see Chapter 8: Indications for Transfusion), patients with SCD



Lowering viscosity by reducing the percentage of sickle hemoglobin (hemoglobin S and hemoglobin C) decreases the likelihood that slow passage through the microcirculation will exacerbate deoxygenation of hemoglobin S or C, precipitating further sickling.

present a unique additional indication for RBC transfusion: decreasing the percentage of sickle hemoglobin.

Hemoglobin S has an altered surface charge so that, when deoxygenated, it polymerizes. The fibers thus created crystallize intracellularly, resulting in increased rigidity of the red cell. This action, in turn, results in increased blood viscosity as well as membrane fragmentation. Repeated cycles of deoxygenation and sickling eventually result in irreversibly sickled red cells.

The increased viscosity further exacerbates the problem because it results in slow transit through the microvasculature, which allows increased oxygen extraction. The resultant decrease in oxygen saturation causes further sickling and a self-perpetuating cycle of sickling, slowed transit time, decreased oxygen saturation, and, of course, further sickling.

The critical determinant for initiation of sickling is the concentration of hemoglobin S in the red cell. In this context, it is important to recognize that both hemoglobin S and hemoglobin C are capable of sickling, whereas hemoglobin F is almost totally resistant to incorporation into sickle dimers. It follows, then, that reducing the percentage of hemoglobin S, hemoglobin C, or both in circulating red cells should reduce viscosity and diminish the risk of sickle events. That hypothesis has proven to be the case, particularly in the prevention of SCD-related stroke.



SCD patients in acute aplastic crisis may have profoundly low (total) hemoglobin levels and may require transfusion or even red cell exchange on an emergent basis.

Aplastic Crisis

Aplastic crises are often associated with infectious diseases, especially parvovirus infection, and most clear spontaneously within 1 to 2 weeks. However, because of the profoundly decreased red cell life span in SCD, red cell aplasia can result in dramatic decreases in hemoglobin concentration, which can acutely fall as low as 2 to 3 g/dL. In such cases, transfusion or even exchange transfusion may be necessary.

Acute Pain Crisis

Occlusion of the microcirculation by irreversibly sickled red cells can affect musculoskeletal and soft tissues, giving rise to fever and bone pain. Acute pain crises (APCs) are often precipitated by hypoxia, acidosis, or infection, although many episodes remain unexplained.

Although there is no evidence that increased hemoglobin concentration per se reduces the severity or duration of APCs, percent hemoglobin S does appear to play a significant role. Microvascular occlusion is rare when hemo-

globin S is less than 50% of the total circulating hemoglobin. Although transfusion to reduce hemoglobin S and to raise hematocrit to 35% may result in rapid resolution of symptoms, conservative management with hydration and analgesics should be tried first. A British guideline for the management of APCs published in 2003 asserts that blood transfusion is typically unnecessary unless the hemoglobin has fallen more than 2 g/dL and is below 5 g/dL. The guideline further advocates that the possibility of splenic or hepatic sequestration or parvovirus infection should be considered if blood transfusion becomes necessary.

When APCs significantly affect the patient's lifestyle, prophylactic transfusion to maintain the percent hemoglobin S at or below 50% for a finite period may be considered. However, a significant risk of recurrence remains when prophylactic transfusions are discontinued.

Acute Splenic Sequestration

In childhood, SCD patients experience splenomegaly as a consequence of chronic sequestration of red cells in the spleen. This sequestration continues until the spleen becomes fibrotic and undergoes "autosplenectomy."

Before autosplenectomy, children with SCD are at risk of sudden, acute splenic sequestration of sickled red cells. Episodes of acute splenic sequestration usually occur within the first 2 years of life and are associated with a precipitous decrease in hemoglobin levels. Hypovolemic shock and death may occur; in fact, acute splenic sequestration is the cause of a large proportion of deaths in early childhood among SCD patients.

Because episodes of acute sequestration tend to recur, it may be necessary to perform splenectomy in patients who have experienced one or more acute episodes and who are at risk for further recurrences. However, transfusion to maintain hemoglobin S below 30% to 40% can prevent further episodes and may obviate the need for surgical intervention.

Acute Chest Syndrome

Acute chest syndrome (ACS) is an acute illness characterized by cough, chest pain, hypoxemia, fever, and pulmonary infiltrates on chest x-ray. Causes for ACS include infection (29%); pulmonary infarction (16%) because of sickling in the microvasculature; and fat embolism, with or without associated infection (9%)—presumably the result



Although transfusion will relieve the pain of acute pain crisis (by reducing percent HbS), patients who receive prophylactic transfusion have a significant risk of recurrence when prophylactic transfusions are discontinued.



In patients with acute splenic sequestration, splenectomy may be required to prevent repeat episodes. Prophylactic transfusion to maintain HbS at <30% to 40% may obviate the need for surgery.



Acute chest syndrome (ACS) can progress rapidly to respiratory failure and even death. ACS can also precipitate neurologic events such as a stroke. History of a recent pulmonary event is the nonneurologic risk factor most predictive of stroke in SCD patients.



Simple transfusion may be sufficient in some cases of acute chest syndrome. Severe or rapidly progressive cases may require erythrocytapheresis (automated red cell exchange), which can result in rapid dramatic improvement.



When conservative management with hydration, analgesics, and alkalinization fails to rapidly resolve priapism, local instillation of epinephrine may be tried. Red cell exchange may also be considered, especially if irrigation fails.

of bony infarction. However, in nearly 46% of cases the cause remains unknown.

ACS may be self-limited when only small areas are involved, but it can progress rapidly and may result in respiratory failure and even death. Furthermore, ACS precipitated a neurologic event in 22% of patients in one study of more than 500 patients with ACS. Indeed, the history of a recent pulmonary event has been described as the *non-neurological* risk factor that is most predictive of stroke in SCD patients.

Treatment of ACS includes intravenous hydration; respiratory support, including supplemental oxygen with or without mechanical ventilation; transfusion to reduce further sickling and to diminish ischemia; and broad-spectrum antibiotics to cover common organisms. In addition, bronchodilators have been recommended even in the absence of audible wheezing.

Even simple transfusion has been shown to improve oxygenation in ACS. However, when progression is rapid, multiple lobes are involved, or there is any sign of respiratory insufficiency, exchange transfusion may be necessary. Exchange transfusion can be accomplished manually or through automated red cell exchange (erythrocytapheresis), which has the advantage of allowing rapid full exchange. In addition, the computer software used by current-generation apheresis machines makes it easy for the operator to specify both desired total hemoglobin level and target percent hemoglobin S levels.

Priapism

A substantial percentage of males with SCD are prone to nonthrombotic obstruction of penile venous outflow by sickle cells, which results in priapism. One study found that as many as 89% of males with SCD will have had at least one episode of priapism by age 20. Prolonged priapism can result in impotence and, thus, constitutes a urologic emergency.

Conservative management with intravenous hydration, analgesia, and alkalinization to relieve acidosis may be used. However, if detumescence does not occur within 2 to 3 hours from onset of symptoms, urologic intervention— aspiration followed by local instillation of epinephrine— may be required. Erythrocytapheresis with a target percent hemoglobin S of less than 30% may also be considered, especially if irrigation fails.

As beneficial as erythrocytapheresis appears to be in the treatment of acute episodes of priapism, one must be alert to the appearance of symptoms or signs that are suggestive of the so-called ASPEN syndrome, which is the acute onset of neurologic signs shortly (1-11 days) after partial exchange transfusion for priapism. (ASPEN is an acronym for Association of Sickle cell disease, Priapism, Exchange transfusion, and Neurologic events.) Headache, seizures, hemiparesis, and obtundation requiring ventilator support have been reported after exchange transfusion for priapism. The proposed mechanisms for those sequelae are either an elevated posttransfusion hematocrit resulting in increased viscosity or coagulation abnormalities resulting from release of activated clotting factors, activated platelets, and cytokines after reperfusion. ASPEN syndrome has been effectively treated with simple transfusion to maintain hemoglobin S levels less than 30%.

Because recurrent priapism is strongly associated with impotence, some physicians advocate chronic transfusion to maintain the percent hemoglobin S below 30% for 6 to 12 months in that setting.

Prophylactic Transfusion in Sickle Cell Disease

The three most common situations in which transfusion has been used prophylactically (in addition to therapeutically) in SCD patients are stroke, pregnancy, and surgery.

Stroke—A Historical Perspective

In SCD, the arterial circulation of the brain is a common site of fibrous intimal hyperplasia and partial vascular occlusion, making SCD patients particularly prone to stroke. Among patients with SCD, 11% have a stroke before age 20 years, with children between the ages of 2 and 5 years being at highest risk. Those figures may underestimate the risk because up to half of cerebral infarcts may be clinically silent, especially early in life. Less is known about the risk of stroke in older patients.

Stroke in SCD patients carries a relatively high mortality rate (20%) and leaves many patients (70%) with residual neurologic deficits. Two-thirds of stroke victims have recurrences, usually within 36 months, and virtually all within 4 years.

Acute stroke episodes are managed by exchange transfusion to reduce percent hemoglobin S to below 30% of total hemoglobin, or possibly even below 20% in the case of severe hemorrhage, if angiography is planned, or both.



ASPEN syndrome (Association of Sickle cell disease, Priapism, Exchange transfusion, and Neurologic events) is a risk when patients receive exchange transfusion to relieve priapism.



SCD patients are particularly prone to strokes, often at an early age. Two-thirds of SCD stroke victims have recurrent strokes.



Exchange transfusion to maintain hemoglobin S at <20% to 30% is effective in acute management of stroke.

Chronic transfusion to maintain hemoglobin S levels at <30% can reduce the risk and severity of recurrent strokes.

Once a patient has had a stroke, transfusion is effective prophylaxis against recurrence. In fact, transfusion to prevent recurrent stroke is now practiced widely. Chronic transfusion programs aimed at maintaining percent hemoglobin S below 30% not only reduce the incidence of recurrent stroke to less than 10% (instead of 66%) but also decrease the severity of recurrences.

Most chronic transfusion programs for the prevention of recurrent strokes aim to maintain hemoglobin S at less than 30% for 3 to 5 years, liberalizing the percentage to 50% thereafter. The optimal duration of prophylactic maintenance transfusion remains uncertain. Most studies reported unacceptably high recurrence rates (greater than 50%) if transfusions were discontinued, even after 5 to 12 years. However, results of several studies suggest that liberalizing the target percent hemoglobin S to 50% or even 60% can minimize recurrence risk while reducing transfusion exposure significantly.

Prevention of First Stroke—the STOP and STOP 2 Trials



Transcranial Doppler ultrasound can predict patients at risk of stroke. In patients at risk, prophylactic transfusion significantly reduces the likelihood of an initial stroke.

All of the preceding data relate to preventing recurrences after an initial stroke. Until the late 1990s, it was impossible to predict which patients were at risk of stroke before their first event. However, transcranial Doppler ultrasound (TCD) has been shown to be a reliable noninvasive predictor of stroke risk. Flow rates on TCD are inversely proportional to vessel diameter; thus, high flow rates correlate with a high risk of stroke.

Using TCD, the Stroke Prevention Trial in Sickle Cell Anemia (STOP) identified 130 children at risk for stroke and randomly assigned them to one of two groups: standard therapy and prophylactic transfusion to maintain their hemoglobin S at less than 30%. The rate of initial stroke was significantly lower (92% less) in the group receiving transfusions on a regular basis; there were 11 strokes in the control group and only one in the transfusion group. Regularly transfused patients who occasionally failed to achieve the target percent of hemoglobin S did not develop strokes, suggesting that brief periods of noncompliance may be well tolerated. The results of the study were so compelling that it was terminated 16 months early.

However, prophylactic transfusion on a regular basis is not without complications. In the STOP study, 10 of the 63 patients in the transfusion group developed alloantibodies directed against red cell antigens, most often E and K, thereby leading the authors to recommend use of RBCs



The authors of the STOP trial recommend use of prophylactic phenotype matching for C, E, and K antigens to reduce the risk of red cell alloimmunization.

phenotypically matched to the recipient for ABO, D, C, E, and K antigens. Furthermore, long-term prophylactic transfusion on a regular basis also carries the risk of iron overload.

Because of concerns about the immediate and cumulative risks associated with chronic transfusion, a follow-on study, the STOP 2 Trial, was conducted. The aim of the STOP 2 Trial was to determine whether prophylactic transfusions can safely be discontinued in patients with abnormal TCD tests who have been maintained on a chronic transfusion regimen with return of TCD findings to normal. TCD examination and clinical surveillance were performed at least every 12 weeks for patients in both groups, with quarterly visits and yearly magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) examinations. The study, which was published at the end of 2005, concluded that patients maintained for at least 30 months on a chronic transfusion regimen with normalization of TCD flow velocity nonetheless showed a high rate of reversion to abnormal TCD velocities and stroke when the prophylactic transfusion regimen was discontinued. Although 20% of patients in the transfusion-halt group experienced neither stroke nor reversion to abnormal TCD velocities, there was no way to identify that subset of patients prospectively. It was noted that both of the patients in the transfusion-halt arm who experienced a stroke had abnormal TCD findings on the examination immediately before stroke.

The authors of the STOP 2 report recommended that further study into alternative regimens be conducted, including randomized trials examining whether the target percent hemoglobin S can safely be liberalized to 50% to 60% after some years of intensive therapy. In the meantime, they recommended that if transfusion is discontinued, patients should undergo frequent TCD studies and receive prompt transfusion if abnormal TCD velocities are observed.

Other recent developments in the area of chronic transfusion therapy for the prevention of stroke include research into the role of MRA in identifying patients at increased risk of stroke. TCD has proven more sensitive than MRA, detecting flow abnormalities indicative of stroke risk before development of abnormal MRA findings. However, when coupled with abnormalities on MRA, abnormal TCD appears to be indicative of even greater risk, leading the authors of a recent study to recommend that MRA be used in the assessment of patients found to be at increased risk



The STOP 2 trial found that discontinuing chronic transfusion even after 30 months was still associated with a high rate of reversion to abnormal TCD velocities and to stroke.



TCD is more sensitive than magnetic resonance angiography (MRA), but MRA may be helpful in further assessing risk in patients with abnormal TCD results.

of stroke by TCD. MRA can also be used when TCD cannot be obtained because of technical difficulties or because the test is not available locally.

Pregnancy

Pregnancy in a mother with SCD carries increased risk for the mother and her fetus. In the mother, risks range from APCs, anemia, and infection to toxemia and even death. The fetus is at risk for growth retardation, prematurity, and fetal demise. The utility of prophylactic transfusions in pregnancy remains controversial.



The efficacy of prophylactic transfusion in pregnant SCD patients remains controversial.

Two studies in the early 1980s compared prophylactically transfused mothers with historical controls and found a significant decrease in morbidity and reproductive loss in the transfused group. In one of the studies, initial partial exchange was used to reduce hemoglobin S to below 60%, followed by maintenance at less than 80%. There were no maternal deaths, reproductive loss was 3%, and overall morbidity was 19% compared with 65% in historical controls. A more aggressive regimen geared at maintaining hemoglobin S at less than 50% for the first trimester and less than 60% thereafter demonstrated similar results.

However, the only fully prospective randomized trial, published in 1988, showed that—after high-risk factors were factored out—there was no difference in perinatal outcome between the group that received prophylactic RBC transfusions and the group transfused only for emergencies. However, the prophylactically transfused group received nearly twice as much blood on average (12 units as opposed to 6.5 units in controls).

Of two more recent studies published in 1995, one showed a trend toward lower sickle complications—but only in the third trimester—in prophylactically transfused women. The other study found no differences between prophylactic and restricted transfusion groups with respect to the rate of preterm labor, infant mortality, maternal mortality, or intrauterine growth retardation. In the latter study, the restricted transfusion group was noted to benefit from decreased “transfusion-related crises” (23% in prophylactic group, 3.1% in restricted transfusion group).

Thus, although there is no clear indication that prophylactic transfusion is beneficial in pregnant SCD patients, clinical practices vary, with some doctors continuing prophylactic transfusions and others following a more restrictive path. An American College of Obstetricians and

Gynecologists (ACOG) Practice Bulletin published in July 2005 and containing practice guidelines for hemoglobinopathies in pregnancy confirmed that controversy still exists over the role of prophylactic transfusions in the management of SCD in pregnancy.

Surgery and Anesthesia

Anesthesia and surgery can be associated with a number of factors known to precipitate sickling, namely, hypoxia, acidosis, venous stasis, and hypothermia. Thus, SCD patients undergoing surgery are at increased risk of perioperative complications, including pulmonary infarction, APCs, infection, and even death. Numerous studies have shown that maintaining hemoglobin S levels less than 60% is safe, provided that the total hemoglobin is not excessive (no greater than 14-15 g/dL).

Historically, many centers transfused preoperative patients to maintain hemoglobin S levels less than 30% to 50%, but there was no consensus about whether a conservative approach (correcting anemia) or an aggressive approach (maintaining percent hemoglobin S less than 30%) was preferable. Vitreoretinal surgery was the one case in which there appeared to be general agreement that prophylactic transfusion is beneficial.

In 1995, the Preoperative Transfusion in Sickle Cell Disease Study Group conducted a multicenter study involving 551 patients undergoing elective surgery. The study compared conservative transfusion (maintaining total hemoglobin greater than 10 g/dL irrespective of percent hemoglobin S) with aggressive management (maintaining 10 g/dL total hemoglobin and hemoglobin S less than 30%). There was no statistically significant difference in complications between the two groups, except that the aggressively transfused group had significantly higher rates of transfusion-related complications. The aggressively treated group used twice as much blood on average, had twice as high a percentage of patients who developed a new alloantibody, and had six times as many hemolytic transfusion reactions. The conclusion from the study was that in the perioperative period, as long as the patient is adequately hydrated, conservative transfusion aimed at maintaining total hemoglobin at 10 g/dL or higher is as effective as aggressive transfusion targeted at maintaining hemoglobin S at less than 30% and a conservative approach results in significant reduction in donor exposures, alloantibody formation, and hemolytic transfusion reactions.



A 1995 multicenter study showed that, if patients are adequately hydrated, a conservative treatment regimen (keep total hemoglobin at >10 g/dL without respect to percent hemoglobin S) was as effective as maintaining both total hemoglobin at >10 g/dL and percent hemoglobin S at <30%.



Although the perioperative transfusion in Sickle Cell Disease Study Group trial recommendations are widely accepted, some centers (most notably Duke) maintain that tight control of perioperative percent hemoglobin S results in fewer postoperative complications.

Taking exception with the conclusions of that study, a group from Duke University performed a retrospective analysis of 130 of its patients treated with a very aggressive perioperative transfusion regimen in which the average total hemoglobin was 11.2 g/dL (± 1.3). The average percent hemoglobin S was 21% ($\pm 10\%$). The authors observed major postoperative events in only 10% of their patients, compared with 31% in the aggressive arm and 35% in the conservative arm of the Preoperative Transfusion in Sickle Cell Disease Study Group report. The Duke group attributed its markedly lower complication rate to meticulous care given to hydration and to the very aggressive transfusion regimen that the group used.

One recent (2005) retrospective study by Fu et al suggested that routine perioperative transfusion might not be required for minor elective surgical procedures in children in the steady state, but even the authors of that study admit that their hypothesis requires confirmation by randomized controlled trials.

In summary, the conclusions of the Preoperative Transfusion in Sickle Cell Disease Study Group report are widely accepted, with many physicians agreeing that it is unnecessary to pursue the percent hemoglobin S; they are focusing attention instead on hydration and maintaining total hemoglobin above 10 g/dL. However, as noted earlier, dissent remains in some quarters. Nonetheless, there is general agreement on the need for adequate hydration and correction of anemia in the perioperative period.

Prevention of Alloimmunization in SCD Patients



Transfused SCD patients (of African ethnicity) have a very high rate of alloimmunization, partly because they are multitransfused but also because of antigenic differences with the donor population, which is predominantly of European ethnicity.

SCD patients have an unusually high rate of alloimmunization—as high as 35% in some studies. This figure reflects, in part, the fact that patients with SCD are multitransfused, but a more important factor is the difference in antigen frequencies between the predominantly Caucasian donor population and non-Caucasian recipients. (See Table 11-1.)

Prophylactically providing SCD patients with phenotypically matched blood has been shown to be effective in preventing formation of alloantibodies, although its cost-effectiveness remains controversial. Because 80% of the antibodies seen in SCD patients are directed against one of four antigens (C, E, K, or Jk^b), some experts recommend prophylactically providing the recipient with RBCs phenotypically matched for those antigens, even before the patient develops any detectable antibodies. Others have proposed more extensive phenotypic matching for the Rh

Table 11-1. Relative Antigen Frequencies (%)

Antigen	European Ethnicity	African Ethnicity
C	68	27
K	9	2
Fy ^a	66	10
Fy ^b	83	23
Fy (a–b–)	rare	68
Jk ^b	73	43
S	55	31

antigens (D, C/c, E/e), K, Fy^a, Fy^b, and S. Still others propose waiting until a patient is shown to be a responder by developing two or more alloantibodies.

Fortunately, it is comparatively easy to obtain an accurate phenotype on an SCD patient, even in the face of recent transfusion. Hemoglobin SS- or hemoglobin SC-containing red cells are resistant to lysis in hypotonic saline, whereas hemoglobin AS and normal red cells are not. Treating the recipient's red cell suspension with hypotonic saline lyses all red cells except those of the recipient, which are the only circulating red cells likely to contain hemoglobin SS or hemoglobin SC.

The use of phenotypically matched blood will not prevent the development of antibodies directed against high-incidence antigens, such as U and Js^b. Furthermore, some experts would argue that, although effective in preventing alloimmunization, phenotypically matched blood may not be cost effective because 65% to 80% of multitransfused SCD patients will not develop antibodies regardless of exposure.

Detection of Delayed Hemolytic Transfusion Reactions in SCD Patients

Delayed hemolytic transfusion reactions can mimic sickle crises, presenting with pain crisis and hemoglobinuria. If the patient's primary caregiver does not maintain a high index of suspicion, hemolysis caused by alloantibody-



Prophylactically providing SCD patients with phenotypically matched red cells can significantly reduce their risk of becoming alloimmunized.

Matching for C, E, and K are most important, but some centers match for the five major Rh antigens as well as for K, Fy^a/Fy^b, Jk^a/Jk^b, M/N, and S/s.



Delayed hemolytic transfusion reactions can mimic SCD crises, so it is crucial to maintain a high index of suspicion for DHTR when recently transfused SCD patients present in apparent sickle cell crisis, especially when hemoglobinemia/uria is seen.

mediated red cell destruction can mistakenly be attributed to a sickle exacerbation, particularly if a sample is not sent to the transfusion service for type and screen.

In the transfusion service, when a new alloantibody is detected in a recently transfused SCD patient, delayed hemolysis should be considered, particularly if the DAT is positive (especially with mixed-field reactivity). It is important to recall, however, that if all transfused red cells have already been cleared, the DAT may be negative in the face of delayed hemolysis.

Identification of new alloantibodies in a patient with multiple prior alloantibodies can present a real challenge. One must make a conscientious attempt to identify the antibody, possibly by sending a sample to a reference laboratory, rather than writing it off as a clinically insignificant antibody (formerly referred to as a high-titer, low-avidity antibody). A positive autocontrol, even if accompanied by a positive DAT, does not prove that the antibody detected is an autoantibody and not an alloantibody. In a recently transfused recipient with a new broadly reactive antibody, the only way of being certain that there is not a significant underlying alloantibody may be through differential allogeneic adsorption techniques.

It may also be difficult to differentiate hemolysis caused by red cell alloantibodies from the "sickle hyperhemolysis syndrome," in which the patient presents with more severe anemia after transfusion than before. Two mechanisms have been proposed to explain sickle hyperhemolysis. The first is removal of antigen-negative autologous red cells by "bystander" hemolysis. The second possible mechanism is transfusion suppression of erythropoietin production, which can result in marked reticulocytopenia. In a patient with a profoundly shortened red cell life span, such as that seen in SCD, the suppression of erythropoietin by transfusion can result in a marked decrease in hematocrit.



Some transfused SCD patients can show evidence of "hyperhemolysis" (anemia more severe after transfusion than before). In some cases, this is due to suppression of reticulocytosis by transfusion, but, in others, there appears to be evidence of "bystander" hemolysis of autologous red cells along with transfused red cells.

Summary

Transfusion in SCD presents a number of unique challenges. Not only is there a need to define when prophylactic transfusion is appropriate and what the target of that prophylaxis should be, but also there is the challenge of preventing the development of multiple alloantibodies in a cost-effective manner. Finally, there is the challenge of determining whether increased hemolysis after transfusion is a consequence of alloantibody or autoantibody, of

increased sickle-related hemolysis, or of the sickle hyperhemolysis syndrome.

Massive Transfusion

The term “massive” is generally applied to transfusions that amount to a full blood volume or more in a 24-hour period. The term is often loosely used whenever an adult receives 10 to 12 or more RBC units in a single day. Patients who are massively transfused encounter a number of problems not seen in patients receiving small amounts of blood.

The foremost problem in massive transfusions is coagulopathy. Although the coagulopathy seen in massively transfused patients is often ascribed to simple dilution, the etiology is more commonly multifactorial. Dilution of coagulation factors and platelets is certainly a factor, but consumption, including disseminated intravascular coagulation, and hypothermia also play a part.

One might expect that replacement of a full red cell volume without transfusion of platelets or plasma would deplete nearly all the platelets and coagulation factors from a hemorrhaging patient, but that is not necessarily the case. Theoretical models predict that more than one-third (37%) of the original blood volume will remain, which explains why it may be possible to transfuse 10 or 12 RBC units and not require platelets or FFP. If a patient starts out with normal levels of coagulation factors and platelets and drops acutely to 37% of those levels, he or she will still have hemostatically adequate levels of both.

Thus, the coagulopathy of massive transfusion is ideally managed by close monitoring of coagulation parameters, which should include fibrinogen level and platelet count as well as prothrombin time (PT) and partial thromboplastin time (PTT). Blood component selection and volume can then be tailored to the measured disturbance in clotting parameters. For example, a patient with elevated PT and PTT accompanied by low fibrinogen might be served better by transfusion of a combination of FFP and Cryoprecipitated AHF rather than by receiving FFP alone. Once replacement blood components are transfused, follow-up coagulation tests can be ordered after a brief interval (15 minutes) to allow equilibration. The efficacy of 15-minute posttransfusion platelet counts is well documented, and the same interval is reasonable for soluble coagulation factors.



“Massive transfusion” refers to transfusion that amounts to a full blood volume or more within 24 hours.

Massive transfusion can be associated with:

- Coagulopathy (partially dilutional)
- Hypothermia
- Hypocalcemia (due to citrate in units)



Management of the coagulopathy of massive transfusion requires close attention to both the platelet count and to the soluble coagulation system (PT, PTT, and fibrinogen).



After therapeutic intervention (transfusion of FFP, platelets, and/or cryo), a full coagulation battery should be obtained and further transfusion guided by those results and by the patient's clinical status. Either piece of data (laboratory results or patient presentation) taken alone may lead to suboptimal management decisions.



One unit of FFP is the equivalent of 2 units of cryo (but in a much larger volume).

It takes 14 cryo-equivalents to raise the fibrinogen of a 70-kg adult by 100 mg/dL in the absence of significant ongoing hemorrhage.

Defining "triggers" for transfusion in the setting of massive blood loss is problematic because such a decision is based on a number of factors, including whether blood loss is ongoing or has begun to taper off. For example, although some physicians prefer to wait for the fibrinogen level to decrease to 80 to 100 mg/dL before transfusing cryo, others prefer to begin cryo when the fibrinogen decreases below 125 mg/dL, assuming massive ongoing bleeding is present. If the bleeding is coming under control, however, a more reasonable trigger might be 100 mg/dL. Likewise, there is considerable variability in setting the "target" fibrinogen level once the decision is made to transfuse. Nonetheless, the aim is to maintain fibrinogen at or above the 100 mg/dL range until hemorrhage is controlled and synthesis can begin to return levels to normal. If major hemorrhage is continuing it may be necessary to transfuse sufficient fibrinogen to raise the patient's fibrinogen level into or nearly to, the normal range in order to ensure the level never falls below 100 mg/dL.

In any event, one must remember that each FFP unit contains approximately the equivalent of two units of cryo and that the calculated requirement for cryo should be adjusted accordingly. A useful rule of thumb is that it takes 14 cryo-equivalents to raise the fibrinogen of a nonbleeding 70-kg adult by 100 mg/dL. Thus, if one needed to raise such a patient's fibrinogen by 100 mg/dL and were planning to give 4 FFP units to correct deficiency of other coagulation factors, the patient would need only 6 units of cryo to achieve the desired fibrinogen because the FFP will contain an amount of fibrinogen equivalent to that found in 8 units of cryo.

Such management assumes an ideal situation in which the turnaround time for STAT coagulation tests is rapid enough to provide laboratory guidance for replacement therapy. In some cases, the bleeding is so profuse, the turnaround time is so long, or both, that laboratory-driven management is not feasible. In those situations, a formulaic approach is sometimes used in which 1 FFP unit is transfused for every 2 to 3 RBC units infused. That ratio is inverted in the setting of liver transplantation, in which case up to 2 FFP units may be required for every RBC unit transfused. The rationale behind the inverted ratio is that most patients continue to produce at least some coagulation factors, whereas liver failure patients are essentially incapable of doing so preoperatively; that inability persists at least through the anhepatic phase of transplantation. Furthermore, patients with liver failure are at increased risk

of disseminated intravascular coagulation because they also have defects in the factors that regulate coagulation, such as antithrombin, protein C, and protein S.

The preceding formulas also provide rough guidelines that can be used to cross-check the composition of blood component replacement even when laboratory monitoring is used.

Regardless of whether transfusion is based on coagulation test results or on a formulaic approach, it is crucial that all elements of the coagulation picture be considered. In a recent Danish study, one-third (13/39) of massively transfused patients were deemed to have received inadequate transfusion replacement therapy, either because they failed to receive platelets or because FFP was not started until the patient had received 20 or more RBC units. Mortality among inadequately transfused patients was significantly higher than in those receiving adequate replacement therapy.

Other problems commonly encountered in massive transfusion are hypocalcemia and hypothermia. Hypocalcemia can result from infusion of citrated blood components at a rate faster than the recipient's liver can metabolize the citrate. Operating rooms in which surgical procedures involving major hemorrhage are performed routinely may even have facilities to monitor ionized calcium directly in the operating theater. Rapid infusion of refrigerated blood can lower the patient's core body temperature significantly, resulting in hypothermia-induced coagulopathy, arrhythmia, or both. The use of a blood warmer at the time of infusion can prevent this occurrence; some rapid-infusion systems incorporate the ability to warm blood as it is being infused. Admixing RBCs with warmed normal saline is an alternative that can be used if the infusion rate imposed by the blood warmer is too slow or if a blood warmer is unavailable.

One emerging topic that merits mention is the off-label use of recombinant Factor VIIa in the setting of massive hemorrhage. At some institutions, recombinant Factor VIIa may be used, most often at a dose of 90 µg/kg, as a "last ditch" effort when a patient continues to have uncontrolled major hemorrhage despite correction of surgically remediable anatomic defects and restoration of coagulation test parameters to normal or near normal by transfusion of FFP, cryoprecipitate, platelets, or a combination of these. However, because such use is off-label, the efficacy and safety have yet to be clearly demonstrated, and optimal dosing has not been established. Furthermore, the product costs nearly



One useful rule of thumb for estimation of FFP requirements is 1 unit of FFP for every 2 to 3 RBC units in most massive transfusion settings, with that ratio inverted in liver transplants.

However, this rule of thumb is *not* a substitute for following coagulation parameters, platelet counts, and patient condition.



Admixing RBCs with warmed normal saline is an alternative when a blood warmer either is not available or delivers blood too slowly for the patient's needs.

one dollar per microgram, dosing is often in the range of 6000 to 7000 micrograms per dose, and third-party payers will often not reimburse for off-label products. Those constraints mean a net cost to the institution of between \$6000 and \$7000 per dose dispensed without reimbursement.

Recombinant Factor VIIa is covered in more detail in Chapter 13: Plasma Derivatives and Recombinant Plasma Proteins.



Platelet refractoriness is defined as failure to achieve an acceptable posttransfusion increment on more than one occasion. Common usage restricts use of the term to cases where the refractoriness has an immunologic basis.



An otherwise healthy adult's platelet count should rise by 30,000 to 60,000/ μL in response to a unit of apheresis platelets (or a 6-unit pool of whole-blood-derived platelets).



CCI, or corrected count increment, is a more precise way of gauging platelet increment because it takes into account the size of the recipient (body surface area) and the platelet content (estimated or measured) of the platelet unit.

Platelet Refractoriness

Defining the Refractory State

The exact definition of platelet refractoriness has remained almost as elusive as the underlying basis for the problem was until the late 1990s. A patient is regarded as refractory to platelets if he or she fails to achieve an acceptable increment in platelet count after transfusion on more than one occasion. Although some physicians use the term "refractory" to mean any failure to achieve an acceptable increment, the term is used here to refer solely to immunologic platelet refractoriness. Other causes of failure to achieve or maintain platelet increment, such as splenomegaly/hypersplenism, fever, sepsis, consumption, or bleeding, are excluded from the following discussion.

An "acceptable" increment in platelet count will depend in part on the clinical setting. Transfusion of 1 unit of apheresis platelets or its equivalent (a 4- to 6-unit pool of platelet concentrates) can be expected to increase an average normal adult's platelet count by 30,000 to 50,000/ μL .

The blood volume of the recipient and the platelet count in the transfused dose will have a direct effect on the achievable platelet increment. One way of normalizing for those factors is to calculate the corrected count increment, or CCI, which incorporates recipient body surface area and absolute platelet content—either measured or anticipated—of the unit as transfused. An acceptable CCI at 10 minutes to 1 hour after transfusion ranges from 5000 to 7500/ μL . Measurement of posttransfusion platelet count on a sample drawn more than 1 hour after transfusion should be avoided because interpretation of such a result may be confounded by other nonimmunologic factors such as consumption, splenic sequestration, or ongoing hemorrhage.

Some transfusion specialists are even more stringent in their definition of refractoriness, in that they require transfusion of ABO-compatible platelets and/or fresh platelets to be demonstrated ineffective before considering a patient to be refractory to platelets. Those requirements reflect the fact that ABO incompatibility between the platelets infused and the recipient's plasma may result in somewhat shortened platelet survival in some patients and that fresher platelets appear to have somewhat better survival.



Although many centers permit transfusion of ABO out-of-group platelets, both ABO compatibility of platelets with recipient serum and the age of the platelet unit(s) can affect platelet response.

Prevention of Platelet Refractoriness

In the late 1990s, immunologic platelet refractoriness was demonstrated to be largely, but not exclusively, related to the presence of HLA antibodies in the recipient. Those antibodies react with HLA Class I antigens on transfused platelets. Antibodies directed against platelet-specific antigens (human platelet antigens), that is, HPA antibodies, appear to play less of a role in most cases. Leukocyte manipulation of cellular blood components by leukocyte reduction or ultraviolet irradiation has been demonstrated to have a significant effect in reducing both alloimmunization to HLA antigens and platelet refractoriness, at least in the (immunosuppressed) leukemic population studied in the Trial to Reduce Alloimmunization to Platelets (TRAP) study. Indeed, the implementation of universal prestorage leukocyte reduction of blood components in Canada has resulted in significant reduction in both the rate of platelet alloimmunization and the incidence of alloimmune refractoriness.



Recipient HLA antibodies (Class I) are the primary mediator of immunologic platelet refractoriness. Platelet-specific antibodies play far less of a role in refractoriness.

Management of the Platelet Refractory Patient

Regardless of how one defines platelet refractoriness, several approaches are possible to improve platelet response in refractory patients. The first step in one approach is to determine whether the patient has evidence of immunologic refractoriness. To make that determination, the recipient is screened for the presence of HLA and platelet-specific antibodies. Alternatively, centers that have a substantial supply of apheresis platelets may choose to perform 8 to 12 platelet crossmatches, using the recipient's serum and donor platelets. Incompatible crossmatches would be regarded as putative evidence of alloimmunization, whereas a patient for whom all platelet crossmatches were compatible would be considered unlikely to be alloimmunized.



Conventional lymphocytotoxicity assays are not sufficiently sensitive to allow detection of HLA alloantibodies in all cases when they are present. Solid-phase testing and MAIPA assays are more sensitive.

One important consideration when testing for HLA antibodies is that conventional lymphocytotoxicity assays are not sensitive enough to detect cases that might be detectable by alternative, more sensitive methods such as solid-phase testing similar to that used in some alternative red cell serologic methods or the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay. Furthermore, it remains unclear how much effort should be spent searching for an immunologic cause (eg, HPA antibodies) in the case of transfusion refractoriness with negative screening tests.

In any event, once evidence of alloimmunization is documented, one of three approaches to transfusion may be used. First, platelet crossmatch may be used to select crossmatch-compatible units. Crossmatch-compatible platelet units are effective about half the time in platelet-refractory patients. Because it is possible that other, less well-defined factors may be in play in any given donor-recipient pairing, it is reasonable to try a second crossmatch-compatible unit from a different donor in a recipient who fails to respond to a single crossmatch-compatible platelet unit. If, however, a recipient fails to achieve an acceptable increment after transfusion of several different crossmatch-compatible platelet units, it would be reasonable to predict that subsequent units selected on the basis of crossmatch alone would be unlikely to yield desired increments. At that point, it may be possible to select HLA-matched units for transfusion. Some centers will go one step farther and perform platelet crossmatches on HLA-selected platelet units and preferentially transfuse units that are both HLA-selected and crossmatch-compatible with the refractory patient. Such an approach addresses the limitations of HLA matching.

Alternatively, some centers prefer to transfuse "HLA-matched" platelets to refractory patients, often without platelet crossmatch. That approach has several inherent problems. The first problem involves patients with chemotherapy-related marrow suppression causing thrombocytopenia. For those patients, one must anticipate the need for HLA-matched platelets and obtain an HLA type on the patient before therapy. Otherwise, once therapy has been started and the marrow is suppressed, there may be too few viable peripheral blood lymphocytes in the patient's circulation to allow HLA typing to be performed at least by conventional methods.

Second, even if an HLA type is available on the recipient, it is often difficult to obtain fully matched platelets,



In patients with evidence of immunologic refractoriness to platelets, the following are treatment options:

- Provide platelets that are crossmatch-compatible with the recipient serum
- Provide Class I HLA-matched platelets
- Screen recipient for HLA antibodies and transfuse antigen-negative platelets
- Combinations of the above

despite the fact that only Class I antigens (HLA-A and HLA-B antigens) need to be matched. In the absence of a full four-antigen or "Grade A" match, one must resort to three-antigen matches. In "Grade B1U" matches, one donor antigen is unknown but presumed to be identical to the antigen that can be typed. "Grade B1X" matches involve a known mismatch of one Class I antigen, but a mismatch that is within a cross-reactive group. Thus, when clinicians order "HLA-matched" platelets, they should be made aware that a full four-antigen match may not be possible. Table 11-2 provides a more complete listing of HLA match grades. Grade C or lower matches are typically avoided.

A third approach, which is gaining favor and which makes intellectual sense, is to handle platelet transfusion in a manner similar to RBC transfusion. One looks for HLA antibody and, if antibody is present, proceeds to identify the antigenic specificity of the antibody. One then selects for transfusion platelet units that lack that particular antigen. Some advocates of this approach suggest that retesting for development of new antibody specificities need be performed no more frequently than monthly.



"HLA-matched" platelets are selected to be the best match available but, depending on match grade, they may not be well matched at all.



Identifying the specificity of a recipient's HLA antibody and then selecting antigen-negative platelet units can improve the likelihood of finding acceptable units when compared with requiring units to be "HLA matched" with the recipient.

Table 11-2. HLA Match Grades

Match Grade	Description
A	4-antigen match Both HLA-A antigens match Both HLA-B antigens match
B	2- or 3-antigen match, but antigen(s) that are not matched are either unknown or the member of a cross-reacting group
B1U	1 antigen unknown
B1X	1 antigen cross-reactive
B2U	2 antigens unknown
B2X	2 antigens cross-reactive
C	3-antigen match, 1 antigen known to be nonidentical and not part of a cross-reacting group
D	2-antigen match, 2 antigens known to be non-identical and not part of a cross-reacting group

The third approach may be combined with the HLA matching. In other words, the best HLA match that also lacks the antigens to which the patient is alloimmunized may be selected for transfusion. This combined approach theoretically could reduce the risk of alloimmunization to additional HLA antigens.

Regardless of the approach taken to select compatible platelets for transfusion, a posttransfusion platelet count should be obtained, and either the platelet increment or the CCI should be observed. Significant improvements in the increment or CCI justify continued use of HLA-selected platelets or crossmatch-compatible platelets. If no improvement in the platelet count increment is seen with selected platelets after a trial of two or three transfusions, clinical factors other than HLA or platelet alloimmunization may be causing the refractoriness. The continued use of HLA-selected or crossmatch-compatible platelets might not be justified. If, however, HLA or platelet alloimmunization is likely or has been confirmed by testing, a better HLA match (perhaps obtained from another blood supplier), an alternative approach, or a combination of approaches may be tried.

Several alternatives have been proposed for the management of the platelet refractory patient who fails treatment and yet has significant bleeding. The first of these is the use of small frequent doses of platelets (eg, 2 to 4 units of platelet concentrates every 6 to 8 hours). A similar approach is the “platelet drip” in which the equivalent of one platelet concentrate is infused slowly every hour (eg, half an apheresis unit transfused slowly over 3 hours). Antifibrinolytic agents such as epsilon-aminocaproic acid or tranexamic acid have been used successfully on occasion to control bleeding in thrombocytopenic patients, evidently working by stabilizing the platelet plug. Finally, there has been at least one anecdotal report of the successful use of recombinant Factor VIIa to control bleeding in a platelet-refractory lymphoma patient with major gastrointestinal bleeding that was unresponsive to all other therapeutic modalities.



Measurement of platelet count 15 minutes to 1 hour after transfusion is critical if one is to reliably determine platelet response.

Measurements taken 12 to 24 hours after transfusion will reflect other factors, such as consumption and peripheral destruction, and do not provide a reliable gauge for the presence or absence of immunologic refractoriness.



Posttransfusion purpura (PTP) is also caused by anti-HPA-1 but, in this case, the affected patient is not a fetus/neonate and the alloantibody behaves like an autoantibody, destroying autologous (antigen-negative) platelets as well as transfused platelets.

Posttransfusion Purpura

Posttransfusion purpura occurs when a transfusion recipient who lacks the platelet antigen HPA-1a develops an alloantibody directed against that antigen in response to transfusion. PTP presents clinically as a reduction, often dramatic, in platelet count during the 3 weeks (range = 1-24 days) after transfusion of platelet-containing blood components

and even after FFP transfusion, although rarely. Although this antibody is, strictly speaking, an alloantibody, it behaves as if it were an autoantibody, destroying the recipient's own antigen-negative platelets as well as transfused antigen-positive ones. The mechanism of platelet destruction in PTP and its therapeutic management are discussed in more detail in Chapter 7: Adverse Effects of Transfusion.

Miscellaneous Disorders: The Role of Platelet Transfusion

The potential role of platelet transfusions in three other situations—idiopathic thrombocytopenic purpura, platelet dysfunction, and heparin-induced thrombocytopenia—merits mention.

ITP results from the autoimmune destruction of platelets; as with autoimmune hemolytic anemia, transfusion should be avoided if at all possible because transfused platelets will be removed as rapidly as autologous platelets. Splenectomy may be performed for patients who are refractory to medical treatment with steroids, IVIG, IV RhIG (effective in Rh-positive ITP patients only), or immunosorbent column apheresis. However, when ITP patients undergo splenectomy, it is unnecessary to transfuse platelets either before or during surgery because the platelet count will rise immediately after removal of the spleen.

Platelet dysfunction can result from medications, uremia, or congenital defects within the platelet. Nonsteroidal anti-inflammatory drugs (NSAIDs), with the exception of aspirin, reversibly affect platelet function. In the case of NSAID-related platelet dysfunction, one need only discontinue the NSAID so that platelet function will return to normal. Aspirin, however, irreversibly poisons (acetylates) platelet cyclooxygenase, rendering the platelets dysfunctional. Because the effect is irreversible, aspirin must be discontinued before platelets are transfused. However, aspirin is rapidly metabolized to salicylic acid, which is only a weak inhibitor of cyclooxygenase. As a consequence, aspirin has a plasma half-life of 15 to 20 minutes; thus, only 1 to 2 hours need pass between the time the aspirin is discontinued and the platelets are transfused. Platelet inhibitors, such as clopidogrel, and GPIIb/IIIa inhibitors are seeing increased use in the effort to reduce thrombotic risk in patients with coronary artery disease. When patients on those agents require emergent cardiac



Prophylactic platelet transfusion is rarely indicated in ITP because transfused platelets are destroyed just as rapidly as autologous platelets.



Aspirin must be discontinued before platelets are transfused—2 hours is sufficient time to clear aspirin before transfusion.

surgery, they are at increased risk of bleeding and may require prolonged platelet support for several days.

The exact cause of uremic platelet dysfunction is uncertain but may be related to platelet hypoxia, to the toxic effect of uremia on platelets, or both. In any event, maintenance of the hematocrit above 25% to 30% appears to ameliorate the effect of uremia on platelet function, as does dialysis to remove uremic toxins. The mechanism underlying the efficacy of increased hematocrit has widely been attributed to the rheostatic effect of red cells displacing platelets to the periphery of the vessel where they are most needed. However, recent evidence that red cell substitutes (which are acellular) also improve uremic platelet function suggests that correction of platelet hypoxia may be more important than rheology. Desmopressin (DDAVP), cryoprecipitate, or both have also been used in the treatment of uremic platelet dysfunction. If DDAVP is used for that indication, the ordering physician should be mindful that because of a recent change to the package insert for this drug, DDAVP is considered contraindicated in patients with moderate to severe renal impairment (creatinine clearance less than 50 mL/minute). Platelet transfusion has no place in the routine treatment of uremic platelet dysfunction because transfused platelets become "uremic platelets" almost immediately after infusion.

Patients with congenital platelet defects have lifelong platelet dysfunction and will require platelet transfusion when bleeding, despite the fact that the quantitative platelet count is normal. Prophylactic use of platelets in that population is usually inadvisable because of the risk of alloimmunization to both HLA and red cell antigens with repeated transfusion exposure.

HIT is best treated by discontinuing the heparin. That action should result in an increase in platelet count within several days. Care must be taken to eliminate all potential sources of heparin, including heparinized catheters and low-dose heparin flushes. Because HIT tends to be accompanied by thrombosis rather than by bleeding, platelet transfusion is rarely indicated.



Because HIT is often associated with thrombosis rather than bleeding, platelet transfusion is usually unnecessary and may be contraindicated.

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Appendix 11-1. The Rosette and Kleihauer-Betke Tests

Fetal Cell Screen (Rosette Test)

The fetal cell screen is based on detection of Rh-positive fetal cells in the circulation of an Rh-negative mother. It is, therefore, of use only in screening for fetomaternal hemorrhage (FMH) in the setting of Rh incompatibility. The fetal cell screen has a sensitivity of 99% to 100% when FMH is 15 mL of red cells or more.

In this test, maternal blood is incubated with anti-D, resulting in antibody coating of any Rh-positive fetal cells present in the maternal circulation. Rh-positive indicator cells are added, and they will form a rosette around fetal cells that are coated with anti-D.

Kleihauer-Betke Test

When the fetal cell screen is positive, the Kleihauer-Betke test can be used to quantitate amount of FMH. Unlike the rosette test, the Kleihauer-Betke test is specific for fetal red cells, regardless of their Rh type.

A blood smear is prepared from the maternal sample and exposed to acid buffer. Fetal hemoglobin resists acid elution, with the result that fetal cells appear intact, whereas adult cells, which are sensitive to acid, appear as ghosts. Two thousand red cells are counted, and the percentage of fetal to maternal cells is calculated. False-positive results can be seen in hereditary persistence of fetal hemoglobin and in a small number (1%-2%) of normal adults.

12

Transfusion Emergencies

GARY STACK, MD, PhD



SOME CLINICAL SETTINGS, SUCH AS ACUTE and life-threatening blood loss, necessitate transfusion on an emergency basis. Transfusion services must have policies and procedures in place to streamline compatibility testing of Red Blood Cell (RBC) units to meet urgent and/or massive transfusion needs. Situations requiring urgent RBC transfusions are more complicated to manage if patients have non-ABO blood group antibodies. Other blood components, such as platelets or plasma, also can be urgently required for patients in some clinical settings. Those involved in the care of such patients, as well as transfusion service personnel, should know when emergency platelet and plasma transfusions are indicated, and also when they may be contraindicated. Another type of emergency exists when shortages of blood components occur. Contingency plans to cope with shortages can help minimize the interruption of needed transfusion services. This chapter discusses some approaches to managing transfusion emergencies.



Transfusion services need policies and procedures that address the following:

- Massive transfusion needs
- Compatibility testing of RBC units in an expedited timeframe
- Indications and contraindications for urgent platelet and plasma transfusions
- Blood shortages and contingency plans

Acute Blood Loss and Hypovolemic Shock

Acute blood loss is classified according to the volume of blood lost and the corresponding clinical manifestations. (See Table 12-1.) A loss of up to 15% of blood volume is classified as a Class I hemorrhage. In otherwise healthy individuals, this results in minimal if any physiologic change

Table 12-1. Classes of Hemorrhage

Class	Blood Loss	Symptoms	Replacement Fluid
I	<15% <750 mL	Possible mild tachycardia (<100 beats per minute)	None
II	15% to 30% 750 to 1500 mL	Increased diastolic blood pressure Decreased pulse pressure Tachycardia (>100 beats per minute) Tachypnea (20-30 breaths per minute) Cool, clammy skin Mild anxiety	Crystalloid first; RBCs rarely needed if hematocrit previously normal
III	30% to 40% 1500 to 2000 mL	Decreased systolic and diastolic blood pressure Decreased pulse pressure Marked tachycardia (>120 beats per minute) Marked tachypnea (30-40 breaths per minute) Oliguria Pale, diaphoretic Delayed capillary refill Anxiety, confusion	Crystalloid first; transfuse RBCs if inadequate response to crystalloid
IV	>40% >2000 mL	Decreased systolic and diastolic blood pressure Decreased pulse pressure Marked tachycardia (>140 beats per minute) Marked tachypnea (>35 breaths per minute) Negligible or no urine output Cold, pale skin Confused, lethargic	Crystalloid and RBCs

because the body has the capacity to compensate for low-volume blood loss. In most cases, vital signs remain unchanged, and there should be no need for fluid replacement therapy. A Class II hemorrhage is defined as an acute loss of 15% to 30% of blood volume. This typically produces modest tachycardia, decreased pulse pressure resulting from elevated diastolic blood pressure, cool extremities, digital cyanosis, anxiety, and restlessness. As blood loss approaches the upper end of a Class II hemorrhage, the patient's extracellular space should be maintained with the infusion of crystalloid solutions.

With a Class III hemorrhage, where 30% to 40% of blood is lost, the patient will likely show signs of shock, including tachycardia, tachypnea, systolic and diastolic hypotension, mental confusion, and anxiety. The patient appears pale and diaphoretic. Some otherwise healthy, nonanemic patients may respond adequately to crystalloid alone for Class III hemorrhage. However, many patients, especially elderly patients, patients with preexisting anemia, or patients at increased risk for organ ischemia, may require red cell replacement. A Class IV hemorrhage, representing a loss of more than 40% of blood volume, is life-threatening and is associated with severe shock. The blood pressure and pulse may be barely detectable. Mortality of about 50% is expected within 15 minutes, if not treated. The blood volume should be restored rapidly, initially with the infusion of crystalloid solution. The restoration of oxygen-carrying capacity of the blood with RBC transfusion is indicated even in otherwise healthy individuals.

Give Crystalloid Solutions First

Oxygen delivery depends on both red cell mass and red cell transport. The latter depends on an adequate intravascular blood volume. Generally, losses of red cell mass can be tolerated better than losses of blood volume. Accordingly, volume replacement, not restoration of the red cell mass, is usually the first priority in acute blood loss. Isotonic crystalloid solutions, such as normal saline or lactated Ringer's solution, are the preferred initial choice for volume expansion. Lactated Ringer's solution has the theoretical advantage of containing lactate, which is metabolized by the liver to bicarbonate, which in turn may be helpful in treating a metabolic acidosis. The volume of crystalloid needed to restore intravascular volume is approximately three to four times the estimated blood loss, eg, 3 to 4 liters of crystalloid per liter of blood loss. This is the result of a rapid distribution of crystalloid solution between the intravascular and extravascular spaces.

There has been debate over whether colloids, such as albumin solutions, should be substituted for crystalloid during initial resuscitation. Colloid is given in lower volumes compared to crystalloid, ie, in a 1:1 ratio relative to the estimated blood loss. Some clinicians have postulated that the increased intravascular oncotic pressure of albumin solutions, for example, should produce a more rapid and enduring expansion of the intravascular volume and a decreased risk of pulmonary edema. However, the most



Volume replacement with crystalloid is a higher priority than transfusion of RBCs in acute hemorrhage.

authoritative study on the subject to date, the Saline vs Albumin Fluid Evaluation (SAFE) study published in 2004, found equivalent 28-day outcomes when normal saline was compared to 4% albumin during trauma resuscitation. Whether there are subsets of patients who might respond better to one or the other resuscitation fluid is the subject of ongoing debate and analysis.

Transfuse RBCs When Oxygen-Carrying Capacity Is Limited

When blood loss is sufficiently severe, preservation of oxygen delivery depends on timely RBC transfusion. One consensus guideline, developed in 2006 by the Inflammation and Host Response to Injury Large-Scale Collaborative Project, advocates RBC transfusion in trauma when the infusion of crystalloid solution has exceeded 30 mL/kg. Even earlier transfusion is advocated for patients who are in severe shock or who have injuries associated with large blood loss, such as vertical shear pelvic fracture or bilateral femur fracture. Arterial pH and serum lactate measured serially during the course of resuscitation can help determine whether oxygen delivery has been adequately restored and guide any further intravascular volume replacement or transfusion.

The timing of RBC transfusion, nevertheless, has been the subject of debate. Some specialists advocate transfusion early during fluid replacement and others argue that it is better to defer transfusion, if possible. The latter viewpoint is based on the fact that increasing the hematocrit with RBC transfusion will increase the viscosity of the patient's blood. Also, storage-related membrane changes appear to make red cells less deformable. The combination of increased viscosity and reduced red cell deformability may slow the microcirculation and decrease the rate of oxygen delivery. In addition, RBC transfusion may not be as effective as expected initially in restoring oxygen delivery to tissues because stored red cells have reduced levels of 2,3-diphosphoglycerate (2,3-DPG). The 2,3-DPG is needed for normal release of oxygen from hemoglobin. Perhaps as a result of these or other effects of transfusion, some studies have shown that blood transfusion is a predictor of poor outcomes in trauma resuscitation independent of shock severity.

Once the patient care team has made a decision to transfuse, it must establish dedicated intravenous access exclusively for infusing blood components and normal sa-



Only normal saline may be mixed with blood components. Lactated Ringer's solution may not clot the blood component and glucose solutions may cause hemolysis.

line (0.9% NaCl). Lactated Ringer's solution, which contains calcium, should never be mixed with or infused through the same line as blood components. The calcium can titrate out the chelating capacity of the citrate anticoagulant in the blood component bag and cause clotting. Glucose and medications also should not be infused through the same line used for transfusion because osmotic effects and chemical incompatibilities may result in hemolysis.

Compatibility Testing Should Proceed as Far as Possible

In emergency transfusion settings, full compatibility testing should be completed before transfusion, if time is available. (See Table 12-2.) ABO- and Rh(D)-identical and crossmatch-compatible RBC units should be transfused, if at all possible. For this to happen, the patient care team should send a blood specimen to the transfusion service as soon as possible during the resuscitation efforts so that ABO and Rh(D) typing, an antibody screen, and cross-matching can be carried out. While full compatibility testing proceeds, which takes about 45 minutes, the patient often can be sufficiently stabilized with crystalloid solutions.

If time does not permit full compatibility testing, some or all levels of compatibility testing may be omitted, depending on the clinical urgency of transfusion. If compatibility testing is abbreviated, the patient's physician must sign an order or a statement indicating that the clinical situation was sufficiently urgent to require blood component release before the completion of testing. In addition, all RBC units should have a label or tag indicating that compatibility testing was not completed. Some options for abbreviated compatibility testing are described below and in



Compatibility testing may be abbreviated if the time for full testing will unduly delay a lifesaving transfusion.

Table 12-2. Time Required for Compatibility Testing

Test	Time
ABO/Rh typing	10-15 minutes
Type and screen	30-45 minutes
Type, screen, and crossmatch	45-60 minutes
Antibody panel	100 minutes to several days



If group A, B, or AB RBC units are issued, the ABO group must be determined on the basis of a current, valid patient specimen.

Table 12-3. It is important to note that if group A, B, or AB RBC units are issued, the ABO group must be determined on the basis of a current, valid patient specimen. It is never acceptable to issue RBC units other than group O whose ABO group was determined only on the basis of prior records.

1. Crossmatched, Type-Specific RBCs without Antibody Screen.

One option is to provide crossmatched, ABO/Rh group-specific RBCs without an antibody screen. This requires about 10 to 15 minutes for ABO/Rh typing of the recipient, followed by about 5 minutes for immediate-spin crossmatching of the ABO/Rh-selected RBC units. The value of the immediate-spin crossmatch is to check the ABO typing result. This option includes no effort to detect clinically significant, non-ABO antibodies in the recipient, so it provides no protection to transfusion recipients who are already alloimmunized to one or more blood group antigens. An antibody screen to detect blood group antibodies should be started as soon as possible but will not be completed until after the RBCs are issued for transfusion. If results indicate or suggest the presence of a clinically significant, non-ABO antibody, the patient care providers should be informed immediately, so that they can decide whether to proceed with transfusion or possibly anticipate a transfusion reaction. Provided time is available,

Table 12-3. Red Blood Cell Testing Scenarios for Emergency Transfusion

Transfusion Urgency	Compatibility Testing Status	ABO/Rh Type Selected	Antibody Screen	Crossmatch	Testing Time* (in minutes)
Lowest	Complete	ABO- and Rh-identical	Yes	Yes	45-60
	Incomplete; no antibody screen	ABO- and Rh-identical	No	Yes	15-20
	Incomplete; no antibody screen or crossmatch	ABO- and Rh-identical	No	No	10-15
	Incomplete; no antibody screen or crossmatch	Group O, Rh-negative	No	No	0

*Testing time includes an "immediate-spin" crossmatch and assumes that there is no history of a blood group alloantibody and that the current antibody screen is negative. Testing time does not include transportation times.

this option is preferred to Option 3 below that uses uncrossmatched, group O, Rh-negative (ie, D-negative) RBCs. Group O, Rh-negative RBCs are best reserved for patients who really need them.

2. Uncrossmatched, Group-Specific RBCs without Antibody Screen. Another option is to issue uncrossmatched, ABO/Rh group-specific RBCs without an antibody screen. Compared to Option 1, this saves time by eliminating the immediate-spin crossmatch. However, this time saving comes at the cost of eliminating a check on the results of ABO/Rh typing. Out of safety concerns, this option should probably be avoided because it increases the risk of an ABO-mismatching error, particularly if no prior ABO typing records are available for the patient. Without the immediate-spin crossmatch or prior records, the entire burden of ABO compatibility is placed on one ABO typing done in a rushed emergency setting. From a safety perspective, either Option 1 or the use of uncrossmatched, group O-negative RBCs (Option 3 below) is preferred. As in Options 1 and 3, clinically significant non-ABO antibodies are not detected in the recipient with Option 2. Complete testing (ie, an antibody screen of the recipient and crossmatching of the issued RBCs) should still proceed after the RBCs are issued. Given the time it takes to transport the RBC unit and set up the unit administration, immediate notification of the patient care providers regarding any detected incompatibility might still be in time to affect the decision to transfuse the issued units.

3. Uncrossmatched Group O, Rh-negative or Group O, Rh-positive RBCs without Antibody Screen. If the patient's situation is so tenuous that even the delay for ABO/Rh typing cannot be tolerated, group O, Rh-negative or group O, Rh-positive RBCs can be transfused without further testing. An immediate-spin crossmatch is typically not performed because ABO incompatibility has been addressed already by using the "universal donor" group O RBCs. As with options 1 and 2 above, no attempt is made to detect non-ABO antibodies. Some institutions issue only group O, Rh-negative RBCs regardless of the gender of the patients, whereas other institutions issue group O, Rh-negative RBCs only to females of childbearing potential and group O, Rh-positive RBCs to all others. All females of childbearing potential should be assumed to be D negative until proven otherwise and should receive Rh-negative RBCs, if at all possible. D alloimmunization must be prevented in females of childbearing potential in order to avoid the possibility of hemolytic disease of the fetus and newborn in



Alloimmunization to the D antigen must be prevented in females of childbearing potential.

future pregnancies. Provision of Rh-negative RBCs for *all* emergency transfusions has the advantage of avoiding D alloimmunization in all recipients, which will make compatibility testing and provision of RBCs to those patients simpler for any future transfusions. The local availability of group O, Rh-negative RBCs and the demographics of the patient population served by the facility will determine if group O, Rh-negative RBCs can or should be made available for all.

In some medical centers, group O, Rh-negative and/or group O, Rh-positive RBCs are available at all times in monitored refrigerators in emergency rooms, trauma centers, or intensive care units. Placing the RBC units at the point of care eliminates transportation delays. However, this ready availability has the drawback of potentially encouraging the overuse of uncrossmatched group O RBCs. As an alternative, several group O units may be prepared and set aside for urgent issue right in the transfusion service itself. These units may be tagged in advance as not having been tested for compatibility, and any relevant computer "sign out" or paperwork may be completed to the extent possible. Tubing segments may be removed in advance and set aside for use in compatibility testing when the RBC units are issued. Advance preparation such as this can significantly reduce the time it takes to issue uncrossmatched group O RBCs and perhaps eliminate the need for monitored refrigerators outside the transfusion service.

Abbreviated Testing Poses Safety Risks

Elimination of the antibody screen significantly shortens the pretransfusion testing time. However, because the crossmatch at many facilities is "immediate spin" only, the antibody screen is often the only test that many transfusion services use to detect non-ABO antibodies. Because some non-ABO antibodies are capable of mediating hemolysis, the elimination of the antibody screen exposes patients to an increased risk of non-ABO hemolytic transfusion reactions. Overall, approximately 1% to 2% of the patients tested by hospital transfusion services have detectable non-ABO antibodies. Of course, the risk of blood group alloimmunization varies from patient to patient, and will be higher in patients previously exposed to foreign red cells through transfusion or pregnancy. Multiparous women, victims of prior trauma, and patients with a history of significant surgery or cancer treatment are among the patients



Elimination of the antibody screen and crossmatch for emergency transfusions increases the risk of non-ABO hemolytic transfusion reactions, because about 1% to 2% of patients have non-ABO blood group antibodies.

with higher-than-average rates of blood group alloimmunization. The highest rate (about 25%) has been observed in multitransfused patients with sickle cell anemia. It is important to note that the use of so-called “universal donor” group O, Rh-negative RBCs does not address compatibility with non-ABO antibodies, unless the antibody happens to be anti-D. Therefore, uncrossmatched, group O RBCs present some risk of hemolysis.

Fortunately, most, although not all, clinically significant non-ABO antibodies are IgG molecules that usually mediate the relatively mild extravascular, as opposed to intravascular, hemolysis. However, even extravascular hemolysis can pose significant morbidity in a patient already compromised by hemorrhagic shock. If a recipient of an emergency transfusion of RBCs is found in subsequent testing to possess a blood group antibody, typing for the implicated red cell antigen(s) should be performed as soon as possible on retained segments of the transfused units so that the extent of a potential hemolytic reaction can be anticipated. This information, as well as the expected clinical significance of the antibody, should be shared with the patient care team immediately.

The use of group O, Rh-negative RBCs for emergency transfusions can be problematic from a blood inventory perspective. Because only about 6% to 7% of the blood supply is group O, Rh-negative, these RBCs are a scarce and valuable resource that should be used judiciously. They need to be conserved, if at all possible, for patients for whom they represent the only transfusion option—ie, group O, Rh-negative females of childbearing potential and group O, Rh-negative patients who have previously formed an anti-D. Therefore, the transfusion service should conduct retrospective audits of requests for uncrossmatched group O RBCs, as well as any abbreviated compatibility testing, in order to detect inappropriate overuse. Abuses of abbreviated compatibility testing should be communicated with the appropriate medical leadership and the Transfusion (aka Blood Usage) Committee.

As noted earlier, the use of ABO group-specific RBCs in an emergency setting can pose a hazard of ABO mismatch. The rushed atmosphere at the patient’s bedside, as well as in the transfusion service, may increase the risk of patient and specimen identification errors as well as laboratory error. Identification systems need to be rigorous and as foolproof as possible to prevent such errors. Although it is always prudent to have a second independent specimen to ABO type if the patient is new to the transfusion service,



Group O, Rh-negative RBCs should be used judiciously in emergency transfusions. Whenever possible, they should be conserved for recipients who cannot receive other blood types, such as group O, Rh-negative females of childbearing potential.

such testing redundancy may not be possible in an emergency. For that reason, group O RBCs are safer components. However, blood inventory levels and the regional availability of group O, Rh-negative RBCs may be limiting factors.

When uncrossmatched, group O RBCs are transfused in emergencies, a small amount of plasma (about 40 mL per unit of additive-solution RBCs) containing anti-A or anti-B is also infused. This usually does not pose a significant risk for non-group-O recipients. The recipient may exhibit a positive direct antiglobulin test result if multiple units are transfused, but usually without significant hemolysis or a clinical reaction. Once the patient's blood type is determined, it is safe to switch to RBCs of the patient's ABO group, provided they are compatible on immediate-spin crossmatch.

Communication with the Patient Care Team Is Key



Open communication between the transfusion service and patient care provider is key to ensuring that the patient's needs are met during transfusion emergencies.

Honest and open communication during transfusion emergencies between the patient care team and the transfusion service is key to the provision of the safest blood possible. The patient's physician needs to make a clear and rational assessment of the patient's condition and the urgency of transfusion. In communicating this to the transfusion service, the physician (or other member of the patient care team) must neither overstate nor understate the urgency. Some providers who are not used to working with the transfusion service in emergency situations may overstate the urgency because they believe such behavior is necessary to ensure that their requests receive priority. They may not be thinking of the risks and drawbacks of abbreviating compatibility testing. If this behavior is recurrent, the transfusion service medical director and technical supervisor should schedule a meeting with the key personnel from the relevant patient care area to review emergency transfusion practices. Discussion at this meeting should give the clinical team confidence in the responsiveness of the transfusion service and encourage open and honest communication in the future.



Despite the goal of never transfusing incompatible blood, any RBCs may be better than no RBCs at all. This is especially true in the massively bleeding patient with alloantibodies for whom antigen-negative blood is not immediately available.

Urgent Transfusion Despite Non-ABO Incompatibility

RBC transfusion sometimes must be administered for urgent clinical reasons even when RBCs are incompatible or untested for compatibility with a blood group alloantibody in the recipient's serum. This may occur when the transfu-

sion service obtains a new positive result on an antibody screen and there is no time to perform an antibody panel. In other cases, a patient may have an alloantibody of known specificity, but the transfusion service has no time to screen for antigen-negative units. If the known red cell alloantibody has a specificity directed against an antigen of high incidence, the transfusion service may not have adequate RBC units in inventory or resources to find antigen-negative units in time to meet urgent transfusion needs. In fact, the rarity of some red cell phenotypes may require a blood center to assist in antibody screening or provide frozen, deglycerolized RBC units of rare phenotype. Such outside assistance may not be available quickly enough to address urgent clinical needs.

The transfusion service physician should discuss the case clearly with a physician responsible for the patient. The urgency of the transfusion needs to be balanced against the risk of a hemolytic transfusion reaction caused by an incompatibility. The perceived risk:benefit ratio may be altered by knowledge of the hemolytic potential of the red cell antibody, if its antigenic specificity is known. For example, blood group antibodies associated primarily with extravascular hemolysis, such as Rh antibodies, may mediate reactions of lesser severity than those associated with intravascular hemolysis. Moreover, alloantibodies directed against antigens of relatively low incidence, such as anti-K, may pose a reduced statistical risk if only 1 or 2 units are transfused. Summary tables such as those in Chapter 5 can help in risk assessment.

Once a decision is made to transfuse incompatible or potentially incompatible RBC units, the patient should be premedicated to lessen the symptoms of a possible reaction. Premedication should include an antipyretic, an antihistamine, and perhaps a corticosteroid. The patient's physician should sign a release form acknowledging awareness of and accepting responsibility for proceeding with transfusion, despite the potential incompatibility. The transfusion service needs to tag or label the RBC unit as incompletely tested for compatibility or as incompatible. If possible, the transfusion should proceed at a slow infusion rate, particularly at the start—eg, no more than about 25 to 50 mL over the first 30 minutes. During the transfusion, the patient needs to be monitored closely for signs and symptoms of a hemolytic reaction. STAT testing of serial plasma and urine specimens for the presence of hemoglobin, as well as direct antiglobulin tests, are useful early markers of hemolysis that can guide the decision to proceed with transfusion.



Incompatible blood should be transfused only with the express knowledge and consent of both the patient's physician and the physician responsible for the transfusion service.



When incompatible blood must be transfused, the patient should be premedicated with antipyretics, antihistamines, and corticosteroids. Infusion of the first 50 mL of each unit should proceed as slowly as possible, and the patient should be closely observed for any sign of a reaction.

Strategies for Emergency Transfusions in Patients with Rh Blood Group Antibodies

If a patient has a previously identified red cell alloantibody against one of the major antigens of the Rh system, it is possible to take advantage of the known Rh haplotype frequencies in the selection of untested RBCs for urgent transfusions. (See Table 12-4.) Because most Rh-negative RBCs have the genotype *ce/ce* and the phenotype D–C–c+E–e+, they should be compatible most of the time with known anti-C, anti-E, and anti-D. Thus, patients with these antibodies should receive Rh-negative RBCs if there is no time to test for and find verified “antigen-negative” RBCs.

Because the c antigen is somewhat less common on Rh-positive than on Rh-negative RBCs, Rh-positive RBCs carry a slight advantage for patients with anti-c. Rh-positive RBCs are preferred for known Rh-positive recipients with anti-c but are highly likely to alloimmunize Rh-negative recipients against the D antigen. For Rh-negative or Rh-untyped recipients, the risk of D alloimmunization may outweigh any other benefit, particularly in females of childbearing potential. It should be remembered also that hemolysis caused by anti-c will ordinarily lead to the milder extravascular, and not intravascular, hemolytic transfusion reactions. Neither Rh-negative nor Rh-positive



When the need for transfusion is urgent and the patient is known to have anti-C or anti-E, Rh-negative RBCs should be selected, because they most likely will be C- and E-negative.

Table 12-4. Emergency Transfusion of Patients with Known Rh Antibodies

Antibody Present	RBC Type to Transfuse*	Comment
Anti-D	Rh-negative	RBC units are pretested for Rh and known to be D-negative.
Anti-E	Rh-negative	Most Rh-negative RBCs are “double dose” c and e cells and lack E.
Anti-c	Rh-positive in known Rh-positive recipients	Most Rh-negative RBCs are c-positive and will be incompatible; a slightly greater percentage of Rh-positive than Rh-negative RBCs will be c-negative.
Anti-C	Rh-negative	Most Rh-negative RBCs lack C antigen.
Anti-e	Rh-identical with patient's type, if known	98% of all donor cells are e-positive, so no real advantage to using Rh-negative or -positive cells exists.

*Rh refers only to the D antigen.

RBCs offer an advantage to patients with anti-e because about 98% of donated RBCs are e-positive.

Massive Transfusion

Streamlined Testing

Massive transfusion is defined as the replacement of a patient's total blood volume or more within 24 hours. In some cases, the ability of the transfusion service to keep up with the patient's transfusion needs in the setting of massive transfusion will be overtaxed. If that occurs, a policy should be in place to abbreviate the testing and documentation requirements in order to expedite the provision of needed blood components.



Patients with a negative pre-transfusion antibody screen who receive massive transfusions (more than one blood volume in 24 hours) can safely receive ABO/Rh-compatible red cells with abbreviated (immediate-spin) crossmatch or repeat ABO typing.

Switching ABO Blood Group in Massive Transfusion

Patients with less common ABO blood groups (ie, B and AB) may receive RBCs of a more common blood group if the RBC inventory of the patient's own type is insufficient. Group AB recipients can receive group A RBCs, and group B recipients can receive group O RBCs. Once hemorrhage slows or stops, the patient can be switched back to the original blood group, if desired, if or when the immediate-spin crossmatch is compatible. Table 12-5 summarizes the acceptable blood group switches and a recommended sequence in which they can be made.

Switching Rh Blood Group in Massive Transfusion

As soon as it becomes apparent that an Rh-negative patient will receive massive RBC transfusions that will exceed the Rh-negative, ABO-compatible RBC inventory, the patient should be switched to Rh-positive RBCs. It is better to preserve the inventory of Rh-negative units for when the hemorrhage slows or stops. Although the transfusion of Rh-positive RBCs to Rh-negative females of childbearing potential generally should be avoided to prevent D alloimmunization and a future risk of hemolytic disease of the fetus and newborn, no patient should be denied life-saving transfusion because of an alloimmunization risk.

If Rh-negative females receive Rh-positive RBCs, they may be given Rh Immune Globulin (RhIG) for prophylaxis against anti-D formation. An intravenous formulation of

Table 12-5. Switching ABO and Rh Types in Massive Transfusion

Recipient Type	Donor ABO/Rh Options				
	1st Choice	2nd Choice	3rd Choice	4th Choice	5th Choice
O Neg	O Neg*	O Pos	—	—	—
A Neg	A Neg*	O Neg*	A Pos	O Pos	—
B Neg	B Neg*	O Neg*	B Pos	O Pos	—
AB Neg	AB Neg*	A Neg*	B Neg*	O Neg*	AB Pos, A Pos, B Pos, O Pos
O Pos	O Pos	O Neg [†]	—	—	—
A Pos	A Pos	O Pos	A Neg [†]	O Neg [†]	—
B Pos	B Pos	O Pos	B Neg [†]	O Neg [†]	—
AB Pos	AB Pos	A Pos	B Pos	O Pos	AB Neg [†] , A Neg [†] , B Neg [†] , O Neg [†]

*The decision to stay with Rh-negative components vs switching to Rh-positive components should be based on 1) anticipated further need for RBC transfusion, 2) availability of Rh-negative components, and 3) urgency of transfusion of other Rh-negative patients. If hemorrhage is ongoing with a continuation of massive transfusion needs, the switch to Rh-positive RBCs should be made as soon as possible.

[†]The use of Rh-negative RBCs for Rh-positive recipients is unlikely to be necessary because about 85% of available banked RBCs are Rh-positive. The more limited supply of Rh-negative components is unlikely to be sufficient to accommodate ongoing massive transfusion needs. They may be given as a stop-gap measure for life-threatening acute anemia, if no other compatible units are available.

RhIG (IV RhIG) is the preferred prophylaxis against anti-D formation for the transfusion of large amounts of Rh-positive RBCs (eg, one or more RBC units). Otherwise, many injections of the intramuscular formulation would be required. According to the manufacturer's recommendations, the IV RhIG can be administered as long as no more than 20% of the patient's red cells are Rh-positive. Above 20%, the risk of a clinically significant extravascular hemolytic reaction rises, and exchange transfusion should be considered. RhIG should not be administered entirely just after transfusion, but rather in injections of 3000 IU (600 mg) every 8 hours until the total dose is given (90 IU/mL of Rh-positive red cells). The IV RhIG should be administered within 72 hours of the Rh-positive transfusion.

Urgent Platelet Transfusions

Platelet Transfusion Emergencies

Patients with severe thrombocytopenia (platelet count <5,000 to 10,000/ μ L) are at risk of life-threatening intracranial hemorrhage and other serious bleeding. Fortunately, fatal or debilitating hemorrhage is rare in the absence of trauma, and the majority of patients will tolerate severe thrombocytopenia with only skin petechiae, purpura, and mild gastrointestinal blood loss. Nevertheless, it is common and prudent practice to transfuse platelets prophylactically in order to maintain patients' platelet counts above 10,000/ μ L.

Immune Thrombocytopenic Purpura

Maintaining adequate levels of platelets in adult patients with immune thrombocytopenic purpura (ITP), however, can be problematic. Thrombocytopenia in these patients results from an increased clearance of platelets mediated by platelet autoantibodies. Unfortunately, these autoantibodies can also mediate the rapid clearance of transfused platelets, causing patients with ITP to be relatively refractory to platelet transfusion. Nevertheless, because some patients with ITP will obtain a transient increase in platelet count despite the autoantibody, a trial of platelet transfusion may be indicated in emergencies. The increment in platelet count can often be augmented and prolonged if intravenous immune globulin (IVIG; 1.0 g/kg) is administered just before or during platelet transfusion. One case report has shown particular success with simultaneous 24-hour continuous infusions of IVIG (1 g/kg over 24 hours) and platelets (1/2 unit apheresis platelets every 4 hours). It should be noted that platelet transfusions in ITP are usually reserved for urgent settings only and do not substitute for other treatment options, including steroids, splenectomy, anti-D in Rh-positive patients, anti-CD20 monoclonal antibodies, danazol, etc. Various mechanisms have been proposed to explain how IVIG works in enhancing the effectiveness of platelet transfusions in ITP. Perhaps the flood of immunoglobulins competes with antibody-coated platelets for Fc receptors on the surface of macrophages in the spleen, thereby preventing the phagocytosis of the platelets. Alternatively or additionally, perhaps the IVIG contains anti-idiotypic antibodies (ie, antibodies to antibodies) that inhibit the platelet antibodies, or there might be suppression of platelet antibody production.



Platelet transfusion may be attempted in patients with ITP who are bleeding, but this is often ineffective. Concurrent administration of IVIG may enhance the effectiveness of platelet transfusion in ITP.



Platelet transfusion is an urgent need in newborns with neonatal alloimmune thrombocytopenia (NAIT) because they are at significant risk for intracranial hemorrhage during the first 24 to 36 hours of life.

Neonatal Alloimmune Thrombocytopenia

Platelet transfusion also should be administered with some sense of urgency in newborn infants with neonatal alloimmune thrombocytopenia. Because the risk of intracerebral hemorrhage is greatest in the first 24 to 36 hours of life, platelet transfusion needs to be administered as soon as possible after birth. Maternal platelets collected by apheresis or prepared from a single-unit whole blood donation are preferred for transfusion because they will always be compatible with the platelet alloantibody. However, if these are not available, randomly selected or antigen-positive platelets have been shown to yield good increases in platelet count about 90% of the time with no adverse effects. Given that the neonate does not make the platelet antibody, there is no possibility of boosting the antibody titer by exposure to more antigen.

Urgent A1voidance of Platelet Transfusion

In some clinical settings, the urgency exists not in transfusing platelets, but in avoiding platelet transfusions. Specifically, platelet transfusions are contraindicated in thrombotic thrombocytopenic purpura (TTP) and heparin-induced thrombocytopenia (HIT).

Thrombotic Thrombocytopenic Purpura

Patients with TTP have a combination of thrombocytopenia and microangiopathic hemolytic anemia. These two defining characteristics of TTP are often accompanied by mental status changes, renal impairment, and fever. When these five signs occur together, as observed in about 40% of patients with TTP, they constitute the "classical pentad." The diagnosis is frequently first suspected when a patient with thrombocytopenia (platelet counts often <50,000/ μ L on presentation) and anemia is noted to have schistocytes (ie, fragmented red cells) on a blood smear. Other typical laboratory results include elevations in lactate dehydrogenase (LDH), indirect bilirubin, creatinine, and reticulocyte count; normal prothrombin and activated partial thromboplastin times; and a negative direct antiglobulin test.

The underlying defect in TTP is an acquired or a hereditary deficiency in von Willebrand factor (vWF) cleaving protease, an enzyme that breaks down unusually large vWF (ULvWF) multimers into smaller multimers. This protease is also known as ADAMTS-13, which is a member of the ADAMTS family of metalloproteinases. ADAMTS stands



Platelets are almost always CONTRAINDICATED in thrombotic thrombocytopenic purpura (TTP), except when the patient is suffering a life-threatening hemorrhage.

for “a disintegrin and metalloproteinase with thrombospondin motifs.” The acquired deficiency of vWF cleaving protease is the result of autoantibodies with inhibitory activity against the ADAMTS-13 protease, whereas the rare congenital form is caused by mutations in the ADAMTS-13 gene. The ULvWF multimers cause abnormal intravascular platelet aggregation and the formation of microthrombi, which clog the microvasculature and damage the endothelium. Red cells suffer mechanical damage and intravascular lysis, presumably during passage through the occluded microvasculature. The result is multiple organ damage, with the effects most noticeable clinically on the kidneys, brain, and sometimes heart. TTP is frequently fatal, if untreated.

Platelet transfusion given to patients with TTP theoretically provides substrate for the formation of more platelet microthrombi (ie, more “fuel for the fire”), and should be considered only if a patient is suffering a life-threatening hemorrhage. Nevertheless, it should be noted that the adverse and fatal outcomes of platelet transfusion in patients with TTP are documented in a few case reports and the risk has not been systematically studied. Some patients with TTP who were given platelets are known to have tolerated the transfusions without consequence. Plasma exchange is the preferred therapy for TTP and is covered below in the section on “Urgent Plasma Transfusion.”

Heparin-Induced Thrombocytopenia

Platelet transfusion also should be avoided in HIT. HIT is characterized by a decrease in platelet counts beginning usually 5 to 10 days after the initiation of heparin therapy. Thrombocytopenia (defined in HIT as a platelet count <100,000/ μ L or a 50% decrease in platelet count from pre-treatment levels) occurs a few days later, typically 7 to 14 days after starting heparin. A rapid-onset form of HIT is characterized by an abrupt (within 24 hours) decrease in platelet counts as soon as a course of heparin is begun. The abrupt form is seen in patients who have been exposed to heparin in the previous 100 days or so. HIT should also be considered whenever a thrombotic event occurs in a patient receiving or recently treated with heparin. Because the underlying pathophysiology involves platelet and endothelial cell activation, HIT is typically associated with thrombotic, rather than bleeding, complications. Patients with HIT have IgG antibodies directed against the complex of heparin with platelet factor-4 (PF4).



HIT is usually associated with thrombotic complications rather than bleeding complications.

PF4 is a small, positively charged molecule present in platelet α -granules, which is secreted upon platelet activation. PF4 binds to and neutralizes negatively charged heparin. When IgG/heparin/PF4 immune complexes form in patients with HIT, they bind to and activate platelets through platelet Fc receptors. The heparin/PF4 antibody also binds to PF4/heparin complexes present on the surface of endothelial cells, which in turn are activated to release tissue factor, an activator of coagulation. The combination of platelet activation and aggregation, along with coagulation activation, gives rise to the thrombocytopenia and thromboses that are seen in patients with HIT. Venous thromboses typically predominate over arterial thromboses.



Platelet transfusion is usually unnecessary and relatively contraindicated in patients with HIT.

Platelet transfusions are relatively contraindicated in patients with acute HIT. This contraindication is based on anecdotal reports of thrombotic events after platelet transfusions. This contraindication rarely affects patient care because bleeding complications are uncommon in HIT and platelet transfusions are not usually indicated. Platelet transfusions in HIT should be limited to serious and perhaps life-threatening bleeding, and prophylactic platelet transfusions should be avoided.

Urgent Plasma Transfusion

Group AB Is the Universal Donor Type for Urgent Plasma Transfusion

On rare occasions, Fresh Frozen Plasma (FFP) is requested before ABO typing of the patient can be completed. Such a request might be made, for example, for the emergency reversal of a warfarin effect in a patient with a life-threatening hemorrhage (see below). In that situation, “universal donor” FFP can be issued. It is important to note that “universal donor” FFP is group AB because it contains no anti-A or -B, whereas “universal donor” RBCs are group O. In the “heat of the moment” during a hemorrhagic emergency, when no time is available for ABO typing, an ordering physician may mistakenly insist on group O FFP, not thinking about the distinction between FFP and RBC universal donor types. The transfusion service should have a policy or procedure to safeguard against filling such an erroneous order and to issue instead group AB plasma when ABO typing results are not available.

Plasma Transfusion or Exchange Is Urgent in TTP

Patients with newly recognized TTP require urgent plasma therapy. Typically, large-volume plasma exchange, achieved through plasmapheresis with plasma as replacement fluid, is performed as soon as possible after diagnosis. If plasma exchange is delayed for any reason, simple plasma transfusion is urgently indicated as a temporizing action. FFP is usually the replacement solution used in plasma exchange for treating TTP. Failure to treat TTP rapidly and aggressively is a negative prognostic factor. The volume and frequency of plasma exchange and dosage of plasma transfusion have not been definitively established. Plasma exchange is often done at a rate of 1.0 to 1.5 plasma volumes daily until platelet counts reach at least the lower limit of the reference range. Patients often overshoot their baseline levels during recovery from TTP, and some centers continue plasma exchange up to a few days longer until this happens to ensure that the recovery is stable. LDH levels can also be followed as a second marker of recovery, in which case therapy might continue until both the platelet count and LDH have stably reached their reference ranges. However, different centers often use somewhat different endpoints for therapy.



FFP or cryoprecipitate-reduced plasma may be given to patients with TTP as an urgent temporizing measure, if plasma exchange will be delayed.

Urgent Warfarin Reversal

Plasma transfusion is indicated for the emergency correction of warfarin-mediated anticoagulation in patients with major bleeding complications that threaten life, limb, or sight. Intracranial, retroperitoneal, intraocular, pericardial, and intramuscular bleeding with compartment syndrome are of greatest concern. Consensus guidelines for warfarin reversal [ie, shortening of a warfarin-prolonged international normalized ratio (INR) or treatment of warfarin-related bleeding] have been developed by the American College of Chest Physicians (ACCP) for various clinical scenarios. (See Table 12-6.) These guidelines employ various combinations of the treatment options known to reverse the effects of warfarin, including dose modification, vitamin K₁, FFP, Factor IX complex (also known as prothrombin complex concentrate or PCC), and recombinant activated Factor VII. (See Table 12-7.) It is important to note that the ACCP guidelines do not recommend the use of FFP or a coagulation factor preparation for the prevention of spontaneous bleeding. Modifications of warfarin dosing, along with vitamin K₁ administration, are advocated instead.

Table 12-6. American College of Chest Physicians Guidelines for Warfarin Reversal*

INR	Clinical Status	Warfarin Dosing	Vitamin K₁	FFP or PCC
Above therapeutic, but <5.0	No significant bleeding	Give lower dose, or omit dose and resume at lower dose when INR is therapeutic	—	—
	≥5.0, but <9.0	Omit 1-2 doses; resume at lower dose	—	—
≥9.0	No significant bleeding	Omit dose	≤5 mg (eg, 2-4 mg) orally; give 1-2 mg second dose at 24 hours, if necessary	—
	No significant bleeding, but bleeding risk is increased, or reversal needed for procedure	Hold; resume at lower dose when INR is therapeutic	5-10 mg orally; give additional dose at 24-48 hours, if necessary	—
Any	Serious bleeding	Hold	10 mg by slow IV infusion; repeat every 12 hours, if necessary	Give FFP; use PCC instead for urgent settings; consider recombinant Factor VIIa in place of PCC
Any	Life-threatening bleeding	Hold	10 mg by slow IV infusion; repeat if necessary	Give PCC; consider recombinant Factor VIIa in place of PCC; repeat if necessary

*Adapted from Ansell J, Hirsh J, Poller L, et al. The pharmacology and management of the vitamin K antagonists. The Seventh ACCP Conference on Antithrombotic and Antithrombolytic Therapy. *Chest* 2004;126:204S-33S.
INR = international normalized ratio; FFP = Fresh Frozen Plasma; PCC = prothrombin complex concentrate, also known as Factor IX complex.

Table 12-7. Options for Reversal of Warfarin

	Withhold Warfarin	Vitamin K ₁	FFP	PCC	Factor VIIa
Content	N/A	Vitamin K ₁	All coagulation factors	Factor IX, plus varying amounts of Factors II, VII, and X, depending on commercial preparation ¹	Recombinant Factor VIIa
Speed of action	Delayed (~4 days)	IV: 3-6 hours ² Oral: within 24 hours	Rapid once infused, but limited by infusion time of large fluid volume	Rapid	Rapid
Volume infused	None	~1 mL, but often diluted in normal saline or dextrose solution ³	~15 mL/kg ⁴	~30 mL ⁴	~10 mL ⁵
Requirement for ABO compatibility	N/A	N/A	Yes	N/A	N/A
Product expense	None	Low	Moderate	High	Highest
Adverse effect	Interruption in therapeutic anticoagulation	Anaphylaxis with IV administration (rare); refractoriness to restart of warfarin with higher doses	Various transfusion reactions ⁶ and infectious risk	Thromboembolic risk	Thromboembolic risk

FFP = Fresh Frozen Plasma; PCC = prothrombin complex concentrate, also known as Factor IX complex; Factor VIIa = recombinant Factor VIIa; N/A = not applicable.

¹Proplex T (Baxter) has a higher content of Factor VII and X activities than other US products and is labeled for both Factor IX and VII content. Other US products are labeled only for Factor IX content.

²This represents time to first effects of bleeding.

³Exact dosing has not been established.

⁴Based on the infusion of 3500 IU of Proplex T.

⁵Based on the infusion of 90 µg/kg in a 70-kg individual (ie, 6300 units).

⁶Allergic reactions are the most common acute, adverse effects of plasma transfusion overall; transfusion-related acute lung injury is the most common serious or life-threatening reaction to plasma transfusion.



FFP should be used to reverse a warfarin effect only if the patient is experiencing a serious or life-threatening hemorrhage or if the patient requires an urgent procedure that has a significant bleeding risk.

If the patient develops major bleeding while taking warfarin, as described above, FFP transfusion along with intravenous vitamin K₁ may be indicated. Specific FFP dosing recommendations have not been established, although 15 mL/kg is often cited in the transfusion literature. FFP transfusion will provide all of the vitamin-K-dependent coagulation factors affected by warfarin in fully functional form. The FFP should be transfused with as little delay as possible. For this reason, transfusion services at large health-care facilities may maintain several units of FFP already thawed at all times in order to eliminate the thawing time, which is the main source of delay. Vitamin K₁ is given intravenously for bleeding emergencies because that is the fastest route of administration. The effects of parenteral vitamin K₁ on bleeding can be seen within 3 to 6 hours, by which time active forms of the vitamin-K-dependent coagulation factors are being endogenously produced. Anaphylactic reactions, although rare, have been reported when vitamin K₁ is given intravenously. For this reason, the intravenous route of administration should be reserved for emergencies, and it is recommended that vitamin K₁ be infused no faster than 1 mg/minute.

If the patient is experiencing an acutely life-threatening hemorrhage, such as an intracranial hemorrhage, the ACCP guidelines call for a more urgent correction of the patient's coagulation status with the "off-label" use of either PCC or recombinant Factor VIIa. This is a somewhat controversial recommendation given that dosing guidelines and the extent of thromboembolic and other complications have not been well established for these products in patients on warfarin. Nevertheless, this recommendation attempts to address the major drawback of FFP transfusion for reversing warfarin in a life-threatening situation: it takes too long to administer. FFP is stored frozen and requires thawing time before use, and the ABO type must be selected for compatibility with the recipient. In addition, a relatively large volume of FFP is required, which slows the speed of administration and puts some patients at risk of volume overload. By comparison, recombinant Factor VIIa and PCC can be more rapidly administered because they do not need to be thawed, do not require ABO matching, and are administered in a smaller volume. Recombinant Factor VIIa has an in-vivo half-life of 2 to 3 hours and the half-life of PCC (like FFP) is that of its shortest lived factor, Factor VII (ie, up to about 6 hours). In order to reverse the effect of warfarin for a longer period than these relatively short-acting agents can sustain, intravenous vitamin K₁ should

be concurrently administered with PCC or recombinant Factor VIIa. In addition, some protocols have called for the concurrent administration of FFP with either PCC or recombinant Factor VIIa in order to ensure that levels of all vitamin-K-dependent factors are adequate for hemostasis during the bridging period before the newly administered vitamin K₁ begins to take effect. However, it is not clear whether this is necessary or, if so, what dose of FFP to use.

PCC has been better studied and more commonly used for reversing the effects of warfarin in Europe than in the United States. There have been no clinical trials studying the reversal of warfarin with PCC products available in the US, dosages have not been established in this setting, and the risk of adverse events in warfarin-treated patients has not been systematically studied. Because the use of high doses of PCC in other clinical settings has been associated with arterial and venous thromboses, the thromboembolic risk of PCC is a particular theoretical concern in patients who require warfarin anticoagulation for a preexisting thrombogenic state. This concern has contributed to reluctance on the part of some practitioners in the United States to use PCC for warfarin reversal.

Several commercial PCCs that contain Factor IX along with varying amounts of the other vitamin-K-dependent coagulation factors—Factors II, VII, and X—are available in the United States. It is important to note that PCCs from different manufacturers do not have equivalent factor levels. Also, although all US preparations are labeled for Factor IX content, only one is also labeled for Factor VII content (Proplex T; Baxter, Deerfield, IL). None are labeled for Factor II and X content. Proplex T, at a Factor IX dose of 25 to 50 IU/kg, was shown to be effective in shortening the INR in patients requiring urgent warfarin reversal in a retrospective review of patients at one institution. However, questions remain whether the INR correction correlates with decreased bleeding and whether other commercial PCCs have similar activity. As noted above, optimal dosing and the rate of adverse effects in this setting have not been determined.

Recombinant Factor VIIa has shown promise in a number of case reports and case series in stopping or preventing bleeding in warfarin-treated patients, including those with intracranial hemorrhage. A wide range of doses has been used (from 5 to 320 µg/kg), and optimal dosing has not yet been established. Apparent effectiveness has been observed in a number of cases with single boluses of relatively low doses in the range of 10 to 40 µg/kg. However,



Indications, dosages, and risks of adverse effects have not fully been established for the use of PCC and recombinant Factor VIIa in reversing warfarin. They should be used with caution in this setting.

there is some evidence that patients with intracranial hemorrhage, who were *not* on warfarin, may have better responses with 160 µg/kg than with 40 or 80 µg/kg. Clearly, questions remain concerning dosing. Unfortunately, accumulating evidence suggests that thromboembolic complications of recombinant Factor VIIa in reversing warfarin and other “off-label uses” in nonhemophilic patients may be greater than observed in patients with hemophilia, for whom recombinant Factor VIIa is licensed. For this reason, recombinant Factor VIIa should be avoided or used cautiously in patients who may have a preexisting increased risk of thrombotic events, such as those with disseminated intravascular coagulation, severe atherosclerotic disease, crush injury, septicemia, or concurrent treatment with PCC or activated PCC. The risk of thromboembolic complications of recombinant Factor VIIa used for reversing warfarin needs further study.

Plasma transfusion, as well as the other options discussed above, is also indicated for emergently reversing warfarin in patients who require urgent invasive procedures that have a significant risk of bleeding. The goal of plasma therapy in this situation should not be the normalization of the INR but, rather, its shortening to approximately <1.5 to 2.0. Achieving correction of the INR to a specific target level may be difficult or impossible with FFP transfusion, and it should not be obsessively pursued. The tolerance of the patient for relatively large volumes of FFP and the 3- to 6-hour half-life of Factor VII are limiting factors. FFP transfusion should begin no sooner than about one Factor VII half-life before the procedure; otherwise, there will be a diminishing therapeutic benefit.

Plasma Is Not Indicated for Urgent Heparin Reversal

Plasma is not useful in reversing the effects of heparin. If anything, FFP transfusion may increase the activity of heparin. Heparin in large part has anticoagulant activity because it binds to and enhances the activity of antithrombin, a natural anticoagulant. Accordingly, patients with a deficiency of antithrombin are refractory to heparin. In such patients, FFP, as well as a commercial antithrombin concentrate, are exogenous sources of antithrombin that can be administered therapeutically to restore heparin responsiveness.

Unfractionated heparin has a short in-vivo half-life of about an hour, and, in nonurgent settings, it is often sufficient to stop the administration of heparin to reverse its effects. However, when urgent reversal of heparin is neces-



If FFP is used for reversing warfarin before an urgent, invasive procedure, it should be administered no sooner than about one Factor VII half-life (ie, 3-6 hours) before the procedure. Factor VII has the shortest *in vivo* half-life of the coagulation factors in FFP and, therefore, is the “rate-limiting” factor.

sary for patients with clinically significant bleeding, protamine sulfate may be administered. Protamine is a basic protein from fish sperm that can bind to and neutralize the anticoagulant effects of heparin, a polyanion. One milligram of protamine sulfate will neutralize 100 units of heparin. The dose of protamine is generally tapered depending on the length of time that has elapsed since the intravenous bolus of heparin was given. For example, a protamine dose of 0.5 mg per 100 units of heparin is administered if the heparin was given 30 minutes previously, and a protamine dose of 0.25 to 0.375 mg per 100 units of heparin is administered if the heparin was given 2 hours previously. If heparin was given by intravenous infusion, only the heparin infused over the preceding several hours needs to be taken into account. This typically requires 25 to 50 mg of protamine sulfate to be given after the heparin infusion has been halted. The effectiveness of heparin neutralization can be monitored with measurement of the activated partial thromboplastin time.

Protamine sulfate has reduced activity in neutralizing the effect of low-molecular-weight heparin (LMWH). At best, about 60% of the activity of LMWH can be neutralized. Nonetheless, protamine is the only antidote to LMWH that has been approved by the Food and Drug Administration. Dosing is calculated differently than for unfractionated heparin; for example, 1 mg of protamine sulfate is given per mg of enoxaparin administered in the previous 8 hours (or 0.5 mg if enoxaparin was given 8-12 hours previously), or 1 mg of protamine sulfate is given per 100 units of anti-Xa activity of dalteparin. There are case reports indicating that the "off-label" use of recombinant Factor VIIa (at doses of 20-30 µg/kg) can be effective in rapidly treating bleeding complications associated with excessive levels of LMWH. The recombinant Factor VIIa does not neutralize the action of LMWH but treats the bleeding. This appears to be a promising option that requires more study.



FFP is ineffective in shortening the aPTT in patients receiving heparin. If anything, FFP may enhance the effect of heparin if the patient's antithrombin level is low.

Blood Component Shortages

RBCs and platelets are often in short supply around holidays, summer vacation times, during prolonged bad weather, or after abnormally high blood component usage at one or more medical centers in a region. Several actions are available to minimize the impact of the blood component shortage on clinical care. For shortages of RBCs of particu-



Blood component shortages may be managed by prospective audits of transfusion appropriations, implementation of restrictive transfusion guidelines, and reduction of blood component doses.

In the event of a shortage of one or more ABO/Rh blood groups, the transfusion service may switch patients to a more plentiful, but still compatible, ABO/Rh blood group (see Table 12-4). However, when the shortage affects the availability of blood components of all ABO/Rh blood groups, a more extensive contingency plan should be put into action—one that should have been mapped out in advance and approved by the medical staff. The first step is to communicate the nature of the shortage to the relevant clinical staff and executive management of the facility and to spell out the steps that will be taken to address the shortage. Possible actions include the following: 1) perform a prospective screen of requests for the component(s) in short supply to ensure that the request is clinically justified and meets the facility's transfusion criteria, 2) if necessary, implement more restrictive guidelines for transfusion of the component(s) in question and defer transfusions not meeting those criteria, 3) reduce the dose issued per patient of the component in short supply (for example, split RBC or apheresis platelet units in half and reduce the pool size of whole-blood-derived platelet concentrates), and 4) if absolutely necessary, postpone elec-

Table 12-8. Key Points to Remember in Transfusion Emergencies

- Volume replacement with crystalloid solutions is the first priority in acute blood loss.
- Compatibility testing of RBCs may be abbreviated for urgent transfusions.
- Uncrossmatched, group O-negative RBCs should be transfused judiciously and only for immediately life-threatening hemorrhage.
- Switching blood groups from ABO-identical to a more plentiful ABO-compatible group can circumvent shortages of less common RBC blood types during massive transfusion and blood shortages.
- Because incompatible RBCs are sometimes better than no RBCs, incompatible transfusions are occasionally necessary.
- Urgent platelet transfusion is necessary for the treatment and prevention of intracerebral hemorrhage for severely thrombocytopenic patients.
- Administration of IVIG before or during platelet transfusion may improve the magnitude and duration of the platelet count increase in patients with ITP.
- Low platelet counts are not always an indication for platelet transfusion. Prophylactic platelet transfusions are contraindicated in TTP and HIT.
- Urgent plasma transfusion or plasma exchange is indicated in the treatment of TTP.
- Urgent plasma transfusion is indicated for the emergency reversal of warfarin.
- Although the “off-label” use of PCC and recombinant Factor VIIa are advocated by some specialists for reversing warfarin to treat life-threatening hemorrhage, optimal dosing and the extent of thromboembolic complications have not yet been established.
- During blood component shortages, transfusion requests should be screened for appropriateness and prioritized according to urgency. Giving smaller doses of blood components per patient may stretch the blood supply.

tive surgery cases that are expected to require transfusion. These actions clearly require effective coordination with the medical, surgical, and administrative leadership of the facility and doubtless require the involvement of a transfusion service physician in screening transfusion requests.

Summary

Transfusion emergencies often involve too little time for complete compatibility testing or too few blood components available during rapid or massive blood loss. Because there is so little time for transfusion service personnel to react during transfusion emergencies, actions must be well planned in advance. Key points to remember in these critical situations are listed in Table 12-8.

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13

Plasma Derivatives and Recombinant Plasma Proteins

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PLASMA DERIVATIVES AND RECOMBINANT

plasma proteins have properties in common. Both groups are proteins, either isolated from specific preparations of proteins using normal human plasma, or the result of advances in recombinant protein technology, derived and formulated for human use. Deficiencies or abnormalities of one or more of these plasma proteins, either inherited or acquired, can have a profound effect on the health and well-being of the patient. Therefore, these proteins are isolated and purified to provide a focused targeting of the abnormal or deficient protein and to achieve maximal benefit to the patient after their therapeutic administration. The two groups differ in origin: plasma derivatives are purified from large pools of human plasma, whereas the recombinant products are synthesized *in vitro* from isolated genes expressed in cell cultures by using recombinant DNA techniques.

This chapter discusses the various deficiencies, products available, processing techniques, indications for use, and potential benefits vs risks to the patient who receives those products. Finally, the chapter addresses recombinant he-



Plasma derivatives are purified from large pools (thousands of units) of human plasma. Recombinant products are synthesized *in vitro* but may contain small amounts of human albumin—depending on the product.

matopoietic growth factors, which can reduce some patients' dependence on blood transfusion.

For some products, information regarding dosages is provided. Such information is included only as a guide for checking the appropriateness of clinicians' requests for plasma derivatives in settings where those products are dispensed by the blood bank. For direct patient care, the clinician should verify dosage recommendations in current product inserts or published literature and should not rely solely on the information provided in this chapter.

Plasma Derivatives

Plasma derivatives are not the same as blood components. Whereas blood components are the cellular and plasma fractions obtained by differential centrifugation of Whole Blood (WB) from a single donor, derivatives are more highly purified protein preparations that are typically obtained from large pools (thousands of liters) of human plasma.

Plasma derivatives include albumin, various Factor VIII and Factor IX preparations, von Willebrand factor (vWF), antithrombin (AT), immune globulins, and a variety of other proteins. In this chapter, only human-derived plasma derivatives will be discussed. However, plasma derivatives for human use may also be obtained from other animals (eg, porcine Factor VIII and equine lymphocyte immune globulin).

Plasma derivatives are manufactured and sold by pharmaceutical companies and are not prepared by blood centers or hospital blood banks. Most are heat- or solvent/detergent-treated, or both, are subjected to chromatography purification methods, and are either micro- or nanofiltered. Those methods are designed to eliminate or substantially reduce the product's viral content and therefore the risk of transmission of infectious agents—including the enveloped viruses [such as human immunodeficiency virus (HIV), the hepatitis B virus (HBV), and the hepatitis C virus (HCV)], the nonenveloped viruses [such as human parvovirus B19, hepatitis A virus (HAV)], and prions [such as those causing transmissible spongiform encephalitis (TSE)].

Although plasma derivatives are often dispensed by the pharmacy for use in hospitals, some plasma derivatives—in particular, the coagulation factor concentrates and Rh Immune Globulin (RhIG)—may be distributed by the hospital blood bank. There is a historic basis for blood banks to



Plasma derivatives are prepared by pooling human plasma, extracting and purifying the desired fraction, and performing one or more virus inactivation steps.

manage the distribution of plasma derivatives because those products often represent highly purified substitutes for blood components such as plasma and Cryoprecipitated Antihemophilic Factor (AHF). In addition, their administration may be closely linked to traditional transfusion therapy. For example, RhIG is administered in order to prevent Rh alloimmunization when Rh-positive Red Blood Cells (RBCs) are passively or actively transfused to Rh-negative recipients. Plasma derivatives are often indicated for complex hematologic disorders that blood bank physicians rather than pharmacists are better trained to monitor for appropriate use. For those reasons and because plasma derivatives carry some of the same risks as blood components, blood bank physicians should know the indications for the use of plasma derivatives and the risks associated with their use.

Preparation of Derivatives

Plasma derivatives are prepared by various methods, all of which include multiple steps of protein extraction and purification, followed by one or more virus inactivation steps. In the United States, plasma used for manufacturing plasma derivatives is most often procured from paid plasmapheresis donors (Source Plasma), but it can also be obtained from single-unit WB donations (Liquid Plasma or Recovered Plasma).

Factor VIII, the least stable of the factors purified into concentrates, is typically prepared from Source Plasma that is frozen shortly after collection. Recovered Plasma may be used to prepare the more stable derivatives, such as albumin and immunoglobulins. Plasma derivatives can be divided into two groups: those that are present in cryoprecipitate and those that are present in the cryosupernatant fluid.

Cryoprecipitation is the first step in preparing Factor VIII or von Willebrand factor. The Factor VIII/vWF:C complex preferentially precipitates in the cold, unlike most other plasma proteins. The remaining cryosupernatant fluid can be used for the fractionation of other plasma proteins, such as Factor IX complex (Factors II, VII, IX, and X), purified Factor IX, the immunoglobulins, antithrombin, and albumin.

Several techniques are used for the subsequent fractionation steps. The techniques differ, depending on the protein to be isolated and the individual manufacturer.

Fractionation techniques include differential precipitation with various chemical precipitating agents, as well as gel,



Types of Plasma Used to Make Derivatives

Source Plasma: Plasma from human blood collected by plasmapheresis and intended for further manufacturing use.

Recovered Plasma: Plasma from single-unit whole blood collections with no restriction on when it was separated from cells or its storage time or temperature.

Liquid Plasma: Plasma from a single donor that has been prepared from whole blood up to 5 days after its expiration and stored at 1–6 °C.

affinity, and immunoaffinity chromatography. Cold ethanol extraction, developed by Edwin Cohn and his coworkers during World War II, is one of the oldest techniques and is still used to manufacture many plasma derivatives. With that technique, thousands of liters of plasma may be pooled and subjected to a series of chemical extraction steps, during which particular plasma proteins sequentially precipitate.

Differential precipitation is based on the solubility of the targeted plasma protein in 1) ethanol concentration, 2) cold temperatures, 3) pH, 4) ionic strength, and 5) protein concentration. The plasma protein fractions resulting from cold ethanol fractionation are shown in Table 13-1.

After the final purification steps, the extracted protein preparations are further subjected to one or more virucidal, bactericidal, and fungicidal treatments intended to reduce the final product's content of HIV, HBV, HCV, and any bacteria or fungi. The goal is to produce a sterile product. However, some virus attenuation is already achieved through the extraction and purification processes. Furthermore, donors are screened at the time of plasma donation for various viral markers.

Virus inactivation treatment generally falls into the following categories: 1) "dry heat" for relatively long periods (72 hours to 6 days); 2) "wet heat," that is, pasteurization or vapor heating; 3) treatment with organic solvents and detergents; and 4) ultrafiltration to remove virions, bacteria, or contaminating particles.

Dry heat appears to be effective in inactivating HIV, whereas HBV and HCV are more resistant. The presence of moisture in the wet heat processes improves the efficiency



Virus inactivation of pooled plasma derivatives can be achieved in one of several ways:

- Dry heat
- Pasteurization (wet heat)
- Treatment with organic solvents and detergents
- Ultrafiltration

Table 13-1. Cohn Cold Ethanol Fractionation of Plasma

Fraction	Key Plasma Proteins Present
I	Fibrinogen; Factor VIII (unless already removed by prior cryoprecipitation)
II and III	Factors II, VII, IX, and X; immunoglobulins G, A, and M
IV	Antithrombin, immunoglobulin M, albumin
V	Albumin

of virus inactivation over inactivation by dry heat. The solvent/detergent treatment [eg, tri-N-butyl phosphate, or TNBP, plus a detergent such as Triton X-100 (Perkin/Elmer, Waltham, MA), Tween-80 (AppliChem, Cheshire, CT), polysorbate, or octoxynol] solubilizes and extracts lipids. As a result, that treatment can inactivate lipid-enveloped viruses such as HIV, HBV, and HCV. Other methods use an ultrafiltration step that is capable of removing virions on the basis of size rather than chemical properties. Often, however, combinations of virucidal treatments are used for improved efficacy.



Although plasma derivatives are subjected to virus inactivation steps, no technique is capable of removing all viral activity all of the time.

Viral Content

US-licensed albumin has not been reported to transmit HIV, HBV, or HCV in half a century of use. The physical separation of virus from albumin, achieved through the cold ethanol precipitation technique coupled with the pasteurization of the product at 60 C for 10 hours, has proven effective in removing infectious viruses. The reduction in viral level through the purification process is important with albumin because pasteurization alone is not capable of eliminating all HBV activity. It is also important that the heating step is applied to the final product, when viral levels have been reduced to their greatest extent, rather than during earlier steps. Thus, albumin safety appears to result from the combination of the physical separation of viruses and the wet heat treatment.

Other blood derivatives have only rarely transmitted HIV, HBV, and HCV since the advent of the virus inactivation techniques that followed the AIDS outbreak. Factor VIII and Factor IX products are not known to have transmitted HIV, HBV, or HCV since virucidal treatments were instituted in the late 1980s. Occasional viral transmission has been documented with other products. For example, plasma protein fraction (PPF) has transmitted HBV in a documented episode resulting from a design flaw in one manufacturing plant. In addition, there have been outbreaks of HCV transmission by intravenous immunoglobulin (IVIG) and RhIG.

Human albumin has also been implicated in the transmission of viruses, and many recombinant proteins used human albumin as a stabilizer. One report demonstrated the presence of TT virus in 23.5% of the first-generation recombinant Factor VIII and IX product lots and in 55.5% of the albumin lots tested as well as in 59% of the patients who received those products. However, the second-gener-

ation (human protein-free) concentrates were negative for TTV. Although infection with TTV in those patients did not appear to be clinically important, it did underscore the fact that no technique at this time is capable of completely removing all of the activity of all viruses.

HAV and parvovirus B19, for example, are more resistant to inactivation with the current techniques than are HIV, HBV, and HCV. Their continuing transmission by blood derivatives remains a concern. Although those two viruses do not usually have the same clinical effect as HIV, HBV, or HCV, their transmission by blood derivatives indicates that current techniques might not eliminate the infectivity of nonenveloped viruses of potentially greater virulence that may emerge in the future.

No evidence exists that human plasma derivatives have transmitted prions, the apparent infectious agents of Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD). It is now considered unlikely that CJD is transmitted by blood derivatives. The lack of evidence of CJD in patients with hemophilia who received large amounts of Factor VIII over a lifetime argues against such transmission.

Variant CJD, however, remains a more active concern, because it has only recently affected humans. Variant CJD is the human equivalent of bovine spongiform encephalopathy, which has been transmitted from cattle to humans in Great Britain probably since the 1980s. There is concern that a large pool of humans with undiagnosed, latent vCJD infections could transmit the disease through blood components or plasma derivatives made from their blood donations. Four instances of probable transfusion transmission of vCJD infection via labile blood products have been identified since 2006. To date, there is no evidence that vCJD has developed in a recipient of any plasma-derived protein products, including immunoglobulins and clotting factors. Despite the lack of known cases, the transmission of vCJD by plasma derivatives cannot be entirely excluded. However, the risk of prion transmission via plasma derivatives is thought to be much lower than transmission via labile blood products. The estimated relatively low levels of any initial plasma infectivity combined with the fractionation techniques likely further reduce prion activity. Moreover, because plasma from thousands of donors is pooled in the preparation of blood derivatives, the large dilution factor may greatly reduce or eliminate the infectivity of plasma from an occasional affected donor. Finally, infectivity appears to be more closely associated with leukocytes than with plasma, which makes transmission through



The risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) by plasma derivatives is not definitely known at this time.

Transmission of classic CJD appears unlikely.

plasma derivatives less likely. However, no conclusive evidence exists regarding vCJD transmission in plasma derivatives as of this writing.

Given that thousands of donors may contribute to the plasma pool from which one batch of factor or protein concentrate is made, the risk of contamination with either known or emerging viruses or other infectious agents that are resistant to current virucidal treatments is higher than for single-donor products or those made from smaller pools. However, smaller batches are inefficient as well as more time consuming and costly from a manufacturing perspective; in the end, they still do not eliminate all infectious risks. Until protein-processing techniques advance to the point where they can readily eliminate or inactivate all viruses and other infectious agents, the infectious potential of blood derivatives is likely to remain a concern.

Plasma Protein Fraction

Description

PPF is available commercially as Plasmanate (Talecris Biotherapeutics, Research Triangle Park, NC). Please refer to the *Physicians' Desk Reference* for specific details.

Preparation. PPF is a less-purified preparation of albumin that is prepared from large pools of human plasma and is produced using the technique of cold ethanol fractionation. Each vial is sterile and heat-treated at 60°C for 10 hours.

Each 100 mL of PPF 5% contains 5 g of selected plasma proteins, approximately 88% albumin, 12% alpha and beta globulins, and no more than 1% gamma globulin, as determined by electrophoresis. The blood group A and B antigens and their corresponding antibodies are at such a low level that routine blood typing procedures should not be affected. The concentration of the proteins and electrolytes is such that PPF is iso-oncotic and isotonic. The approximate electrolyte concentrations of PPF are sodium 145 mEq/L, potassium 0.25 mEq/L, and chloride 100 mEq/L. The solution is buffered with sodium carbonate and stabilized with sodium caprylate, sodium N-acetyltryptophan, or both.

Use. PPF, like albumin, is used in the treatment of shock resulting from burns, infections, surgery, trauma, and any other cause of an acute loss of plasma fluids. The recommended infusion rate of PPF is not to exceed 10 mL/min. PPF can be infused with WB or RBC units.



Plasma protein fraction (PPF) is a less purified preparation than albumin that can be used for the same indications as albumin. Hypocoagulability and hypotension have been reported with PPF.



Rapid infusion of PPF may be associated with hypotension, particularly when used perioperatively.

Risks. PPF, like albumin, should not be mixed with protein hydrolysates or solutions containing alcohol. There are reports that the rapid infusion of PPF has produced hypotension in patients undergoing surgery, as well as during the pre- and postoperative periods. Blood pressure should be monitored during use. If hypotension occurs, the infusion rate must be slowed; if acute hypotension occurs, it must be halted. The blood pressure will likely normalize spontaneously after those measures. If it does not, vasopressors may be used to correct the hypotension.

Hypotensive reactions have been attributed to the presence of prekallikrein activator, bradykinin, and sodium acetate in the PPF. Rapid infusions of PPF at rates >10 mL/min, in particular, have been associated with such reactions. There is a significant risk of hypotensive reaction with the intra-arterial administration of PPF to patients on cardiopulmonary bypass (CPB). PPF does not contain coagulation factors; if given in large amounts, it can cause a dilutional coagulopathy. Flushing, urticaria, back pain, nausea, and headache may occur.

The usual minimum effective dose in adults is 250 to 500 mL. Dosage will vary, however, depending on the individual case and the patient's response to therapy.

Albumin

Description

Albutein (Grifols, Los Angeles, CA), Flexbumin (Baxter Healthcare, Deerfield, IL), Human Albumin (ABO Pharmaceuticals, Mission Viejo, CA), and Plasbumin-20 (Talecris) are the albumin products available in the United States. Please refer to the *Physicians' Desk Reference* for specific details.

Albumin is a highly soluble globular protein, accounting for 70% to 80% of the colloid pressure of plasma. It serves as the primary transport protein and binds naturally occurring, therapeutic, and toxic materials in the circulation. Albumin is distributed throughout the extracellular water, and more than 60% of it is located in the extravascular fluid compartment. The total body albumin is 320 g in a 70-kg person. It has a circulating life span of 15 to 20 days, with a turnover of approximately 15 g/day.

Preparation. Albumin is prepared from pooled human venous plasma by using the Cohn cold ethanol fractionation method. Human physiologic pH of the solution is obtained with the addition of sodium bicarbonate, sodium

hydroxide, or both. Stabilizers, such as sodium caprylate or sodium *N*-acetyltryptophan, are added to the final product. The total sodium concentration in the various human albumin products ranges from 130 to 160 mEq/L. The solution is sterilized by bacterial filtration and is pasteurized at 60°C for 10 hours. The final protein product contains at least 96% albumin. It is available as an isotonic 5% solution and as hypertonic, or concentrated, 20% or 25% solutions. The percentage refers to the amount of albumin (weight/volume) in the solution. For example, albumin 5% contains 5 g of human albumin per 100 mL of solution.

Use. The 5% (5 g/dL) solution is osmotically equivalent to normal human plasma. This concentration is used to treat hypovolemic shock and as an adjunct in hemodialysis, plasmapheresis, and CPB procedures so it can maintain normal colloid osmotic pressure.

The 20% and 25% (20 and 25 g/dL) albumin solutions are hyperoncotic—the oncotic equivalent of approximately five times the normal concentration of albumin in human plasma. As a result, use of the hyperoncotic albumin solutions will expand the intravascular circulation by three to four times the volume of albumin administered to a patient. It acts quickly, redistributing fluid from the interstitial spaces into the vascular circulation within 15 minutes. The extra fluid reduces hemoconcentration and decreases blood viscosity. When hyperoncotic albumin solutions are used to prevent or treat hypovolemic shock, additional crystalloids must be given to the patient if he or she is dehydrated. Critically ill patients with hypovolemia, burns, or hypoproteinemia showed a 6% increased risk of death when albumin was used in their treatment, as compared with patients who did not receive albumin.

Albumin may be administered, often in conjunction with exchange transfusion, in an attempt to bind and detoxify unconjugated bilirubin during treatment of neonatal hyperbilirubinemia. Albumin may be useful in treating edema in patients with severe nephrosis who are receiving steroids or diuretics. It has also been used to induce diuresis in patients with acute, but not chronic, nephrosis. During renal dialysis, 20% to 25% albumin solution may be used for an acute volume or oncotic deficit, but the patient should be monitored closely for signs of circulatory overload. When the albumin deficit is the result of excessive protein loss, the effect of albumin administration will be temporary unless the underlying disorder is reversed. In most cases of excessive catabolism, or protein loss, increasing the patient's nutritional replacement of amino acids, protein, or



Albumin is used as a plasma volume expander and to maintain normal colloid osmotic pressure in shock, severe burns, and acute hypoproteinemia.



Albumin is generally not indicated for postoperative hypoproteinemia, paracentesis, acute liver failure, or the treatment of chronic nephrosis or chronic cirrhosis.

both and concurrently treating the underlying disorder will restore normal plasma albumin levels more effectively than albumin solutions.

Risks. Usually, the use of albumin is not indicated or justified for postoperative hypoproteinemia, paracentesis, or acute liver failure. Albumin is of no value in the treatment of chronic nephrosis or chronic cirrhosis. The use of albumin is contraindicated in patients with severe anemia or cardiac failure in the presence of normal or increased intravascular volume as well as in patients with a history of allergic reactions to albumin. There is a risk of potentially fatal hemolysis and acute renal failure from the inappropriate use of sterile water as a diluent for 20% or 25% albumin. Acceptable diluents include 0.9% sodium chloride or 5% dextrose in water. Table 13-2 compares the two main sources of albumin (PPF and albumin) as to their purification processes, the percentage of albumin present, the rate at which they can safely be infused, and the other proteins present in the final products.

Factor VIII Concentrates

Description

Factor VIII concentrates are indicated for the treatment or prevention of hemorrhage in patients with hemophilia A or acquired Factor VIII deficiency. Hemophilia A is a sex-linked recessive trait that causes a variable deficiency of Factor VIII coagulant activity. Characteristic laboratory

Table 13-2. Albumin Sources

Product	Treatment	Albumin Content (%)	Infusion Rate	Comment
Plasma protein fraction (PPF)	Pooled plasma, cold ethanol fractionation, pasteurized	>88%	<10 mL/min, intravenous only	Contains up to 12% alpha and beta globulins and up to 1% gamma globulins
Albumin	Pooled plasma, Cohn cold ethanol fractionation, filtered, pasteurized	>96%	No restriction, intravenous only	More highly purified than PPF; contains up to 4% alpha and beta globulins

findings include a prolonged activated partial thromboplastin time (aPTT), normal prothrombin time (PT), and decreased Factor VIII activity. The severity of the disease for an individual patient depends on the level of Factor VIII activity. Patients with severe disease typically have Factor VIII activity levels below 1%, whereas those with mild disease have Factor VIII activity levels ranging from 6% to 30%. Specific Factor VIII replacement recommendations for different clinical settings are shown in Table 13-3.

Table 13-3. Guidelines for Factor VIII Replacement Therapy

Hemorrhagic Event	Loading Dose IU Factor VIII:C/kg	Desired Peak Level (% of normal)	Frequency of Administration
Mild – Mild superficial or early hemorrhages – Early joint or muscle bleeds – Severe epistaxis	15 IU Factor VIII:C/ kg of body weight	Approximately 30% of normal	Give one dose. If one dose is not sufficient, give half the loading dose once or twice daily for 1 to 2 days.
Moderate – Advanced joint or muscle bleeds – Mouth, pharyngeal, or neck hematoma (without airway compromise) – Tooth extraction – Severe abdominal pain	25 IU Factor VIII:C/ kg of body weight	Approximately 50% of normal	Follow by 15 IU Factor VIII:C/kg every 8 to 12 hours for first 1 to 2 days to maintain Factor VIII:C plasma level at least 30% of normal. Then give same dose once or twice a day for up to 7 days, or until adequate wound healing.
Life-threatening – Major operations – Gastrointestinal bleeding – Neck, tongue, or pharyngeal hematoma with potential airway compromise – Intracranial, intrathoracic, or intra-abdominal bleeding – Fractures	40-50 IU Factor VIII:C/kg of body weight	80-100%	Follow by 20 to 25 IU Factor VIII:C/kg every 8 hours to maintain plasma levels at 80% to 100% of normal for 7 days. Then continue the same dose once or twice a day for another 7 days to maintain the Factor VIII:C level at 30% to 50% of normal.

Preparation. Products containing Factor VIII are available in varying degrees of purity. The plasma-derived Factor VIII products considered to be of higher purity are produced using monoclonal antibody-based affinity chromatography, and the Factor VIII products derived from synthetic, or recombinant, technology are considered to be of the highest purity. Because albumin is often added after the purification steps as a stabilizing agent, the specific activity of the final product is not necessarily indicative of the actual purity obtained by the extraction technique used for any given product.



The desired level of Factor VIII activity depends on clinical factors such as the following:

- Use in prophylaxis
- Bleeding site
- Severity of bleeding

Dosage. Monitoring the plasma Factor VIII activity is recommended as a means of precisely monitoring the replacement therapy. Doses should be titrated according to the patient's clinical response. Dosage calculations for coagulation factors are determined in part by their percent recovery in the intravascular space after intravenous administration. In the case of Factor VIII, the intravascular recovery is about 80%. For dosage calculations, the assumption of 100% recovery is often made.

The level of Factor VIII activity is defined by the World Health Organization (WHO) as follows: 1 international unit (IU) of AHF equals the Factor VIII activity found in 1 mL of fresh pooled human plasma; that is, the concentration of Factor VIII in pooled normal plasma is 1 IU per mL. This concentration of Factor VIII is also defined as 100% of normal. The expected in-vivo, peak AHF level, expressed as IU/dL of plasma or percentage of normal, can be calculated by multiplying the dose administered per kilogram of body weight (IU/kg) by two. For example, a dose of 1400 IU AHF administered to a 70-kg patient, that is, 20 IU/kg ($1400/70$), should be expected to cause a peak postinfusion AHF increase of $20 \times 2 = 40$ IU/dL, or 40% of normal.

If a 70-kg patient with severe hemophilia has an initial Factor VIII level of 2% and the desired activity is 50%, the dosage can be calculated as follows:

$$\begin{aligned} \text{Total dose to be administered in} \\ \text{IU} &= 70 \text{ kg} \times [(50\% - 2\%)/2] = 1680. \end{aligned}$$



1 IU of a coagulation factor is defined as the amount of factor activity in 1 mL of normal plasma.

A more precise calculation of the dose to be administered can be calculated by the following formula:

$$\begin{aligned} \text{Total dose} &= (\text{Patient mass} \times 70 \text{ mL/kg}) \times (1 - \text{Hct}) \times \\ &\quad (\text{desired activity} - \text{current activity}) \end{aligned}$$

The manufacturer's recommended dosage calculation as described in the product insert is somewhat different be-

cause it is based on the assumption that the plasma volume is a fixed 50 mL/kg. That higher estimate of plasma volume takes into account larger relative plasma volumes in the pediatric population; it avoids underdosing for patients with lower hematocrits.

Both methods are estimates based on population averages. Because Factor VIII intravascular recovery and half-life may vary from patient to patient, it is important to measure Factor VIII levels in order to verify that the desired hemostatic level is being attained. Because the intravascular half-life of Factor VIII is about 12 hours, dosage intervals are repeated at 8- to 12-hour intervals. In that way, doses can be targeted to produce intravascular levels twice that desired at the trough.



It is important to measure levels because the Factor VIII intravascular recovery and half-life will vary between patients, and even in the same patient depending on the clinical condition or if he or she is developing a Factor VIII antibody.

Low-Purity Factor VIII

Description

Cryoprecipitated AHF (cryo) is categorized as a blood component rather than as a blood or plasma product derivative because it is produced from single-unit WB donations. The production process is not complex, and additional purification processes are not used.

Use. Cryo is the only product available with concentrated fibrinogen. Its primary clinical use is the intravenous supplementation of fibrinogen. Cryo contains Factor VIII:C, vWF, and fibrinogen. Each unit of cryo prepared from a single blood donation should contain a minimum of 80 IU of Factor VIII and 150 mg of fibrinogen. Cryo also contains vWF, fibronectin, and other plasma proteins. For that reason, cryo was traditionally used as a source of Factor VIII:C for patients with hemophilia until the commercial Factor VIII products became available and were safer to use. The therapy of choice for severe hemophilia A is one of the recombinant Factor VIII products. Cryo is now used only as a last resort for that purpose. Although it is less appropriate now that commercial Factor VIII concentrates containing vWF are available, cryo was used as a source of vWF in patients with severe vWF deficiencies. Cryo has also been used topically as a fibrin sealant, although commercial preparations are now available. Cryo may be used to ameliorate the platelet dysfunction associated with uremia.



The correct term is Cryoprecipitated AHF. However, in practice many workers use the shorthand term "cryo."

Intermediate-Purity Factor VIII

Description

Humate-P (CSL Behring, King of Prussia, PA), Koäte -DVI (Talecris), Alphanate (Grifols), and Hemofil M (Baxter Healthcare) are some of the nonrecombinant Factor VIII concentrates available in the United States. Please refer to the *Physicians' Desk Reference* for specific details.

Concentrates in this category are prepared from the cold insoluble fraction (cryoprecipitate) of pooled human plasma either as Source Plasma (Alphanate and Hemofil M) or as Fresh Frozen Plasma (FFP) (Humate-P and Koäte -DVI). The different manufacturers use various methods to achieve a sterile, stable, purified, and dried or lyophilized concentrate of human AHF. The final products are vials labeled with the amount of Factor VIII activity as defined by the WHO (1 IU equals 1 mL of fresh pooled human plasma), with further differences between the products being the presence and activity level of activated vWF and protein C.

Factor VIII concentrates may be further purified with gel chromatography, affinity chromatography, or monoclonal antibody-based affinity chromatography. The monoclonal antibodies used in affinity chromatography can have specificity either for Factor VIII or for vWF because the two factors are bound to each other as a complex.

The monoclonal-based affinity chromatography technique yields the most highly purified of the nonrecombinant products.

Humate-P

Preparation. Humate-P is a stable, purified, sterile, lyophilized concentrate of AHF and vWF made from the cryoprecipitation of pooled FFP. Humate-P is heat-treated in an aqueous solution at 60°C for 10 hours. The result is a product that contains a low amount of nonfactor proteins; the fibrinogen amount is ≤ 0.2 mg/mL. The albumin level is 8 to 16 mg per vial. Each vial contains the labeled amount of Factor VIII activity and von Willebrand factor:Ristocetin cofactor (vWF:RCOF) activity expressed in IU. On reconstitution, each mL of Humate-P contains 40 to 80 IU Factor VIII activity and 72 to 224 IU vWF:RCOF activity.

Use. Because Humate-P contains the high-molecular-weight multimers of vWF, it is approved for use in treating patients with von Willebrand disease (vWD) or Factor VIII deficiencies. However, controlled clinical trials have not been conducted to evaluate the safety and efficacy of pro-



Most of the intermediate-purity Factor VIII products contain undetermined levels of blood group isoagglutinins that can cause a progressive anemia and/or a risk of hemolysis when given in large volumes or frequently repeated doses to patients with group A, B, or AB blood types.



Humate-P was originally developed to act as a concentrated source of Factor VIII, but it also contains significant amounts of von Willebrand factor. It is indicated for the treatment of hemophilia A or von Willebrand disease.

phylactic dosing to prevent spontaneous or excessive bleeding in patients with vWD.

Risks. There are no known contraindications for the use of Humate-P. However, because Humate-P contains blood group isoagglutinins, there is a risk of hemolysis when it is used with certain blood group antigens, as previously discussed. Thromboembolic events have been reported in patients who have vWD and who are receiving coagulation factor replacement therapy in conjunction with known risk factors for thrombosis. In those cases, caution should be exercised. Rare cases of temperature rises and allergic reactions have been reported, including urticaria, rash, pruritus, chest tightness, and edema.

Koāte-DVI

Preparation. Koāte-DVI is purified from pooled FFP according to the methods of modification and refinement first described by Hershgold, Pool, and Pappenhaben. The concentrated human AHF is treated with TNBP and polysorbate 80, then purified by a gel permeation chromatography step, which serves the dual purposes of reducing the amount of TNBP and polysorbate 80 and of increasing the purity of the Factor VIII. The product is heated in lyophilized form in the final container at 80°C for 72 hours. When reconstituted as directed, the final product contains approximately 50 to 150 times as much factor as an equal volume of fresh plasma. The specific activity, after the addition of human albumin, is in the range of 9 to 22 IU/mg protein.

Use. Although Koāte-DVI contains naturally occurring vWF, which is co-purified as a part of the manufacturing process, the drug has not been investigated for efficacy in the treatment of vWF deficiency. It is indicated only for the treatment of classic hemophilia A.

Risks. There are no known contraindications to the use of Koāte-DVI. However, the drug contains levels of blood group isoagglutinins that may cause progressive anemia in patients of blood groups A, B, or AB when large or frequently repeated doses are required.



Koāte-DVI may also contain vWF, but it has not been investigated for efficacy in the treatment of vWD. It is indicated only for treatment of hemophilia A.

Alphanate

Preparation. Alphanate is prepared from pooled human plasma by cryoprecipitation and fractional solubilization, with further purification using a cross-linked agarose heparin-binding affinity column that binds the Factor VIII:C/vWF

complex. The product is treated with a solvent (TNBP) and a detergent (polysorbate 80). It is heated at 80 C for 72 hours, then filtered. Alphanate contains human albumin as a stabilizer. When reconstituted, Alphanate results in a concentrate with a specific activity of at least 5 IU Factor VIII:C/mg total protein.

Use. Although Alphanate contains naturally occurring vWF, which is co-purified as a part of the manufacturing process, the drug has not been investigated for efficacy in the treatment of vWF deficiency. It is indicated only for the treatment of classic hemophilia A or acquired Factor VIII deficiency.

Risks. No contraindications to the use of Alphanate are known. Adverse drug reactions include urticaria, fever, chills, nausea, vomiting, headache, somnolence, or lethargy. If mild allergic reactions, chills, nausea, or stinging at the infusion site occur, a product from a different lot should be administered when additional AHF is necessary.

Alphanate contains blood group isoagglutinins that can cause progressive anemia. In cases where massive doses were given to patients of blood groups A, B, or AB, acute hemolytic anemia, an increased bleeding tendency, or hypofibrinogenemia has occurred. When large or frequent doses are required, the patient should be monitored for signs of intravascular hemolysis and decreasing hematocrit.

Hemofil M

Preparation. Hemofil M is prepared by the method M process from pooled human plasma by using immunoaffinity chromatography with a murine monoclonal antibody to Factor VIII:C; that process is followed by an ion exchange chromatography step. Preparation of Hemofil M involves the use of the organic solvent TNBP and a detergent (octoxynol 9). The final product is lyophilized. The result is a concentrated form of AHF with a specific activity range of 2 to 20 AHF IU/mg of total protein. A maximum of 12.5 mg/mL of human albumin and not more than 0.1 ng of mouse protein is present in each vial.

Use. Hemofil M is indicated for the prevention and control of hemorrhagic episodes in patients with hemophilia A and in those patients with Factor VIII inhibitors not exceeding 10 Bethesda units (BU) per mL. It is not indicated for the treatment of vWD.

Risks. Contraindications include a known hypersensitivity to mouse protein and natural rubber latex. Additional rare allergic reactions to albumin have also been reported,



The Bethesda unit (BU) is a measure of the activity of Factor VIII inhibitors. One BU is the reciprocal of the dilution of an inhibitor patient's plasma that neutralizes 50% of the Factor VIII in normal plasma. Thus, 2 BU means that a 1:2 dilution of patient plasma will neutralize 50% of normal Factor VIII activity.

including nausea, fever, chills, or urticaria. The processing of Hemofil M significantly reduces the presence of blood-group-specific antibodies in the final product, thereby mitigating the risk of hemolysis.

High-Purity Factor VIII, Recombinant

Description

Kogenate FS (Bayer HealthCare Pharmaceuticals, Tarrytown, NY), Recombinate (Baxter), and ReFacto (Wyeth, Madison, NJ) are some of the recombinant AHF products available in the United States. Please refer to the *Physicians' Desk Reference* for specific details.

This group of factors has been manufactured using recombinant DNA technology; their only intended use is to treat classic hemophilia A. The human Factor VIII gene is introduced into either baby hamster kidney (BHK) cells or a genetically engineered Chinese hamster ovary (CHO) cell line. Those cells secrete recombinant AHF into the cell culture medium, which contains human plasma protein solution (HPPS) and recombinant insulin. The purification processes for these products may include a solvent/ detergent virus inactivation step, ion exchange chromatography, monoclonal antibody immunoaffinity chromatography, and other chromatographic steps to further purify the recombinant Factor VIII and to remove contaminating substances. Some products have undergone additional measures to reduce the risk of TSE. The final product is often lyophilized. Each vial contains the labeled amount of recombinant Factor VIII activity in IU.

Contraindications may include a known intolerance or allergic reactions to constituents of the product, including albumin or the mouse or hamster proteins. Symptoms may include the development of hives, localized or general urticaria, wheezing, and hypotension during use of the product. Circulating neutralizing antibodies to the Factor VIII antigen may develop during treatment. As a precaution, patients should be monitored for the development of antibodies. When an antibody is present, the dosage requirement for the patient will be variable, and it can be determined only by monitoring the patient's clinical response and the circulating Factor VIII levels after treatment.

Kogenate FS

Preparation. Kogenate FS is produced by growing BHK cells in a culture medium containing HPPS and recombi-



Recombinant Factor VIII products do not contain any other coagulation factors; their only use is to treat classic hemophilia A.



Recombinant Factor VIII preparations currently available in the United States include Kogenate FS, Recombinate, and ReFacto.



Kogenate FS is stabilized with glycine, sucrose, and histidine in place of the usual albumin. Thus, it is acceptable to strict Jehovah's Witnesses who find derivatives containing human albumin unacceptable.



Kogenate FS does not contain vWF.

nant insulin. The revised purification and formulation processes eliminate the need to add human albumin. The purification processes include a solvent/detergent virus inactivation, ion exchange chromatography, monoclonal antibody immunoaffinity chromatography, and other chromatographic steps designed to further purify by removing any remaining contaminating substances. Reducing the risk of TSE involves Fraction II and III separation steps for HPPS and an anion exchange chromatography step. Studies conducted by the manufacturer provide reasonable assurance that the agents of CJD/vCJD infectivity, if present in low levels in the starting material, would be removed during processing. The stabilizers used are glycine, sucrose, and histidine, in place of the traditional albumin. The final product is lyophilized. Kogenate FS with BIO-SET is a needle-less, self-contained system with a prefilled syringe containing diluent for reconstitution.

Use. Kogenate FS is indicated only for the treatment of bleeding disorders arising from a demonstrated deficiency of Factor VIII activity. Kogenate FS does not contain vWF or any other clotting factors, and it cannot be used to treat disorders arising from deficiencies in those factors.

Risks. The use of Kogenate FS is contraindicated when the patient has a known intolerance of, or allergic reactions to, constituents of the preparation, such as known hypersensitivity to mouse or hamster proteins. Circulating neutralizing antibodies to Factor VIII may develop, and the patient should be monitored for that possibility. Also, antibodies to mouse or hamster proteins may develop. Serious anaphylactic reactions requiring emergency care have been reported.

Recombinate

Preparation. Recombinate is produced by cells of the genetically engineered CHO cell line. The recombinant AHF is purified by a series of chromatography columns, including the use of a monoclonal antibody directed against Factor VIII. Human albumin is added as a stabilizing agent. The detergent polysorbate 80 is used to treat the product, which is lyophilized.

Use. Recombinate is indicated only for the treatment of bleeding disorders arising from a demonstrated deficiency of Factor VIII activity. Although vWF is coexpressed by the CHO cells and helps stabilize the product, it is not produced in amounts that would have any clinical relevance for patients with vWF deficiency; furthermore, it is not indicated for the treatment of vWD.

Risks. Contraindications to the use of Recombinate include known hypersensitivities to human albumin; mouse, hamster, or bovine proteins; and natural rubber latex.

ReFacto

Preparation. ReFacto is produced by the cells of a genetically engineered CHO cell line; those cells secrete B-domain-deleted recombinant Factor VIII. The culture medium contains human albumin and insulin. The purification process includes chromatography, addition of the detergent polysorbate 80 as well as sucrose and L-histidine for stabilization; sterilization; and finally lyophilization.

Use. ReFacto is indicated for the control and prevention of hemorrhagic episodes and for surgical prophylaxis in patients with a demonstrated deficiency of Factor VIII. Although ReFacto is indicated for short-term prophylaxis to reduce the frequency of spontaneous bleeding episodes, the effect of regular routine prophylaxis on long-term morbidity and mortality is unknown. ReFacto is not indicated for the treatment of vWD.

Risks. Contraindications to the use of ReFacto are known hypersensitivities to human albumin, mouse or hamster proteins, or other materials in the product. There have been reports of less-than-expected results or lack of effect after the infusion of ReFacto, mainly among patients receiving prophylactic treatment. Such effects have occurred in patients with inhibitors, as well as in patients with no evidence of inhibitors. Monitoring the plasma Factor VIII:C activity is important to ensure an adequate therapeutic response, particularly in the setting of surgical prophylaxis and major bleeds.



ReFacto, a recombinant Factor VIII preparation, is *not* indicated for the treatment of vWD.

von Willebrand Factor Concentrate

Description

Humate-P (CSL Behring) is the only commercially available, plasma-derived product licensed for use in the United States for treatment of patients with vWD. Please refer to the *Physicians' Desk Reference* for specific details.

vWF is a protein that circulates in the plasma as a family of multimeric molecules with a wide range of molecular weights. vWF proteins with the highest molecular weights are the most hemostatically effective. They mediate platelet adhesion to damaged endothelial surfaces and also transport Factor VIII. As a result, a deficiency or an abnormality of the vWF protein(s) results in varying de-



Humate-P, a Factor VIII concentrate prepared from pooled human plasma, contains significant amounts of high molecular weight vWF in addition to Factor VIII and is licensed by the FDA for treatment of vWD.

grees of abnormal platelet plug formation and partial deficiency of Factor VIII. The laboratory values are a prolonged bleeding time and aPTT. Additional laboratory evaluation demonstrates a specific deficiency in the level of vWF, often measured as ristocetin cofactor activity because vWF is required for the platelet agglutination by ristocetin *in vitro*.

The vWF syndromes are the most common of the major inherited coagulation abnormalities. They are usually autosomal dominant and manifest as a spectrum of qualitative and quantitative abnormalities of vWF. Because mild cases of vWF syndrome have low to moderate levels of endogenous vWF, they can often be treated by desmopressin (DDAVP), which releases the endogenous stores of Factor VIII and vWF. If DDAVP is not an option, and in the absence of a suitably therapeutic, virus-inactivated concentrate, vWF syndrome can be treated with cryo.

Use. One commercial Factor VIII concentrate, Humate-P, is licensed by the Food and Drug Administration (FDA) for use in vWD because of its content of biologically active, high-molecular-weight vWF multimers. Humate-P is indicated for use in adult and pediatric patients with vWD for the treatment of spontaneous or trauma-related bleeding, and it is usually efficacious for patients with all types of vWD. However, DDAVP—not Humate-P—should be considered for the initial treatment for those types of vWD that are responsive to DDAVP—usually Type I, sometimes Type IIa, and rarely Type III. Desmopressin is contraindicated in Type IIb.

Dosage. The appropriate dose of vWF varies with the vWD type and clinical setting. The product insert should be consulted for specific recommendations. Typical doses are 40 to 80 IU of vWF:RCOF per kilogram of body weight, administered every 8 to 12 hours. Because Humate-P is also labeled in terms of Factor VIII IU, it is important not to confuse ristocetin cofactor activity IU with Factor VIII IU. Please note that 40 to 80 IU vWF:RCOF corresponds to 16 to 32 IU of Factor VIII.

The half-life of administered vWF is about 10 hours. See Table 13-4 for a summary of treatment options for vWD.

Anti-Inhibitor Coagulant Complex

Description

FEIBA VH (Baxter Healthcare) is the only anti-inhibitor coagulant complex (AICC) product available in the United

Table 13-4. Guidelines for the Treatment of von Willebrand Disease

vWD Classification	Hemorrhagic Event	Loading Dose	Frequency of Administration
Type 1 Mild (Baseline vWF:RCoF activity >30%)	Major Severe or refractory epistaxis, GI bleeding, CNS trauma, traumatic hemorrhage	40 to 60 IU/kg of body weight	Give 40 to 50 IU/kg every 8 to 12 hours for 3 days to maintain nadir level of vWF:RCoF >50%. Then give same dose once daily for up to 7 days.
	Minor Epistaxis, oral bleeding, menorrhagia	40 to 50 IU/kg of body weight	Give one or two doses (should be sufficient).
Moderate or Severe (Baseline vWF:RCoF activity <30%)	Major Severe or refractory epistaxis, GI bleeding, CNS trauma, hemarthrosis, traumatic hemorrhage	50 to 75 IU/kg of body weight	Give 40 to 60 IU/kg every 8 to 12 hours for 3 days to maintain nadir level of vWF:RCoF >50%. Then give the same dose once daily for up to 7 days of treatment. Monitor and maintain Factor VIII:C levels according to the guidelines for hemophilia A therapy (refer to Table 13-3).
	Minor (Clinical indications as above)	40 to 50 IU/kg of body weight	Give one or two doses (should be sufficient).
Type 2 (all variants) and Type 3	Major (Clinical indications as above)	60 to 80 IU/kg of body weight	Give 40 to 60 IU/kg every 8 to 12 hours for 3 days to maintain nadir level of vWF:RCoF >50%. Then give same dose once daily for up to 7 days of treatment. Monitor and maintain Factor VIII:C levels according to the guidelines for hemophilia A therapy (refer to Table 13-3).

Note: 40 to 80 IU of von Willebrand factor ristocetin cofactor corresponds to 17 to 33 IU of Factor VIII in Humate-P.
GI = gastrointestinal; CNS = central nervous system; vWF:RCoF = von Willebrand factor ristocetin cofactor activity.



Anti-Inhibitor Coagulant Complex (AICC) is an activated Factor IX complex that can be used to bypass the intrinsic coagulation pathway but must be used *only* to treat patients with Factor VIII or IX inhibitors.

States; please refer to the *Physicians' Desk Reference* for specific details.

FEIBA VH, an anti-inhibitor coagulant complex, is derived from Source Plasma, PPF, or both with Factor VIII inhibitor-bypassing activity. Processing of the product includes vapor heat and excess pressure for 10 hours at 60 C, followed by increased excess pressure and vapor heat at 80 C for 1 hour. It contains mainly nonactivated Factors II, IX, and X, as well as Factor VII, primarily in the activated form. AICC has the ability to bypass the intrinsic coagulation pathway, which depends on Factors VIII and IX. That ability results from the presence of already activated Factor VIIa of the extrinsic pathway or of already activated Factor Xa, which is present at the intersection point of the intrinsic and extrinsic pathways of the coagulation cascade.

The product contains approximately equal unitages of Factor VIII inhibitor-bypassing activity and prothrombin complex factors. There are between 1 and 6 units of Factor VIII coagulant antigen, but only traces of factors of the kinin-generating system. In vitro, FEIBA shortens the aPTT of plasma-containing Factor VIII inhibitor. That activity is expressed in arbitrary units called immunizing units. One immunizing unit of activity is defined as the amount of FEIBA VH (or AICC) that shortens the aPTT of high-titer Factor VIII inhibitor reference plasma to 50% of the blank value.

Use. FEIBA VH is indicated for the control of spontaneous bleeding or bleeding during surgical interventions in hemophilia A and B patients with inhibitors. It has been used effectively in patients with acquired inhibitors to Factors VIII, XI, and XII, and in a patient with vWD with an inhibitor. FEIBA VH must be used only in patients with circulating inhibitors to one or more coagulation factors. An inadequate response to treatment with FEIBA VH can result from an abnormal platelet count or impaired platelet function.

Risks. The use of FEIBA VH is contraindicated in patients who are known to have a normal coagulation mechanism, and the product should not be used to treat bleeding episodes resulting from coagulation factor deficiencies. It should not be given to patients with significant signs of disseminated intravascular coagulation (DIC) or fibrinolysis. Anamnestic responses to the presence of Factor VIII in FEIBA VH, with a corresponding rise in the antibody titers, have been observed in 20% of the patients with preexisting Factor VIII inhibitors.

Treatment of Inhibitors to Factor VIII and Factor IX. Patients with inhibitor activity less than 5 BU may be treated with factor concentrates instead of AICC because, at that level of inhibitor, the infusion of higher-than-usual amounts of factor concentrate may neutralize the inhibitor. For levels of inhibitor activity between 5 and 10 BU, factor concentrate or AICC may be administered.

However, for patients with histories of inhibitor titers >10 BU (that is, for "high responders"), factor administration is likely to evoke an anamnestic response. The rise in inhibitor level in such patients will likely render further factor therapy ineffective. If the inhibitor is already present at a level >10 BU, AICC should be used, not factor concentrate. (See Table 13-5.)

Alternative Treatments. Three alternative treatments to AICC exist for patients with neutralizing Factor VIII or IX antibodies: recombinant Factor VIIa, porcine Factor VIII, and Factor IX complex concentrates. Recombinant Factor VIIa is used for patients with either Factor VIII or IX inhibitors. Porcine Factor VIII may be useful for patients with Factor VIII inhibitors as long as it does not cross-react with the patient's antihuman Factor VIII antibody or the patient develops an antibody to the porcine factor.



Alternatives to AICC that can be used for patients with Factor VIII and Factor IX inhibitors include:

Recombinant Factor VIIa
Porcine Factor VIII
Factor IX complex concentrates

Table 13-5. Guidelines for Therapy for Factor VIII or IX Inhibitors

Patient's Inhibitor Titer	Clinical Situation		
	Minor Bleeding	Major Bleeding	Surgery (Emergency)
<5 BU	AHF	AHF	AHF
5 to 10 BU	AHF, AICC, porcine Factor VIII, Factor VIIa	AHF, AICC, porcine Factor VIII, Factor VIIa	AHF, AICC, porcine Factor VIII, Factor VIIa
>10 BU	AICC, porcine Factor VIII, Factor VIIa (not AHF)	AICC, porcine Factor VIII, Factor VIIa (not AHF)	AICC, porcine Factor VIII, Factor VIIa (not AHF)

Note: An inadequate response to treatment may result from an abnormal count and/or impaired platelets.
BU = Bethesda unit; AHF = antihemophilic factor; AICC = anti-inhibitor coagulant complex.

Factor IX Concentrates

Description



AlphaNine SD, Profilnine SD, and BeneFIX all contain Factor IX and are indicated for the treatment of the hemophilia B (Factor IX deficiency).

The Factor IX-containing products available in the United States include AlphaNine SD (Grifols), Profilnine SD (Grifols), and BeneFIX (Wyeth). Please refer to the *Physicians' Desk Reference* for specific details.

Factor IX deficiency (hemophilia B, Christmas disease) is clinically indistinguishable from Factor VIII deficiency. Both are sex-linked disorders with similar laboratory findings, as both cause a prolonged aPTT in the presence of a normal PT and bleeding time. The diagnosis and treatment use the specific monitoring of Factor IX activity.

The formula for calculating the dosage of Factor IX is similar to that for Factor VIII, as the WHO International Standard for the Factor IX IU is the amount of Factor IX activity present in 1 mL of pooled, normal human plasma. The primary difference is that only half of the infused Factor IX dose is recovered in the intravascular space. Therefore, the number of units to be given needs to be doubled. The biologic half-life is 18 to 24 hours, so doses are given one or two times per day; the dose and treatment schedules will vary, however, depending on the severity and type of bleeding and the patient's response to therapy.

Various intermediate- and high-purity Factor IX concentrates are commercially available. The intermediate-purity Factor IX preparations are known as Factor IX complex concentrates. They are also referred to as prothrombin complex concentrates. Those preparations are "complexes" of vitamin-K-dependent clotting factors and, therefore, also contain varying amounts of Factors II, VII, and X, depending on the product.

Preparation. The Factor-IX-containing products derived from human plasma are first separated by fractionation, then purified with diethylaminoethanol (DEAE)-cellulose adsorption. They are then solvent/detergent-treated, and they may be virus-filtered. Despite the presence of the other coagulation factors, the content of Factor IX complex concentrates is standardized and labeled according to Factor IX activity only. Therefore, the content of Factors II, VII, and X in each lot is variable and unregulated. The Factor IX potency is expressed in IU.

Use. The various Factor IX and Factor IX complex preparations are to be used for Factor IX replacement therapy in hemophilia B only. However, Factor IX complex concentrates have also been used for other indications because of the presence of other vitamin-K-dependent coagulation

factors. However, because those products contain nontherapeutic levels of Factors II, VII, and X, they are *not* indicated for the treatment of Factor II, VII, or X deficiencies, nor can they be used for the reversal of warfarin anticoagulant-induced hemorrhage. In addition, they are not indicated for the treatment of hemophilia A in patients with inhibitors to Factor VIII.

Dosage. The dosage for Factor IX replacement depends on the clinical setting, as shown in Table 13-6. Factor IX has a larger volume of distribution than Factor VIII has because it is distributed roughly equally in the intravascular and extravascular spaces. As a result, only about 50% of the administered dose of Factor IX is recovered in the intravascular space, compared with about 80% for Factor VIII. Therefore, to achieve an equivalent percentage, dosage calculations must provide about twice as much Factor IX as Factor VIII to increase the level of circulating factor. As with Factor VIII and all other coagulation factors, the level of Factor IX in pooled normal plasma is by definition 1 IU/mL. In adults, 1 IU of Factor IX increased the circulating



Only about half of the administered dose of Factor IX is recovered in the vascular spaces, compared with 80% of Factor VIII. Thus, twice the amount of Factor IX is required to achieve an equivalent percentage increase.

Table 13-6. Guidelines for Factor IX Replacement Therapy

Hemorrhagic Event	Desired Circulating Level of Factor IX (% of normal)	Frequency of Administration	Duration of Therapy
Minor			
– Uncomplicated hemarthroses – Soft tissue and superficial muscle bleeding	20% to 30%	Every 12 to 24 hours	1 to 2 days
Moderate			
– Intramuscular bleeding – Soft tissue bleeding with dissection – Mucous membrane – Dental extractions – Hematuria	25% to 50%	Every 12 to 24 hours	Until bleeding stops and healing begins (about 2 to 7 days)
Major			
– Surgery – CNS, pharynx, retropharynx, or retroperitoneal bleeding	50% to 100%	Every 12 to 24 hours	About 7 to 10 days

CNS = central nervous system

activity of Factor IX by 0.4 to 1.4, depending on the lot and the product. The following formula is a guide because the amount required to achieve hemostasis will vary with each patient and will depend on the circumstances in which it is used. Therefore, the Factor IX level of each patient should be monitored frequently during replacement therapy.

$$\text{Amount of Factor IX} = \frac{1 \text{ IU/kg}}{\text{desired increase in Factor IX (\%)}} \times \text{body weight (kg)}.$$

Intermediate-Purity Factor IX

AlphaNine SD

Preparation. AlphaNine SD is a purified formulation of Factor IX derived from pooled human plasma. The processing includes DEAE chromatography and solvent/detergent treatment, followed by dual affinity chromatography; the final purification step consists of nanofiltration and lyophilization. Each vial contains a minimum of 150 IU Factor IX/mg protein.

Use. AlphaNine SD is indicated only for the prevention and control of bleeding in patients with a demonstrated Factor IX deficiency. Even though the product contains low nontherapeutic levels of Factors II, VII, and X, it is not indicated for the treatment of Factor II, VII, or X deficiencies.

Risks. No contraindications to the use of this product are known. Warnings are based on reports of incidences of thrombosis or DIC after the administration of Factor IX complexes that contained high levels of Factors II, VII, and X. Patients having surgery and patients with liver disease in particular should be monitored closely for signs or symptoms of DIC. Allergic-type hypersensitivity reactions, including anaphylaxis, have been reported in patients receiving Factor IX products. In previously untreated patients, it is possible that anaphylaxis may occur after a median exposure of 11 days. Nephrotic syndrome has been reported after immune tolerance induction with all Factor IX products in hemophilia B patients who have Factor IX inhibitors and a history of allergic reactions to Factor IX.

Profilnine SD

Preparation. Profilnine SD is a nonactivated Factor IX complex of vitamin-K-dependent clotting factors derived from pooled human plasma and purified by DEAE cellulose adsorption. The product is treated with an organic solvent (TNBP) and the nonionic detergent (polysorbate 80),



Intermediate-purity Factor IX concentrates (prothrombin complex concentrates) contain variable amounts of Factors II, VII, and X but are only standardized with respect to their content of Factor IX.

sterilized, and lyophilized. In addition to Factor IX (labeled as IU), the complex contains Factor II (prothrombin), Factor X (Stuart-Prower factor), and low levels of Factor VII (proconvertin).

Use. Profilnine SD is indicated for the prevention and control of bleeding in patients with hemophilia B. Despite containing low nontherapeutic levels of Factors II, VII, and X, the product is not indicated for the treatment of Factor II, VII, or X deficiencies. Mild to moderate hemorrhages may be treated with a single dose to raise the plasma Factor IX level to 20% to 30%. With more serious bleeding, the level should be raised to 30% to 50%. Generally, infusions are required daily. Surgery requires that the Factor IX level be raised to 30% to 50% and maintained for at least 1 week after the operation.

Risks. There are no known contraindications for the use of Profilnine SD. The administration of Factor IX complex concentrates is associated with the development of thrombosis or DIC, particularly in those patients who receive prolonged treatment or who have known liver disease. Allergic reactions may occur, and they may be treated by slowing the rate of infusion or using a different lot.



Intermediate-purity Factor IX concentrates have been associated with thrombosis and disseminated intravascular coagulation (DIC), but high-purity Factor IX concentrates have low thrombotic potential.

High-Purity Factor IX, Recombinant

BeneFIX

Preparation. BeneFIX is produced by a genetically engineered CHO cell line. Those cells secrete recombinant Factor IX into a cell culture medium that does not contain any proteins derived from animal or human sources. A chromatography purification process, membrane filtration, and lyophilization are used to yield an active Factor IX product of high purity. The specific activity of BeneFIX is ≥ 200 IU per milligrams of protein, and each vial nominally contains 250, 500, or 1000 IU of recombinant Factor IX.

Use. The high-purity Factor IX preparation is useful only for Factor IX replacement in patients with demonstrated hemophilia B and in patients with acquired Factor IX deficiencies. BeneFIX is not indicated for the treatment of other factor deficiencies (eg, Factors II, VII, VIII, and X), the treatment of patients who have hemophilia A with inhibitors to Factor VIII, the reversal of warfarin-induced anticoagulation, or the treatment of bleeding caused by low levels of liver-dependent coagulation factors.

Risks. Contraindications for the use of BeneFIX are in patients with a known hypersensitivity to hamster protein or with a sensitivity to latex. Allergic-type hypersensitivity

reactions have been reported, frequently in close temporal association with the development of Factor IX inhibitors. In addition, because thromboembolic complications have been reported after the use of Factor IX complexes, the use of Factor-IX-containing products, including recombinant Factor IX products, may be potentially hazardous in patients with signs of fibrinolysis and in patients with DIC.

Factor VII

Description

NovoSeven (Novo Nordisk Health Care AG, Bagsvaerd, Denmark) is the only commercially available product licensed for use in the United States for treatment of patients with Factor VII deficiency. Please refer to the *Physicians' Desk Reference* for specific details.

Factor VII is a vitamin-K-dependent glycoprotein that promotes hemostasis by activating the extrinsic pathway of the coagulation cascade. After activation, Factor VIIa, when complexed to tissue factor, activates coagulation Factor X to Factor Xa, as well as coagulation Factor IX to Factor IXa. Factor Xa, in complex with other factors, then converts prothrombin to thrombin, which in turn converts fibrinogen to fibrin, resulting in the formation of a hemostatic plug and inducing local hemostasis. By activating Factor X, clotting can be initiated independently of the intrinsic coagulation cascade clotting factors, thus bypassing the need for Factors VIII and IX. Accordingly, the product has important therapeutic value in the treatment of patients who have hemophilia A or B with inhibitors, which are neutralizing antibodies to Factors VIII and IX.

Preparation. NovoSeven is produced by the expression of the coding sequence of the Factor VII gene and the production of the human inactive form of Factor VII by BHK cells into the culture medium (containing newborn calf serum). The inactive, single-chain form of Factor VII is proteolytically converted by autocatalysis to the biologically active double-chain form, activated Factor VII, during a chromatographic purification process. The product is then sterilized and lyophilized. The final formulation has no added human albumin but does contain trace amounts of hamster proteins, bovine immunoglobulin, gamma class (IgG) (from the calf serum used in cell culture), and mouse IgG (from an immunoaffinity purification step).

Use. NovoSeven is indicated for use in patients with Factor VII deficiencies. It is also approved and indicated for the treatment of bleeding episodes or for the prevention



Recombinant Factor VII is approved for the treatment of patients with:

- Factor VII deficiency
- Inhibitors to Factor VIII or Factor IX

of bleeding during surgical interventions or invasive procedures in patients who have hemophilia A or B with or without the presence of Factor VIII or Factor IX neutralizing antibodies. Because neutralizing antibodies can develop, patients being treated for Factor VII deficiency should have the prothrombin time, Factor VII coagulant activity, and bleeding (if any) monitored before and after the administration of NovoSeven to ensure that the expected effects of treatment are achieved.

Pharmacokinetic studies of the effect of a single dose of NovoSeven in patients with either hemophilia A or B exhibited dose-proportional behavior, with a product half-life of 2.3 hours, and a median in-vivo plasma recovery of 44% and of 20% in patients with Factor VII deficiency. The normal Factor VII plasma concentration is 0.5 µg/mL. Factor VII levels of 15% to 25% (0.075-0.125 µg/mL) are generally sufficient to achieve hemostasis. A 70-kg individual with Factor VII deficiency would require a NovoSeven dose in the range of 16 to 27 µg/kg to achieve sufficient Factor VII plasma levels for hemostasis. Hemostasis should be evaluated to determine the effectiveness of the product in individual patients and to provide a basis for the modification of the dose and the treatment schedule. Coagulation parameters do not necessarily correlate with or predict the effectiveness of NovoSeven.

Dosage. In patients with hemophilia A or B with inhibitors, bleeding episodes can be managed if the recommended dose of 90 µg/kg is given every 2 hours by bolus infusion until hemostasis is achieved. If hemostasis is not achieved, the treatment is judged to be inadequate. Doses between 35 and 120 µg/kg have been used successfully in trials, so the dose and the administration interval may be adjusted according to the severity of the bleeding and the degree of hemostasis achieved. The minimal effective dose has not been established. Most patients who were reported to have adverse experiences received more than 12 doses.

For severe bleeding, dosing may continue at 3- to 6-hour intervals after hemostasis is achieved in order to maintain the hemostatic plug. The appropriate duration of posthemostatic dosing has not yet been studied. Therefore, the duration of posthemostatic dosing should be minimized, and the patients should be closely monitored.

For prophylactic control of bleeding during surgical intervention or invasive procedures, an initial dose of 90 µg/kg should be given immediately before the start of the procedure and repeated at 2-hour intervals for the duration of the surgery. Postsurgical boluses for minor surgeries should



The starting dose of NovoSeven in bleeding patients with Factor VIII or Factor IX inhibitors is generally 90 µg/kg by bolus injection every 2 hours until hemostasis has been achieved.



In patients with congenital Factor VII deficiency, dosage is generally lower and less frequent (15-30 µg/kg every 4-6 hours).

occur at 2-hour intervals for the first 48 hours and should continue at 2- to 6-hour intervals until healing has occurred. After major surgery, boluses should be given at 2-hour intervals for 5 days, followed by 4-hour intervals until healing has occurred. Additional bolus doses may be administered if required. However, the recommended dose schedule should not be increased, even in the case of a lack of clinical effect, because of the lack of information on the additional risks that may be incurred.

In patients with congenital Factor VII deficiency, the dose range of NovoSeven to treat bleeding episodes or to prevent significant bleeding during invasive procedures or surgical intervention is 15 to 30 µg/kg every 4 to 6 hours until hemostasis is achieved. The minimal effective dose has not yet been determined, and effective treatment has been achieved with doses as low as 10 µg/kg. Because a patient's response to treatment with NovoSeven clearly varies, the dose and frequency should be adjusted according to the individual's clinical response.



NovoSeven is contraindicated in patients with known hypersensitivity to mouse, hamster, or bovine proteins. In addition, it should be used with care in patients who may be predisposed to thromboembolic events, such as those with crush injury, advanced atherosclerotic disease, septicemia, and surgeries associated with increased release of tissue factor (ie, neurosurgery, genitourinary surgery).

Risks. Contraindications for use of NovoSeven include patients with known hypersensitivities to mouse, hamster, or bovine proteins. Although the risk of thrombosis is generally considered to be low in patients with hemophilia, the risk increases with the use of NovoSeven. However, the extent of the risk of arterial and venous thrombotic adverse events is not known. Patients who receive NovoSeven for the previously mentioned indications should be monitored closely for signs or symptoms of activation of the coagulation system or thrombosis. If clinical signs of thrombosis occur or if there is laboratory confirmation of intravascular coagulation, the dosage should be reduced or stopped, depending on the patient's clinical condition.

There have been no adequate and well-controlled studies in pregnant women; therefore, NovoSeven should be used only if the potential benefit for the mother justifies the potential risk to the fetus. It is not known whether NovoSeven is excreted in human milk.

Warnings. Reports indicate that the use of NovoSeven in patients with increased circulating tissue factor or with predisposing coagulopathy appears to have an increased risk of an adverse event, including, but not limited to, arterial thromboembolic events, myocardial ischemia, myocardial infarction, cerebral ischemia and infarction, and death. Even in patients with hemophilia, the use of NovoSeven should be carefully considered when thromboembolic predisposing conditions are present, such as DIC, advanced atherosclerotic disease, crush injury, septicemia,

surgeries in which there may be increased tissue factor release (genito-urinary surgery or neurosurgery), and intracerebral hemorrhage. NovoSeven should be used cautiously in patients undergoing concomitant treatment with activated or nonactivated prothrombin complexes.

Although abundant case reports demonstrate the benefits of NovoSeven treatment to individual patients for a variety of conditions (among patients who do not have Factor VIII or IX inhibitors or Factor VII deficiencies), the use of NovoSeven under those conditions is considered to be "off-label." During the postmarketing phase of NovoSeven, after its release and its use in both labeled and unlabeled indications, a number of adverse events were voluntarily reported. Those events involved patients with known situational and consumptive coagulopathies and without known coagulopathies. Among the adverse reactions reported were 1) thromboembolic events, including myocardial infarction or ischemia, cerebral infarction or ischemia, thrombophlebitis, arterial thrombosis, and deep vein thrombosis and related pulmonary embolism, and 2) isolated cases of hypersensitivity reactions, including anaphylaxis.

No studies of NovoSeven have fully identified the patients who can safely use the product, the minimum or maximum doses, the dosing interval schedule, or a reliable method for monitoring the product's effectiveness in achieving hemostasis. For all of those reasons, the use of NovoSeven in situations other than those for which it has been approved should be approached with caution.

Protein C

Description

Xigris (Eli Lilly and Company, Indianapolis, IN) is the only commercially available product of activated protein C (APC) licensed for use in the United States. Please refer to the *Physicians' Desk Reference* for specific details.

Protein C is a vitamin-K-dependent protein that is normally produced and that circulates as a zymogen. Once it becomes activated after enzymatic cleavage, APC exerts its antithrombotic effect by inhibiting Factors Va and VIIIa. In-vitro data suggest a number of roles for APC. It appears to have indirect profibrinolytic activity through its ability to inhibit plasminogen activator inhibitor-1. In addition, APC may exert an anti-inflammatory effect by modulating the chemotactic response of leukocytes to inflammatory cyto-



Anecdotal reports suggest that recombinant Factor VIIa may be of benefit in other situations (eg, trauma); however, such "off-label" use of NovoSeven in patients other than those with Factor VIII or IX inhibitors and those with congenital Factor VII deficiency has been inadequately studied. The use of NovoSeven in "off-label" conditions should be approached with caution.



Xigris is indicated for mortality reduction in patients with severe sepsis who are at significant risk of death. The mechanism by which Xigris exerts its effect remains unclear.

kines when bound to the leukocyte cell surface APC receptor. Data also suggest that leukocyte-bound APC may reduce interactions between leukocytes and the microvascular endothelium. In-vitro bacterial phagocytosis by APC-bound leukocytes and monocytes is not affected.

Preparation. Xigris is a recombinant form of human APC, or drotrecogin alfa (activated). It is a serine protease with the identical amino acid sequence as human plasma-derived APC with the same sites of glycosylation, although there are some differences in the glycosylation structures. Xigris is prepared by using an established human cell line. Those cells possess the complementary DNA for the inactive human protein C zymogen, and they secrete the protein into the fermentation medium. To that nutrient-rich medium is added the antibiotic geneticin sulfate, and fermentation is allowed to take place. Human protein C is enzymatically activated by cleavage with thrombin and is subsequently purified. The product is then sterilized and lyophilized. Geneticin sulfate is not detectable in the final product.

Xigris may affect the aPTT assay. Therefore, if Xigris is present in patient plasma samples, it may interfere with one-stage coagulation assays that are based on the aPTT, such as Factor VIII, IX, and XI assays, with the result of the assay having a lower apparent concentration than the actual concentration of the factor. Xigris does not interfere with one-stage factor assays that are based on the PT, such as Factor II, VII, and X assays.



Xigris may interfere with testing that is based on the aPTT (Factor VIII, IX, XI assays). However, it does not interfere with assays based on the prothrombin time (Factor II, VII, X).

Use. Xigris is indicated for the reduction of mortality in adult patients who have severe sepsis and who have a significant risk for death; sepsis is associated with acute organ dysfunction. Studies of patients with severe sepsis who were given Xigris infusions over 48 or 96 hours demonstrated a dose-dependent decline in D-dimer and interleukin 6 (IL-6), as compared with a placebo. The Xigris-treated patients experienced more rapid declines in circulating plasminogen activator inhibitor-1 (PAI-1) levels, thrombin-antithrombin levels, prothrombin F1.2, and IL-6. Those patients also experienced more rapid increases in circulating protein C and antithrombin levels as well as a normalization of plasminogen. The specific mechanism by which Xigris exerts its effect on survival in patients with severe sepsis is not completely understood.

The first international, multicenter, randomized, double-blind, placebo-controlled trial in patients with severe sepsis was terminated early after an interim analysis of the data showed a significantly lower mortality in patients receiving Xigris (25%) vs those receiving placebo (31%) ($p =$

0.005). The reductions in mortality corresponding with the Xigris treatments were most apparent in the older patients, in patients with serious underlying diseases that predated their sepsis, and in patients with more severe physiologic disturbances (ie, the sickest patients who have the greatest risk of dying from sepsis). In those patients, small differences were detected in the plasma clearance of Xigris with regard to age, gender, hepatic dysfunction, renal dysfunction, and obesity. However, dose adjustment based on those factors did not appear to be required. The efficacy of Xigris has not been established in patients with a lower risk of death or in those with end-stage renal disease; the latter were excluded from the study. Data from a placebo-controlled clinical trial in pediatric patients did not establish the efficacy of Xigris.

Risks. Bleeding is the most common serious adverse effect associated with Xigris therapy. Because Xigris increases the risk of bleeding, its use is contraindicated in patients for whom bleeding could be associated with a high risk of significant morbidity or death—primarily those patients at risk for internal bleeding or bleeding into the brain or spinal cord. Those clinical situations include, but are not limited to, active internal bleeding, recent trauma associated with a risk of life-threatening bleeding, recent hemorrhagic stroke or severe head trauma, intracranial or intraspinal surgery, intracranial neoplasm, mass lesion, cerebral herniation, and presence of an epidural catheter.

For patients who are taking medication or who have underlying conditions that impair coagulation, the risk of bleeding could be compounded by Xigris. Its use, therefore, should be carefully considered before therapy is initiated. Concurrent use of medications such as heparin, warfarin, aspirin, or other platelet inhibitors, as well as the recent administration of thrombolytic therapy, could be problematic. Among the clinical conditions that must be considered are recent gastrointestinal bleeding, an intracranial arteriovenous malformation or aneurysm, a known bleeding diathesis, a chronic severe hepatic disease, a platelet count $<30,000/\mu\text{L}$, and a prothrombin time-international normalized ratio (INR) >3 , or any other condition in which bleeding would be hazardous or difficult to manage.

Antithrombin

Description

The commercially available antithrombin products licensed for use in the United States include Thrombate III (Talecris),



Xigris is associated with increased risk of bleeding complications and is contraindicated in patients for whom bleeding could be associated with significant morbidity or death.



Concurrent use of Xigris with other medications that impair coagulation (eg, heparin, warfarin, aspirin, or other platelet inhibitors) requires careful consideration of potential benefits vs bleeding risk.



The term "antithrombin III" is used mainly in historical context. By international agreement, the term antithrombin has replaced the term antithrombin III.

Argatroban (GlaxoSmithKline, Pittsburgh, PA), and Arixtra (Fonda BV, Oss Noord Brabant, the Netherlands). Please refer to the *Physicians' Desk Reference* for specific details.

AT, also known as heparin cofactor, is normally synthesized by the liver. It is a serine protease inhibitor, inactivating serine proteases such as thrombin and Factors IXa, Xa, XIa, and XIIa. AT acts by forming a covalent bond at the serine site, which induces a conformational change, thereby inactivating the target factor. Heparin greatly accelerates (about 1000 times) the AT activity by inducing a conformation change in the AT protein, allowing for easier access to the binding site and enhancing the bonding between AT and thrombin. The AT effect of heparin requires sufficient levels of AT. The half-life of purified AT is approximately 60 to 90 hours, but it will be shortened when replacement is for consumptive conditions.

The diagnosis of hereditary AT deficiency should be based on a clear family history of thrombosis, decreased plasma AT levels, and exclusion of an acquired deficiency. AT levels in plasma may be measured by amidolytic assays that use synthetic chromogenic substances, by clotting assays, or by immunoassays. Immunoassays will not detect all hereditary AT deficiencies and should not be the only method used to diagnose an inherited disorder.



Patients with hereditary deficiency of antithrombin are prone to thromboembolic phenomena.

The hereditary deficiency of AT in its heterozygous state affects about 1 in 2000 to 5000 individuals in the general population; its pattern of inheritance is autosomal dominant. Spontaneous episodes of thromboembolic events may be associated with AT levels of about 40% to 60% of normal. These episodes usually appear after age 20, with the risk increasing with age, surgery, pregnancy, and delivery. By age 50, more than 85% of affected individuals have suffered at least one thrombotic episode; in about 60%, thrombosis is recurrent. During pregnancy, the frequency of thromboembolic events approaches 70%, and several studies have reported the benefits of using AT during pregnancy in women with hereditary deficiency.



AT deficiency is common; spontaneous episodes of thromboembolic events may occur with AT levels of 40% to 60%. By age 50, greater than 85% of individuals with moderately reduced AT levels will have suffered at least one thrombotic episode.

Because of its pattern of inheritance, the AT level in neonates of parents with hereditary AT deficiency should be measured immediately after birth. There have been reports of fatal neonatal thromboembolic events, including the formation of aortic thrombi. In neonates, the plasma levels of AT are normally low (averaging about 60%), and they may be even lower in premature infants. Therefore, a reliable diagnosis is more challenging and the findings should be discussed with an expert on coagulation in the pediatric population.

Patients with deficiencies in AT, whether congenital or acquired, are prone to thromboembolic complications. Acquired deficiencies in AT can be caused by low production (from liver disease or malnutrition), protein loss (from nephrotic syndrome and certain gastrointestinal conditions), and accelerated consumption (from DIC, preeclampsia, surgery, or trauma). They are also associated with the use of heparin, L-asparaginase, and oral contraceptives.

AT is stable in plasma, so deficiencies can be easily treated with FFP, or with Liquid or Thawed Plasma. Various AT products are available, including an AT concentrate and two synthetic direct thrombin inhibitors. Recombinant and transgenic sources are under investigation.

Thrombate III

Preparation. Thrombate III (Talecris) is an antiprotease concentrate prepared from pooled human plasma by modifications and refinements of the cold ethanol fractionation technique developed by Cohn. The product is heat-treated in solution at 60°C for not less than 10 hours, filtered, and lyophilized. Each vial is labeled with the amount of AT in IU, as standardized against a WHO AT reference preparation.

Use. AT supplementation is indicated for patients with hereditary AT deficiency as prophylaxis against, or as management of, venous thromboembolic events associated with surgical or obstetric procedures. Dosage should be determined by the individual clinical response and should be based on the pretherapy plasma AT level, with the goal of achieving a level of 120%. However, recovery may vary, so when therapy is started, levels should be drawn at baseline and 20 minutes after infusion. Additional doses can be calculated on the basis of the recovery of the first dose. Thereafter, the plasma levels of AT should be monitored at least every 12 hours. In some situations when there may be an increased consumption, such as surgery, hemorrhage, acute thrombosis, or the concurrent use of heparin, the AT levels may need to be checked more frequently. Dosages and dosing intervals should be adjusted as needed to meet each patient's particular needs. The formula for calculating the AT dosage follows:

$$\text{Units required (IU)} = [(\text{desired} - \text{baseline AT level}) \times \text{weight (kg)}]/1.4.$$

If heparin is used in conjunction with Thrombate III, a reduced dosage of heparin is recommended to avoid bleed-



Acquired AT deficiency can be associated with
Low production (liver disease, malnutrition)
Protein loss (nephrotic syndrome, gastrointestinal loss)
Consumption (DIC, trauma, surgery, preeclampsia)
Drugs (heparin, L-asparaginase, oral contraceptives)



The currently available AT products vary greatly in their therapeutic dosage levels and dosing schedules, but all of them are contraindicated in patients with overt major bleeding.



If heparin is used in conjunction with Thrombate III, the dose of heparin should be reduced to avoid bleeding complications.

ing. AT is being evaluated for use in a variety of clinical settings. Other probable and possible indications that are based on studies to date are summarized in Table 13-7.

Argatroban

Action. Argatroban (GlaxoSmithKline) is a synthetic, direct thrombin inhibitor derived from L-arginine that reversibly binds to the thrombin active site and that does not require the cofactor AT for antithrombotic activity. Argatroban is highly selective for thrombin and has little or no effect on related serine proteases such as trypsin, Factor Xa, plasmin,

Table 13-7. Indications for Antithrombin*

FDA-approved indications (clinical data suggest efficacy)

- Congenital antithrombin deficiency
- Perioperative period
- Postsurgical prophylaxis for deep vein thrombosis
- Acute thromboembolism
- Pregnancy: delivery and abortion
- Neonates with congenital antithrombin deficiency

Probable indications (data suggest improvement in laboratory and clinical measures)

- Neonates born to mothers with congenital antithrombin deficiency or with strong family history of thrombosis
- Disseminated intravascular coagulation caused by sepsis, trauma, burns, pregnancy
- Heparin resistance associated with low antithrombin
- L-asparaginase therapy
- Extracorporeal circulation (cardiopulmonary bypass, hemodialysis)
- Hepatic artery thrombosis after orthotopic liver transplantation

Possible indications (data suggest improvement in laboratory values without proven clinical efficacy)

- Veno-occlusive disease
- Orthotopic liver transplantation
- LeVeen peritoneovenous shunt
- Chronic hepatic insufficiency

Under investigation

- Nephrotic syndrome
- Antithrombin deficiency resulting from gastrointestinal loss (inflammatory bowel disease, protein-losing enteropathy)
- Pregnancy: pre-eclampsia, gestational hypertension, and acute fatty liver of pregnancy
- Neonatal respiratory distress syndrome

*Adapted from Bucur SZ, Levy JH, Despotis GJ, et al. Uses of antithrombin III concentrate in congenital and acquired deficiency states. *Transfusion* 1998;38:481-98

or kallikrein. It is capable of inhibiting the action of both free and clot-associated thrombin. Argatroban acts as an anticoagulant by the inhibition of thrombin-catalyzed or thrombin-induced reactions, including the activation of Factors V, VIII, and XIII; fibrin formation; activation of protein C; and platelet aggregation. Argatroban does not interact with heparin-induced antibodies. To date, antibody formation to Argatroban has not been reported, despite eight patients receiving multiple doses.

Pharmacology and Metabolism. Argatroban is distributed primarily in the extracellular fluid but is 54% protein bound to circulating albumin and alpha₁-acid glycoprotein and to a lesser extent to other human serum proteins. Metabolism of Argatroban is mainly mediated by the liver by hydroxylation and aromatization of the chemical's 3-methyltetrahydroquinoline ring. The primary metabolite still is able to exert AT activity, but it is three to five times weaker and is found at low levels in plasma—between 0% and 20% as compared with the parent drug. The terminal half-life of Argatroban ranges between 39 and 51 minutes. It is excreted primarily in the feces, presumably through biliary secretion.

Use. Argatroban has been approved for use as an anticoagulant for the prophylaxis or treatment of thrombosis in patients with heparin-induced thrombocytopenia and thrombosis syndrome (HITTS) as well as for those patients with or at risk for HITTS who are undergoing percutaneous coronary intervention.

During the clinical trials conducted in patients with HITTS, most patients recovered their platelet count within 3 days, with the recovery defined as an increase in the count >100,000/ μ L or at least 1.5 times the baseline. In addition, the rate of major bleeding events and intracranial hemorrhage in patients who have HITTS and who were undergoing percutaneous coronary intervention was 1.8%, as compared with 3.1% in the placebo arm of the trial (placebo plus standard dose, weight-adjusted heparin). A more detailed account of the results of the clinical trials conducted in patients who have HITTS and who were treated with Argatroban vs more traditional therapy appears in the *Physicians' Desk Reference*.

Dosage. Generally, therapy with Argatroban is monitored by aPTT. Steady-state levels are typically attained within 1 to 3 hours after the initiation of therapy.

When patients with HITTS but without liver impairment are treated, the recommended initial dose is 2 μ g/kg/min, administered as a constant infusion. A baseline aPTT should



Argatroban is a direct thrombin inhibitor that acts by reversibly binding to the thrombin active site, without requiring cofactor AT for its antithrombotic activity.



Argatroban is used for prophylaxis or treatment of thrombosis in patients with HITTS.



Argatroban therapy is monitored by measurement of aPTT.

be obtained before the initiation of therapy and then checked at 2 hours to confirm that the patient has attained the desired therapeutic range. The dose can be adjusted as clinically indicated, but it should not exceed 10 µg/kg/min. The desirable steady-state range for the APTT is considered to be 1.5 to 3 times the initial baseline level, not to exceed 100 seconds.

During percutaneous coronary interventions in patients with HITTS, an infusion dose of 25 µg/kg/min should be started, with an additional bolus of 350 µg/kg given over 3 to 5 minutes. Ten minutes after completion of the bolus, the activated clotting time (ACT) should be checked. The therapeutic ACT range is between 300 and 450 seconds. If the ACT is >300 seconds, the procedure can begin. If the ACT is <300 seconds, an additional bolus of 150 µg/kg should be administered, and the infusion rate increased to 30 µg/kg/min and the ACT checked in 10 minutes. If the ACT is >450 seconds, the infusion rate should be decreased to 15 µg/kg/min and the ACT should be checked in 10 minutes. Once a therapeutic ACT is achieved, the infusion dose should be continued during the procedure.

For patients with HITTS and moderate liver impairment, the initial dose should be reduced to 0.5 µg/kg/min. That recommended dosage is based on the fourfold reduction in Argatroban's clearance rate, as compared with the clearance rate in patients with normal liver function.

When Argatroban is coadministered with warfarin, a loading dose of warfarin should not be used. The INR will be prolonged beyond that produced by warfarin alone. The reason for the combined effect on the INR is not clear because the reduction in Factor Xa activity is consistent with that expected for warfarin therapy alone.

No dosage adjustment is necessary in patients with renal dysfunction as it is in patients with hepatic dysfunction.

Risks. Argatroban is contraindicated in patients with overt major bleeding and in patients experiencing hypersensitivity reactions to this product or any of its components. All parenteral anticoagulants should be discontinued before administration of Argatroban. Caution should be exercised, with close monitoring of the laboratory values and the clinical condition of the patient, when Argatroban is used in patients with liver impairment. The safety and effectiveness of the coadministration of Argatroban with thrombolytic agents, aspirin, acetaminophen, glycoprotein IIb/IIIa antagonists, other antiplatelet agents, and any anticoagulants other than warfarin have not yet been established.



When argatroban is coadministered with warfarin, a loading dose of warfarin should *not* be used because it could result in a supratherapeutic INR.

Arixtra

Pharmacology and Metabolism. Arixtra is a sterile solution containing fondaparinux sodium, a synthetic specific inhibitor of activated Factor X. It is packaged as a single-dose, injectable solution in a prefilled syringe that is affixed with an automatic needle protection system. Each dose contains 2.5 mg, 5 mg, 7.5 mg, or 10 mg of fondaparinux sodium in an isotonic solution.

The antithrombotic activity following administration of Arixtra is the result of AT-mediated selective inhibition of Factor Xa. When bound to AT, Arixtra potentiates (about 300 times) the innate neutralization of Factor Xa by AT, thus inhibiting thrombin formation and thrombus development. The anti-Xa activity of the drug increases with increasing drug concentration, reaching maximum values in approximately 3 hours.

Once administered as a subcutaneous injection, Arixtra is rapidly and completely absorbed (absolute bioavailability is 100%), and it is distributed mainly in the intravascular space. Arixtra is eliminated virtually unchanged in urine. In patients with normal renal function, approximately 77% of the product is eliminated within 72 hours. The elimination half-life is 17 to 21 hours.

Clinical Studies. In a randomized, double-blind clinical trial in patients undergoing prophylactic treatment against thromboembolic events after hip fracture surgery, Arixtra subcutaneous injection of 2.5 mg/day was compared with an enoxaparin sodium subcutaneous injection of 40 mg/day, with the treatment continuing for 7 ± 2 days. Under those conditions, venous thromboembolism (VTE) of 8.3% was associated with Arixtra, as compared with 19.1% for enoxaparin, for a relative risk reduction of 56%. Major bleeding episodes occurred in the 2.2% of the patients taking Arixtra vs 2.3% of the patients receiving enoxaparin. The results of trials that examined prophylaxis of VTE after hip replacement surgery and knee replacement surgery demonstrated similar results.

A noncomparative unblended trial examined the relative risk of VTE when Arixtra vs placebo was used for an extended period (21 ± 2 days). The incidence of VTE in patients treated with Arixtra was 1.4% vs 35% for patients treated with placebo, for a relative risk reduction of 95.9% ($p = 0.0001$). Major bleeding in the patients was 2.4% for Arixtra and 0.6% for placebo.

Treatment for the prophylaxis of VTE after abdominal surgery in patients at risk for thromboembolic complications, as well as treatment for deep vein thrombosis and



Arixtra is a synthetic specific inhibitor of activated Factor X. Its antithrombotic properties result from AT-mediated selective inhibition of Factor Xa.

pulmonary embolism, demonstrated similar results when compared with traditional therapies.

Use. Arixtra injection is indicated for prophylactic treatment of VTE in patients who were undergoing hip fracture surgery, including extended prophylaxis; in patients who were undergoing hip or knee replacement surgery; and in patients who were undergoing abdominal surgery and who are at risk for thromboembolic complications. However, Arixtra should be used with caution in older patients because there is an increasing risk of major bleeding with increasing age (>65 years).

Arixtra has not been fully evaluated for use during pregnancy or in lactating females. The safety and effectiveness of Arixtra in pediatric patients have not been established.

Dosage. In patients undergoing treatment with fondaparinux sodium injection once daily for symptomatic deep vein thrombosis, the desired peak of steady-state plasma concentration is approximately 1.2-1.26 mg/L and is reached about 3 hours after dosing. The mean minimum steady-state plasma concentration ranges from 0.46 to 0.62 mg/L. The doses to be administered once daily are as follows: 5 mg for body weight <50 kg, 7.5 mg for body weight = 50-100 kg, and 10 mg for body weight >100 kg.

Because Factor Xa levels cannot be used to monitor Arixtra therapy, periodic routine complete blood counts, serum creatinine level, and stool occult blood tests are recommended during the course of treatment with Arixtra injection.

Warnings. Arixtra is contraindicated in patients with severe renal impairment because they are at an increased risk for major bleeding episodes. In patients undergoing chronic intermittent hemodialysis, data suggest that the clearance rate of Arixtra can increase by 20%.

Arixtra prophylactic therapy is contraindicated in patients with a body weight <50 kg. The occurrence of a major bleeding episode was doubled in those patients as compared with patients with a body weight ≥ 50 kg.

Patients with active major bleeding, bacterial endocarditis, thrombocytopenia with platelet antibodies, and a known hypersensitivity to fondaparinux sodium are not candidates for Arixtra therapy.

Like other anticoagulants, Arixtra should be used with extreme caution in patients with an increased risk of hemorrhage. Among the conditions associated with increased risk are: congenital or acquired bleeding disorders; active ulcerative and angiodysplastic gastrointestinal disease; hemorrhagic stroke; the period shortly after brain, spinal, or



Factor Xa levels *cannot* be used to monitor Arixtra therapy.



Arixtra is contraindicated in patients with severe renal impairment because they would be at increased risk of bleeding.

ophthalmologic surgery; and concomitant treatment with platelet inhibitors.

There is a boxed warning against using Arixtra during neuraxial anesthesia and when a postoperative indwelling epidural catheter is in place. Spontaneous postmarketing reports have described several cases of epidural or spinal hematoma that have occurred in association with the use of Arixtra.

Moderate thrombocytopenia (platelet counts between 100,000/ μ L and 50,000/ μ L) occurred at a rate of 3% after the administration of Arixtra, whereas the rate of occurrence for severe thrombocytopenia was 0.2%. The platelet counts of patients receiving Arixtra should be monitored closely, and its use should be discontinued if the platelet count drops below 100,000/ μ L.

There is no known antidote for Arixtra injection, and an overdose may lead to hemorrhagic complications.

Table 13-8 lists the available coagulation factors and describes production methods, indications for use, and important characteristics.

Immune Globulin

Description

The commercially available immune globulin products licensed for use in the United States include Vivaglobin (CSL Behring), GamaSTAN SD (Talecris), Flebogamma (Grifols), Gammagard SD (Baxter), and Gamunex (Talecris). Please refer to the *Physicians' Desk Reference* for specific details.

Preparation. Immune globulin products contain all of the immune globulins, but the gamma globulin—the IgG antibody fraction of human plasma—is further isolated and purified.

Immune globulin is prepared from large plasma pools from many donors by the cold ethanol fractionation technique. Additional measures for purification may be used, depending on the specific manufacturer, including solvent/detergent treatments, pasteurization, precipitation, chromatography, and different types of filtration. Immune globulins are prepared from unselected donors or from selected donors with known high titers of antibodies to specific antigens (eg, anticytomegalovirus, anti-D, and antitetanus). The products obtained from the selected donors are known as hyperimmune globulins.

Use. Immune globulin products are available in formulations for subcutaneous, intramuscular, or intravenous ad-



Immune globulin preparations are available for intramuscular and intravenous use.

The intramuscular preparation is indicated for prophylaxis or modification of several viral infections, including hepatitis A, rubella, rubella (in susceptible females exposed to rubella in the first trimester of pregnancy), and varicella-zoster (if varicella-zoster immune globulin is unavailable).

Table 13-8. Available Coagulation Factor Concentrates

Product Type	Product	Approved Indications	Comment
Blood component; human plasma, centrifuged and frozen	Cryoprecipitated Antihemophilic Factor	Fibrinogen replacement, Factor VIII:C, vWF replacement IV use only	Contains 150 mg fibrinogen; 80 IU of Factor VIII; vWF; fibronectin, and low levels of other proteins; human albumin
Factor VIII; pooled human plasma, cold insoluble-fractionated, pasteurized	Humate-P	Factor VIII and vWF replacement IV use only	Contains therapeutic levels of vWF:RCo; blood group isoagglutinins; human albumin
Factor VIII; pooled human plasma, cold insoluble-fractionated, S/D-treated, dry-heated	Koate -DVI	Factor VIII replacement IV use only	Contains some vWF; blood group isoagglutinins; human albumin
Factor VIII; pooled human plasma, fractional solubilized, affinity column-purified, S/D-treated	Alphanate	Factor VIII replacement IV use only	Contains some vWF and protein C; blood group isoagglutinins; human albumin
Factor VIII; pooled human plasma, purified with both immunoaffinity and ion exchange chromatography, S/D-treated	Hemofil M	Factor VIII replacement IV use only	Contains some mouse protein; trace blood group isoagglutinins; latex; human albumin
Factor VIII, recombinant; S/D-treated; purified with ion exchange, immunoaffinity, and other chromatographic steps	Kogenate FS	Factor VIII replacement IV use only	Contains mouse and hamster proteins; human albumin
Fraction II + III and anion exchange chromatography for the HPPS			

Table 13-8. Available Coagulation Factor Concentrates (continued)

Product Type	Product	Approved Indications	Comment
Factor VIII, recombinant; purified by a series of chromatographic columns, including an immunoaffinity column	Recombinate	Factor VIII replacement IV use only	Contains nontherapeutic amount of vWF; mouse, hamster, and bovine proteins; latex; human albumin
Factor VIII, recombinant, B-domain-deleted; purified by chromatography	ReFacto	Factor VIII replacement IV use only	Contains mouse and hamster proteins; human albumin
Factor vWF:RCoF; pooled human plasma, cold insoluble-fractionated, pasteurized	Humate-P	vWF and Factor VIII replacement IV use only	Contains high-molecular-weight multimers of vWF:RCoF; therapeutic levels of Factor VIII; blood group isoagglutinins; human albumin
Anti-inhibitor coagulant complex; source plasma and/or plasma, vapor-heated	FEIBA VH	Treatment for Factor VIII, Factor XI, Factor XII, and vWF inhibitors IV use only	Contains Factor IIa, Factor VIIa, and Factor Xa; trace factors of the kinin generating system; human albumin
Factor IX complex; pooled human plasma, fractionated by DEAE chromatography, S/D-treated, purified by dual-affinity chromatography, nano-filtered	AlphaNine SD	Factor IX replacement IV use only	Contains low levels of Factor II, Factor VII, and Factor X; human albumin
Factor IX complex; S/D-treated, pooled human plasma, fractionated by DEAE chromatography	Profilnine SD	Factor IX replacement IV use only	Contains low levels of Factor II, Factor VII, and Factor X; human albumin
Factor IX, recombinant; chromatography-purified, membrane-filtered	BeneFIX	Factor IX replacement IV use only	Contains trace amounts of hamster protein; latex

(continued)

Table 13-8. Available Coagulation Factor Concentrates (continued)

Product Type	Product	Approved Indications	Comment
Factor VIIa, recombinant; chromatography-purified	NovoSeven	Factor VII replacement, and Factor VIII and Factor IX inhibitor treatment IV use only	Contains mouse, hamster, and bovine proteins; does not contain human albumin
Protein C, activated, recombinant; human cell line, fermented, purified	Xigris	Protein C replacement in adult patients with severe sepsis at risk for death without end-stage renal disease or risk of internal bleeding, bleeding into the brain or spinal cord IV use only	Contains human proteins, including albumin
Antithrombin, serine protease inhibitor; pooled human plasma, Cohn cold ethanol-fractionated, pasteurized	Thrombate III	Hereditary antithrombin-III deficiency IV use only	Contains human proteins, including albumin
Antithrombin, synthetic—direct antithrombin activity; derived from L-arginine	Argatroban	Anticoagulant for prophylaxis or treatment of thrombosis in patients with HITTS who do not have overt major bleeding IV use only	Does not contain animal or human proteins
Antithrombin, synthetic—specific inhibitor of Factor Xa; fondaparinux sodium	Arixtra	Prophylactic treatment of VTE in adult patients undergoing surgery for hip fracture, hip or knee replacement, or abdominal surgery without severe renal impairment or risk for major bleeding IV use only	Does not contain animal or human proteins

IV = intravenous; vWF = von Willebrand factor; RCoF = ristocetin cofactor; S/D = solvent/detergent; HPPS = human plasma protein solution; DEAE = diethylaminoethanol; HITTS = heparin-induced thrombocytopenia and thrombosis syndrome; VTE = vascular thrombotic event.

ministration. The distinctions are important because products intended for intramuscular use must not be administered intravenously unless otherwise specified. They may cause severe hypotensive reactions similar to anaphylaxis because of their content of complement-activating aggregates of immunoglobulins. It is extremely important to realize that all of the products containing immunoglobulins contain at least some immunoglobulin A (IgA). When immunoglobulins are used to treat individuals who are deficient in IgA, those patients may develop IgA antibodies that can cause severe and even fatal anaphylactic hypersensitivity reactions.

Vivaglobin

Preparation. Vivaglobin is a polyvalent human, normal immunoglobulin intended for subcutaneous infusion. It is a 16% protein solution, of which at least 96% is immunoglobulin G. The distribution of the IgG subclasses is roughly equivalent in proportion to that of normal plasma. Vivaglobin is indicated for the treatment of patients with primary immune deficiency.

Vivaglobin is manufactured from pooled human plasma by cold alcohol fractionation and pasteurized by heating at 60°C for 10 hours. It is not chemically altered or enzymatically degraded.

Risks. As with all immune globulin products, Vivaglobin should not be used to treat patients with a history of anaphylactic or severe systemic response to immune globulin preparations or patients with an immunoglobulin A deficiency, particularly those patients with known antibody against IgA.

GamaSTAN SD

Preparation. Immune globulin for intramuscular use is available as a 15% to 18% protein solution. This product is intended only for intramuscular administration. It is solvent/detergent-treated and is heated at 30°C for 6 hours. Further processing steps are precipitation, filtration, and finally ultrafiltration and diafiltration.

Use. Passive immunization with GamaSTAN is indicated for hepatitis A prophylaxis or modification by decreasing the clinical severity of infection if it is given before or within 2 weeks of exposure. It is also indicated for prophylaxis or modification of measles (rubeola), if it is given within 6 days of exposure. The product is particularly indicated for susceptible household contacts of pa-



All of the immunoglobulin-containing products contain immunoglobulin A (IgA). Patients who are IgA-deficient may develop IgA antibodies when treated with these products. These antibodies can cause severe and possibly fatal anaphylactic hypersensitivity reactions.



Immunoglobulin preparations designed for intramuscular use should *not* be administered intravenously because of the risk of severe hypotensive reactions.

tients with measles, especially if the contacts are under the age of 1 year. The risk of serious complications is highest in that population. For immunocompromised children exposed to measles, GamaSTAN should be given immediately.

It also may be of benefit in reducing the likelihood of infection and fetal damage in susceptible, but not previously immunized, women exposed to rubella in the first trimester of pregnancy. In addition, it may be considered for use in passive immunization against varicella-zoster virus in immunosuppressed individuals, but only if varicella-zoster immune globulin is unavailable. Finally, although intramuscular preparations may be used for immunoglobulin replacement therapy in immunodeficient patients, intravenous preparations are more often used for that indication. GamaSTAN may not prevent chronic infections of the external secretory tissues, such as those in the respiratory or gastrointestinal tract.

Risks. GamaSTAN and measles vaccine should not be given at the same time. GamaSTAN is not indicated for the prophylaxis of hepatitis B infection. Also, it should not be used for routine prophylaxis or treatment of rubella, poliomyelitis, mumps, or varicella-zoster infections. It is not indicated for treatment of allergy or asthma in patients with normal levels of immunoglobulin.

Flebogamma, Gammagard SD, and Gamunex

Preparations. Preparations include 5% and 10% protein solutions as well as freeze-dried or lyophilized powders ready for reconstitution with sterile water. IgG antibodies represent 90% to 98% of the total protein present in the various preparations. Techniques have been developed to prevent immunoglobulin aggregation so that most of the immunoglobulins in those formulations are present as monomers. This practice minimizes complement activation that occurs with the infusion of aggregated immunoglobulins and thereby decreases hypotensive, anaphylactic-like reactions.

Advantages. The advantages of the intravenous preparations over the intramuscular preparation include: 1) a shortened time to peak intravascular level, 2) the possibility of administering larger doses, 3) avoidance of hematoma formation from intramuscular injections in thrombocytopenic or coagulopathic patients, and 4) avoidance of discomfort or pain from intramuscular injections.

Use. A major indication for the use of intravenous immune globulin (IVIG) is the augmentation or replacement



IVIG can be used to treat primary immunodeficiency, acquired hypogammaglobulinemia, immune thrombocytopenic purpura (ITP), and Kawasaki syndrome and also as an alternative to plasmapheresis for Guillain-Barré syndrome.

of IgG antibody levels in patients with primary immunodeficiency diseases or acquired hypogammaglobulinemia, as in B-cell chronic lymphocytic leukemia, marrow transplantation, or pediatric HIV infection. IVIG is also used as an immunomodulating agent in the treatment of immune thrombocytopenic purpura. When administered with aspirin within 10 days of the onset of Kawasaki syndrome, IVIG decreases the incidence of coronary artery abnormalities in that disorder. In addition, IVIG treatment has been found to be as effective as plasmapheresis in shortening the recovery time in patients with Guillain-Barré syndrome.

Table 13-9 lists the available immune globulin products, purification methods, indications, methods of administration, concentration of immune globulin, and their use in the treatment of specific disorders.

Hyperimmune Globulins

Description

Immune globulins can be prepared from plasma obtained from human donors with high titers of specific immunoglobulins. These immune globulin preparations are known as hyperimmune globulins. Current FDA-approved products follow: Rh immune globulin (intramuscular and intravenous), rabies immune globulin, cytomegalovirus immune globulin, tetanus immune globulin, varicella-zoster immune globulin, respiratory syncytial virus immune globulin, and hepatitis B immune globulin.

Indications

The major indication for hyperimmune globulins is the immediate postexposure prophylaxis of infection with specific infectious agents (see Table 13-10). Hyperimmune globulin is also available for preventing Rh alloimmunization after the intravenous exposure of Rh-negative individuals to Rh-positive red cells.

Hyperimmune globulins are administered to individuals who do not have, or may not already have, humoral immunity to the infectious agent in question. They provide passive immunity by supplying neutralizing antibodies that can prevent or lessen the severity of infection during the period before the patient develops active immunity. Generally, for maximum effectiveness, hyperimmune globulins should be administered shortly before or as soon as possi-



Hyperimmune globulin preparations are available for immediate postexposure prophylaxis of rabies, tetanus, and hepatitis B.

Table 13-9. Immune Globulins

Product Type	Product	Route of Administration	Concentration of Ig	Indications	Dose/Timing
Immune globulin; pooled plasma, cold alcohol-fractionated, pasteurized	Vivaglobin	SC	16% protein solution, with minimum of 96% immunoglobulin G	Primary immune deficiencies	Once a week 100 to 200 mg/kg, adjust accordingly
Immune globulin; pooled plasma, cold ethanol-fractionated, S/D-treated, pasteurized, precipitated, filtered, ultrafiltered, diafiltered	GamaSTAN SD	IM	15% to 18% protein solution	Primary immune deficiencies	Double dose first, then at least 0.66 mL/kg every 3 to 4 weeks
				Hepatitis A exposure	0.02 mL/kg before or soon after exposure <2 weeks
				Hepatitis A travel <3 months	0.02 mL/kg
				Hepatitis A travel >3 months	0.06 mL/kg (repeat every 4 to 6 months)
				Measles (rubella)	0.25 mL/kg if exposure is <6 days
				Varicella	If VZIG is unavailable, 0.6 to 1.2 mL/kg
				Rubella (may be of benefit)	0.55 mL/kg

Immune globulin: pooled plasma, cold alcohol-fractionated, pasteurized, PEG-precipitated, purified with ion-exchange chromatography	Flebogamma IV	50 mg protein/mL with >99% immunoglobulin G	Primary immune deficiencies	300 to 600 mg/kg every 3 to 4 weeks
Immune globulin: pooled plasma, Cohn-Oncley fractionated, ultrafiltered, purified with ion-exchange chromatography, S/D-treated	Gammagard SD IV	50 mg protein/mL with >90% immunoglobulin G	Primary immune deficiencies B-cell CLL	300 to 600 mg/kg every 3 to 4 weeks 400 mg/kg every 3 to 4 weeks
			ITP	1 g/kg, but up to three doses on alternate days if required
			Kawasaki syndrome	Either single dose of 1 g/kg, or dose of 400 mg/kg for 4 consecutive days
Immune globulin: pooled plasma, cold ethanol-fractionated, caprylate-precipitated, filtered, purified with anion-exchange chromatography	Gamunex IV	9% to 11% protein/mL with >98% gamma globulin	Primary immune deficiencies ITP	300 to 600 mg/kg

SC = subcutaneous; IM = intramuscular; IV = intravenous; VZIG = varicella-zoster immune globulin; PEG = polyethylene glycol; CLL = chronic lymphocytic leukemia; ITP = idiopathic thrombocytopenic purpura.

Table 13-10. Hyperimmune Globulins

Product	Route	Time of Initial Infusion (for approved indications)
Cytomegalovirus immune globulin	IV	Within 72 hours of kidney transplantation for attenuation of CMV disease in CMV-seronegative recipients of CMV-seropositive kidneys
Hepatitis B immune globulin	IM	Within 12 hours of birth for perinatal exposure; within 24 hours of percutaneous exposure; within 14 days of sexual contact
Rabies immune globulin	IM	As soon as possible after rabies exposure and at the time of the first dose of vaccine
Rh immune globulin	IM	Within 72 hours of Rh-incompatible delivery, miscarriage, abortion, or transfusion to Rh-negative individuals; also at 26 to 28 weeks of gestation in Rh-negative females
Rh immune globulin IV	IV or IM	Rh IVIG may be given IV or IM for suppression of Rh alloimmunization but is only given IV for ITP
Respiratory syncytial virus immune globulin	IV	Administration monthly during RSV season (typically early November through April) to children <24 months old with bronchopulmonary dysplasia or history of premature birth
Tetanus immune globulin	IM	As soon as possible after receiving a significant wound that may be contaminated with tetanus spores in individuals whose prior immunization against tetanus toxoid is unknown or uncertain
Varicella-zoster immune globulin	IM	Within 96 hours of exposure; given to eligible (see product insert) immune-compromised individuals only

CMV = cytomegalovirus; IV = intravenous; IM = intramuscular; ITP = immune thrombocytopenic purpura; RSV = respiratory syncytial virus.

ble after exposure to the infectious agent. Hyperimmune globulin for rabies, tetanus, and hepatitis B prophylaxis may be administered at the same time as the first dose of the vaccine without significant interference with subsequent development of active immunity. Repeated doses of hyperimmune globulins, however, may interfere with vaccine effectiveness.

Rh Immune Globulin

Products for intramuscular administration include RhoGAM (Ortho-Clinical Diagnostics, Raritan, NJ), MICRhoGAM (Ortho), HyperRHO S/D (Talecris), and HyperRHO S/D Mini-Dose (Talecris). Rhophylac (CSL Behring) is intended

for intravenous use only. WinRho SDF (Cangene Corporation, Winnipeg, Manitoba, Canada) is used for intramuscular or intravenous administration, depending on the clinical situation.

Preparation. RhIG consists of immunoglobulin, gamma class (IgG) prepared from pooled human plasma from donors with enhanced levels of polyclonal antibody directed against the D antigen of the Rh blood group system. RhoGAM is the trade name of the first commercial preparation of RhIG. The pooled hyperimmune plasma undergoes fractionation by a modified cold alcohol procedure; then an ultrafiltration step is incorporated into the processing. Now that other commercial products have become available, the name "RhoGAM" should not be used as a generic term when referring to other RhIG preparations. The product Hyper RHO is solvent/detergent-treated; is heated at 30 C for at least 6 hours; and then is precipitated, filtered, ultrafiltered, and diafiltered.

Use. Formulations of RhIG intended for either intramuscular or intravenous administration are used to prevent anti-D formation in Rh-negative individuals who are exposed parenterally to Rh-positive RBCs. Exposure may occur for the following reasons: 1) fetomaternal hemorrhage during delivery, abortion, amniocentesis, or abdominal trauma in Rh-negative mothers with Rh-positive fetuses or neonates; or 2) transfusion of Rh-positive red cells to Rh-negative recipients. Rh IVIG has more recently been used for Rh-positive patients with immune thrombocytopenic purpura (ITP) as an alternative to IVIG.

Advantages. Rh IVIG has an advantage over intramuscular preparations of RhIG in two clinical settings: 1) when the patient has been exposed to relatively large amounts of Rh-positive red cells that would necessitate a large number of painful intramuscular injections, and 2) when the patient has a bleeding diathesis that predisposes him or her to hematoma formation from intramuscular injections. Furthermore, only Rh IVIG should be used for patients with ITP. The intramuscular formulation can be administered only by intramuscular injection, but the intravenous formulation may be administered either intravenously or intramuscularly. (See Table 13-11.)

Timing of Administration. RhIG should be administered as soon as possible, preferably within 72 hours of a parenteral exposure to Rh-positive red cells in the setting of either pregnancy or transfusion. If not given by that time, it should still be administered later (up to 28 days in the postpartum setting), although the efficacy with late ad-



Rh Immune Globulin (RhIG) is available for IM and IV use. The primary use of the IM preparation is in preventing the sensitization of females of childbearing potential to the D antigen. The IV preparation can be used for this purpose but is also administered to Rh-positive individuals for the treatment of ITP.

Table 13-11. Rh Immune Globulin

Product Type	Product	Administration Route	Concentration of Anti-D	Indications	Dose/Timing
Rh Immune Globulin; pooled D-immune plasma, cold alcohol-fractionated, detergent-treated, ultrafiltered; latex-free	RhoGAM	IM only	Approximately 300 µg or 1500 IU/vial	Suppression of isoimmunization Obstetric: Mother D- pregnancy/delivery of D+ infant – abortion or threat – ectopic pregnancy – fetomaternal hemorrhage Transfusion: D+ blood or blood products	Best if within 72 hours and exposed to <15 mL D+ blood, then give 1500 IU if exposed to >15 mL D+ blood, give 1500 IU/15 mL D+ blood, plus an additional 1500 IU
Rh Immune Globulin; same purification methods as for RhoGam	MICRhoGAM	IM only	Approximately 50 µg or 250 IU/vial	Obstetric: Pregnancy is terminated up to and including 12 weeks' gestation Transfusion: <2.5 mL D+ blood/blood product	Best if within 72 hours and exposed to <2.5 mL D+ blood, then give 250 IU
Rh Immune Globulin; pooled D-immune plasma, anion-exchange chromatography, S/D-treated, and virus-filtered	WinRho SDF	IV only	Approximately 300 µg or 1500 IU/vial	Treatment of ITP: (if spleen and D+) – children: chronic or acute ITP – adults: chronic ITP – children or adults: ITP secondary to HIV infection	Initial dose: 250 IU/kg single/day or divided dose/2 days; If Hb <10 g/dL, give reduced dose of 125 to 200 IU/kg Maintenance dose: 125 to 300 IU/kg based on individual response, platelet and Hb levels

Rh Immune Globulin (continued)	IV or IM	Suppression of isoimmunization Obstetric: – 28 weeks' gestation – postpartum – RhD+ infant – RhD– infant – threatened abortion – invasive sampling at <34 weeks' gestation – abortion, invasive sam- pling, or any other manip- ulation at >34 weeks' gestation	Best if within 72 hours and <17 mL fetomaternal hemorrhage – give 1500 IU – give 600 IU – not needed – give 1500 IU – give 1500 IU repeat this dose every 12 weeks dur- ing pregnancy – give 600 IU
	IV	Transfusion: –D+ Whole Blood –D+ RBCs	If IV: 3000 IU/8 hours give 45 IU/mL of blood give 90 IU/mL of RBCs
	IV	–D+ Whole Blood –D+ RBCs	If IM: 6000 IU/12 hours give 60 IU/mL of blood give 120 IU/mL of RBCs

(continued)

Table 13-11. Rh Immune Globulin (continued)

Product Type	Product	Admini-stration Route	Concentration of Anti-D	Indications	Dose/Timing
Rh Immune Globulin; pooled D-immune plasma, S/D-treated, chromatographic process steps, nanofiltered	Rhophylac	IV only	1500 IU/syringe	Suppression of D isoimmunization Transfusion: D+ blood or blood products Obstetric: Mother RhD- pregnancy/delivery of RhD+ infant – abortion or threat – ectopic pregnancy – fetomaternal hemorrhage Treatment of ITP (see WinRho) Additional Guidelines: – 28 weeks' gestation – postpartum – D+ infant – D- infant – threatened abortion – abortion, invasive sampling, or any other manipulation at >15 weeks' gestation	Best if within 72 hours and <15 mL D+ exposure, give 1500 IU If >15 mL D+ blood exposure, give 1500 IU/15 mL of estimated D+ blood infused plus an extra 1500 IU – give 1500 IU – give 1500 IU – not necessary – give 1500 IU – give 1500 IU

Rh Immune Globulin; pooled RhD- immune plasma, cold ethanol-fractionated, S/D-treated, pasteurized, precipitated, filtered, ultra-filtered, and diafiltered	HyperRHO SD Full Dose	IM only	≥ 1500 IU/vial	Suppression of isoimmunization Transfusion: D+ blood or blood products Obstetric: Mother RhD- pregnancy/delivery of RhD+ infant – abortion or threat – ectopic pregnancy – fetomaternal hemorrhage Additional Guidelines: – 28 weeks' gestation – postpartum – RhD+ infant – RhD- infant – threatened abortion – abortion, invasive sampling, or any other manipulation at >15 weeks' gestation	Best if within 72 hours and <15 mL D+ exposure, give 1500 IU If >15 mL RhD+ blood exposure, give 1500 IU/ 15 mL of estimated D+ blood infused plus an extra 1500 IU
Rh Immune Globulin; same purification methods as for HyperRho SD	HyperRHO SD Mini-dose HyperRho SD	IM only	≥ 250 IU/vial	Suppression of isoimmunization: D- females at the time of abortion <12 weeks' gestation	Best if given within 72 hours

IM = intramuscular; IV = intravenous; IU = international units; ITP = idiopathic thrombocytopenic purpura; HIV = human immunodeficiency virus; S/D = solvent/detergent; Hb = hemoglobin.

ministration is expected to be reduced. In the setting of pregnancy and childbirth, the administration of RhIG or Rh IVIG is also recommended at 26 to 28 weeks of gestation for Rh-negative mothers when the father is Rh negative or his Rh status is unknown. It should be administered again within 72 hours after delivery if the infant's red cells test as Rh positive. The blood bank physician should be prepared to make indications and dosage recommendations regarding RhIG or Rh IVIG after the transfusion of Rh-positive red cells to Rh-negative recipients. RhIG or Rh IVIG is recommended in that setting for all females of childbearing potential to prevent hemolytic disease of the fetus and newborn in future pregnancies.

RhIG may be administered to other patients, men as well as women, to prevent anti-D formation that could complicate compatibility testing by causing a positive red cell antibody screen. However, the long-term advantage of preventing a positive antibody screen needs to be balanced against the short-term and transient prospect of a positive antibody screen attributable to the RhIG itself until it has been cleared from the circulation.

Rh Immune Globulin may be administered to Rh-negative males as well as females after the exposure of the patient to Rh-positive red cells to prevent the formation of anti-D, and to avoid possible issues with blood product availability for later transfusions. Regardless of the reason for administration, the presence of RhIG does complicate compatibility testing. The presence of the anti-D and other red cell antibodies, often present in detectable levels in RhIG products, causes an iatrogenic positive red cell antibody screen that persists until the antibodies have been cleared from the patient's circulation, in approximately 1 to 3 months, depending on a number of factors including the total dose administered.

Positive red cell antibody screens necessitate further workup (antibody panels) to rule out other alloantibodies. The goal should be to avoid positive antibody screens at the time of the most intensive transfusion therapy. Therefore, for patients other than females of childbearing potential, the use of RhIG or Rh IVIG should be investigated on a case-by-case basis, taking into account the patient's age, the patient's clinical status and prognosis, and the likelihood of further transfusion in the short term vs the long term. For elderly patients and those with poor prognoses, the prevention of Rh alloimmunization is not usually a clinical priority.

Rh IVIG is approved for the treatment of ITP.



Administration of RhIG complicates red cell compatibility testing because the passive anti-D will result in a positive antibody screen.



WinRho SDF (RhIG for IV administration) is approved for treatment of ITP in *Rh-positive patients*.

Rh IVIG

Rh IVIG, unlike the intramuscular preparation, is indicated for the treatment of acute and chronic childhood ITP, adult chronic ITP, and ITP secondary to HIV infection. Patients with ITP must be Rh positive and nonsplenectomized in order to benefit from treatment with Rh IVIG. Platelet counts in such patients typically rise within 1 or 2 days of treatment, peak at 7 to 14 days, and remain elevated for about 30 days. Response rates are generally in the range of 80% to 90%. The mechanism of action is believed to be the formation of anti-D-coated red cells that bind to and that block Fc receptors on phagocytes in the spleen. Because the Fc receptors are not available to bind and mediate the clearance of antibody-coated platelets, the platelet count rises.

Dosage. The dose of RhIG depends on the clinical situation. In the setting of pregnancy, one full dose of RhIG (1500 IU, or 300 µg) should be administered at 26 to 28 weeks of gestation, within 3 days of the following, or both: threatened abortion with continuation of pregnancy, abortion or termination of pregnancy at or beyond 13 weeks of gestation, amniocentesis, chorionic villus sampling, percutaneous umbilical blood sampling, and delivery of an Rh-positive neonate.

One full dose of RhIG is adequate to prevent Rh alloimmunization to 15 mL of Rh-positive red cells or 30 mL of Rh-positive WB. If the total fetal hemorrhage into the maternal circulation exceeds 15 mL of Rh-positive red cells or 30 mL of Rh-positive WB, one or more additional doses of RhIG is indicated, where the total dose is:

$$\text{No. of full doses} = \frac{\text{estimated fetomaternal hemorrhage}}{\text{(mL of Rh-positive red cells)}} \times \frac{1}{15 \text{ mL}}$$

or

$$\text{No. of full doses} = \frac{\text{estimated fetomaternal hemorrhage}}{\text{(mL of Rh-positive WB)}} \times \frac{1}{30 \text{ mL}}$$

Fractional doses should be rounded up to the next whole number. When multiple doses are indicated, they may be administered sequentially as long as they are given within 72 hours of exposure.

If spontaneous or induced abortion or termination of an ectopic pregnancy occurs before the 12th week of gestation, a minidose of RhIG (HyperRHO S/D Mini-Dose S/D



The RhIG dose required for prevention of sensitization of an Rh-negative mother by her Rh-positive fetus can be calculated as follows:

Determine the volume of fetal *whole blood* in the mother's circulation by solving for X:

$$\frac{\text{fetal red cells}}{\text{maternal red cells}} = \frac{X}{\text{maternal EBV}} \quad (80 \text{ mL/kg})$$

Divide estimated volume of Rh-positive whole blood by 30 mL to obtain number of full doses

or MICRhoGAM) may be administered instead of a full dose. The amount of anti-D in a minidose is approximately one-sixth of the full dose and is sufficient for suppressing the immune response to 2.5 mL of Rh-positive red cells or 4.0 mL of Rh-positive WB. The 12th week of gestation is the cutoff for the use of a minidose because the volume of red cells in a 12-week-old fetus does not exceed 2.5 mL.

For the treatment of ITP, only RhIG administered intravenously is approved in the United States. Rh IVIG has been shown to increase platelet counts in nonsplenectomized, D-positive patients with ITP, usually within 1 or 2 days, and peaking within 7 to 14 days after initiation of therapy. However, the initial response and the duration of the response to therapy are highly individualistic. The drug's mechanism of action is not completely understood but is thought to be caused by the coating of the D-positive red cells with the anti-D, forming antigen-antibody complexes that bind to the Fc receptors of the cells in the reticuloendothelial system, and sparing the binding of the antibody-coated platelets.

Some of the contraindications to the use of Rh IVIG for the treatment of ITP are identical to those of any immunoglobulin-containing product: it should not be administered to infants, to any individuals known to have had an anaphylactic or a severe systemic reaction to human globulin or albumin, or to individuals with IgA deficiency because the potential exists for the development of life-threatening IgA antibodies.

The potential benefit of using Rh IVIG for the treatment of ITP, as when using any product with human globulins, must be weighed against the potential for hypersensitivity reactions. Several drugs are available and have demonstrated remarkable results in the treatment of diseases that are the result of the formation of autoantibodies, including ITP. Please consult the *Physicians' Desk Reference* for specific details of those drugs.

For suppressing Rh alloimmunization after RBC transfusion, the intramuscular dose is, again, one full dose per 15 mL of Rh-positive red cells or 30 mL of Rh-positive WB to which the patient has been exposed. The product potency is expressed in IU in comparison to the WHO standard. A 1500-IU (300- μ g) vial contains sufficient anti-RhD to effectively suppress the immunizing potential of approximately 17 mL of D-positive red cells.

Risks. Rh IVIG should be administered with caution in patients with hemoglobin levels <8 g/dL, particularly if a substantial amount of circulating red cells are Rh positive.



RhIG for IV administration was developed to treat ITP in Rh-positive patients. It can also be used to prevent Rh alloimmunization after inadvertent administration of Rh-positive red cells (or platelet units containing red cells) to Rh-negative individuals.

That situation could result in severe anemia. In addition, Rh IVIG should be administered only if the amount of Rh-positive red cells in circulation is estimated to be <20%. Greater amounts increase the likelihood of an extravascular hemolytic reaction.

Fibrin Sealant

Products available in the United States include Tisseel VH Kit (Osterreichisches Institut fur Haemoderivate Ges.m.b.H., Vienna, Austria) and Hemaseel APR Kit (Haemacure, Montreal, Quebec, Canada). Fibrin sealant, also known as fibrin glue, is an agent that is applied topically to tissue in surgical sites to promote hemostasis or adhesion. It consists of two blood derivatives: a fibrinogen preparation and a thrombin preparation, which are mixed together just before use. The fibrinogen and thrombin in those products are obtained from large pools of human plasma.

The fibrinogen is prepared from the cryoprecipitate of Source Plasma, and the thrombin source is the prothrombin in anti-inhibitor coagulant complex (ie, an activated Factor IX complex). Both the fibrinogen and prothrombin preparations used in fibrin sealant undergo virucidal treatment by vapor heating. Immediately before use, the fibrinogen is mixed with bovine aprotinin, a fibrinolysis inhibitor, and the prothrombin preparation is mixed with a calcium chloride solution to stimulate the conversion to thrombin. Finally, the fibrinogen/aprotinin solution is mixed with the thrombin/calcium chloride solution to generate fibrin, which has sealant properties when applied topically.

At many hospitals, fibrin sealant is still prepared from individual units of blood-bank-supplied allogeneic cryo mixed just before use with bovine thrombin. The number of donor exposures is significantly less with such preparations, although no virucidal treatment is carried out. Autologous cryo can also be made for use in preparing autologous fibrin sealant, which should be the safest product available with regard to infectious risk. However, commercial preparations have the advantage of a standardized fibrinogen content, making them more uniform, quality-controlled products.

Alpha₁-Proteinase Inhibitor

Prolastin (Talecris) is the only product available in the United States. Alpha₁-proteinase inhibitor, also known as alpha₁-antitrypsin, is isolated from large pools of plasma by cold ethanol fractionation techniques.



Fibrin sealant consists of fibrinogen and thrombin that are mixed together immediately before use and applied topically as a hemostatic agent, most notably in cardiothoracic and neurosurgical procedures.

Alpha₁-proteinase inhibitor is indicated for chronic replacement therapy in certain individuals with inherited alpha₁-antitrypsin deficiency. The most severely affected individuals with alpha₁-antitrypsin deficiency have alpha₁-proteinase inhibitor levels <35% of normal. Those individuals may suffer from a slowly progressive, severe emphysema that is usually fatal. The alpha₁-proteinase inhibitor is the principal inhibitor of neutrophil elastase, which breaks down elastin. Emphysema is believed to result from the chronic unopposed action of neutrophil elastase in the lungs—an action that may occur in the absence of adequate levels of alpha₁-proteinase inhibitor.

Alpha₁-antitrypsin is administered intravenously once a week at a recommended dose of 60 mg/kg of body weight. Eligible patients are only those with early evidence of emphysema and with the most severe forms of alpha₁-antitrypsin deficiency.

Recombinant Hematopoietic Factors

Recombinant Human Granulocyte and Granulocyte/Macrophage Colony-Stimulating Factors

Description

Recombinant human granulocyte colony-stimulating factor (G-CSF) products include Neupogen (Amgen, Thousand Oaks, CA) and Neulasta (Amgen). The only recombinant human GM-CSF product licensed in the United States is Leukine (Bayer Schering Pharma AG, Berlin, Germany).

G-CSF and GM-CSF are growth factors that stimulate the proliferation of granulocyte only or both granulocyte and macrophage-committed marrow progenitor cells and their further differentiation into mature granulocytes and macrophages. G-CSF is a lineage-specific factor that stimulates the production and functional activation of neutrophils but has a minimal direct effect on other hematopoietic cells. In contrast, GM-CSF is a multilineage factor that affects not only the neutrophil and monocyte/macrophage production but also the megakaryocytic and erythroid lineages. GM-CSF stimulates the progenitor known as granulocyte-erythrocyte-macrophage-megakaryocyte colony-forming cell to differentiate into precursors for the individual cell lineages. It also promotes the further differentiation and proliferation of mature granulocytes, eosinophils, basophils, and mono-



G-CSF and GM-CSF stimulate the proliferation of granulocyte and granulocyte-macrophage progenitor cells, respectively. These preparations can be used in treatment of postchemotherapy neutropenia and also to stimulate production of neutrophil precursors before collection of hematopoietic progenitor cells for transplantation.

cytes, and it stimulates the function of mature neutrophils, eosinophils, and monocytes.

Preparation. The coding portions of G-CSF and GM-CSF genes are isolated and inserted into the bacterium *Escherichia coli* or the yeast *Saccharomyces cerevisiae*, respectively, in order to produce recombinant versions of those factors for pharmaceutical use (rHuG-CSF and rHuGM-CSF).

Use. rHuG-CSF and rHuGM-CSF are indicated for use after myelosuppressive chemotherapy in cancer patients to speed the recovery of neutrophil levels and to reduce the risk of infections. They are also used after marrow transplantation to enhance the engraftment and to speed the production of neutrophils in order to reduce the risk of posttransplant infections. They may be used before leukapheresis for the collection of hematopoietic progenitor cells (HPCs) in the setting of autologous and allogeneic HPC transplantation in order to increase the number of progenitor cells in the donor's circulation and to improve collection yields.

Risks. The toxic side effects of GM-CSF are reportedly greater than those of G-CSF, particularly at doses above 5 g/kg/day. The effects are probably caused by the stimulatory effect of GM-CSF on monocyte/macrophage activation. The toxicity, which is seen most often on first doses and is generally self-limited, is characterized by dyspnea, flushing, tachycardia, hypotension, and musculoskeletal pain.

Recombinant Human Interleukin-11

Description

Interleukin-11 (Neumega, Wyeth) is a thrombopoietic growth factor that stimulates the proliferation of megakaryocyte progenitor cells as well as megakaryocyte maturation and platelet production.

Preparation. The coding portion of the IL-11 gene has been engineered using recombinant DNA techniques to permit the production of a fully bioactive, nearly complete recombinant human IL-11 (rhIL-11) in *E. coli*. The recombinant protein is 177 amino acids long, which is one shorter than the native protein, and it lacks only one amino-terminal proline residue.

Use. rhIL-11 is FDA-licensed for administration after myelosuppressive chemotherapy to patients who have nonmyeloid malignancies and who are at high risk of severe thrombocytopenia. The product is used to reduce the severity of the thrombocytopenia and the need for platelet transfusions. Recommendations for use are limited to pa-



Recombinant human interleukin-11 is FDA-licensed for reduction of the severity of thrombocytopenia and the need for platelet transfusion in patients at high risk of severe chemotherapy-induced thrombocytopenia. It is not indicated in the setting of myeloid malignancies after myeloablative chemotherapy, however.

tients who have experienced severe thrombocytopenia in earlier chemotherapy or who are receiving chemotherapy regimens with high risk for severe thrombocytopenia (eg, high-dose regimens).

Administration of rhIL-11 decreases the percentage of patients who require platelet transfusions. For example, about 20% of breast cancer patients who received rhIL-11 after chemotherapy required platelet transfusion, as compared with about 50% of the control patients. Platelet counts rise between 5 and 9 days after administration and continue to rise for up to a week after discontinuation of dosing.

Risks. rhIL-11 is not recommended for routine use for the reduction of thrombocytopenia in all patients who have nonmyeloid cancers and who are receiving chemotherapy, only those at particularly high risk for platelet transfusion. Also, it is not indicated in the setting of myeloid malignancies after myeloablative chemotherapy. For example, after autologous marrow transplantation for myeloid malignancy, rhIL-11 administration neither reduces platelet transfusion requirements nor shortens the time to platelet engraftment.

Dosage. The recommended adult dose is 50 µg/kg given by subcutaneous injection once daily, and the recommended pediatric dose is 75 to 100 µg/kg. Administration should begin 6 to 24 hours after the completion of chemotherapy and should continue until the platelet count is >50,000/µL or for 21 days, whichever comes first. The administration of rhIL-11 should be discontinued at least 2 days before the start of any subsequent chemotherapy.



Two recombinant thrombopoietin (TPO) preparations have been produced. Recombinant TPO preparations have shown promise in elevating platelet counts in patients with nonmyeloid malignancies undergoing chemotherapy.

Recombinant Human Thrombopoietin

Description

Thrombopoietin (TPO) is a constitutively hepatic-produced endogenous molecule that stimulates platelet production. Endogenous TPO acts on early stages of hematopoiesis by stimulating the marrow pluripotential stem cell as well as early megakaryocyte, myeloid, and erythroid progenitors. TPO also stimulates late maturation in the megakaryocyte lineage, resulting in increased platelet production.

Preparation. Endogenous TPO is also known as *Mpl* ligand. This terminology derives from the original research that led to the discovery that the oncogene *v-mpl* of the murine myeloproliferative leukemia virus was a truncated version of the TPO receptor gene. Subsequent investigation led to the isolation of the full-length human cellular

homologue of *v-mpl*, that is, the human TPO receptor. That finding in turn led to the isolation of the TPO gene. Because TPO is the ligand of the TPO receptor and *Mpl* is the TPO receptor, TPO can be thought of as the *Mpl* ligand.

The coding region of the TPO gene has been used to produce synthetic recombinant versions of the natural molecule. Two different TPO preparations have been produced and used in human trials. Neither preparation at present is FDA-licensed.

One preparation is a full-length, glycosylated molecule called recombinant human thrombopoietin (rHuTPO), which is produced in CHO cells. The other preparation, called recombinant human megakaryocyte growth and development factor (rHuMGDF), is a recombinant truncated version (amino acids 1-163) of the native molecule that consists predominantly of the TPO receptor-binding domain. It is both glycosylated and PEGylated (ie, conjugated with polyethylene glycol) and is produced in *E. coli* (PEG-rHuMGDF). The polyethylene glycol is coupled to the polypeptide product to protect it from degradation. Both preparations appear to have similar in-vivo biologic activity.

Use. Promising preliminary results have been obtained with TPO preparations in elevating platelet counts in chemotherapy of nonmyeloid malignancies (eg, ovarian cancer) and in myelodysplastic syndrome, HIV-related thrombocytopenia, and thrombocytopenia of liver disease. Somewhat unexpectedly, the effects of administered TPO seem to be limited to the megakaryocyte lineage, that is, only platelet counts are increased, despite studies that indicate multilineage effects of endogenous TPO. Additional clinical outcome studies are under way to clarify the nature and magnitude of the benefit of exogenous TPO in those and various other clinical settings.

Unfortunately, trials of both TPO preparations have revealed no clinical benefit in decreasing platelet transfusion requirements after myeloablative chemotherapy for the treatment of acute leukemia or during marrow transplantation. Such results may be caused by the already high circulating endogenous TPO levels in such patients. TPO is produced constitutively in the liver and appears to be regulated by its binding to circulating platelets. When platelet counts are normal, TPO is bound to platelets to a greater extent and therefore becomes less biologically available. However, when platelet counts are low, the unbound and biologically active TPO levels are higher. That mechanism is a simple yet elegant feedback system for raising the TPO level when it is needed most. However,



Trials of both TPO preparations have revealed *no* clinical benefit in reducing transfusion requirements after myeloablative chemotherapy in acute leukemia or during marrow transplantation.

when that mechanism has already maximally elevated endogenous TPO levels, there may be limited added value of administering exogenous TPO, at least in some clinical settings.

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14

Therapeutic Apheresis

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THE TERM APHERESIS DERIVES FROM THE Greek, meaning “to carry away.” The technique of apheresis involves removing whole blood through a central or peripheral venous catheter, separating the desired portion from the remaining blood, and removing the desired fraction while returning the remainder. Plasma (plasmapheresis), white cells (leukapheresis), red cells (erythrocytapheresis), platelets (plateletapheresis), and even hematopoietic progenitor cells (HPC-A) can be removed by apheresis. Apheresis can be used for therapy (therapeutic apheresis) or to facilitate collection of a blood component, such as platelets or granulocytes, from an allogeneic donor. This chapter primarily discusses therapeutic apheresis.

The simplest of apheresis machines consists of a large-bore intravenous catheter connected to a spinning centrifuge bowl. Whole blood is drawn from the donor or patient into the centrifuge bowl, in which the denser elements (red cells) settle to the bottom, with less dense elements such as white cells and platelets overlying the red cell layer and finally with plasma at the very top and overlying all three cellular components. In plasmapheresis, the plasma layer is collected into a collection bag, and the red cells and buffy coat layers are returned to the patient after being mixed with a plasma substitute, usually 5% albumin or a mixture of normal (0.9%) saline and 5% albumin. Because all current apheresis equipment uses advanced



Apheresis is a technique in which whole blood is taken from a person and separated extracorporeally, allowing the desired portion (eg, plasma) to be removed and the remainder returned to the individual.

microcomputer technology, it is a simple matter to adjust the system to collect platelets, red cells, white cells, or HPC-A instead of plasma.

If, instead of returning plasma substitute to the patient, one returns allogeneic plasma, the technique is called “plasma exchange” rather than “plasmapheresis.” Likewise, although the process by which sickled red cells are removed is often called “erythrocytapheresis,” it is probably more accurately described as “red cell exchange” because the removed sickled red cells are replaced by allogeneic red cells rather than by red cell substitute. Note that the preceding semantic distinctions are not universally applied. Both terms are used interchangeably by many in the apheresis community.

Most early apheresis machines used the spinning bowl technique. This method has the disadvantage of requiring alternate draw and return cycles instead of permitting continuous flow apheresis. As a result, the patient has a significant volume of red cells in the extracorporeal circuit at the end of each draw cycle, which must be returned before a new draw cycle can be started. The exact amount of extracorporeal volume will depend on the size of the bowl, but it is not uncommon to have 225 mL of red cells—the equivalent of 1 unit—outside the patient’s circulation immediately before return.

Newer technology significantly reduces the extracorporeal volume by using one of several continuous-flow devices to supplant the earlier generation intermittent-flow machines described above. Continuous-flow devices use a circular spinning plastic belt as a collection chamber. Centrifugal force pushes red cells to the outside of the belt, with lighter elements arraying themselves closer to the center of the circle. A tube is positioned in the belt at levels designed to collect the desired component while allowing the remainder to be returned to the patient. Virtually all apheresis machines used in the United States are designed for continuous-flow collection.



Early apheresis machines required alternating draw and return cycles, but current technology permits continuous flow during apheresis procedures.



Apheresis can be used to remove undesirable substances, such as unwanted antibodies or HbS-containing red cells, from a patient’s bloodstream. It can also be used to collect a single desired blood component (eg, platelets) while returning the rest to the patient.

Uses of Apheresis

Therapeutic apheresis can be used to remove undesired antibodies or even lipids from the circulation, to reduce excess leukocytes or platelets in patients with myeloproliferative disorders, and to automatically and rapidly exchange sickled red cells with normal red cells from an allogeneic donor.

In addition, marrow progenitor cells can be mobilized into the peripheral blood through the use of granulocyte colony-stimulating factor (G-CSF), which directly stimulates marrow production of myeloid precursors; the use of cyclophosphamide, which acts indirectly by reducing white count and thus inducing feedback stimulation of hematopoiesis; or both. Brisk hematopoiesis results in "spillover" of HPCs into the peripheral blood, where they can be collected by apheresis, frozen, and later used for progenitor cell rescue after myeloablative therapy.

Table 14-1 contains a synoptic list of diseases amenable to treatment by apheresis. Appendix 14-1 contains a comprehensive summary of disease states for which apheresis has been used, divided into four categories according to the strength of evidence supporting use of apheresis.

Table 14-1. Diseases for which Apheresis or Plasma Exchange Is Indicated

Apheresis/plasma exchange: standard therapy

- Chronic inflammatory demyelinating polyneuropathy
- Cryoglobulinemia
- Cutaneous T-cell leukemia-lymphoma (photopheresis)
- Familial hypercholesterolemia (immunoabsorbent column apheresis)
- Goodpasture's syndrome
- Guillain-Barré syndrome
- Hyperviscosity
- Idiopathic thrombocytopenic purpura—immunoabsorbent column apheresis
- Leukostasis (associated with leukemic hyperleukocytosis)
- Myasthenia gravis (including Eaton-Lambert syndrome)—in crisis or prethymectomy
- Posttransfusion purpura
- Sickle cell anemia—in crisis
- Thrombocytosis
- Thrombotic thrombocytopenic purpura—plasma exchange

Apheresis/plasma exchange: adjunctive therapy

- Marrow-ablative therapy requiring "progenitor cell rescue" (peripheral blood progenitor cell transplant)
- Rapidly progressive glomerulonephritis (antineutrophil cytoplasmic antibody-positive)

Practical Considerations



Apheresis requires venous access through a large-bore peripheral or central vein. The catheter used must be stiff enough to sustain the high flow rates involved.

Vascular Access

A number of practical considerations are involved in the decision about whether a patient or donor should undergo apheresis. First, one must have adequate vascular access through intravenous lines of sufficient caliber and stiffness to sustain the high flow rates required. A single line may be used for intermittent-flow devices, which can draw and return through the same line. Continuous-flow devices usually require separate lines to draw whole blood from the donor or patient and to return the portions that are not being collected or removed.

When apheresis is planned on an occasional basis, as with platelet donors or with red cell exchange for sickle crisis, large-bore intravenous line(s) that are introduced through antecubital vein(s) and are removed immediately after the procedure may suffice. Femoral vein access is an option that permits slightly longer-term access in patients at bed rest, but the risk of thrombosis associated with the prolonged presence of a femoral catheter limits use of that site to a maximum of several days. If multiple procedures are planned over a period of days to weeks, it may be necessary to use a central vein, most often the internal jugular, to introduce a catheter designed to remain in place for a period of weeks to months.



Replacement fluids used for apheresis must be FDA-approved for use with blood components. Approved fluids include:

- Normal saline (0.9% USP)
- 5% albumin
- (Allogeneic) human plasma (FFP or cryo-reduced)

Replacement Fluid

The second practical concern is the choice of replacement fluid. In all cases, the fluid chosen must be approved by the Food and Drug Administration (FDA) for use with blood components because it will be admixed with red cells before the return phase. That requirement functionally limits the options to three: normal saline (0.9%), plasma, and 5% albumin.

At first glance, normal saline would appear to be the obvious choice because it is the fluid used most often in blood transfusion. The disadvantage to using normal saline alone is that it equilibrates rapidly with the extravascular space. Thus, one must either add colloid in the form of 5% albumin or replace removed plasma with approximately three times the volume of normal saline. A one-volume plasmapheresis would necessitate the use of 9 L of normal saline to replace the 3 L removed in a 70-kg patient. As a consequence, replacement for plasma removed through

plasmapheresis is usually accomplished with 5% albumin alone or with a 50-50 mixture of normal saline and 5% albumin. The latter is typically accomplished by alternating a bag of normal saline with a bag of 5% albumin.

Replacement with plasma carries with it the same infection risks as simple transfusion of allogeneic plasma. Therefore, replacement with plasma rather than saline and albumin is indicated only in the setting of plasma exchange for thrombotic thrombocytopenic purpura (TTP) or for liver failure. In those cases, the replacement plasma is felt to contain a vital element lacking in the patient. In the case of TTP, that constituent is the ADAMTS-13 protease responsible for cleaving von Willebrand factor. Plasma exchange in liver failure is used to replace the coagulation factors not being produced by the failed liver. In the latter situation, the volumes that would need to be infused by simple transfusion to accomplish such a replacement would result in intolerable fluid overload were the deficient plasma not being removed at the same time.



If normal saline is used without colloid, the volume replaced must be three times that removed because saline equilibrates rapidly with the extravascular space.

Patient's History and Medications

A third consideration is evaluation of the patient's history and medications. In the case of therapeutic apheresis, one must determine that the patient has a disease that is amenable to treatment by apheresis. Anyone undergoing apheresis—donor or patient—must be deemed capable of sustaining the fluid shifts associated with apheresis without developing hypotension or myocardial ischemia.

Medication history is vital for several reasons. First, certain medications—particularly antibiotics and anticoagulants—can be removed by apheresis and should be given immediately after apheresis rather than before the procedure. Certain drugs, most notably angiotensin-converting enzyme (ACE) inhibitors, may interfere with apheresis. ACE inhibitors not only block conversion of angiotensin I to angiotensin II but also inhibit the breakdown of bradykinin. In the setting of apheresis, the inhibition of bradykinin catabolism can result in profound hypotension. Therefore, it is often recommended that ACE inhibitors be withheld for at least 24 hours before apheresis procedures. ACE inhibitors are contraindicated in immunoabsorbent column (ProSORBA column) apheresis because of the risk of anaphylaxis. Finally, plasma cholinesterase is removed along with other plasma constituents during plasmapheresis. The diminution in cholinesterase activity can result in prolonged apnea if the patient undergoes general anesthe-



ACE inhibitors can interfere with apheresis because they inhibit breakdown of bradykinin. The inhibition of bradykinin catabolism can result in profound hypotension.

ACE inhibitors should be withheld for at least 24 hours before apheresis procedures.



The amount of volume that can be in the extracorporeal portion of the circuit at any time should not exceed 15% of the patient's total blood volume. In the case of intermittent flow apheresis this can be estimated by

$$\text{Extra-corporeal volume} = \frac{\text{bowl volume} \times 0.8}{\text{patient Hct}}$$

sia with suxamethonium shortly after apheresis. That possibility has prompted some practitioners to recommend allowing at least 72 hours to lapse between plasmapheresis and suxamethonium anesthesia.

Extracorporeal Volume

Fourth, safety concerns dictate that the maximum permissible extracorporeal volume should not exceed 15% of the individual's total estimated blood volume, meaning that one must know both the patient's (or donor's) weight and hematocrit in order to calculate the extracorporeal volume, particularly when using an intermittent-flow apheresis machine. For adults, total blood volume is estimated at 70 mL/kg body weight. In an intermittent-flow apheresis device, the hematocrit of the bowl contents is approximately 80%. In other words, the extracorporeal volume can be estimated by multiplying 0.8 times the bowl volume and dividing by the patient's hematocrit:

$$\text{Extracorporeal volume} = \frac{\text{bowl volume} \times 0.8}{\text{patient hematocrit}}$$

Bowl volumes usually average 225 mL, although smaller bowls are available for pediatric use. Use of continuous-flow devices that minimize the extracorporeal volume obviates the need for such strict calculation.



A 1.0 volume exchange will remove about 65% of most plasma constituents; 1.5-volume exchange removes 80%; and 2-volume exchange removes 88%.

Percentage of Constituents Removed

Fifth, one must consider the percentage of normal and abnormal blood constituents that are removed. For plasmapheresis or plasma exchange, a 1.0-volume exchange removes about 65% of most plasma constituents, whereas a 1.5-volume exchange removes approximately 80%. A single 2.0-volume exchange removes about 88% of plasma constituents. Note that increasing the volume of exchange beyond 1.0 to 1.5 volumes has very little effect on removal of plasma constituents, including pathologic substances, thus explaining why plasmapheresis is usually limited to 1.0- to 1.5-volume exchanges per procedure.

Because most coagulation factors are lost at approximately the same rate, hemostatic balance between procoagulant and anticoagulant forces is maintained. Coagulation factors are rapidly synthesized in most patients, so depleted factors are replaced within 2 or 3 days after

exchange. As a consequence, clotting parameters such as PT (prothrombin time), aPTT (activated partial thromboplastin time), and fibrinogen level generally need to be measured only every 2 to 3 days rather than daily, even in patients undergoing daily plasmapheresis. Most plasma constituents return to pretreatment levels in 48 to 72 hours, but fibrinogen and complement lag somewhat and immunoglobulins (IgM and IgG) will likely remain low even after 2 weeks.

Cellular blood constituents are also decreased by apheresis, but continuous-flow devices minimize the red cell loss to approximately 50 mL lost in the tubing per procedure. Platelet counts decrease at a rate of 25% to 30% per procedure, but endogenous synthesis replaces lost platelets within 2 to 4 days, except in patients with hypoplastic or aplastic marrow.



Continuous flow devices reduce red cell losses to approximately 50 mL per procedure. Platelet counts, on the other hand, decrease at a rate of 25% to 30% per procedure.

Follow-up Testing

One final practical consideration is the timing of follow-up laboratory tests, if indicated. It is possible to measure coagulation parameters and chemistries immediately after apheresis, but obtaining the results on the following morning or immediately before the next procedure might yield results that are clinically more useful—reflecting not only equilibration between the intra- and extravascular compartments but also recovery that is based on synthesis of replacement constituents. When following lactate dehydrogenase (LDH) levels in patients with TTP, timing of follow-up tests is particularly important. Removal of LDH during the procedure can leave a false impression of improvement if measurement is performed immediately after exchange; measuring LDH immediately before the patient's next procedure will better reflect the amount of ongoing hemolysis.



Measurement of postprocedure coagulation parameters and chemistries is better performed the morning after the procedure or immediately before the next procedure.

Risks of Apheresis and Obtaining Consent

Compared with many diagnostic and therapeutic procedures, the risks of apheresis are small but nonetheless real. Risks and benefits must be carefully discussed with the patient (or donor), and consent must be obtained before beginning apheresis. The risks associated with apheresis can be divided into three main categories: line-related, procedure-related, and anticoagulant-related.

Line-Related Risks



Line-related risks of apheresis include bleeding and infection.

Line-related risks include hemorrhage and infection. Hemorrhage can occur when the line is introduced, when it is removed, or while it is in place. The risk of major hemorrhage is less with peripheral lines, which are more readily observed for signs of excessive bleeding and amenable to local compression should bleeding occur. Central lines cannot be directly observed and can sustain significant hemorrhage before the presence of bleeding is apparent. Placement of thoracic central lines also carries the risk of traumatic pneumothorax, so it is advisable to obtain a chest x-ray immediately after central line placement. Obviously, bleeding is a greater risk in patients receiving systemic anticoagulant therapy and in patients with marked thrombocytopenia. Nonetheless, patients with profoundly low platelet counts caused by TTP can and do survive central line placement as long as the placement procedure is performed cautiously, often under radiographic guidance by the interventional radiology service.

Infection is always a risk, albeit small, whenever the integrity of the skin is breached by an intravenous needle. Of more concern is infection associated with lines maintained in place for months at a time. Aseptic technique must be used whenever the line is accessed. If the patient is undergoing intermittent apheresis as an outpatient, it is particularly important for the patient to be carefully instructed in aseptic techniques for maintaining line patency. Failure of aseptic technique has the potential to be catastrophic, resulting in bacteremia, endocarditis, and even frank septic shock. Fortunately, infectious complications are rare.



Procedure-related risks of apheresis include:

- Hypotension, myocardial infarction associated with fluid shifts
- Anemia, thrombocytopenia
- Reduced levels of coagulation factors, other proteins

Risks if replacement fluid is allogeneic plasma:

- Transfusion-transmitted (viral) disease
- Transfusion-related acute lung injury

Procedure-Related Risks

Procedure-related risks include decreases in red cell and platelet counts plus lowered levels of proteins, particularly coagulation factors. In addition, even in a procedure that is euvolemic overall (fluid volume removed is equal to fluid volume replaced), significant fluid shifts are continually taking place. Such shifting is particularly true with intermittent-flow procedures that involve relatively large extracorporeal volumes. However, even continuous-flow procedures involve significant equilibration between the intravascular space and the extravascular compartment. Hypotension and even myocardial infarction, although uncommon, can be associated

with apheresis procedures, especially in patients with underlying coronary disease. A diligent cardiac history is imperative, but even relatively young patients can have unrecognized coronary artery disease. Any complaint of chest pain or dyspnea during an apheresis procedure or shortly thereafter should be carefully investigated.

Finally, procedures that involve replacement of removed plasma with allogeneic plasma carry the same risks as any transfusion of plasma, including anaphylactoid reaction, transfusion-related acute lung injury (TRALI), and the transmission of infectious disease. Those conditions are discussed in detail in Chapter 7: Adverse Effects of Transfusion.

Anticoagulant-Related Risks

While circulating extracorporeally, the patient's blood must be anticoagulated. Citrate is the anticoagulant of choice in most cases, although heparin may be used in some.

Upon return to the patient, the infused citrate is processed by the patient in a number of ways (including dilution and redistribution, metabolism in the liver, and excretion by the kidneys), so that significant in-vivo anticoagulation does not occur, unlike when heparin is used. Usually, citrate toxicity is limited to perioral tingling, cramping of the extremities, or both. However, citrate binds (chelates) calcium, so markedly elevated levels of citrate are capable of resulting in hypocalcemia, arrhythmia, and even frank tetany.

In most cases, the symptoms of citrate toxicity can be relieved by stopping the procedure transiently and allowing the infused citrate to be metabolized or excreted with or without the use of oral calcium supplements. More severe symptoms or symptoms that persist despite these interventions should be treated with intravenous calcium infusion. Patients who are most prone to citrate toxicity are small patients, especially those receiving large volumes of citrated blood components, and patients with liver dysfunction, which interferes with metabolism of citrate. Because collection of HPC-A typically involves processing large volumes of blood (12-20 L), many centers use prophylactic intravenous calcium during progenitor cell collections.

Arrhythmia can also be precipitated by cold, so some centers use blood warmers for apheresis in selected patients, especially when collecting from small children.



Anticoagulant-related risks include citrate toxicity. This is usually limited to perioral tingling, but may rarely progress to arrhythmia, seizures, or even frank tetany resulting from hypocalcemia.

Therapeutic Plasmapheresis

Plasmapheresis is most commonly used to remove pathogenic antibodies [eg, in Guillain-Barré syndrome (GBS), myasthenia gravis (MG), and Goodpasture's syndrome (GPS)], immune complexes (eg, in immune thrombocytopenic purpura), or the cryoglobulins of cryoglobulinemia. In certain disease states, plasmapheresis is more effective when combined with immunosuppressive therapy.

Use in Guillain-Barré Syndrome and Chronic Demyelinating Polyneuropathy

Guillain-Barré syndrome is an acute immune-mediated demyelinating polyneuropathy that presents as ascending paralysis, usually symmetrical, accompanied by absence of deep tendon reflexes. The onset of GBS symptoms is often preceded by respiratory or gastrointestinal infection, including an association with *Campylobacter jejuni* infection. GBS has also been reported after vaccination for influenza. Electrodiagnostic studies can be used to establish the diagnosis.

The causative antibody in GBS is directed against gangliosides. Several studies have shown intravenous immune globulin (IVIG) and plasmapheresis to be equally effective in treating GBS. Thus, some centers rely primarily on IVIG, whereas others prefer to use every-other-day plasmapheresis as primary therapy. In patients treated with plasmapheresis, a follow-up course of IVIG is sometimes instituted if the patient experiences difficulty in maintaining gains achieved through plasmapheresis. However, such sequential treatment is of dubious efficacy and is not recommended. Indeed, a recent practice parameter promulgated by the American Academy of Neurology's Quality Standards Subcommittee concluded that treatment with either plasmapheresis or IVIG "hastens recovery from GBS. [But] combining the two treatments is not beneficial." At present, insufficient data exist to support use of immunoadsorption apheresis (described later in this chapter) for treatment of GBS. Steroids no longer play a role in treating GBS.

Autonomic instability is one of the hallmarks of GBS, so particular attention must be paid to monitoring vital signs during plasmapheresis for GBS.

Often characterized as a form of "chronic GBS," chronic inflammatory demyelinating polyneuropathy (CIDP) also appears amenable to treatment with plasmapheresis, as does the slowly progressive polyneuropathy associated



Autonomic instability is a hallmark of Guillain-Barré syndrome (GBS), so careful monitoring of vital signs is crucial when removing the causative antibody by plasmapheresis.

with IgM antibody directed against myelin-associated glycoprotein (MAG) antibodies. In the latter case, immunosuppressive drugs such as cyclophosphamide are often used as an adjunctive therapy.

Use in Myasthenia Gravis

In MG, the causative autoantibody is directed against the acetylcholine receptor, resulting in proximal weakness of the muscles of the eye, mouth, and throat. Presenting symptoms include ptosis and diplopia, dysphagia, and dyspnea. Pharmacologic treatment with pyridostigmine (Mestinon, Valeant Pharmaceuticals International, Costa Mesa, CA) is the mainstay of treatment for MG. A variety of different immunosuppressive medications, including corticosteroids, azathioprine, and cyclosporine, may be added when pyridostigmine alone fails to relieve symptoms.

Plasmapheresis may be used as an acute, short-term treatment in cases of respiratory crisis and rapidly progressive disease, as well as in patients being prepared for thymectomy. IVIG is an alternative that, like plasmapheresis, provides a means of rapid immunomodulation in similar settings (eg, myasthenic crisis, prethymectomy).

Because pyridostigmine can be removed by plasmapheresis, it is advisable to hold the dose until after the procedure, if possible. Even with that precaution, there remains a risk of postprocedure respiratory depression, particularly in patients presenting with respiratory symptoms, rapidly progressive disease, or both. Close monitoring of respiratory status in the hours immediately after plasmapheresis for MG is advisable. Eaton-Lambert syndrome, which is acute onset myasthenia associated with carcinoma of the lung, is also amenable to treatment with plasmapheresis.

Use in Goodpasture's Syndrome

Pulmonary hemorrhage and renal failure are the presenting findings of Goodpasture's syndrome, which results from antibody against glomerular basement membrane (GBM). Circulating GBM antibodies are detectable in the vast majority of cases, and cross-reactivity of those antibodies with alveolar basement membrane is felt to be the cause of the pulmonary hemorrhage. The exact trigger for production of GBM antibodies remains unclear. Plasmapheresis is the mainstay of therapy for GPS, but it must be accompanied by high-dose corticosteroids and cyclophosphamide



Plasmapheresis can be used to remove the acetylcholine receptor antibody that causes myasthenia gravis (MG). Postprocedure respiratory depression is a risk in patients with respiratory involvement by MG and those with rapidly progressing disease.



Plasmapheresis can be used to remove anti-GBM in Goodpasture's syndrome.

to suppress autoantibody production. Immunosuppressive medications are continued beyond the cessation of plasmapheresis to ensure that antibody production remains controlled.

Use in Dysproteinemia: Cryoglobulinemia, Hyperviscosity Syndromes, Waldenstrom's Macroglobulinemia, and Multiple Myeloma

Cryoglobulins, which are immunoglobulins that precipitate in the cold and redissolve when warmed, can be seen in association with hepatitis B or hepatitis C infection, with lymphoproliferative diseases such as multiple myeloma and Waldenstrom's macroglobulinemia, or with autoimmune diseases. Cryoglobulin-induced immune complexes can result in vasculitis, synovitis, or proliferative glomerulonephritis. In addition, hyperviscosity can be seen with high concentrations of cryoglobulins. Finally, immunoglobulin paraprotein may form complexes with clotting factors and may result in bleeding.

Response to plasmapheresis depends in part on the immunoglobulin class of the cryoglobulin, with better responses being noted with IgM or mixed cryoglobulins than with IgG cryoglobulins. Once the underlying disease process responsible for cryoglobulin production is determined, specific therapy must be initiated—for example, antiviral therapy for hepatitis C, antibiotics for bacterial infection-associated cryoglobulinemia, or immunosuppressive drugs for autoimmune or lymphoproliferative states.

Hyperviscosity caused by very high plasma immunoglobulin concentrations can be seen in Waldenstrom's macroglobulinemia and occasionally in multiple myeloma. Typical presentation is with elevated plasma viscosity (three to four times that of water), accompanied by a variety of neurologic findings that can range from dizziness, vertigo, ataxia, confusion, and headache to dementia, stroke, or even coma. Fundoscopic examination may reveal venous dilatation, hemorrhage, or exudates, or a combination of them. Measured viscosity demonstrates a poor correlation with symptoms when comparing one patient to another, but viscosity correlates well with clinical signs and symptoms when measured serially in the same patient.

The pathologic immunoglobulin in Waldenstrom's macroglobulinemia is IgM in class and, thus, is predominantly intravascular, making it particularly amenable to removal by plasmapheresis. Typically, the hyperviscosity of the condition responds to one or two 1.0-volume exchanges,



Hyperviscosity associated with Waldenstrom's macroglobulinemia or with multiple myeloma (MM) responds well to plasmapheresis, but the underlying condition must be treated to prevent recurrent episodes.

although maintaining a response requires treatment of the underlying disorder with appropriate chemotherapy.

Multiple myeloma itself does not respond to plasmapheresis, but hyperviscosity is amenable to treatment by apheresis. Hyperviscosity associated with myeloma may require more aggressive apheresis than is needed for Waldenstrom's macroglobulinemia because the immunoglobulins involved (IgG most commonly, followed by IgA) are rarely confined to the intravascular compartment but are also found in the extravascular space where they are less amenable to removal by plasmapheresis.

Use in Posttransfusion Purpura

Posttransfusion purpura (PTP) develops when a human platelet antigen-negative patient develops antibody directed against a high-incidence platelet antigen (eg, HPA-1a) as the result of allogeneic stimulation. PTP manifests clinically as a decrease in platelet count during the 3 weeks after red cell or platelet transfusion in a patient with prior allogeneic exposure either through transfusion or pregnancy.

Because the antibody is an alloantibody, one would expect that the patient's own platelets would be spared—but this assumption appears not to be the case. High-dose IVIG is considered the treatment of choice for PTP. Plasmapheresis to remove the offending antibody is generally reserved for those patients who fail to respond to IVIG therapy.

The mechanism and treatment of PTP are discussed at more length in Chapter 7: Adverse Effects of Transfusion.



Plasmapheresis and IVIG have been used, with varying degrees of success, to treat posttransfusion purpura (PTP).

Therapeutic Plasma Exchange

The term "plasma exchange" is used instead of "plasmapheresis" when the replacement fluid is allogeneic plasma rather than plasma substitute such as normal saline or albumin. Because replacement with plasma carries the same risks of transfusion reaction (most notably, TRALI and anaphylaxis) and the same risk of infectious disease transmission as any other transfusion of Fresh Frozen Plasma (FFP), plasma exchange is indicated only when the replacement plasma contains an essential constituent lacking in the recipient. Note also that the volumes used in plasma exchange are generally much larger than those used in simple FFP transfusion (3 L or 12 units for plasma exchange



The volumes of plasma used in plasma exchange (3 liters or 12 units) are much larger than simple FFP transfusion (4-6 units).

vs 4 to 6 units for simple transfusion), meaning that each plasma exchange results in far more donor exposures for the recipient.

Thrombotic Thrombocytopenic Purpura



TTP results from functional deficiency of ADAMTS-13, the metalloprotease responsible for cleaving von Willebrand factor multimers. Most commonly, this functional deficiency is the result of inhibition by an antibody.

The primary indication for plasma exchange is TTP. Before the advent of treatment with plasma exchange, TTP had a fatality rate in excess of 90%. Aggressive plasma exchange regimens have reduced the fatality rate in TTP to 10% to 15%, although a substantial proportion of responders (30-40%) will have one or more relapses.

Over the past decade, major strides have been made in defining the pathogenesis of TTP, which now appears to result from a deficiency of the metalloprotease (known as a ADAMTS-13) responsible for cleaving von Willebrand factor multimers. The functional deficiency of ADAMTS-13 is often, but not always, the consequence of inhibition of enzyme activity by an autoantibody. Whatever its cause, deficiency of ADAMTS-13 results in the accumulation of unusually large von Willebrand factor (ULvWF) multimers in the circulation. Until recently, it had been thought that the ULvWF multimers were pathogenic and that their presence resulted in spontaneous platelet aggregation in areas of shear stress, such as the microvasculature. Recent work suggests instead that platelets bind to vWF as it emerges from the endothelial cell. In the absence of normal cleavage of the vWF-platelet strands by ADAMTS-13, they go on to form the nidus of platelet-rich microthrombi. Thus, therapeutic plasma exchange works not only by replacing ADAMTS-13 activity but also by removing whatever pathogenic substance—most likely autoantibody—is present. That phenomenon could help to explain why plasma exchange has proven more effective than simple plasma infusion in the treatment of TTP.

In any event, the hallmark of TTP is the presence of platelet-rich thrombi in the microvasculature. TTP presents with the following pentad of clinical findings:

1. Microangiopathic hemolytic anemia, with schistocytes on examination of peripheral blood smear
2. Thrombocytopenia
3. Neurologic dysfunction, ranging from confusion to coma
4. Renal dysfunction, with rising blood urea nitrogen (BUN) and creatinine
5. Fever

Although the first two signs are always seen in TTP, the latter three are variably present.



Thrombotic thrombocytopenic purpura (TTP) presents with a pentad of signs and symptoms:

Microangiopathic hemolytic anemia
Thrombocytopenia
Neurologic dysfunction
Renal dysfunction
Fever

Because the soluble coagulation system is not involved in the pathogenesis of TTP, there are no associated abnormalities of PT or PTT, and D-dimer is not elevated. Those properties allow differentiation of TTP from disseminated intravascular coagulation, which can also present with microangiopathic hemolytic anemia and thrombocytopenia. The absence of a positive direct antiglobulin test differentiates TTP from Evans syndrome—autoimmune hemolytic anemia accompanied by autoimmune thrombocytopenia.

Confounding the diagnosis of TTP, however, is the fact that not all patients with TTP exhibit all of the classic findings at the time of presentation. Furthermore, measurement of ADAMTS-13 levels is available at many institutions only as a send-out test, resulting in extended turnaround time that limits its utility in the acute management of possible TTP.

However, because untreated TTP is nearly always fatal, one must maintain a high index of suspicion whenever a patient presents with acute onset thrombocytopenia accompanied by hemolytic anemia with peripheral schistocytosis, and emergent plasma exchange should be seriously considered in this situation. Platelet transfusion is contraindicated in TTP because transfused platelets merely “fuel the fire” by being available for entrapment in further platelet-rich microthrombi.

Another condition that closely resembles TTP is hemolytic uremic syndrome (HUS), which presents with microangiopathic hemolytic anemia, thrombocytopenia, and renal dysfunction in the absence of neurologic signs and of fever. Classic HUS is seen in the pediatric population, often after an episode of bloody diarrhea, particularly in association with enteropathic Shiga toxin-producing *Escherichia coli* (*E. coli* O-157) infection. Patients with HUS do not lack ADAMTS-13 and, likewise, do not have autoantibody inhibitors of ADAMTS-13. HUS in the pediatric population is typically self-limited, with 91% of children surviving without plasma exchange. However, in adults, making a distinction between TTP and HUS has less utility and may be detrimental. In adults, a presentation that appears to represent HUS may be a subset of full-blown TTP and, thus, would require rapid initiation of plasma exchange therapy. Given that the majority of deaths from TTP occur within the first few days of presentation, one should not delay plasma exchange merely because the entire pentad is not evident initially.

In the early 1990s, a large prospective Canadian study examined the question of whether plasma exchange is superior to plasma infusion in the treatment of TTP. That



The differential diagnosis of microangiopathic hemolytic anemia with thrombocytopenia includes:

TTP
DIC
Evans syndrome



Classic childhood HUS often follows an episode of bloody diarrhea caused by Shiga toxin-producing *E. coli* (*E. coli* O-157:H7) and does not involve deficiency of ADAMTS-13.



TTP is best treated with plasma exchange, which
 Removes autoantibody inhibitors of ADAMTS-13 if present
 Replaces the vWF-cleaving protease ADAMTS-13 missing in TTP patients

In patients for whom plasma exchange is contraindicated, a less effective alternative is simple plasma infusion.

study concluded that therapeutic plasma exchange was superior to plasma infusion, although it is not absolutely clear whether the improved response was due to plasma exchange per se or the larger volume of plasma that was infused in the plasma exchange group (3 L vs 1 L daily). Be that as it may, plasma exchange remains the mainstay of therapy for TTP, and daily exchanges should be continued until LDH levels normalize and the platelet count rises to normal and stabilizes there for 2 consecutive days.

In most cases, high-dose corticosteroid therapy accompanies plasma exchange, particularly in situations where TTP appears to be the result of autoantibody formation. During treatment, the patient's clinical status, especially neurologic function, should be closely monitored and daily laboratory work should include, at a minimum, the following: hemoglobin/hematocrit, platelet count, LDH level, and creatinine.

Some TTP patients who fail to respond to plasma exchange with allogeneic FFP replacement receive Plasma Cryoprecipitate Reduced (also known as cryo-supernatant plasma or "cryo-poor" plasma), the supernatant that remains after Cryoprecipitated Antihemophilic Factor (AHF) is prepared from a unit of FFP. Indeed, on the basis of the theory that cryo-poor plasma is relatively depleted of vWF, some centers prefer to use cryo-poor plasma from the outset. However, there is no definitive evidence for a therapeutic advantage to the use of cryo-poor plasma.

Antiplatelet agents such as aspirin and dipyridamole are sometimes used to reduce platelet aggregation, but the benefit of those drugs in TTP is uncertain and they carry the risk of enhancing bleeding tendencies in some patients. Chemotherapeutic agents (eg, vincristine, cytoxan) and splenectomy have been tried with varying success in patients who are refractory to standard plasma exchange. The most recent addition to the immunosuppressive armamentarium for treating TTP is rituximab, a monoclonal antibody directed against CD20. Its mechanism of action is felt to be the destruction of CD20+ precursors of the B cells that produce the ADAMTS-13 autoantibody. Thus far, evidence for efficacy has been solely in the form of case reports detailing its use in small numbers of patients with refractory TTP.



Patients with acute fulminant liver failure can be treated with plasma exchange (to replace the coagulation factors they cannot synthesize) while awaiting liver transplantation.

Use in Fulminant Hepatic Failure with Possible Liver Transplantation

Another use for plasma exchange is in patients who have acute (fulminant) liver failure and who are awaiting liver

transplantation. In that case, the patient is unable to synthesize coagulation factors and to remove toxins that are normally detoxified by the liver. Plasma exchange removes some of those plasma-bound toxins and, more important, allows replacement of large volumes of coagulation-factor-containing plasma without resulting in fluid overload. In that setting, plasma exchange can help keep the recipient alive until liver transplantation can be accomplished.

Therapeutic Cytapheresis

Cytapheresis can be used to reduce pathologically elevated white cell or platelet counts; to exchange sickle-hemoglobin-damaged red cells for normal red cells; and to collect platelets, granulocytes, or both from healthy donors for transfusion into allogeneic recipients. When used for the removal or collection of white cells, hydroxyethyl starch (ie, hetastarch) is required to facilitate separation of the buffy coat from the red cell layer.

Leukostasis (sludging of white cells in the microcirculation) can occur when white cell counts in peripheral blood reach very high levels or when relatively large numbers of blasts are circulating. Leukocytes are less deformable than red cells are, and, as a consequence, blood viscosity rises significantly as the leukocrit (fractional volume of leukocytes) increases. Of most concern in patients at risk for leukostasis is the risk of stroke caused by diminished cerebral blood flow. Thus, the onset of neurologic symptoms, even simple clouding of the sensorium, in patients at risk for leukostasis should trigger consideration of emergent leukapheresis.

In chronic myelogenous leukemia (CML), the elevated white count is predominantly mature white cells, and patients with CML can sustain very high white cell counts (often in excess of $200,000/\mu\text{L}$) without leukostatic manifestations as long as the number of circulating blasts is low. In contrast, patients with acute myelogenous leukemia (AML) may be symptomatic and at risk of stroke at total white counts as low as $50,000/\mu\text{L}$ to $100,000/\mu\text{L}$. In reality, a total white count of $50,000/\mu\text{L}$ is extraordinarily high for a patient with AML, most of whom have normal or near-normal total white counts with high percentages of circulating blasts. Leukemic lymphoblasts are smaller than leukemic myeloblasts, and leukemic lymphocytes are even smaller still. It has been suggested that this disparity may account for the fact that patients with lymphocytic



Leukostasis associated with very high white counts (in CML) or with very high blast counts (in AML or ALL) can be treated with leukapheresis.

Leukapheresis can also be used to reduce total white cell count before chemotherapy, thus reducing the risk of tumor lysis syndrome.



Patients with chronic lymphocytic leukemia rarely require leukapheresis for leukostasis.

leukemias, particularly chronic lymphocytic leukemia, rarely require leukapheresis for leukostasis.

Leukapheresis may be also used to reduce total leukocyte count before chemotherapy and to reduce the risk of tumor lysis syndrome.

Cytapheresis for Allogeneic Blood Collection



Apheresis platelets have two advantages over pooled whole-blood-derived platelets:

- Fewer donor exposures
- Can be leukocyte reduced during collection instead of requiring postcollection leukocyte reduction.



Current apheresis technology permits collection of platelets that are leukocyte reduced during collection, eliminating the need for postcollection or bedside leukocyte filtration.

Cytapheresis can also be used to collect platelets, red cells, plasma, and granulocytes from allogeneic donors. Apheresis platelets (once known as single-donor platelets) have several advantages when compared with pooled platelet concentrates derived from whole blood donations. First, a single apheresis platelet unit contains approximately the same number of platelets as are found in 6 units of pooled platelet concentrates. Thus, a patient receives five fewer donor exposures from an apheresis unit than from a comparable "dose" of pooled platelets. Second, apheresis platelets can be leukocyte reduced in the process of collection, obviating the need for leukocyte filtration after collection.

Red cells and plasma may be collected by apheresis as well. For red cells, it is possible to collect double units at a single time, provided the donor meets qualifications. Up to 2 allogeneic or autologous red cell units may be removed every 16 weeks by an automated apheresis method. Volume depletion is minimized with saline infusion. Likewise, it is often possible to collect double plasma units (so-called jumbo plasma units) during a single apheresis procedure. Transfusing both aliquots of the larger-volume units to the same recipient cuts the recipient's donor exposure in half.

Recently, apheresis machines that allow simultaneous collection of all three constituents (red cells, platelets, and plasma) have become available. In cases of multiple simultaneous component collection by apheresis, the qualifications of the donor and the combined volume limits of red cells and plasma removed should follow the FDA criteria specified for the particular device being used.

Furthermore, current apheresis technology permits the removal of leukocytes during the process of collecting cellular blood components. A component collected by that methodology will contain leukocyte levels well below the level required to qualify as leukocyte reduced (less than 5×10^6 leukocytes per bag). In addition to markedly reducing the risk of alloimmunization to HLA antigens and, thus, reducing the risk of inducing platelet refractoriness, leuko-

cyte reduction is beneficial in reducing the risk of cytomegalovirus transmission by transfused cellular blood components. Also, prestorage leukocyte reduction has the advantage of removing leukocytes before they can produce activation products such as cytokines. Thus, prestorage leukocyte reduction of apheresis platelets during collection can reduce the likelihood of febrile nonhemolytic transfusion reactions beyond the reduction achievable by poststorage bedside leukocyte filtration. The indications for leukocyte reduction are discussed at greater length in Chapter 1: Blood Component Preparation and in Chapter 8: Indications for Transfusion.

Not all apheresis equipment is capable of leukocyte reduction, so the label on a cellular apheresis unit will specify that it is leukocyte reduced if the procedure has been done by the donor center. Platelets and Red Blood Cell (RBC) units that have been leukocyte reduced at collection do not require use of a leukocyte-reduction filter at the time of transfusion but must be transfused through a standard (170-micron) blood filter (standard blood infusion set) to remove fibrin strands and other particulate matter.

Granulocyte collection requires the use of hydroxyethyl starch to promote a cleaner separation between the buffy coat and the red cell layer. In the past, corticosteroids alone were used to mobilize neutrophils in the donor, but recent work suggests that treating donors with both steroids and G-CSF results in higher-yield granulocyte products. Granulocytes must be transfused as soon as possible after collection and are ineffective if more than 24 hours have elapsed since collection. Furthermore, the indications for granulocyte transfusion are few and often controversial. Thus, granulocyte collection by apheresis is an uncommon occurrence in most institutions. The indications for granulocyte transfusion are discussed in Chapter 8: Indications for Transfusion.

As beneficial as apheresis collection is in reducing donor exposures, most plasma and RBC units are still derived from whole blood donations that require less time on the part of the donor and less equipment to perform the collection. Since the adoption of a requirement for bacterial contamination testing of all platelet products, there has been a trend in some areas of the United States to collect more apheresis platelets and fewer whole-blood-derived platelets. Testing of a single apheresis unit is less expensive than testing the six individual units that will eventually compose a pool of whole-blood-derived platelets.



Granulocyte collection by apheresis requires the use of hydroxyethyl starch to provide a cleaner separation between the buffy coat and the red cell layer.

Special Techniques: Immunoabsorption Apheresis and Photopheresis



Immunoabsorbent column apheresis can be used to treat immune thrombocytopenic purpura (ITP) and refractory cases of rheumatoid arthritis.



Photopheresis involves subjecting collected leukocytes to UV irradiation in the extracorporeal circuit in the presence of a drug (psoralen) that can be photoactivated and returning them to the patient. Photopheresis can be used to treat cutaneous T-cell lymphoma.

Two highly specialized apheresis techniques that merit discussion are immunoabsorbent column apheresis and photopheresis.

Immunoabsorbent column plasmapheresis involves passing the plasma through a column containing purified staphylococcal protein A bound to a silica matrix or, alternatively, to agarose. Staphylococcal protein A has a high affinity for IgG and immune complexes containing IgG and can be used to remove them from the plasma. After the autologous plasma is circulated through the column, it is returned to the patient. Initially developed for the treatment of idiopathic thrombocytopenic purpura (ITP), the staphylococcal protein A immunosorbent column has also been recently approved by the FDA for treatment of rheumatoid arthritis resistant to, or intolerant of, other disease-modifying therapies. A different adsorption technique, low-density lipoprotein (LDL) apheresis, uses antibodies or other substances, such as dextran, to selectively remove LDL cholesterol, apo-B-containing lipoproteins, or both in the treatment of familial hypercholesterolemia.

In photopheresis, peripheral blood leukocytes are collected by apheresis, subjected to extracorporeal ultraviolet (UV) irradiation in the presence of a drug (psoralen) that can be photoactivated, and reinfused into the patient. The psoralen either can be given to the patient orally several hours before the procedure or can be added extracorporeally. Photopheresis is FDA approved for treatment of cutaneous T-cell lymphoma, although the exact mechanism by which psoralen-UV treatment works remains uncertain.

HPC-A Collection

Although detailed discussion of stem cell collection and processing is beyond the scope of this book, it is impossible to discuss apheresis without at least mentioning its role in collection of HPC-A, also commonly referred to as peripheral blood progenitor (stem cell) collection.

Following mobilization by G-CSF (and cyclophosphamide in the case of autologous collection), HPCs can be collected by cytapheresis, frozen, and later reinfused as a form of progenitor cell “rescue” for patients after marrow ablative therapy. The reinfused HPC-A cells engraft and repopulate the recipient’s marrow in much the same way that



Hematopoietic cells can be mobilized by G-CSF and chemotherapy, collected by apheresis, and used for stem cell “rescue” of patients after marrow ablative therapy.

HPCs from marrow (HPC-M) do in marrow transplantation. HPC-A transplantation has several advantages over HPC-M transplantation, including ease of collection from the donor. HPC-M collection requires general anesthesia, but HPC-A collection can be performed without anesthesia. In addition, when compared with HPC-M transplants, HPC-A transplants exhibit more rapid engraftment of both neutrophils and platelets, thereby reducing the time during which the recipient is at risk from neutropenia and thrombocytopenia.

HPC-A can be collected from the patient and stored for later reinfusion after myeloablative chemotherapy (autologous transplantation), or they may be obtained from an allogeneic donor selected on the basis of a six-antigen HLA match (matched for A, B, and DR antigens). The allogeneic donor can be either a blood relative of the recipient or a matched unrelated donor. In the case of autologous transplantation, the patient receives chemotherapy (usually including cyclophosphamide) as well as G-CSF to mobilize progenitors into the peripheral blood. Mobilization regimens in allogeneic donors typically use G-CSF alone.

In any event, once the HPCs mobilize into the donor's peripheral blood, as manifested by a rise in white cell count, HPC-A collection is begun. Because it is possible to use flow cytometry to enumerate stem cells, which are CD34+, some institutions will obtain a peripheral blood CD34+ count before collection to ensure that adequate numbers of HPCs are circulating. Between 12 and 20 liters of blood are processed through the apheresis machine over a period of 2 to 4 hours and the HPCs are collected, while the remainder of the donor's blood constituents are returned to him or her. A CD34+ count is obtained on a sample from the collection bag at the end of collection, and a decision is made about whether further collections are needed to achieve the requisite target dose. Typical minimum collection targets are expressed as 5×10^6 (ie, 5 million) CD34+ cells/kg of recipient body weight. In some donors (or donor-patients), a single collection suffices, but, in other cases, it may take several collections on successive days to collect the target dose.

At the end of each collection, the HPCs in the collection bag are frozen in dimethyl sulfoxide (DMSO) and plasma or albumin using a controlled rate freezer. DMSO and controlled rate freezing are used to help prevent progenitor cell lysis during freezing and thawing. The recipient then undergoes myeloablative therapy followed by infusion of the preserved cells. The frozen HPCs are thawed at the patient's bedside and infused immediately.



HPC-A collections can be from the patient (autologous) or from an allogeneic donor selected on the basis of a six-antigen HLA match.



HPC-A collection targets are typically in the range of 5×10^6 (5 million)/kg of recipient body weight.



HPC-A collections are frozen in DMSO and plasma or albumin using a controlled rate freezer.

Additional techniques that can be applied to the HPC-A collection before freezing include positive selection of CD34+ cells, which results in a product enriched in HPCs, and tumor purging of autologous collections to remove a significant proportion of the circulating tumor cells in the collection. Finally, allogeneic HPC-A collections can be depleted of T cells in an attempt to reduce the likelihood that the recipient will develop graft-vs-host disease. However, T-cell depletion is without negative consequences because it can be associated with an increased risk of graft failure as well as a reduced "graft-vs-leukemia" effect.

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Appendix 14-1. Indication Categories for Therapeutic Apheresis*

Disease	Procedure	Indication Category
Renal and metabolic diseases		
Antiglomerular basement membrane antibody disease	Plasma exchange	I
Rapidly progressive glomerulonephritis	Plasma exchange	II
Hemolytic uremic syndrome	Plasma exchange	III
Renal transplantation		
Rejection	Plasma exchange	IV
Sensitization	Plasma exchange	III
Recurrent focal glomerulosclerosis	Plasma exchange	III
Heart transplant rejection	Plasma exchange	III
Photopheresis		
Acute hepatic failure	Plasma exchange	III
Familial hypercholesterolemia	Selective adsorption	I
	Plasma exchange	II
Overdose or poisoning	Plasma exchange	III
Phytanic acid storage disease	Plasma exchange	I
Autoimmune and rheumatic diseases		
Cryoglobulinemia	Plasma exchange	II
Idiopathic thrombocytopenic purpura	Immunoabsorption	II
Raynaud's phenomenon	Plasma exchange	III
Vasculitis	Plasma exchange	III
Autoimmune hemolytic anemia	Plasma exchange	III
Rheumatoid arthritis	Immunoabsorption	II
	Lymphoplasmapheresis	II
	Plasma exchange	IV
Scleroderma or progressive systemic sclerosis	Plasma exchange	III
Systemic lupus erythematosus	Plasma exchange	III
Lupus nephritis	Plasma exchange	IV
Psoriasis	Plasma exchange	IV
Hemolytic diseases		
ABO-mismatched marrow transplant	RBC removal (marrow)	I
	Plasma exchange (recipient)	II

(continued)

Appendix 14-1. Indication Categories for Therapeutic Apheresis* (continued)

Disease	Procedure	Indication Category
Erythrocytosis or polycythemia vera	Phlebotomy	I
	Erythrocytapheresis	II
Leukocytosis and thrombocytosis	Cytapheresis	I
Thrombotic thrombocytopenic purpura	Plasma exchange	I
Posttransfusion purpura	Plasma exchange	I
Sickle cell diseases	RBC exchange	I
Myeloma, paraproteins, or hyperviscosity	Plasma exchange	II
Myeloma or acute renal failure	Plasma exchange	II
Coagulation factor inhibitors	Plasma exchange	II
Aplastic anemia or pure red cell aplasia	Plasma exchange	III
Cutaneous T-cell lymphoma	Photopheresis	I
	Leukapheresis	III
Hemolytic disease of the fetus and newborn	Plasma exchange	III
Platelet alloimmunization and refractoriness	Plasma exchange	III
	Immunoabsorption	III
Malaria or babesiosis	RBC exchange	III
AIDS	Plasma exchange	IV
Neurologic disorders		
Chronic inflammatory demyelinating polyradiculoneuropathy	Plasma exchange	I
Acute inflammatory demyelinating polyradiculoneuropathy	Plasma exchange	I
Lambert-Eaton myasthenia syndrome	Plasma exchange	II
Multiple sclerosis		
Relapsing	Plasma exchange	III
Progressive	Plasma exchange	III
	Lymphocytapheresis	III
Myasthenia gravis	Plasma exchange	I
Acute central nervous system inflammatory demyelinating disease	Plasma exchange	II
Paraneoplastic neurologic syndromes	Plasma exchange	III
	Immunoabsorption	III

Appendix 14-1. Indication Categories for Therapeutic Apheresis* (continued)

Disease	Procedure	Indication Category
Demyelinating polyneuropathy with IgG and IgA	Plasma exchange	I
	Immunoadsorption	III
Sydenham's chorea	Plasma exchange	II
Polyneuropathy with IgM (with or without Waldenstrom's macroglobulinemia)	Plasma exchange	II
Immunoadsorption		III
Cryoglobulinemia with polyneuropathy	Plasma exchange	II
Multiple myeloma with polyneuropathy	Plasma exchange	III
POEMS syndrome	Plasma exchange	III
Systemic (AL) amyloidosis	Plasma exchange	IV
Polymyositis or dermatomyositis	Plasma exchange	III
	Leukapheresis	IV
Inclusion-body myositis	Plasma exchange	III
	Leukapheresis	IV
Rasmussen's encephalitis	Plasma exchange	III
Stiff-person syndrome	Plasma exchange	III
PANDAS	Plasma exchange	II
Amyotrophic lateral sclerosis	Plasma exchange	IV

*Modified with permission from Smith JW, Weinstein R, Hillyer KL, et al. Therapeutic apheresis: A summary of current indications categories endorsed by the AABB and the American Society for Apheresis. *Transfusion* 2003;43:820-2.

POEMS = polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin lesions; PANDAS = pediatric autoimmune neuropsychiatric disorders; Category I = standard acceptable therapy; Category II = sufficient evidence to suggest efficacy usually as adjunctive therapy; Category III = inconclusive evidence of efficacy or uncertain risk/benefit ratio; Category IV = lack of efficacy in controlled trials.

Managing a Hospital Transfusion Service: Role of the Medical Director

GARY STACK, MD, PhD



TRANSFUSION SERVICE PHYSICIANS HAVE A wide variety of clinical, administrative, managerial, regulatory, and quality oversight responsibilities. First and foremost, they must be expert clinicians in the specialty of transfusion medicine and must be capable of providing clinical consultation on transfusion issues such as transfusion indications and the recognition and treatment of transfusion reactions. In conjunction with technical supervisory staff and administrative support, they must be capable of managing budgets and personnel for the transfusion services that they oversee. They provide quality oversight of the transfusion program at their facilities, so they should be knowledgeable about the principles of quality management and performance improvement.

Transfusion medicine is not only a clinical specialty; it also involves product "manufacturing." Blood bank physicians must be conversant with the requirements of a variety of accreditation organizations, such as AABB, the College of American Pathologists (CAP), and The Joint Commission (TJC, formerly the Joint Commission on Accreditation of Healthcare Organizations). If their facilities prepare blood components, they must also be familiar with licensing and inspection requirements of the Food and Drug Administration (FDA).

The breadth of these and other responsibilities is perhaps unique among medical specialties, causing some to say that blood bank and transfusion service medical directors are “all things to all people”—an exaggeration that makes a point. The discussion that follows reviews a few of the key issues with which physicians who direct hospital transfusion services or blood banks should be familiar. A full discussion of transfusion service management, however, is beyond the scope of this chapter.



The major roles of a transfusion service medical director are to protect the patient, the blood supply, the transfusion service, and the health-care facility.

Key Duties of Transfusion Service Physicians

A listing of the key duties of transfusion service physicians is provided in Table 15-1. A physician who oversees a hospital transfusion service provides leadership to the technical and clerical staff of the service, represents the transfusion service to the health-care institution as a whole, and serves as chief consultant and chief quality officer to his or her institution in the medical specialty of transfusion medicine. From a reductionist perspective, the transfusion service director provides leadership in striving to achieve four goals: 1) protecting the patient, 2) protecting the blood supply, 3) protecting the transfusion service, and 4) protecting the health-care facility. Achieving those goals is a complex multifactorial process involving many staff members throughout the hospital at numerous levels of training.

The first goal, protecting the patient, requires that safe, efficacious, and compatible blood components are available for transfusion when a patient needs them. The development and implementation of policies and procedures for blood component preparation, labeling, storage, testing, and administration by well-trained staff members help ensure that this end is achieved. Appropriate indications for transfusion should be defined on the basis of the latest medical and scientific findings, with a goal of maximizing risk-benefit ratios for patients. Implementation of policies and procedures for patient monitoring after a transfusion is required to detect transfusion reactions so that they can be treated in a timely fashion. Quality control and performance improvement programs provide a means to monitor and improve quality.

The second goal, protecting the blood supply, is achieved when the transfusion service medical director provides good stewardship for blood component use. That effort requires developing monitoring and educational ef-

Table 15-1. Key Duties of the Transfusion Service Medical Director*

Area of Responsibility	General Examples	Specific Examples
Policy development and approval	Pretransfusion testing sample acquisition	Phlebotomy Phlebotomist training Sample requirements (including labeling) Testing of specimens obtained in advance of surgical procedure Assurance of blood when expected
	Pretransfusion testing systems	Compatibility testing Emergency release of blood components
Transfusion administration	Qualification and training of transfusionists	Requirements at the time of administration
	Development and approval of policies	Requirements for reporting adverse events Education of transfusionists
Clinical involvement	Clinical consultation	Unexpected antibodies and/or provision of appropriate component Transfusion reactions Reports Clinical guidance
		Transfusion indications and audits Transfusion alternatives Massive transfusion
		(Continued)

Table 15-1. Key Duties of the Transfusion Service Medical Director* (Continued)

Area of Responsibility	General Examples	Specific Examples
Clinical involvement (continued)	Direct patient care	Therapeutic phlebotomy Therapeutic apheresis Apheresis collection of stem cells and special components
General administration	Development and approval of policies	Qualification of blood component suppliers Management of autologous unit issues Interaction with leadership of transfusion committee Ensuring special component provision Oversight of transfusion service quality system Budget development Support of culture to detect near misses Involvement in hospital quality system Involvement in training (as appropriate for institution) Appropriate, functional, and effective audit system Staffing plan development and advocacy for appropriate staffing
Education	Development and approval of educational tools	Education of technologists, residents, fellows, medical students Education of hospital professional staff Medical education and leadership

*Adapted from Szczepiorkowski ZM, AuBuchon JP. The role of physicians in hospital transfusion services. *Transfusion* 2006;46:862-7.

forts that ensure the judicious ordering of transfusions for appropriate medical indications.

Achieving the third goal, protecting the transfusion service, entails advocating for adequate resources for the transfusion service to carry out its missions. It also involves representing the transfusion service well when interfacing with the facility's clinical and administrative staffs and executive leadership.

The fourth goal, protecting the health-care facility, requires maintenance of a high standard of care as well as protection of the public reputation, legal standing, and financial status of the facility. That goal is accomplished when the preceding three goals are achieved in a cost-effective manner.

The following sections present some practical recommendations and information relevant to achieving those goals as well as a brief discussion of some of the ethical issues that arise when goals conflict.

Automatic Consultations

Some transfusion orders issued by the medical staff to the transfusion service, as well as certain other transfusion-related issues, should automatically be considered as requests for consultation from the transfusion service physician, regardless of whether a request for consultation is formally made. However, the degree to which the transfusion service physician is involved in such cases depends on whether she or he is a full-time transfusion medicine specialist and on the extent of his or her other responsibilities. Consultations and reviews may be performed concurrently or retrospectively. Concurrent reviews are preferable because they present the opportunity to intervene in the clinical decision-making process. However, concurrent review is not always possible because of the blood bank physician's other responsibilities and time demands. Table 15-2 lists some situations that might trigger a review by or consultation with the blood bank physician.

Whereas some triggering scenarios—such as the evaluation of a suspected adverse outcome of transfusion—will be universal, others will vary out of necessity from one institution to another. Local issues such as the likelihood of certain transfusion requests being inappropriate, the availability of services, and the cost will determine whether intervention is indicated. For example, in a large tertiary care hospital with a busy oncology service, it is possible



Investigation of requests by the blood bank physician for unusual blood components or unusual amounts of common products may enable suggestion of a more appropriate component or regimen.

Table 15-2. Automatic Consultations

Topic	Activity of Transfusion Service Medical Director
Any suspected transfusion reaction	Interprets the laboratory test results and the clinical signs and symptoms to diagnose a reaction or rule one out. Makes recommendations for recipient premedication, component modifications, and speed of infusion for future transfusions.
Any other morbidity or mortality thought to be related to transfusion	Investigates possible causal links to transfusion. Presents findings and conclusions to medical staff members, such as at clinical rounds and morbidity and mortality conferences. Reports transfusion-related mortality to the Food and Drug Administration.
Request for any unusual amount of a component (eg, larger dose of platelets than usual)	Investigates unusual requests because they may represent inappropriate orders or may indicate a problem case that the transfusion service may need to prepare for.
Increased number of transfusion requests for a given patient	Investigates to determine if it is an inappropriate order or a problem case for which the transfusion service needs to stock extra components. Multiple platelet transfusion requests may indicate platelet refractoriness for which HLA-matched or crossmatched platelets should be considered.
Platelet transfusion request for a patient with possible thrombotic thrombocytopenic purpura (TTP) or heparin-induced thrombocytopenia (HIT)	Intervenes to advise requesting physician that platelet transfusion is usually contraindicated in TTP and HIT.
Request for crossmatched or HLA-matched platelets	Verifies that the patient is responding inadequately to platelet transfusion and not obviously because of factors other than platelet/HLA alloimmunization. Calculates a corrected count increment or similar measure of response to platelet transfusion to determine the patient's eligibility for special platelet products.
Request for more RBC units than is called for in the maximum surgical blood order schedule (MSBOS)	Investigates to determine if the request indicates a lack of familiarity of a new surgeon or surgical house officer with the institution's MSBOS or whether this could be a problem case (eg, a patient with a preexisting coagulopathy) that the transfusion service needs to prepare for.

that the transfusion service will have an in-house blood irradiator and may automatically irradiate all cellular blood components issued to an oncology ward. In that situation, it would not make sense to screen requests for irradiated blood components from that ward. At a smaller facility, however, irradiated blood components may be available only by ordering them from a distant blood center, perhaps at significant cost and with substantial delay to patient care. If there is a pattern of inappropriate orders at the smaller facility, screening requests for irradiation would be justifiable.



The blood bank physician should be involved whenever the decision is made to transfuse Rh-positive cellular blood components to an Rh-negative patient. If indicated, appropriate immunopro-phylaxis (Rh Immune Globulin) may be recommended.

Transfusion Service and Clinical Rounds

Transfusion Service Rounds

The transfusion service director should conduct laboratory rounds daily at facilities with high transfusion caseloads. Less frequent rounds may be appropriate for transfusion services with a lower workload, provided a transfusion service physician is available at other times as needed. Ideally, rounds should be made at the same time each day so that a predictable routine can be established and random disruptions of technical work can be avoided. Rounds logically should be led by a technical supervisor and should be attended at least by the supervisory staff, the blood bank physician(s), and fellows or resident(s), if applicable. The agenda for rounds should be structured with the order of topics being the same each time. Items that might be discussed at rounds are listed in Table 15-3. Errors, accidents, and customer complaints may be discussed, although additional time needs to be dedicated to those and other quality assurance issues outside daily laboratory rounds.



Making rounds in the laboratory on a daily basis and on the wards when appropriate ensures that the blood bank physician is aware of problems as they emerge and can play a consultative role in the management of patient care problems.

Clinical Rounds

If clinical issues or problems arise that require further investigation or communication between the transfusion service and the clinical staff, the transfusion service physician may consider conducting clinical or ward rounds. For example, it may be helpful or necessary to visit the patient wards or clinics to interview and examine patients who have suffered transfusion reactions. Discussions with the clinicians and nursing staff members who witnessed the

Table 15-3. Sample Agenda for Transfusion Service Rounds

Item	Lead Presenter
Review of antibody panels	Technical supervisor
Review of atypical results on blood group typing	Technical supervisor
Review of reported transfusion reactions	Technical supervisor
Release of Rh-positive cellular components to Rh-negative recipients	Technical supervisor
Transfusion of uncrossmatched RBCs	Technical supervisor
Massive transfusion cases	Technical supervisor
Upcoming potential problem cases	Technical supervisor
Blood bank errors/accidents/occurrences (although further in-depth review needs to be held in a dedicated meeting)	Technical supervisor
Unusual or special requests for transfusion or testing	Technical supervisor
Review of patient responses to special products, eg, HLA-matched or crossmatched platelets, based on clinical and laboratory data	Blood bank physician
Follow-up information and clinical updates on patients with unusual needs or problems	Blood bank physician

reaction may be helpful as well, particularly if it was not well documented in the medical record. In fact, if the medical record is in paper rather than electronic form, actual ward rounds are necessary for reading the patient's chart and for filing a transfusion reaction report (see Fig 15-1) or other note. Ward rounds are also a particularly useful way to investigate possible delayed hemolytic transfusion reactions in newly admitted patients who present with positive antibody screens and direct antiglobulin test results and with no recent transfusion history at the current facility. Sometimes, interviewing patients directly is the best and may be the only way to learn if they have been recently transfused at other medical facilities. Finally, the transfusion service physician may wish to join the clinical team at patient care rounds to discuss any of a variety of transfusion issues, such as those listed in Table 15-2, involving specific patients.

TRANSFUSION REACTION REPORT					
PATIENT NAME: _____			SOC. SEC. NO: _____		
DATE/TIME OF REACTION: _____			TIME TX STARTED/STOPPED: _____ / _____		
COMPONENT: _____ DONOR UNIT NO.: _____			VOLUME INFUSED: _____		
SERVICE: _____ LOCATION: _____ RES/BEEPER: _____			ATTENDING: _____		
PREVIOUS TRANSFUSION REACTION(S) (DATE/TYPE): _____ _____					
BLOOD BANK INVESTIGATION					
BLOOD BAGS & FORMS RETURNED:		LABORATORY TESTS:	POST-TX	PRE-TX	CULTURES ORDERED (PER LAB MED RES):
<input type="checkbox"/> YES	<input type="checkbox"/> NO	Hemolysis*			_____
<input type="checkbox"/> Records, tags, component are correct		DAT: Poly*			_____
<input type="checkbox"/> Discrepancy noted (Describe): _____		IgG*			_____
		C3*			_____
		Antibody Screen			_____
		Repeat ABO			_____
		Repeat X-match			_____
Visual Inspection of Unit:		*Required testing			
<input type="checkbox"/> No abnormality noted		_____			
<input type="checkbox"/> Discoloration		_____			
<input type="checkbox"/> Aggregated/clumped cells		_____			
<input type="checkbox"/> Other: _____		_____			
MEDICAL DIRECTOR INVESTIGATION					
VITAL SIGNS:				CLINICAL HISTORY:	
DATE	PreTx	Rxn	15-24 HR Post-Tx	_____	
TIME				_____	
TEMP				_____	
BP				_____	
HR				_____	
RR				_____	
SYMPTOMS/SIGNS (Circle all that apply):					
<input type="checkbox"/> Fever (____°C rise) <input type="checkbox"/> Chills <input type="checkbox"/> Hypotension <input type="checkbox"/> Dyspnea <input type="checkbox"/> Urticaria <input type="checkbox"/> Localized / Generalized <input type="checkbox"/> Flushing Other: _____					
CLINICAL ACTIONS TAKEN (Circle all that apply):					
<input type="checkbox"/> Transfusion: Discontinued / Slowed / Restarted <input type="checkbox"/> Patient Cultures Ordered: _____ Medications/Fluids Administered: _____ Other: _____					
Additional Testing Ordered: <input type="checkbox"/> YES <input type="checkbox"/> NO					
CONCLUSION					
REACTION TYPE:			RECOMMENDATIONS:		
<input type="checkbox"/> ACUTE HEMOLYTIC			<input type="checkbox"/> PREMEDICATE WITH: _____		
<input type="checkbox"/> DELAYED HEMOLYTIC/SEROLOGIC			<input type="checkbox"/> USE LEUKOCYTE REDUCTION FILTERS		
<input type="checkbox"/> SEPTIC			<input type="checkbox"/> TRANSFUSE AT SLOWER RATE		
<input type="checkbox"/> FEBRILE, NON-HEMOLYTIC; <input type="checkbox"/> PROBABLE <input type="checkbox"/> POSSIBLE			<input type="checkbox"/> TRANSFUSE SPECIAL COMPONENTS: _____		
<input type="checkbox"/> ALLERGIC: <input type="checkbox"/> PROBABLE <input type="checkbox"/> POSSIBLE			_____		
<input type="checkbox"/> OTHER: _____			_____		
<input type="checkbox"/> UNLIKELY RELATED TO TRANSFUSION			_____		
COMMENTS: _____ _____					
/			LAB. MED. RESIDENT	ATTENDING	DATE

Figure 15-1. Example of a transfusion reaction investigation report.

Quality Assurance



A nonpunitive approach should be taken for error reporting and corrective action; errors are opportunities.

The transfusion service director needs to take the lead and to be an enthusiastic driving force for implementing and maintaining an effective quality system. Such a system should reduce errors, improve customer service, boost employee morale, and increase cost efficiency. A nonpunitive approach needs to be taken for error reporting and for the development of corrective action plans. A punitive approach results in unreported errors and lost opportunities for performance improvement. Follow-up on corrective action plans is key. Individuals who work in transfusion services and other laboratories, especially those who are at supervisory levels, often are perfectionists who may be tempted to view errors as failures. Although the statement has become a cliché, it is important that errors be seen as opportunities. The attitude should be fostered that occasional errors are inevitable, but the failure to address the root cause of errors is unacceptable.

Root Cause Analysis

Root cause analysis (RCA) is a structured and formalized approach to investigating the underlying causal factors of a performance failure. RCAs include the creation of an action plan to prevent future similar occurrences. The key steps in an RCA are: 1) collection of data, 2) interview of key personnel involved in the error or incident, 3) charting causes, 4) identification of root causes, and 5) development of an action plan for changes that will reduce the risk of such occurrences in the future. RCAs emphasize systems and processes rather than individual performance. An individual's performance is better addressed in performance evaluation, competency assessment, and disciplinary action processes. The action plan of an RCA assigns responsibility for who will implement the various changes, establishes time frames, and includes provision for follow-up and for monitoring the changes to ensure that they happen. RCAs organized under the auspices of a hospital quality management service are particularly useful for investigating problems that involve multiple departments of a hospital. An RCA of large scope or complexity may be best carried out by a multidisciplinary team of individuals.

RCAs use a variety of data collection and analysis techniques. One example is the Pareto analysis, which is a tool to set priorities for corrective actions. The analysis is based

on the Pareto principle, also known as the 80/20 rule, which states that 80% of problems are often caused by only 20% of the potential sources of error. A corollary to that principle states that corrective action should be focused on the top 20% of causes because that will address 80% of the errors. The data display in a Pareto analysis is often a bar graph, where the height of each bar represents the percentage of a particular error or problem attributable to one specific cause. The bars are added up from left to right until 80% of the total errors are reached. Corrective efforts are focused on the causes that led to that 80%.



The Pareto principle, or 80/20 rule, states that 80% of consequences stem from 20% of the causes.

Another tool is the Ishikawa diagram, also called the fishbone or cause-and-effect diagram. This tool provides a way to display and categorize the various potential causes of a single problem. The diagram is drawn to resemble the skeleton of a fish; hence, the name "fishbone diagram." A horizontal line (ie, the spine) is first drawn, and the problem being analyzed is written at the end of the line to the right. The main categories of possible causes of the problem are represented as "bones" branching off from the spine. Often three to six (commonly four) such branches slanting leftward are displayed—with an equal number drawn above and below the spine. Typical sets of labels for those four branches are "manpower, methods, materials, and machinery" or "equipment, policies, procedures, and people." The main categories should be customized, however, to the problem at hand.

Possible specific causes of the problem being investigated are drawn on horizontal lines branching off from the slanted main branches. More specific causes are drawn as slanting branches off the secondary horizontal branches, and so on. The subdivision into increasingly more specific causes may continue as far as possible, but, usually, not more than four or five levels are practical. The Ishikawa diagram is particularly useful for record-keeping during brainstorming sessions with a group of people. It is a visualization tool that allows the ideas of the group to be collected in a systematic and organized way.

Report of Sentinel Events

Hemolytic transfusion reactions involving the administration of blood or blood components having "major blood group incompatibilities," whether they are fatal or not, are considered "sentinel events" that are subject to TJC review. A sentinel event is defined by TJC as "an unexpected

occurrence involving death or a serious physical or psychological injury, or the risk thereof." The seriousness of sentinel events calls for them to be investigated immediately and for action to be taken to help prevent them in the future. The investigation should be in the form of an RCA (see previous section), and an action plan must be developed and implemented to address the root cause(s). TJC-accredited health-care organizations are encouraged, but not required, to report reviewable sentinel events to TJC. If the sentinel event is reported to TJC or if TJC learns of the sentinel event by other means, a report containing the RCA and action plan must be submitted to TJC within 45 calendar days of the known occurrence of the hemolytic reaction. Otherwise, records will be subject to review at the time of the next TJC survey of the facility.

Severe adverse reactions, including but not limited to septic reactions caused by bacterial contamination of a component, should be reported to the blood supplier as soon as possible. Prompt reporting is important because, depending on the etiology of the adverse reaction, the blood supplier may choose to quarantine or withdraw other components made from the same donation. In addition, the information may be useful for the blood supplier's own performance improvement program. All transfusion-related deaths must be reported to the FDA as soon as possible by telephone and within 7 days in writing.



Transfusion-related fatalities must be reported by telephone or e-mail to the FDA as soon as possible, with a follow-up written report within 7 days.

Review of Standard Operating Procedures

Accrediting organizations usually require that the transfusion service director or designee annually review the standard operating procedures (SOPs). The transfusion service director should work closely with the technical supervisor to ensure that the SOPs are up to date, reflect current practice, and cover all necessary activities and topics. For CAP purposes, the annual review may be delegated to the transfusion service supervisor, although the transfusion service medical director should, nevertheless, still personally review the SOPs periodically. Each SOP reviewed should be documented by signature or initials and the date. It is not adequate to merely sign a cover page for the entire procedure manual.

Market Withdrawal, Recall, and Look-Back

A “market withdrawal” is an effort on the part of the blood donor center to retrieve a questionable unit of a blood component that may still be in the inventory of a hospital transfusion service. As defined by the FDA, market withdrawal is a removal or correction of a product whose distribution was either a minor violation or no violation of FDA regulations. The underlying problem in a market withdrawal is considered beyond the control of the blood component manufacturer. A market withdrawal may occur, for example, when a donor notifies the blood center after a donation that he or she remembers a disqualifying aspect in his or her medical history not reported on the original donor questionnaire.

By contrast, a “recall” is a removal or correction of a marketed product that does violate FDA regulations. The goal is to retrieve a unit before transfusion, when there is reason to doubt its safety. Transfusion services need to act on recall notifications immediately. If the component had already been transfused when the notification was received, the transfusion service physician in most situations should inform the patient’s physician about the possible risk to the patient and the possible need for follow-up of the patient.

“Look-back” refers to the effort to identify recipients of blood from a particular donor. It usually refers to efforts to identify and notify recipients who received blood components from a donor before he or she was known to test positive for a transfusion-transmitted infection. Look-back investigations are conducted because of the possibility that the donor, who is known to be infected now, was also infected at the time of previous donations. By identifying and notifying the transfusion recipients of past donations, the transfusion service gives them the opportunity to seek testing or treatment (if applicable) for possible infection. FDA-initiated look-backs have been conducted as a result of the introduction of new screening tests for transfusion-transmitted infections (eg, human immunodeficiency virus and hepatitis C virus). Look-backs are also a research tool and have been used, for example, to investigate recipients of blood components from donors whose blood has been implicated in transfusion-related acute lung injury.

The transfusion service director may assist with or be given the responsibility to oversee an FDA-mandated look-back at his or her facility. The blood supplier will provide the hospital transfusion service with the donor number of



Look-back refers to efforts to identify and notify recipients of blood components collected from donors who subsequently tested positive for transfusion-transmitted infectious diseases, most notably, HIV and hepatitis C.

the component implicated, which is used to look up the recipients of those units. This scenario underscores the need for the transfusion service to maintain accurate, complete, and accessible records of blood component disposition. If indicated, the transfusion service director, the designated look-back coordinator, or the recipient's physician will send a letter of notification or otherwise contact the recipient about the possible exposure to a transfusion-transmitted infection and about any recommended follow-up.

Change Management



A transfusion service may experience changes in many areas, including but not limited to:

- Laboratory procedures
- Suppliers
- Equipment or technology
- Nursing practices
- Physician practices

One of the most challenging aspects of managing a transfusion service—or any organizational unit—is managing the process by which changes are made. Changes typical to transfusion medicine include, but are not limited to, changes to laboratory procedures, suppliers and equipment, nursing practice at the bedside, and ordering practices of physicians.

To proceed smoothly, change must be well thought out and planned. It is key that an organizational perspective is adopted while implementing change. The scope of the effect of a planned change should be assessed, and those people and positions potentially affected should be informed and have input into the change before it occurs. It may be necessary for a project champion to be appointed as well as a transition team with representation of all affected departments. An implementation plan should be written if changes have wide consequences, particularly when staff members or activities outside the transfusion service need to be coordinated. The implementation plan should include intermediary milestones and target dates for their completion. It should include a description of which staff members require training, as well as how and when the training will be accomplished.

A validation plan should also be devised. Validation includes a run-through of new procedures or testing of new equipment under as close to real-world conditions as possible before “going live” with the changes. Quality assurance staff members should be involved in designing and reviewing the implementation plan. Of course, it is important to closely track errors and incidents during and immediately after implementation of a change because that is when errors are most likely to occur.

Blood Usage or Transfusion Committee

A standing hospital committee should provide oversight of blood usage and other transfusion-related issues at each health-care facility that performs transfusions. This blood usage or transfusion committee should meet at least every 3 to 4 months. The committee should be composed of the transfusion service director and other transfusion service physicians (if applicable); the transfusion service supervisor, chief technologist, or laboratory manager (or all of them); the apheresis service supervisor (if applicable); physician representatives from major clinical services that use transfusion services [eg, medicine (usually a hematologist or an oncologist), surgery, anesthesiology, and pediatrics]; a nursing representative; a nursing educator; a representative of the medical center's quality management department; and possibly a hospital lawyer. It is usually preferable for the chairperson of the committee to be a clinician other than the transfusion service physician. Such a selection helps in reinforcing the concept for the committee members that they are participating in a truly hospital-wide committee rather than only a transfusion service activity. Also, it relieves the transfusion service physician(s) of any appearance of conflict of interest and generally ensures that at least one clinician (ie, the chairperson) will be seriously engaged in transfusion issues rather than merely being a passive audience member. That attitude sets a good example for the rest of the membership.

Typical responsibilities of the committee are shown in Table 15-4. The transfusion service director should use the blood usage committee as one means to communicate



When establishing and enforcing blood utilization guidelines, it is imperative to have input from the clinical services that will be transfusing the component(s) in question. Indeed, it may be desirable that the chairperson of the blood transfusion committee tasked with such oversight be a clinician rather than a member of the blood bank staff.

Table 15-4. Responsibilities of a Blood Usage or Transfusion Committee

- Develop local transfusion guidelines and audit criteria.
- Monitor the appropriateness of transfusion practice by performing or overseeing blood utilization audits.
- Review the wastage of blood components.
- Review the adequacy of the local blood supply.
- Review transfusion service workload and adequacy of resources.
- Review transfusion reactions with the goal of assessing whether the reactions were reported, diagnosed, and treated appropriately and whether they were preventable.
- Review new transfusion-related products and services in order to determine whether they should be obtained by the facility.
- Review hospital-wide transfusion policies or practices.
- Develop contingency plans to address shortages of blood components.

with the medical staff about inappropriate transfusion practices; the adequacy of transfusion service resources; and changes in transfusion service policies, procedures, products, and services. The blood usage committee should have a key role in educating the hospital staff about transfusion issues.

Blood Transfusion and Wastage Audits

Health-care facilities that administer transfusions should maintain a mechanism for monitoring and discouraging the inappropriate use or wastage of blood components. This function is important for improving patient safety, controlling costs, and meeting regulatory requirements. The transfusion service physician(s) must take the lead in promoting the appropriate use of transfusion therapy. Transfusion guidelines and audit criteria should be developed for the local facility, ideally using a consensus development process with relevant practitioners in clinical departments. The blood usage committee can oversee such tasks, although the transfusion service physician initially may need to bring the issue to the committee's attention. Proposed or draft criteria should be presented to, and approved by, the medical staff through appropriate channels for each health-care facility, such as through a medical staff executive committee made up of clinical department directors. Transfusion guidelines and audit criteria must be updated periodically. Various consensus guidelines have been published by different professional societies and organizations that are useful resources for development of local guidelines.

Transfusions not meeting criteria, as identified by the auditing efforts of the transfusion service or a quality management department (or both), should be presented to, and reviewed by, the blood usage committee. Transfusions or component wastage identified by the committee as unjustified should be brought to the attention of the ordering physician, his or her departmental director, and the quality management service for educational purposes and for use in performance improvement activities.

Audits for transfusion appropriateness can be conducted concurrently or retrospectively. Concurrent review involves a screening step during or immediately after the blood order. That step may be accomplished in a variety of ways, including a telephone call by the transfusion service physi-



Transfusion guidelines should be reviewed periodically and revised as needed.

cian, a review of laboratory results in the hospital computer, or the use of computer audit software. Concurrent reviews are advantageous because they provide the possibility of intervening and changing inappropriate transfusion orders. The drawbacks to concurrent audits include possible delays in urgent transfusions and the appearance of requiring the ordering physician to ask for "permission." The discussion over transfusion appropriateness should not be adversarial. If there is disagreement, the "final call" in most cases should be made by the patient's physician, unless the patient may be harmed. The transfusion medicine physician is playing an advisory and educational role in such a setting. Inappropriate requests can always be brought to the blood usage committee later for a retrospective review.

Retrospective audits involve review of the patient's charts at some time after the transfusion. Such audits have the disadvantage of not intervening in order to change an inappropriate request, but they have the advantage of being batched and conducted at a time more convenient for the auditor. Unfortunately, the medical record does not always reflect the factors that influenced the decision to transfuse, often necessitating further input from the requesting physician. If the audit is performed at a considerable time after the transfusion, the decision-making process may be difficult to reconstruct. For that reason, clinicians should be encouraged to document the indications for transfusion in the medical record at the time of transfusion.

Retrospective audits can be performed in several steps. For example, the hospital computer system may be used to identify cases in which laboratory data did not meet predetermined criteria for transfusion. The transfusion service staff or quality management staff of the institution could also perform such a review manually. Cases not meeting laboratory criteria could be referred to the transfusion or blood usage committee, whose members would review the medical record, taking into account not only laboratory data but also other clinical factors. The transfusion service physician or blood usage committee chairperson may attempt to obtain additional clinical information from the ordering physician before committee review. The committee should discuss each case, arrive at a decision on its appropriateness, and give feedback to the ordering physician and possibly to the hospital's quality management department and the clinical department of the ordering physician for potential use in performance improvement activities.



Concurrent review of blood utilization takes place when the order is processed and either before the blood is issued (prospective review) or shortly thereafter. Retrospective audits involve review of the patient's chart at some time after transfusion.

Figure 15-2 provides an example of an audit worksheet with transfusion guidelines for use in manual review by the technical or physician staff. Figures 15-3 and 15-4 are examples of letters that may be sent to physicians for clarifying information about transfusion appropriateness or blood component wastage.

Development of a Maximum Surgical Blood Order Schedule

The transfusion service medical director in conjunction with the surgical departments should develop a standard or maximum surgical blood order schedule (MSBOS). (See Fig 15-5.) The schedule indicates the number of Red Blood Cell (RBC) units that the transfusion service will crossmatch or type and screen for specific surgical procedures. The MSBOS eliminates the need for surgeons to order blood components for each case, standardizes transfusion service efforts for similar cases, prevents last-minute blood orders that give too little time to the transfusion service, and avoids situations in which surgeons may order too few or too many blood components.

The MSBOS can be objectively derived from a review of surgical transfusion practice in each institution. For example, the provision of crossmatched units, as opposed to "type and screen" or "no routine order," is appropriate if RBC transfusion is required for 10% or more of the cases of that particular procedure. The number of units to crossmatch may be determined as the number of units that would meet the total operating room demands for a specific procedure 95% of the time. For procedures that, on average, require transfusion occasionally but in no more than 10% of cases, it may be sufficient to type and screen the patient rather than prepare crossmatched units. For procedures that rarely, if ever, require transfusion, the case may be listed on the MSBOS as "no routine order," and the transfusion service need not type, screen, or crossmatch units for such cases.

Those criteria need not be followed rigidly, however, and other factors may need to be taken into account. Such considerations include the proximity of the transfusion service to the operating room, the speed of transportation of blood components from the transfusion service to the operating room, and the ability and availability of transfusion service staff members to conduct urgent crossmatching for



The Maximum Surgical Blood Order Schedule (MSBOS) attempts to improve patient care and blood inventory management by standardizing the number of blood units that are crossmatched for patients undergoing common surgical procedures.

TRANSFUSION AUDIT WORKSHEET										
Pt. Name:	SS#:				Location/Svc:					
Patient's MD/Attending:					Resident:					
TRANSFUSION DATA					PRE-TX LAB RESULTS					
COMPONENT	DONOR / POOL #	#UNITS	DATE	TIME	HCT	PLTS	PT	aPTT	FIB	
Tech's Comments _____ _____ _____										
Tech's Initials: _____										
MEDICAL DIRECTOR INVESTIGATION:										
Patient History: _____ _____ _____ _____										
TRANSFUSION APPROPRIATE:	YES	NO	<input type="checkbox"/> Letter sent to attending (Dated: _____)							
WASTAGE JUSTIFIED:	YES	NO								
Lab. Med. Resident/Date					Lab. Med. Attending/Date					
BLOOD USAGE COMMITTEE REVIEW:			TRANSFUSION APPROPRIATE:		YES	NO	<input type="checkbox"/> Letter sent to attending, service chief, QM (Dated: _____)			
WASTAGE JUSTIFIED:			YES	NO						
REVIEW CRITERIA FOR TRANSFUSION:										
<p>A. RED BLOOD CELLS</p> <ol style="list-style-type: none"> 1. Hematocrit <25% (or hemoglobin <8.0 g/dL) not due to chronic anemia and when alternative therapies are unavailable. 2. Hematocrit <28% (or hemoglobin <9.0 g/dL) with high risk of ischemic end organ damage. 3. Symptomatic anemia. 4. Acute blood loss with significant changes in blood pressure or pulse. <p>B. FRESH FROZEN PLASMA</p> <ol style="list-style-type: none"> 1. Bleeding or planned invasive procedure when the PT and/or aPTT are >1.5 times the control (and the aPTT is not prolonged due to heparin). 2. Emergency reversal of coumadin effect, only when it would be unsafe to await corrective by intravenous Vitamin K1 or discontinuing coumadin. 3. Documented deficiencies of isolated coagulation factors (e.g. Factor XI) in the setting of bleeding or a significant risk of bleeding, when a concentrate is unavailable. 4. Documented deficiencies of antithrombin III, protein C or protein S in patients undergoing surgery or who require heparin for treatment of thrombosis when a concentrate is available. 5. Thrombotic thrombocytopenic purpura. <p>C. PLATELETS</p> <ol style="list-style-type: none"> 1. Platelet count <10,000/μl not due to immune thrombocytopenic purpura or thrombotic thrombocytopenic purpura. 2. Platelet count <50,000/μl with bleeding or a planned invasive procedure. <p>D. CRYOPRECIPITATE</p> <ol style="list-style-type: none"> 3. Platelet count <100,000/μl with active, life threatening bleeding. 4. Documented or suspected platelet dysfunction in the setting of abnormal bleeding or a planned invasive procedure. 5. Post-cardiopulmonary bypass with abnormal bleeding. <p>E. ADDITIONAL SPECIAL CIRCUMSTANCES REQUIRING REVIEW</p> <ol style="list-style-type: none"> 1. Wastage of blood components. 2. Transfusion of >10 platelet concentrates (or >2 apheresis units) into a given patient in a 24 hour period. 3. Transfusion of platelet concentrates into a patient with immune thrombocytopenic purpura or thrombotic thrombocytopenic purpura. 4. Request for irradiated blood components. 5. Requests for CMV seronegative components. 6. Requests for HLA-compatible or crossmatch-compatible platelets. 7. Adverse effects associated with transfusion. 7. Mortality associated with transfusion. 										
12/11/00										
Figure 15-2. Example of a worksheet for a transfusion audit.										

Dear Dr. _____:

The (your institution) transfusion policy requires review of blood component administration to determine whether transfusions are administered according to guidelines approved by the medical staff (see attached). Transfusions administered outside these guidelines are investigated by the blood usage committee for discussion and review regarding their appropriateness.

The transfusion indicated below, for which you were the attending physician of record, appeared not to conform with the transfusion guidelines on preliminary review. Since transfusion decisions are often influenced by data not apparent on chart review, I am inviting you to provide additional information in support of your decision to transfuse. This information should be submitted to me before our next blood usage committee meeting, which takes place on _____. If we receive no further information from you, the committee will conduct a final discussion on this case with the information it already has at hand. Please send your comments in writing to (your name and department), drop them off at (your room number), or send them electronically to (your e-mail address). If you have questions, you may call me at (your telephone number).

The intent of our reviews is educational with a goal of improving the appropriateness of blood transfusions and patient safety. The committee welcomes your input.

Thank you for your cooperation.

Sincerely,

(your title)

Encls.

Justification or clarifying information is requested for the transfusion(s) indicated below:

Patient: _____

Hospital ID#: _____

Date/Time of Transfusion: _____

Component(s) Transfused: _____

Review Criterion Not Met (Preliminary): _____

cc: Director, (name of physician's clinical department)
Blood Bank Supervisor

Figure 15-3. Example of a letter to physicians requesting information for retrospective transfusion audits.

Dear Dr. _____:

The blood usage committee and the blood bank review all instances of blood component wastage, ie, blood components that are signed out from the blood bank but discarded when not transfused. As you know, because blood components are a valuable and often limited resource, we must strongly discourage their wastage.

We are currently investigating the blood component wastage indicated below. Our records identify you as the attending physician for this patient. Because changes in clinical circumstances may justify last-minute cancellations of transfusion, I am requesting that you provide me with clarifying information regarding why blood components were ordered but not transfused, in this case. I ask that you please reply before the date of the next blood usage committee meeting, which will be held on _____.

As you may know, blood components issued from the blood bank but not transfused may be reissued for another patient only if they have been returned to the blood bank within 30 minutes of original issue. (Exception: RBCs for the OR stored in a blood bank monitored refrigerator). Once pooled, platelets and cryoprecipitate outdate in 4 hours, and after thawing, plasma has only a 24-hour outdate. Due to the generally low demand for platelets, plasma, and cryoprecipitate at our medical center, these components are rarely reissued to another patient once they have been pooled or thawed and usually are discarded. For these reasons, blood components should only be requested for pooling, thawing, or pickup from the blood bank when the patient is ready to receive the transfusion immediately.

Sincerely,

(your title)

Justification or clarifying information is requested for the blood component wastage indicated below:

Patient: _____

Hospital ID #: _____

Component(s) Wasted: _____

Date/Time of Blood Component Request: _____

cc: Director, (name of physician's clinical department)
Blood Bank Supervisor

Figure 15-4. Example of a letter to physicians requesting information on component wastage.

	No. units	GENERAL SURGERY (Continued)	No. Units
CARDIOTHORACIC			
01. A-V Fistula	T&S	09. Colonoscopy	NRO
02. Angiogram	T&S	10. Colostomy or Colostomy Closure	T&S
03. Angioplasty (PTCA)	T&S	11. Empyema – Drainage	T&S
04. Atrial Septal Defect (ASD), Repair	4	12. Excision, Squamous Cell Carcinoma	NRO
05. Cardiac Catheterization	T&S	13. Exploratory Laparotomy	T&S
06. Coronary Artery Bypass Graft (CABG)	4	14. Gastrectomy	2
07. Coronary Artery Bypass Graft (CABG), Redo	6	15. Gastrostomy, Percutaneous	T&S
08. Coronary Artery Bypass Graft plus Valve Replacement	6	16. Gastrostomy, Feeding Tube	T&S
09. Esophagectomy (also Esophagogastrectomy)	4	17. Harrmann Procedure	2
10. Intra-Aortic Balloon Insertion	T&S	18. Hemipelvectomy	6
11. Lobectomy (lung)	2	19. Hemorrhoidectomy	NRO
12. Mediastinoscopy	T&S	20. Hepatectomy	4
13. Mitral Commissurotomy	4	21. Herniorrhaphy	
14. Pacemaker Insertion, or Repair of Replacement	T&S	a. Femoral, Inguinal, or Ventral	NRO
15. Pericardectomy	2	b. Incisional	NRO
16. Pneumonectomy	2	c. Umbilical	NRO
17. Pulmonary Valvulotomy	4	22. Hiatus Hernia Repair	T&S
18. Thoracic Aneurysm	6	23. Hysterectomy	T&S
19. Thoractomy (with or without Lobectomy)		24. Ileostomy	T&S
20. Trachcostomy	NRO	25. Ileostomy Closure	T&S
21. Valve Replacement		26. LeVeen Shunt	T&S
a. Aortic	4	27. Liver Biopsy, Percutaneous	NRO
b. Aortic, Redo	6	28. Lobectomy, Hepatic	4
c. Mitral	4	29. Mastectomy	
d. Mitral, Redo	6	a. Simple	T&S
22. Ventricular Septal Defect (VSD) Repair	6	b. Modified	T&S
GENERAL SURGERY		c. Radical	T&S
01. Abdominal-Perineal Resection (AP Resection)	2	30. Pancreatectomy	2
02. Appendectomy	NRO	31. Parathyroidectomy	T&S
03. Axillary Node Biopsy	NRO	32. Pilonidal Cystectomy	NRO
04. Belsey Fundoplication (like hiatus Hernia)	T&S	33. Shunt	
05. Breast Biopsy	NRO	a. Distal Splenorenal (Warren)	4
06. Carpal Tunnel Release	NRO	b. Meso-caval	4
07. Cholecystectomy		c. Porto-caval	4
a. Laparoscopic	NRO	d. Porto-caval H graft	4
b. Open, with or without Common Duct (CD) Exploration (Gall Bladder)	T&S	34. Splenectomy	T&S
08. Colectomy		35. Sympathectomy	T&S
a. Hemicolectomy	2	36. Tenckhoff Catheter	
b. Total	2	a. Insertion	T&S
c. Sigmoid	2	b. Removal	T&S
		37. Thyroidectomy	T&S

Figure 15-5. Excerpt from a maximum surgical blood order schedule. (Courtesy Gary Stack, MD, PhD, VA Connecticut Healthcare System, West Haven, CT.)

cases that exceed the MSBOS. Distant locations, the absence of a reliable, fast transport system (eg, on-demand couriers or a pneumatic tube system for blood components), or an inadequate staff to handle urgent testing may justify making more crossmatched units available than might otherwise be the case.

After the transfusion service has developed its recommendations for the MSBOS, the transfusion service direc-

tor should give the schedule to the chief or director of surgery and to the surgical specialty directors for review, discussion, and consensus development. The MSBOS should inspire confidence by the surgical staff that components will be available as needed, yet it should not appease unreasonable demands for more components than are justified. The MSBOS should reduce unnecessary crossmatches by the transfusion service staff and should allow the transfusion service to stock fewer RBC units overall. It may also decrease the risk of transfusions to misidentified recipients because the number of excessive untransfused units in the operating room will be reduced.

Staffing Plans

In order to manage the transfusion service and meet the requirements of accrediting organizations, the transfusion service should have a staffing plan. Such a plan details the staffing requirements and staffing mix; that is, how many employees with what level of training are needed to meet the current or projected workload for each shift that the transfusion service is open. As much as possible, the staffing plan should be based on expected defined productivity for each employee in relation to the transfusion service's workload. The goal of the staffing plan is to optimize productivity while maintaining sufficient redundancy of staff members to allow for peak workload demands and temporary staff vacancies.

The staffing plan should also include a narrative description of the scope of services of the transfusion service and what services are offered at what times, including what services are purchased from outside the facility. It should contain an organizational chart showing reporting relationships. The position titles or classification, as well as the total full-time employee equivalents devoted to each position classification, should be shown on the chart or on a separate listing.

A comprehensive staffing plan should provide contingencies for vacant positions. For example, a contingency plan could include 1) crosstraining of staff members from other areas of the laboratory to provide temporary coverage, 2) use of overtime, 3) possible redesign of processes or a change in services, or 4) perhaps the use of contracted employees from an employment agency.



A staffing plan should identify how many employees with what level of training are needed to meet workload requirements.

Budgets and Costs

Development of an Annual Budget

Fiscal planning by an institution requires that the transfusion service develop and submit an annual operating budget before the start of each fiscal year. The budget may include costs associated with the following items: 1) salaries and benefits for the transfusion service staff, 2) procurement or production of blood components, 3) reagents and supplies, 4) service contracts and repairs for equipment, and 5) other expenses that vary from institution to institution. Each succeeding year's budget can be based on the current year's budget to date with adjustments for expected salary, cost of living, and promotion increments; the projected changes in the cost of blood components from a blood supplier; the projected changes in staffing levels; the projected changes in products or services offered (eg, converting to apheresis platelets from whole-blood-derived platelet concentrates); and projected changes in the institution's clinical programs (eg, opening a liver transplant program).

It is important to track test workload, blood components purchased, and other categories of expenses at least quarterly. In that way, the transfusion service director can monitor trends that will allow a more accurate budget projection for the coming year. Frequent tracking is also important for determining whether the budget is within guidelines for the current year and whether additional resources are needed. Justification with documentation of unanticipated expenses is, of course, necessary when requesting additional funding for the remainder of the current year or for a succeeding year. Such requests may need to include evidence that the transfusion service leadership is taking steps to control costs as best as it can while maintaining quality and providing the level of services required clinically.



More accurate budget projections can be made when the transfusion service director monitors trends in testing workload, component/reagent purchases, and other expense categories.

Terminology

The transfusion service physician should be familiar with the following laboratory-related accounting terminology:

Capital budget: Funding for the purchase of equipment and elements of the physical plant (eg, buildings). The capital budget is kept separate from the annual operating budget because it is used to acquire assets with lifetimes that exceed 1 year.

Direct cost: Expenses directly related to the provision of a product or service, such as wages, benefits, reagents, supplies, and equipment. Direct costs can be fixed or variable.

Fixed cost: Expenses that do not change with workload volume. For example, the cost of janitorial services usually does not vary with transfusion service workload.

Full cost: All costs associated with an activity, a service, or a product, including wages, benefits, space, utilities, supplies, administrative support, and building maintenance.

Full-time equivalents (FTEs): The equivalent of a full-time employee who works 40-hour weeks. For example, two half-time (20 hours/week) employees (0.5 FTE each) are equal to 1 FTE. Sometimes, an FTE is called an FTEE; that is, a full-time employee equivalent.

Indirect cost: Expenses not directly related to the provision of a particular product or service. Indirect costs contribute to and support the overall operation. Indirect costs can be fixed or variable. Examples of indirect costs are maintenance, utilities, administration, and depreciation.

Marginal cost: The cost to produce one additional test, product, or service. Marginal cost may include only reagent and supply costs for a laboratory test because indirect costs and personnel costs are already accounted for in the performance of the core workload. Marginal cost assumes that no additional employees are needed and that no more equipment is necessary for the additional work.

Variable cost: Expenses that change with workload volume. Examples of variable costs are those associated with reagents and supplies. As more tests are performed, more reagents and supplies will be consumed and purchased.



Blood bank physicians, as well as chief technologists, need to be familiar with a number of laboratory-related accounting terms.

Equipment and Personnel Recruitment Requests

Each institution has its own procedure for handling requests for resources, such as new or replacement equipment, and for personnel recruitment. Mechanisms should exist for emergency equipment requests (when critical equipment fails and urgently needs replacing) and for routine, usually annual, capital equipment requests and purchases. Two keys for improved success with resource requests are 1) performing a “make or buy” analysis and 2) citing quality or customer service deficiencies that have already resulted or will result from failure to obtain the requested resource. A “make or buy” analysis entails a cost comparison between obtaining resources to make or provide a product or service in-house and buying a service or

product from an outside source. For example, if someone requested the purchase of a gamma-irradiator to irradiate cellular blood components in his or her own transfusion service, it is important to compare that cost vs the cost of purchasing already irradiated blood from a blood supplier over the expected life span of the requested irradiator. Financial cost, however, should not always be the final determinant. Even if it costs more to irradiate in-house than to purchase already irradiated components, the transfusion service director might justify the purchase of a gamma-irradiator on the basis of improving the quality of patient care by eliminating delays in obtaining irradiated components from a distant blood supplier.

Inspections and Accreditation



Most inspections and accreditation visits are now unannounced.

According to provisions of the Public Health Service Act and the Federal Food, Drug, and Cosmetic Act, the FDA conducts inspections of establishments that manufacture blood or blood components for human use, including blood banks, blood centers, and donor centers. The FDA transferred the inspection of hospital transfusion services, however, to the Centers for Medicare and Medicaid Services (CMS). Transfusion services are considered by the FDA to be laboratories that perform compatibility testing but that do not collect allogeneic or autologous blood or process whole blood into components. The CMS has in turn granted certain laboratory accrediting organizations such as AABB, CAP, and TJC authority (ie, “deemed status”) to inspect hospital transfusion services in its place. All programs involve inspections of the hospital transfusion service every 2 years, as required by the Clinical Laboratory Improvement Amendments (CLIA ’88). Transfusion services and clinical laboratories need to be accredited by only one organization with CMS deemed status, although many transfusion services maintain both CAP and AABB accreditation. Key features of inspection and accreditation programs are described next and are compared in Table 15-5.

AABB

The AABB uses staff assessors and volunteer transfusion specialists, both physicians and medical technologists, who are selected and trained to be assessors. Unlike CAP accreditation, hospital transfusion services that are AABB

Table 15-5. Comparison of Inspection and Accreditation Programs

	AABB	CAP	FDA	TJC*
Inspectors or assessors	AABB staff and/or selected volunteer transfusion specialists	Peers	FDA staff	TJC staff
Interval between inspections	2 years	2 years	2 years	2 years
Source of standards, checklists, or regulations	AABB Standards	CAP Transfusion Medicine Checklist	Code of Federal Regulations	TJC Standards
Date of inspection	Unannounced, in final quarter before accreditation expiration	Unannounced, within 6 months of accreditation expiration	Unannounced	Unannounced within 6 months of accreditation expiration

*Refers to TJC laboratory accreditation program only.

accredited are not automatically expected to provide assessors. During the on-site assessment, the AABB assessor determines whether the transfusion service is in compliance with the current edition of *AABB Standards for Blood Banks and Transfusion Services*. All standards relevant to a facility must be adhered to for it to obtain accreditation. On-site assessments are unannounced and occur at some time in the final quarter of the 2-year accreditation cycle. The AABB requires that a corrective action plan be submitted within 30 days of the assessment date for each nonconformance.

CAP

A CAP inspection is usually carried out by a peer physician or medical technologist from another similar clinical laboratory that is accredited by CAP. Laboratories that are CAP accredited are expected to provide inspectors for the peer inspection process. Peer inspection has the advantage of being an educational and information-sharing process for both the inspected facility and the inspector. The drawback is that there can be some lack of uniformity in the in-



Accreditation by AABB, CAP, or TJC must be renewed every 2 years. Inspections or surveys occur unannounced near the end of each 2-year accreditation cycle.

spection process, despite significant measures taken by the CAP to ensure consistency.

For some smaller facilities whose staffing levels do not permit them to contribute inspectors to the program, the CAP may send CAP staff inspectors, who usually have a medical technology background. A transfusion service inspection by the CAP typically is performed as part of an inspection of the entire clinical laboratory. The date of the inspection is unannounced, but the transfusion service knows that the inspection will be carried out at some point during the 6 months before the 2-year anniversary date assigned to the facility by the CAP.

The transfusion service is inspected to determine whether it is in compliance with CAP Transfusion Medicine Checklist items. Noncompliance with checklist items that the CAP considers the most important are designated as "Phase II deficiencies." Noncompliance with items considered to be of somewhat lesser importance are designated "Phase I deficiencies." A response must be sent to the CAP within 30 days of the inspection if there are Phase I or II deficiencies. Phase II deficiencies require a written corrective action plan with documentation of implementation. Phase I deficiencies require a response, but not documentation.

TJC

TJC conducts a survey and accreditation program for entire health-care organizations. Some aspects of the institution-wide survey involve the transfusion activity of the facility, such as requirements for ensuring proper patient identification. However, detailed inspections of the transfusion service comparable to CAP and AABB assessments are not part of the larger institutional survey, unless the institution specifically participates in TJC laboratory accreditation program. Like CAP and AABB accreditation programs, TJC's laboratory accreditation program meets CLIA requirements, has deemed status with CMS, and is conducted on a 2-year cycle.

The survey assesses compliance with laboratory standards found in TJC's *Comprehensive Accreditation Manual for Pathology and Clinical Laboratory Services*. The surveyors are paid TJC staff members, not volunteers, with a medical technology background who have passed TJC certification examination. Like the CAP, TJC carries out unannounced surveys sometime in the final 6 months of each 2-year accreditation cycle (although the initial survey is

announced). The movement toward unannounced inspections has been prompted by a Government Accountability Office report that expressed concerns about the effect of prior notification of inspection dates on the integrity of the inspections. Announced inspection dates, as opposed to surprise inspections, have the potential to encourage last-minute preparations rather than continuous compliance with standards.

FDA

FDA inspections, which are unannounced, are also on a biennial cycle. Inspectors are paid FDA staff members. FDA-licensed blood establishments are subject to full inspections, which may last several days, depending on the size and scope of operations. Hospital transfusion services that are registered, rather than licensed, with the FDA are often subject to more limited inspections, which are proportional to their reduced complexity of operations. A transfusion service must be registered with the FDA if it 1) processes blood components, such as washing RBCs; 2) prepares cryoprecipitate-reduced plasma; 3) prepares leukocyte-reduced blood components in the laboratory (bedside filtration does not require registration); 4) irradiates blood components; or 5) is a military or Veterans Administration facility. Hospital transfusion services that are not registered with the FDA are not subject to FDA inspections.

Preparation for Inspections

Without doubt, the best way to prepare for inspections is to meet standards and checklist items on a continuous basis, not only on the day of the inspection. Interim self-assessments (as required by the CAP) and mock inspections can help transfusion services strive for a continuous state of compliance. However, standards and checklists frequently change from one accreditation visit to the next. Although each transfusion service's management team should stay abreast of those changes as they occur, some degree of specific preparation for an inspection is nevertheless prudent.

The most time-efficient approach is to first focus on items with which the transfusion service is most likely to be noncompliant. Accordingly, the first priority should be to review deficiencies that were reported on the previous inspection (or on the midcycle self-assessment in the case



The best way to prepare for an inspection is to maintain compliance on a continuous basis.

of the CAP) and to make sure they have been definitively corrected. Inspectors are instructed to specifically check that old deficiencies have indeed been addressed. The second priority is to focus on any new or modified checklist items or standards. New items are a focus for inspectors because those items have a relatively high probability of being overlooked by the laboratory being inspected. The third priority is to review a list, if available, of items that the accrediting organization most commonly finds to be deficient across all laboratories inspected. Such a list is usually available from CAP. Finally, as time permits, the laboratory being inspected should verify that it remains in compliance with preexisting checklist items or standards with which it was compliant previously. Those items should have become parts of ongoing laboratory operations and are likely still in compliance.

Ethical Considerations



The four key principles of medical ethics are: 1) patient autonomy, 2) beneficence, 3) nonmaleficence, and 4) justice.

Practitioners of transfusion medicine face their own unique set of ethical dilemmas. For example, during times of blood shortage, blood components may have to be rationed. Rationing raises the issue of which patients qualify for scarce blood components. Another related ethical dilemma occurs when the massive transfusion of one critically ill patient so depletes the local blood supply that other patients' transfusions might have to be deferred. In that situation, the transfusion service medical director may have to insert himself or herself into the situation by posing the question and forcing a decision about what takes precedence: the transfusion needs and chances of survival of one patient or the needs and well-being of several others.

In another scenario, a new blood-processing technique may become available to make blood safer from transfusion-transmitted diseases or other adverse outcomes. Although the technique may be licensed for clinical use, the long-term effects on the health of transfusion recipients cannot be assessed because the technology has not been in use long enough. Does the transfusion medicine specialist decide to implement it immediately because it has the potential to prevent known risks of transfusion today, or does that specialist wait until its safety is better documented in the long term? What makes a decision such as this difficult is that it must be made before all the facts can be known.

If one is to put those and other ethical issues in perspective, it is useful to consider them in light of the basic principles of medical ethics. The four principles most often cited by medical ethicists are: 1) patient autonomy, 2) beneficence, 3) nonmaleficence, and 4) justice. Some medical ethicists also add other principles such as truthfulness, honesty, confidentiality, and dignity. Those principles do not dictate what decisions to make in a specific situation, but they do provide guidance. Thus, it is worth considering them in a little more detail.

The principle of autonomy, simply put, means that patients have the right to choose or refuse their treatment. Also, patients with diminished autonomy should be protected. Respect for autonomy in transfusion medicine, for example, is shown by obtaining the recipient's consent before transfusion or by respecting a patient's informed decision to refuse transfusion on religious grounds.

Beneficence means that health-care workers have an obligation to help patients and to secure their well-being. A transfusion-related example is that many efforts are made to ensure that blood components are available for patients when they need them and that the components are prepared in a way that optimizes their potency.

Nonmaleficence means that health-care providers make every effort to minimize the possible harms. Examples in transfusion medicine include the testing of donated blood for transfusion-transmitted infectious agents, testing of platelets for bacterial contamination to prevent septic transfusion reactions, and provision of leukocyte-reduced or irradiated blood components to prevent other types of adverse outcomes.

Justice means that the benefits, risks, and costs of health care will be distributed fairly and that patients with similar needs will be treated similarly. An example of justice applied in the transfusion setting is allowing an HIV-infected patient to donate autologous blood before surgery rather than forbidding the donation out of concern that the infected unit might be transfused mistakenly to the wrong patient.

Ethical dilemmas arise when ethical principles conflict with each other in a particular situation. For example, limiting the amount of blood components provided to the massive transfusion recipient who was depleting the local blood supply could harm that patient. However, failure to limit the availability of blood components for that one patient could cause harm to other patients who also require transfusion. The situation makes it difficult or impossible to provide beneficence and nonmaleficence to everyone si-



Ethical dilemmas arise when ethical principles conflict in a particular situation.

The transfusion medicine director should not try to resolve these dilemmas alone.

multaneously. The principles of justice and beneficence for the HIV-infected patient who is allowed to donate autologous blood conflicted with the principle of nonmaleficence for other patients or staff members who might be exposed to the HIV-infected blood.

It is important that the transfusion medicine physician not bear the burden of such dilemmas alone. In fact, the role of the transfusion specialist is typically to first pose the question to the appropriate decision-makers, such as the patient's physician and family. Other key decision-making groups or individuals include the hospital ethics committee, the hospital chief of staff and clinical chiefs, the hospital executive management, and the hospital legal counsel. Decisions about whether to implement a new technology that offers benefits, along with known and perhaps unknown risks, should be made institutionally and involve those other decision-makers. The role of the transfusion medicine physician is to present a fair and honest assessment of risks and benefits to key advisory or decision-making groups and individuals and to strive for an institutional consensus.

Another important way to deal with ethical dilemmas is to develop policies ahead of time, when possible, that address what actions to take in specific situations. For example, for blood shortages that may require rationing of blood components, it is helpful to develop contingency plans that the medical staff members agree to ahead of time (see Chapter 12: Transfusion Emergencies). Such plans avoid rushed decisions in the heat of the moment, give ample opportunity for ethical review, and facilitate input and buy-in by the medical staff.

It should be noted that since the mid-1980s, after the discovery of HIV transmission by transfusion, the field of transfusion medicine has increasingly adopted an ethical approach consistent with the "precautionary principle." This principle states that if the potential consequences of an action are severe or irreversible, precautionary measures should be taken even if scientific certainty of cause and effect has not been established. Adoption of the precautionary principle gives priority to achieving nonmaleficence. For example, at the time of the initial AIDS outbreak, the medical community generally took the approach that until it was clearly shown that AIDS was transmitted by transfusion, either no or limited precautionary measures were indicated. In fact, there was some concern at the time that any precautionary measures that disqualified some individuals from donating blood could do harm by causing blood shortages. An ethical dilemma arose: achieving nonmaleficence by



The precautionary principle states that if the potential consequences of an action are severe or irreversible, precautionary measures should be taken even if scientific certainty of cause and effect has not been established.

avoidance of a known risk came into conflict with avoidance of an unknown risk. Today's more precautionary approach to potential new hazards gives more emphasis to assuming the worst until proven otherwise.

Suggested Reading

Role of Transfusion Service Physicians

AuBuchon J. The role of transfusion medicine physicians: A vanishing breed? *Arch Pathol Lab Med* 1999;123:663-7.

Barnes A. Transfusion practice in a private hospital. *Arch Pathol Lab Med* 1989;113:296-9.

Goodnough LT. What is a transfusion medicine specialist? *Transfusion* 1999;39:1031-3.

McCullough J. The role of physicians in blood centers. *Transfusion* 2006;46:854-61.

Popovsky MA. The transfusion medicine consultant and hospital transfusion committee. *Vox Sang* 1998;74(Suppl 2):391-3.

Snyder EL. The academic hospital blood bank director: All things to all people. *Arch Pathol Lab Med* 1989;113:300-6.

Szczepiorkowski ZM, AuBuchon JP. The role of physicians in hospital transfusion services. *Transfusion* 2006;46:862-7.

Recalls and Market Withdrawals

Ramsey G. Managing recalls and withdrawals of blood components. *Transfus Med Rev* 2004;18:36-45.

Transfusion Committee

Haynes SL, Torella F. The role of hospital transfusion committees in blood product conservation. *Transfus Med Rev* 2004;18:93-104.

Saxena S, Shulman IA, eds. The transfusion committee: Putting patient safety first. Bethesda, MD: AABB, 2006.

Shulman IA, Saxena S. The transfusion services committee—responsibilities and response to adverse transfusion events. *Hematology (Am Soc Hematol Educ Program)* 2005:483-90.

Laboratory Management

Bozzo P, ed. Cost-effective laboratory management. Philadelphia: Lippincott-Raven, 1998.

Gillon R. Medical ethics: Four principles plus attention to scope. *Br Med J* 1994;309:184-90.

Travers EM. Clinical laboratory management. Baltimore, MD: Williams & Wilkins, 1997.

Appendix

A. National and International Organizations

The following list of organizations of interest to blood bank and transfusion medicine professionals is not meant to be all inclusive. Data are accurate as of May 2007.

AABB

8101 Glenbrook Rd.
Bethesda, MD 20814-2749
Phone: (301) 907-6977
Fax: (301) 907-6895
URL: <http://www.aabb.org>

American Association of Tissue Banks
1350 Old Chain Bridge Rd. (Suite 450)
McLean, VA 22101
Phone: (703) 827-9582
Fax: (703) 356-2198
URL: <http://www.aatb.org>

American Hemochromatosis Society
4044 W. Lake Mary Blvd., Unit #104,
PMB 416
Lake Mary, FL 32746-2012
Phone: (407) 829-4488
Fax: (407) 332-1284
URL: <http://www.americanhs.org>

American Medical Technologists

10700 West Higgins Rd., Suite 150
Rosemont, IL 60018
Phone: (847) 823-5169, (800) 275-1268
Fax: (847) 823-0458
URL: <http://www.AMT1.com>

American Red Cross

Biomedical Services
2025 E Street, NW
Washington, DC 20006
Phone: (202) 303-4498
URL: <http://www.redcross.org>

American Society for Apheresis

375 West 5th Ave., Suite 201
Vancouver, BC, V5Y 1J6
Canada
Phone: (604) 874-4366
Fax: (604) 874-4378
URL: <http://www.apheresis.org>

American Society for Blood and Marrow Transplantation

85 W. Algonquin Rd., Suite 550
Arlington Heights, IL 60005
Phone: (847) 427-0224
Fax: (847) 427-9656
URL: <http://www.asbmt.org>

American Society for Clinical Laboratory Science

6701 Democracy Blvd., Suite 300
Bethesda, MD 20817
Phone: (301) 657-2768
Fax: (301) 657-2909
URL: <http://www.ascls.org>

American Society for Clinical Pathology

33 W. Monroe St., Suite 1600
Chicago, IL 60603
Phone: (800) 267-2727
Fax: (312) 541-4998
URL: <http://www.ascp.org>

American Society of Hematology

1900 M Street, NW, Suite 200
Washington, DC 20036
Phone: (202) 776-0544
Fax: (202) 776-0545
URL: <http://www.hematology.org>

**American Society of Pediatric Hematology/
Oncology**

4700 W. Lake Ave.
Glenview, IL 60025-1485
Phone: (847) 375-4716
Fax: (877) 734-9557
URL: <http://www.aspbo.org>

America's Blood Centers

725 15th Street, NW, Suite 700
Washington, DC 20005
Phone: (202) 393-5725
Fax: (202) 393-1282
URL: <http://www.americasblood.org>

**Blood and Marrow Transplant Information
Network**

2900 Skokie Valley Rd., Suite 104
Highland Park, IL 60035
Phone: (888) 597-7674, (847) 433-3313
Fax: (847) 433-4599
URL: <http://www.bmtnews.org>

**Canadian Blood and Marrow Transplant
Group**

375 West 5th Ave., Suite 201
Vancouver, BC V5Y 1J6
Canada
Phone: (604) 874-4366
Fax: (604) 874-4378
URL: <http://www.cbmtg.org>

**Center for International Blood and Marrow
Transplant Research**

8701 Watertown Plank Rd.
P.O. Box 26509
Milwaukee, WI 53226
Phone: (414) 456-8325
Fax: (414) 456-6530
URL: <http://www.ibmtr.org>

Centers for Disease Control and Prevention

1600 Clifton Rd.
Atlanta, GA 30333
Phone: (404) 639-3534 or (800) 311-3435
URL: <http://www.cdc.gov>

Clinical and Laboratory Standards Institute

(Formerly the National Committee for Clinical
Laboratory Standards)
940 West Valley Rd., Suite 1400
Wayne, PA 19087-1898
Phone: (610) 688-0100
Fax: (610) 688-0700
URL: <http://www.cls.org>

Clinical Laboratory Management Association

989 Old Eagle School Rd., Suite 815
Wayne, PA 19087
Phone: (610) 995-2640
Fax: (610) 995-9568
URL: <http://www.clma.org>

College of American Pathologists

325 Waukegan Rd.
Northfield, IL 60093-2750
Phone: (847) 832-7000 or (800) 323-4040
Fax: (847) 832-8000
URL: <http://www.cap.org>

Food and Drug Administration

Center for Biologics Evaluation and Research
5600 Fishers Lane
Rockville, MD 20857
Phone: (888) 463-6332
<http://www.fda.gov/cber/index.html>

Foundation for the Accreditation of Cellular Therapy
c/o University of Nebraska Medical Center
986065 University Medical Center
Omaha, NE 68198-6065
Phone: (402) 559-1950
Fax: (402) 559-1951
URL: <http://www.unmc.edu/Community/fahct/Default.htm>

International Society for Apheresis
c/o Department of Surgery
Shiga University of Medical Science
Seta Tsukinowa-cho
Otsu, Shiga 520-2192
Japan
Fax: +81(77) 548-2240
URL: <http://www.shiga-med.ac.jp/~hqsurge1/ISFA.html>

International Society for Cellular Therapy
(Formerly the International Society for Hematotherapy and Graft Engineering)
375 West 5th Ave., Suite 201
Vancouver, BC V5Y 1J6
Canada
Phone: (604) 874-4366
Fax: (604) 874-4378
URL: <http://www.celltherapysociety.org>

International Society of Blood Transfusion
Jan van Goyenkade, 11
1075 HP Amsterdam
The Netherlands
Phone: +31 (0) 20 679 3411
Fax: +31 (0) 20 673 7306
URL: <http://www.isbt.web.org>

International Society on Thrombosis and Haemostasis
610 Jones Ferry Rd., Suite 205
Carrboro, NC 27510
Phone: (919) 929-3807
Fax: (919) 929-3935
URL: <http://www.med.unc.edu/isth/welcome>

The Joint Commission
(Formerly The Joint Commission on Accreditation of Healthcare Organizations)
One Renaissance Blvd.
Oak Brook Terrace, IL 60181
Phone: (630) 792-5000
Fax: (630) 792-5005
URL: <http://www.jointcommission.org>

National Heart, Lung, and Blood Institute
Building 31, Room 5A48
31 Center Drive, MSC 2486
Bethesda, MD 20892
Phone: (301) 435-0080
URL: <http://www.nhlbi.nih.gov>

National Hemophilia Foundation
116 West 32nd Street, 11th Floor
New York, NY 10001
Phone: (212) 328-3700 or (800) 42-HANDI
Fax: (212) 328-3799
URL: <http://www.hemophilia.org>

National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-4000
URL: <http://www.nih.gov>

National Library of Medicine
8600 Rockville Pike
Bethesda, MD 20894
Phone: (301) 594-5983 or (888) FIND-NLM
Fax: (301) 402-1384
URL: <http://www.nlm.nih.gov>

National Marrow Donor Program
3001 Broadway St. NE, Suite 500
Minneapolis, MN 55413-1753
Phone: (800) 526-7809
Fax: (612) 627-8125
URL: <http://www.nmdp.org>

Plasma Protein Therapeutic Association
PPTA North America (formerly the American Blood Resources Association)
147 Old Solomons Island Rd. (Suite 100)
Annapolis, MD 21401
Phone: (410) 263-8296
Fax: (410) 263-2298
URL: <http://www.pptaglobal.org>

World Apheresis Association

c/o Dr. P. Coppo
Hôpital Cochin
Unité de Médecine Transfusionnelle
27, rue du Faubourg Saint-Jacques
75679 Paris Cedex 14
France
Phone/fax: +33 1 42 08 80 15
URL: <http://www.worldapheresis.org>

World Federation of Hemophilia

1425 René Lévesque Boulevard West,
Suite 1010
Montréal, Quebec
Canada, H3G 1T7
Phone: (514) 875-7944
Fax: (514) 875-8916
URL: <http://www.wfh.org>

World Health Organization

Headquarters Office
Avenue Appia 20
CH-1211 Geneva 27
Switzerland
Phone: +41 22 791 21 11
Fax: +41 22 791 31 11
URL: <http://www.who.int>

B. State And Regional Associations

California Blood Bank Society
<http://www.cbbsweb.org>

Florida Association of Blood Banks
<http://www.floridaabb.org>

Heart of America Association of Blood Banks
<http://www.haabb.org>

Illinois Association of Blood Banks
<http://www.ilabb.org>

Indiana State Association of Blood Bankers
<http://www.isabb.org>

Kentucky Association of Blood Banks
<http://www.kabb.org>

Massachusetts Association of Blood Banks

<http://www.mabbweb.org>

Michigan Association of Blood Banks

<http://www.mabb.org>

Mid-Atlantic Association of Blood Banks

<http://www.maabb.org>

Minnesota Association of Blood Banks

<http://www.mnabb.org>

New York State, Blood Bank Association of

<http://www.bbanys.org>

North Carolina Association of Blood Bankers

<http://www.ncabb.org>

Ohio Association of Blood Banks

<http://www.oabb.org>

South Central Association of Blood Banks

<http://www.scabb.org>

C. Journals

The following is a list of journals of interest to blood bank and transfusion medicine professionals. It is not all inclusive, and the reader is encouraged to consult the National Library of Medicine's Internet Grateful Med web site at <http://www.nlm.nih.gov> for additional resources.

American Journal of Hematology

<http://www.interscience.wiley.com>

Blood

<http://www.bloodjournal.org>

Blood Cells, Molecules, and Diseases

<http://www.academicpress.com/bcmd>

Blood Coagulation and Fibrinolysis

<http://www.bloodcoagulation.com>

British Journal of Haematology

<http://www.blackwellpublishing.com/journals/bjh>

Journal of the American Medical Association
<http://jama.ama-assn.org>

Journal of Clinical Apheresis
<http://www.interscience.wiley.com>

Pathophysiology of Haemostasis and Thrombosis
<http://www.karger.com/pht>

Transfusion
<http://www.transfusion.org>

Transfusion Medicine
<http://www.blackwellpublishing.com/journal.asp?ref=0958-7578&site=1>

Transfusion Medicine Reviews
<http://www.tmreviews.com>

Transfusion and Apheresis Science
<http://www.elsevier.com>

Vox Sanguinis
(Journal of the International Society of Blood Transfusion)
<http://www.blackwellpublishing.com/journals/vox>

D. OTHER COMMUNICATIONS

AABB Weekly Report, AABB News, Smart Brief
<http://www.aabb.org>

Blood Weekly
<http://www.newsrx.com/newsletters/Blood-Weekly.html>

E. INTERNET INFORMATION RESOURCES

Because the Internet is an ever-changing resource, readers should not consider this list to be all inclusive.

BloodLine (resource for hematology education and news)
<http://www.bloodline.net>

BloodMed (peer-reviewed resource for hematology education, practice, and research)
<http://www.bloodmed.com>

BloodNet (information on blood donation, blood-related disorders, professional organizations, and blood-related research)
<http://www.bloodnet.org>

Hematology on MedMark (lists hematology associations/societies, centers/institutes/labs, departments/ divisions, education/training, information for consumers, general information, guides/guidelines, images/atlasses, information sources, journals/news/publications, other related resources and organizations)
<http://medmark.org/hem/hem2.html>

Mednets (Information on hematology/oncology associations, journals, online resources, clinical information, diseases, news and research, centers of excellence, and other information)
<http://www.mednets.com>

National Archives and Records Administration (Federal Register)
<http://origin.www.gpoaccess.gov/fr>

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