Protocol for the Examination of Hematologic Malignancies in Bone Marrow

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| **Version:** Bone Marrow 4.0.0.0 | **Protocol Posting Date:** February 2019 |
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**Accreditation Requirements**

The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

**This protocol is intended to be used for the following procedures AND tumor types:**

|  |  |
| --- | --- |
| **Procedure** | **Description** |
| Bone marrow trephine biopsy | Includes specimens designated non-targeted bone marrow biopsy and touch preparations |
| Bone marrow aspiration | Includes non-targeted bone marrow aspiration, clot section, and aspirate smear preparations |
| **Tumor Type** | **Description** |
| Myeloproliferative neoplasms  Mastocytosis  Myelodysplastic syndromes  Myelodysplastic/myeloproliferative neoplasms  Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement  Myeloid neoplasms with germline predisposition  Blastic plasmacytoid dendritic neoplasm  Acute myeloid leukemia  Acute leukemia of mixed/ambiguous lineage  Acute lymphoblastic leukemia/lymphoma  Mature B-cell neoplasms with leukemic presentation (excluding plasma cell myeloma)  Mature T- and NK-cell neoplasms with leukemic presentation | Includes most primary myeloid malignancies, acute leukemias, and mature B-cell, T-cell, and NK-cell neoplasms with frequent leukemic presentation. Disease entities in this protocol are based on the 2017 revised fourth edition World Health Organization classification and include provisional entries. |

**The following should NOT be reported using this protocol:**

|  |
| --- |
| **Procedure** |
| Extramedullary biopsy specimens |
| Peripheral blood smears (without bone marrow material) |
| **Tumor Type** |
| Plasma cell myeloma (consider the Plasma Cell Neoplasm protocol) |
| Histiocytic disorders involving the bone marrow |
| Non-neoplastic diseases of the bone marrow |
| Secondary marrow involvement by lymphoma or metastatic cancer (consider the Hodgkin or non-Hodgkin Lymphoma Protocols) |

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The pathologist responsible for issuing the diagnostic bone marrow report included in the patient’s medical record and forming the basis of treatment decisions is encouraged to fill out the synoptic report. The pathologist may need to include data elements generated at an external facility (e.g. reference lab) for inclusion in the synoptic report. In these scenarios, it is understood that the pathologist is not assuming responsibility for the technical accuracy of such external data.

CAP Bone Marrow Protocol Summary of Changes

Version 4.0.0.0

**The following data elements were added:**

Clinical Context

Peripheral Blood Complete Blood Cell Count

## Bone Marrow Cellularity

Bone Marrow Blasts

Bone Marrow Lymphocytes

Biomarker Information

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| --- |
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**Surgical Pathology Cancer Case Summary**

Protocol posting date: February 2019

**BONE MARROW: Final Integrated Diagnosis**

**Note: This case summary is recommended for reporting hematologic malignancies in bone marrow but is NOT REQUIRED for accreditation purposes. Core data elements are bolded to help identify routinely reported elements.**

**Note: If the Integrated Diagnosis section is not applicable, proceed to Histological Assessment summary.**

**Select a single response.**

**Final Integrated Diagnosis (Note A)**

## Myeloproliferative neoplasms

\_\_\_ Chronic myeloid leukemia, *BCR-ABL1* positive

\_\_\_ Chronic neutrophilic leukemia

\_\_\_ Polycythemia vera

\_\_\_ Primary myelofibrosis

\_\_\_ Essential thrombocythemia

\_\_\_ Chronic eosinophilic leukemia, NOS

\_\_\_ Myeloproliferative neoplasm, unclassifiable

### Mastocytosis

### \_\_\_ Systemic mastocytosis

### \_\_\_ Mast cell leukemia

Myelodysplastic syndromes (MDS)

\_\_\_ Myelodysplastic syndrome with single lineage dysplasia

\_\_\_ Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia

\_\_\_ Myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia

\_\_\_ Myelodysplastic syndrome with multilineage dysplasia

\_\_\_ Myelodysplastic syndrome with excess blasts-1

\_\_\_ Myelodysplastic syndrome with excess blasts-2

\_\_\_ Myelodysplastic syndrome with isolated del(5q)

\_\_\_ Myelodysplastic syndrome, unclassifiable

## \_\_\_ Refractory cytopenia of childhood

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

\_\_\_ Chronic myelomonocytic leukemia

\_\_\_ Atypical chronic myeloid leukemia, *BCR-ABL1-*negative

\_\_\_ Juvenile myelomonocytic leukemia

\_\_\_ Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis

\_\_\_ Myelodysplastic/myeloproliferative neoplasm, unclassifiable

Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement

\_\_\_ Myeloid/lymphoid neoplasm with *PDGFRA* rearrangement

\_\_\_ Myeloid/lymphoid neoplasm with *PDGFRB* rearrangement

\_\_\_ Myeloid /lymphoid neoplasm with *FGFR1* rearrangement

\_\_\_ Myeloid /lymphoid neoplasm with *PCM1-JAK2*

Acute myeloid leukemia (AML) and acute leukemias of ambiguous lineage

\_\_\_ Acute myeloid leukemia, NOS

\_\_\_ Acute myeloid leukemia with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*

\_\_\_ Acute myeloid leukemia with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*

\_\_\_ Acute promyelocytic leukemia with *PML-RARA*

\_\_\_ Acute myeloid leukemia with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*

\_\_\_ Acute myeloid leukemia with t(6;9)(p23;q34.1);*DEK-NUP214*

\_\_\_ Acute myeloid leukemia with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*

\_\_\_ Acute myeloid leukemia (megakaryoblastic) with t(1;22)(p13.3;q13.1);*RBM15-MKL1*

\_\_\_ Acute myeloid leukemia with *BCR-ABL1*

\_\_\_ Acute myeloid leukemia with mutated *NPM1*

\_\_\_ Acute myeloid leukemia with biallelic mutations of *CEBPA*

\_\_\_ Acute myeloid leukemia with mutated *RUNX1*

\_\_\_ Acute myeloid leukemia with myelodysplasia-related changes

\_\_\_ Therapy-related myeloid neoplasm

\_\_\_ Acute myeloid leukemia with minimal differentiation

\_\_\_ Acute myeloid leukemia without maturation

\_\_\_ Acute myeloid leukemia with maturation

\_\_\_ Acute myelomonocytic leukemia

\_\_\_ Acute monoblastic/monocytic leukemia

\_\_\_ Pure erythroid leukemia

\_\_\_ Acute megakaryocytic leukemia

\_\_\_ Acute basophilic leukemia

\_\_\_ Acute panmyelosis with myelofibrosis

\_\_\_ Acute undifferentiated leukemia

\_\_\_ Mixed phenotype acute leukemia with t(9;22)(q34.1;q11.2); *BCR-ABL1*

\_\_\_ Mixed phenotype acute leukemia with t(v;11q23.3); *KMT2A* rearranged

\_\_\_ Mixed phenotype acute leukemia, B/myeloid, NOS

\_\_\_ Mixed phenotype acute leukemia, T/myeloid, NOS

\_\_\_ Mixed phenotype acute leukemia, NOS

\_\_\_ Acute leukemia of ambiguous lineage, NOS

\_\_\_ Transient abnormal myelopoiesis (TAM) associated with Down syndrome

\_\_\_ Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

\_\_\_ Blastic plasmacytoid dendritic cell neoplasm

Precursor lymphoid neoplasms

\_\_\_ B-lymphoblastic leukemia/lymphoma, NOS

\_\_\_ B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*

\_\_\_ B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A* rearranged

\_\_\_ B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*

\_\_\_ B-lymphoblastic leukemia/lymphoma with hyperdiploidy

\_\_\_ B-lymphoblastic leukemia/lymphoma with hypodiploidy

\_\_\_ B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3); *IGH-IL3*

\_\_\_ B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*

\_\_\_ B-lymphoblastic leukemia/lymphoma, *BCR-ABL1-*like

\_\_\_ B-lymphoblastic leukemia/lymphoma with iAMP21

\_\_\_ T-lymphoblastic leukemia/lymphoma

\_\_\_ Early T-cell precursor lymphoblastic leukemia

\_\_\_ NK-lymphoblastic leukemia/lymphoma

Mature B-cell neoplasms

\_\_\_ Chronic lymphocytic leukemia/small lymphocytic lymphoma

\_\_\_ B-cell prolymphocytic leukemia

\_\_\_ Hairy cell leukemia

\_\_\_ Lymphoplasmacytic lymphoma

\_\_\_ Other mature B-cell neoplasm (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mature T and NK cell neoplasms

\_\_\_ T-cell prolymphocytic leukemia

\_\_\_ T-cell large granular lymphocytic leukemia

\_\_\_ Chronic lymphoproliferative disorder of NK-cells

\_\_\_ Aggressive NK-cell leukemia

\_\_\_ Systemic EBV-positive T-cell lymphoma of childhood

\_\_\_ Hepatosplenic T-cell lymphoma

\_\_\_ Adult T-cell leukemia/lymphoma

\_\_\_ Other mature T/NK-cell neoplasm (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Other

\_\_\_ Other histologic type not listed (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined

**Surgical Pathology Cancer Case Summary**

Protocol posting date: February 2019

**BONE MARROW: Histologic Assessment**

**Note: This case summary is recommended for reporting hematologic malignancies in bone marrow but is NOT REQUIRED for accreditation purposes. Core data elements are bolded to help identify routinely reported elements.**

**Select a single response unless otherwise indicated.**

Clinical

## Clinical context

\_\_\_ New diagnosis, untreated

\_\_\_ New diagnosis, treatment status unknown

\_\_\_ Follow up sample

\_\_\_ Other (specify, or state if unknown): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## Procedure (select all that apply) (Note B)

\_\_\_ Bone marrow aspiration

\_\_\_ Bone marrow aspirate clot

\_\_\_ Bone marrow core biopsy

\_\_\_ Bone marrow core touch preparation (imprint)

\_\_\_ Other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## Peripheral Blood Complete Blood Cell Count

White blood cell count: \_\_\_ x103 / µL

Neutrophils: \_\_\_%

Monocytes: \_\_\_%

Lymphocytes: \_\_\_%

Eosinophils: \_\_\_%

Basophils: \_\_\_%

Blasts: \_\_\_%

Other cells: \_\_\_%

Cell type (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_

Hemoglobin: \_\_\_ g/dL

Platelets: \_\_\_ x103 / µL

## Bone Marrow Morphology (Note C)

## Bone Marrow Cellularity: \_\_\_%

**Bone Marrow Blasts: \_\_\_ %**

**Bone Marrow Lymphocytes (report for lymphoid malignancies): \_\_\_ %**

## Dysplasia (report for myeloid malignancies)

\_\_\_ Absent

\_\_\_ Present (select all that apply)

\_\_\_ Granulocytic lineage

\_\_\_ Erythroid lineage

\_\_\_ Megakaryocytic lineage

Special Stains (Note D)

## Iron stain (report for myeloid malignancies)

\_\_\_ No ring sideroblasts detected

\_\_\_ Positive for ring sideroblasts (specify percent of erythroid precursors): \_\_\_\_\_%

\_\_\_ Stain not evaluable (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Reticulin/Trichrome stains (fibrosis grade) (report if applicable)**

## \_\_\_ MF-0

\_\_\_ MF-1

\_\_\_ MF-2

\_\_\_ MF-3

**Histologic Group**

### \_\_\_ Myeloproliferative neoplasm

### \_\_\_ Mastocytosis

\_\_\_ Myelodysplastic syndrome (MDS)

\_\_\_ Myelodysplastic/myeloproliferative neoplasm (MDS/MPN)

\_\_\_ Acute myeloid leukemia (AML)

\_\_\_ Blastic plasmacytoid dendritic cell neoplasm

\_\_\_ Precursor lymphoid neoplasm (acute lymphoblastic leukemia/lymphoma)

\_\_\_ Mature B-cell neoplasm

\_\_\_ Mature T and NK cell neoplasm

\_\_\_ Other histologic group not listed (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined

**Biomarker Studies (Note D)**

\_\_\_ Testing performed (see Bone Marrow Biomarker Reporting Template)

\_\_\_ Pending

\_\_\_ Not performed

\_\_\_ Not applicable

Comment(s)

**Bone Marrow Biomarker Reporting Template**

Protocol posting date: February 2019

**BONE MARROW: Biomarker Template**

**Note: This case summary is recommended for reporting hematologic malignancies in bone marrow but is NOT REQUIRED for accreditation purposes.**

## Biomarker Studies (Note D)

Immunohistochemistry

\_\_\_ Immunophenotype of neoplastic cells (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Not performed

Flow Cytometry (select all that apply)

\_\_\_ No aberrant populations identified

\_\_\_ Positive for aberrant myeloid blast population

\_\_\_ Immunophenotype (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Positive for aberrant lymphoid blast population

\_\_\_ Immunophenotype (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Positive for aberrant mixed phenotype blast population

\_\_\_ Immunophenotype (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Positive for aberrant mature B-cell population

\_\_\_ Immunophenotype (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Positive for aberrant mature T-cell population

\_\_\_ Immunophenotype (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Positive for other aberrancy (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Immunophenotype (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Not performed

*Note: Specify immunophenotype or refer to separate report*

Cytogenetics

\_\_\_ Normal diploid karyotype

\_\_\_ Abnormal karyotype

\_\_\_ Karyotype (specify: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Not performed

*Note: Specify karyotype or refer to separate report*

Fluorescence in situ Hybridization

\_\_\_ Normal pattern (specify panel): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Abnormal pattern (specify panel): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Not performed

Molecular Diagnostics

## *BCR-ABL1* Transcript by RT-PCR Testing (select all that apply)

\_\_\_ Absent

\_\_\_ Present

\_\_\_ Present transcript type p210

\_\_\_ Present transcript type p190

\_\_\_ Present transcript type, other (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *CEBPA* (mono-allelic) Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *FLT3*-ITD *(*internal tandem duplication) Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *FLT3* p.D835 *(*tyrosine kinase domain)Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *JAK2* p.V617F Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *MYD88 p.L265P* Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *NPM1* Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *PML-RARA* Transcript by RT-PCR Testing

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *RUNX1* Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## *SF3B1* Mutation

\_\_\_ Absent

\_\_\_ Present (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Cannot be determined (explain): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## Specify Other Mutations (repeat as needed)

\_\_\_ Absent for other mutation(s) (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_ Present for other mutation(s) (specify): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Comments:

**Explanatory Notes**

## Introduction

## The aim of this protocol is to improve the completeness, clarity, and portability of bone marrow reporting in routine clinical practice settings, while being mindful of the wide range of practices in which the data in the report are generated and disseminated. Diagnostic workup of hematologic neoplasms requires the integration of data from multiple sources, including microscopic evaluation, flow cytometry immunophenotyping, cytogenetic analysis, and molecular testing. These requirements are reflected in the World Health Organization (WHO) classification of hematolymphoid malignancies. While this protocol emphasizes diagnostic data elements, it should be noted that many markers considered previously to be diagnostic in nature now provide the basis for frontline treatment decisions thus obscuring the boundaries of diagnostic and biomarker testing (e.g. CD20 and CD33 expression, *BCR/ABL1* fusion, *JAK2*p.V617F and *FLT3* mutations, etc.).

## This protocol is based to a large extent on the following documents:

## Revised 4th edition of the WHO classification.1

## CAP bone marrow synoptic reporting guidelines for hematologic neoplasms.2

## CAP/ASH clinical practice guidelines on initial diagnostic workup of acute leukemia.3

## CAP templates for reporting results of biomarker testing.4,5

In a disease group where complementary tests may on occasion yield results that challenge the notion of succinct reporting (e.g. complex karyotype, complex mutational profile, etc.), a midway stance was adopted in this protocol to translate complex results into simpler terms that best inform treatment decisions and risk stratification. The synoptic report might thus on occasion lack the full scope of complexity of a given patient’s neoplasm, requiring reliance on source documentation. Previously published templates for reporting biomarker testing for myeloproliferative neoplasms (MPN) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) provide more in-depth details compared to the corresponding generic entries in the present protocol. The choice between the generic entries and the more detailed MPN and CLL/SLL templates, where applicable, is left to local discretion.

**Integrated Diagnosis**

It is understood that the preferred timepoint at which this synoptic report is to be completed might vary by practice patterns and could be influenced by myriad factors, including the information technology environment. Notwithstanding, in the interest of medical record clarity and to avoid effort redundancy, *it is recommended that this synoptic report be completed when all tests required for definitive WHO classification are completed*. In other words, the use of this protocol would be most appropriate as a template for an “integrated report” in cases with hematologic malignancy, rather than for use as a standard template for morphology-based reporting. To this end, this protocol is structured in a “layered” reporting format as follows:

Layer 1: Integrated diagnosis (incorporating all tissue-based information)

Layer 2: Histological assessment

Layer 3: Biomarker studies

## References

1. Swerdlow HS, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thele J eds. *World Health Organization Histological Classification of Tumors of* *Haematopoietic and Lymphoid Tissues* (ed 4th (rev)). Lyon, France: IARC Press; 2017.
2. Sever C, Abbott CL, de Baca ME, et al. Bone Marrow Synoptic Reporting for Hematologic Neoplasms: Guideline From the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med.* 2016;140(9):932-949.
3. Arber DA, Borowitz MJ, Cessna M, et al. Initial Diagnostic Workup of Acute Leukemia: Guideline From the College of American Pathologists and the American Society of Hematology. *Arch Pathol Lab Med*. 2017;141(10):1342-1393.
4. Kelley TW, Arber DA, Gibson C, et al. Template for Reporting Results of Biomarker Testing of Specimens From Patients With Myeloproliferative Neoplasms. *Arch Pathol Lab Med*. 2016;140(7):675-677, www.cap.org/cancerportocols.
5. Duncavage E, Advani RH, Agosti S, et al. Template for Reporting Results of Biomarker Testing of Specimens From Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. Arch Pathol Lab Med. 2016, www.cap.org/cancerportocols.

## Sample Type and Clinical Data

Bone marrow evaluation is a critical part of the evaluation of patients suspected of having a hematologic malignancy. At initial presentation, sampling should ideally entail a core (trephine) biopsy and aspiration, with procurement of sufficient material for microscopic evaluation, flow cytometry immunophenotyping, cytogenetics, and molecular studies. It is advisable that a portion of the aspirate material be used to prepare a formalin-fixed paraffin-embedded clot sample (cell block). While this protocol is intended primarily for reporting of bone marrow specimens, applicable elements can be used for reporting extramedullary hematologic neoplasms if needed.

Although the availability of clinical and laboratory data may be limited in certain practice settings, efforts to ensure that the pathologist has at their disposal as much pertinent information as possible to inform their diagnostic assessment are highly encouraged. This premise has been endorsed jointly by the College of American Pathologists and the American Society of Hematology.1 Inclusion of pertinent clinical and laboratory data in the bone marrow synoptic report is an evidence-based strong recommendation2, which often also serves as a basis for classification or subclassification on certain hematologic malignancies.

## References

1. Arber DA, Borowitz MJ, Cessna M, et al. Initial Diagnostic Workup of Acute Leukemia: Guideline From the College of American Pathologists and the American Society of Hematology. *Arch Pathol Lab Med*. 2017;141(10):1342-1393.
2. Sever C, Abbott CL, de Baca ME, et al. Bone Marrow Synoptic Reporting for Hematologic Neoplasms: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med.* 2016;140(9):932-949.

## Morphology

Bone marrow aspirate smears stained with Wright Giemsa or May-Grunwald-Giemsa stain should be used to perform a 500-cell differential count to enumerate bone marrow blasts (including promonocytes where pertinent) and other marrow cellular elements excluding megakaryocytes. Adequate aspirate smears are also a prerequisite for optimal evaluation for dysplasia. If aspirate smears are limited, touch preparations might provide an alternative; on these a 300-cell differential is recommended. A 200-cell differential is recommended on peripheral blood smears. In instances where sample adequacy does not permit an adequate manual count, the number of blasts and other elements in the bone marrow may be estimated based on ancillary studies, particularly immunohistochemistry, and this would be specified in the pathology report. Descriptors for sample adequacy should be included in the bone marrow report. In this protocol, entries for dysplasia and the percentage of bone marrow blasts and lymphocytes are recommended, as applicable. For instance, reference to dysplasia or the percentage of blasts might not be necessary in a sample involved by chronic lymphocytic leukemia/small lymphocytic lymphoma.

Age-matched bone marrow cellularity should be estimated on 3-4 µm thick sections of formalin-fixed paraffin-embedded tissue sections of the core biopsy and/or clot preparation stained with hematoxylin and eosin, whichever is deemed most representative in a given sample. Normal ranges of bone marrow cellularity vary with age, as described in Table 1.1

**Table 1. Normal ranges of bone marrow cellularity.**

|  |  |
| --- | --- |
| **Age (years)** | **% Hematopoietic area** |
| 20-30 | 60-70 |
| 40-60 | 40-50 |
| ≥70 | 30-40 |

References

1. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica*. 2005;90(8):1128-1132.

## Ancillary and Biomarker Studies

## Special Stains

A number of cytochemical stains may be utilized in the evaluation of hematologic neoplasms. An iron (Prussian blue) stain is required for assessment of stainable iron in erythroid precursors and the identification of ring sideroblasts.1 Stainable iron is best evaluated on an aspirate smear or a touch preparation in cases for which such preparations are available. Evaluation of stainable iron on biopsy specimens should be avoided because of limited visualization of ring sideroblasts and the impact of decalcification on iron content. Aberrant cytoplasmic periodic acid-Schiff (PAS) positivity, either diffuse or granular, is a characteristic of dysplasia in erythroid precursors. Cytochemical detection of myeloperoxidase is a rapid and cost-effective tool in the initial workup of acute myeloid leukemia. Notwithstanding, although useful and practical for lineage determination in some instances, cytochemical stains are no longer required for the diagnostic workup of most hematologic neoplasms.

The assessment of bone marrow fibrosis requires a good-quality reticulin stain.2 The WHO classification recommends the use of trichrome stain in samples with moderate or severe fibrosis (see below). Bone marrow fibrosis grading scheme is summarized in Table 2.

**Table 2. Semi-quantitative grading of bone marrow fibrosis.**

|  |  |
| --- | --- |
| **Myelofibrosis grade** | **Description** |
| MF-0 | Scattered linear reticulin with no intersections (crossovers) corresponding to normal BM. |
| MF-1 | Loose network of reticulin with many intersections, especially in perivascular areas. |
| MF-2 | Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis. |
| MF-3 | Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers consistent with collagen, usually associated with osteosclerosis. |

Fiber density should be assessed only in hematopoietic areas.

In grades MF-2 or MF-3 an additional trichrome stain is recommended.

## Immunophenotyping

Immunophenotyping of bone marrow specimens can be performed by flow cytometry or immunohistochemistry for diagnostic evaluation and for biomarker assessment.3 Both techniques provide diagnostic, prognostic, and therapy-guiding data elements, and each technique has advantages and disadvantages. Flow cytometry is rapid (hours), quantitative, and allows multiple antigens to be evaluated on the same cell simultaneously. Flow cytometry is the gold standard for minimal residual disease detection in patients with acute leukemia.

Immunohistochemistry permits correlation of antigen expression with architecture and cytomorphology, and it can be performed on archival material.

**Cytogenetics, Fluorescence in situ Hybridization, and Molecular Genomics Studies**

Cytogenetic and molecular data are integral to the evaluation of patients with primary bone marrow neoplasms. Cytogenetic analysis typically entails conventional karyotyping and FISH. Conventional karyotyping requires viable cells. FISH may be performed on metaphase spreads from karyotyping studies or on air-dried, fresh unfixed aspirate or touch preparation slides. Array comparative genomic hybridization (aCGH) is used as an adjunct tool to detect copy number changes in certain conditions. Unlike conventional karyotyping, aCGH does not require viable cells.

The WHO classification recognizes certain cytogenetic abnormalities as sufficient for a diagnosis of acute myeloid leukemia with myelodysplasia-related change if the bone marrow blast percentage is greater than 20% and the patient has no history of antecedent cytotoxic therapy. These abnormalities are summarized in Table 3.

**Table 3. Myelodysplasia-related cytogenetic changes.**

|  |
| --- |
| Complex karyotype (3 or more abnormalities) |
| Unbalanced abnormalities |
| -7/del(7q)  del(5q)/t(5q)  i(17q)/t(17p)  -13/del(13q)  del(11q)  del(12p)/t(12p)  idic(X)(q13) |
| Balanced abnormalities |
| t(11;16)(q23.3;p13.3)  t(3;21)(q26.2;q22.1)  t(1;3)(p36.3;q21.2)  t(2;11)(p21;q23.3)  t(5;12)(q32;p13.2)  t(5;7)(q32;q11.2)  t(5;17)(q32;p13.2)  t(5;10)(q32;q21.2)  t(3;5)(q25.3;q35.1) |

The advent of next-generation sequencing (NGS) has altered the landscape of molecular diagnostics. Mutation profiling using gene panels that range from tens to hundreds of genes is increasingly becoming widespread, providing valuable diagnostic, prognostic, and therapy-guiding data. Mutation profiling may be done at initial diagnosis or at subsequent timepoints such as at transformation or relapse. There are no definitive approaches to synoptic reporting of NGS-based mutation profiling results. In this protocol, entries for mutation data on key genes are included in the generic portion of the document. Inclusion of mutation results for other genes is kept at the discretion of the pathologist completing the synoptic report.

References

1. Della Porta MG, Travaglino E, Boveri E, et al. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. *Leukemia* 2015;29: 66–75.
2. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica*. 2005;90(8):1128-1132.
3. Khoury JD, Wang WL, Prieto VG, et al. Validation of Immunohistochemical Assays for Integral Biomarkers in the NCI-MATCH EAY131 Clinical Trial. *Clin Cancer Res*. 2018;24(3):521-531.

**Sample synoptic reports**

Pathologists may use synoptic reporting tools incorporated into pathology informatics systems or use custom template forms to incorporate the data elements and responses into cancer pathology reports. The following are examples of synoptic reports using this version of the protocol.

Example 1

Integrated Diagnosis: Hairy cell leukemia

Procedure: Bone marrow aspiration, clot, and core biopsy

Peripheral blood complete blood cell count:

White blood cell count: 2.3 x 103/µL

Hemoglobin: 10.4 g/dL

Platelets: 112 x 103/µL

Bone Marrow Cellularity: 20%

Bone Marrow Lymphocytes: 30%

Immunohistochemistry: Positive for annexin A1 and BRAF p.V600E

Flow cytometry: Aberrant B-cell population: Positive for CD11c, CD19, CD20, CD22, CD25, CD103, CD123, kappa; negative for CD5, CD10, lambda

Cytogenetics: Normal diploid karyotype

Molecular Diagnostics: Other mutation testing performed:

Positive: *BRAF* p.V600E

Example 2

Integrated Diagnosis: Chronic myelomonocytic leukemia-1

Procedure: Bone marrow aspiration, clot, and core biopsy

Peripheral blood complete blood cell count:

White blood cell count: 13.7 x 103/µL

Monocytes: 21%

Blasts: 1%

Hemoglobin: 11.3 g/dL

Platelets: 68 x 103/µL

Bone Marrow Cellularity: 70%

Bone Marrow Blasts: 6%

Dysplasia: Present; erythroid, megakaryocytic

Flow cytometry: Positive for other aberrancy: CD56+monocytes

Cytogenetics: Normal diploid karyotype

Fluorescence in situ hybridization: Negative for *BCR/ABL1* fusion

Molecular Diagnostics: Negative for *JAK2* p.V617Fmutation

Other mutation testing performed:

Positive: *NRAS* p.G12D