Protocol for the Examination of Specimens From Patients With Hematopoietic Neoplasms Involving the Bone Marrow*

Version: BoneMarrow 3.1.0.0  Protocol Posting Date: January 2018
This protocol is NOT required for accreditation purposes

*This protocol applies to bone marrow specimens involved by primary hematopoietic neoplasms. The plasma cell neoplasm protocol is recommended for reporting bone marrow samples with plasma cell myeloma.

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.

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Accreditation Requirements
This protocol can be utilized for clinical care purposes, but is not required for accreditation purposes.

CAP Laboratory Accreditation Program Protocol Required Use Date: Not applicable

CAP Bone Marrow Protocol Summary of Changes

v3.1.0.0
The following data elements were modified:
Histologic Type
Surgical Pathology Cancer Case Summary

Protocol posting date: January 2018

BONE MARROW: Aspiration, Core (Trephine) Biopsy

Note: This case summary is recommended for reporting bone marrow specimens, but is not required for accreditation purposes.

Select a single response unless otherwise indicated.

Specimen (select all that apply) (Note A)
___ Peripheral blood smear
___ Bone marrow aspiration
___ Bone marrow aspirate clot (cell block)
___ Bone marrow core (trephine) biopsy
___ Bone marrow core touch preparation (imprint)
___ Other (specify): ___________________________
___ Not specified

Procedure (select all that apply)
___ Aspiration
___ Biopsy
___ Other (specify): ___________________________
___ Not specified

Aspiration Site (if performed) (select all that apply) (Note B)
___ Right posterior iliac crest
___ Left posterior iliac crest
___ Sternum
___ Other (specify): ___________________________
___ Not specified

Biopsy Site (if performed) (select all that apply) (Note B)
___ Right posterior iliac crest
___ Left posterior iliac crest
___ Other (specify): ___________________________
___ Not specified

Histologic Type (Note C)

Note: The following is a partial list of the 2017 World Health Organization (WHO) classification and includes those neoplasms seen in bone marrow specimens. The list does not include provisional entities.

___ Histologic type cannot be assessed

Myeloproliferative Neoplasms
___ Chronic myelogenous leukemia, BCR-ABL1 positive
___ Chronic neutrophilia leukemia
___ Polycythemia vera
___ Primary myelofibrosis
___ Essential thrombocytemia
___ Chronic eosinophilic leukemia, not otherwise specified (NOS)

+ Data elements preceded by this symbol may be clinically important but are not yet validated or regularly used in patient management.
**Mastocytosis**

- Systemic mastocytosis
- Mast cell sarcoma

**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

**Myelodysplastic/Myeloproliferative Neoplasms**

- 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

**Myelodysplastic Syndromes**

- Myelodysplastic syndrome with single lineage dysplasia
- Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia
- Myelodysplastic syndrome with ring sideroblasts and multi-lineage lineage dysplasia
- Myelodysplastic syndrome with multilineage dysplasia
- Myelodysplastic syndrome with excess blasts
- Myelodysplastic syndrome with isolated del(5q)
- Myelodysplastic syndrome, unclassifiable

**Myeloid Neoplasms with Germline Predisposition**

- Acute myeloid leukemia with germline CEBPA mutation
- Myeloid neoplasms with germline DDX41 mutation
- Myeloid neoplasms with germline RUNX1 mutation
- Myeloid neoplasms with germline ANKRD26 mutation
- Myeloid neoplasms with germline ETV6 mutation
- Myeloid neoplasms with germline GATA2 mutation

**Acute Myeloid Leukemia (AML) With Recurrent Genetic Abnormalities**

- Acute myeloid leukemia with t(8;21)(q22;q22.1); RUNX1-RUNX1T1
- Acute myeloid leukemia with inv(16)(p13.1;q22) 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)(q21.3;q26.2) 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 mutated NPM1
- Acute myeloid leukemia with mutated CEBPA
- Acute myeloid leukemia with myelodysplasia-related changes

**Acute Myeloid Leukemia, NOS**

- Acute myeloid leukemia with minimal differentiation
- Acute myeloid leukemia without maturation
- Acute myeloid leukemia with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia

* Data elements preceded by this symbol may be clinically important but are not yet validated or regularly used in patient management.
Pure erythroid leukemia
Acute megakaryocytic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis

Myeloid Proliferations Related to Down Syndrome
- Transient abnormal myelopoiesis associated with Down syndrome
- Myeloid leukemia associated with Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

Acute Leukemias of Ambiguous Lineage
- 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, rare types (specify type): ______________
- Acute leukemia of ambiguous lineage, NOS

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 (hypodiploid ALL)
- B lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.1); IGHL/HIL3
- 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

Mature B-Cell Neoplasms
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- Lymphoplasmacytic lymphoma
- Intravascular large B-cell lymphoma
- HHV8-positive DLBCL, NOS
- Burkitt lymphoma

Mature T- and NK-cell Neoplasms
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Aggressive NK-cell leukemia
- Systemic EBV-positive T-cell lymphoma of childhood
- Adult T-cell leukemia/lymphoma
- Hepatosplenic T-cell lymphoma

Histiocytic and Dendritic Cell Neoplasms
- Histiocytic sarcoma
- Langerhans cell histiocytosis

* Data elements preceded by this symbol may be clinically important but are not yet validated or regularly used in patient management.
___ Langerhans cell sarcoma
___ Interdigitating dendritic cell tumor
___ Follicular dendritic cell sarcoma
___ Fibroblastic reticular cell tumor
___ Disseminated juvenile xanthogranuloma

___ Other (specify): ____________________________

+ Additional Pathologic Findings
+ Specify: ______________________________________

+ Cytochemical/Special Stains (Note D)
+ ___ Performed
  + Specify stains and results: ____________________________
+ ___ Not performed

Immunophenotyping (flow cytometry and/or immunohistochemistry) (Note E)
___ Performed, see separate report: ____________________
___ Performed
  + Specify method(s) and results: ________________________
___ Not performed

Cytogenetic Studies (Note F)
___ Performed, see separate report: ____________________
___ Performed
  + Specify method(s) and results: ________________________
___ Not performed

+ Fluorescence In Situ Hybridization (Note F)
+ ___ Performed, see separate report: ____________________
+ ___ Performed
  + Specify method(s) and results: ________________________
+ ___ Not performed

+ Molecular Genetic Studies (Note F)
+ ___ Performed, see separate report: ____________________
+ ___ Performed
  + Specify method(s) and results: ________________________
+ ___ Not performed

+ Comment(s)
Explanatory Notes

A. Specimen
Complete evaluation of hematopoietic disorders involving the bone marrow requires integration of data from multiple sources, including the clinical history, pertinent laboratory studies (e.g., complete blood count [CBC], serum lactate dehydrogenase [LDH], and beta-2-microglobulin levels, serum protein electrophoresis, and immunofixation results), and a satisfactory peripheral blood smear and bone marrow specimen. In most instances, the latter entails a bone marrow aspirate specimen, an aspirate clot preparation (cell block), and a bone marrow core (trephine) biopsy. Touch preparations (imprints) of the biopsy specimen are also very helpful and highly recommended. The World Health Organization (WHO) classification recommends performing a 200-cell differential count on peripheral blood smears and a 500-cell differential count on bone marrow aspirate specimens in the evaluation of hematopoietic disorders. This will allow adequate evaluation of the cellular elements within the peripheral blood and bone marrow.

In addition, submission of appropriate bone marrow material for flow cytometry immunophenotyping, cytogenetic studies, fluorescence in situ hybridization (FISH), and molecular studies is often necessary. The guidelines that follow are suggested for handling of bone marrow specimens:

- The number of stained and unstained peripheral blood, bone marrow aspirate, and bone marrow core biopsy touch preparation smears should be recorded.
- The length of the bone marrow core biopsy(s) should be recorded.
- For conventional cytogenetic studies, a sterile bone marrow aspirate specimen received in a sodium heparin tube is ideal, but fresh specimens submitted in saline or RPMI transport medium may be appropriate.
- For immunophenotyping by flow cytometry, a bone marrow aspirate specimen received in an EDTA tube (lavender top tube) or an ACD tube (yellow top tube) is required.
- Decalcification: Bone marrow core biopsy specimens require decalcification, and care must be taken not to under- or over-decalcify the specimen, as that might impact the quality of histologic sections and could interfere with the outcome of immunohistochemical staining.
  - Acid decalcification procedures are most commonly done using formic acid.
  - EDTA decalcification circumvents DNA degradation but is slower than acid-based decalcification, thus its use is thus more limited.
- Fixation:
  - Formaldehyde (formalin; 10% neutral buffered) is the most commonly used fixative for bone marrow samples; it is suitable for most ancillary tests, including molecular/genetic studies, colorimetric in-situ hybridization, and immunohistochemistry-based immunophenotyping.
  - Zinc formalin or B5 fixatives may produce superior cytologic detail but are not suitable for DNA extraction and may impair some immunostains. B5 has the additional limitation of requiring proper hazardous-materials disposal.
  - Over-fixation (i.e., more than 24 hours in formalin, more than 4 hours in zinc formalin or B5) should be avoided for optimal immunophenotypic reactivity.

Care must be taken to ensure that high-quality specimens and sections are obtained for each bone marrow specimen. This often requires coordination with oncologists and radiologists in setting where they perform bone marrow sampling to ensure that appropriate triage of aspirate material is exercised.

B. Aspiration/Biopsy Site
Bone marrow sampling (aspiration and core biopsy) is usually performed at the right and/or left posterior iliac crests. Aspirations and biopsies may be unilateral or bilateral, depending on the clinical indication for bone marrow sampling, as well as the preference of the oncology provider. Occasionally, the anterior iliac crest may be an appropriate choice in certain patients. The use of sternal aspirations is rare and should only be considered as a last resort option in very limited situations.

C. Histologic Type
This protocol recommends assigning histologic type based on the 2017 WHO classification of hematolymphoid neoplasms. Originally published in 2001 and revised and updated in 2008, this classification incorporates the morphologic, immunophenotypic, cytogenetic, and molecular findings into the final diagnosis. Whereas histologic
examination remains of paramount importance, many neoplasms will require the use of ancillary studies for precise diagnosis and subclassification. It may not be possible to provide a specific lymphoma diagnosis with bone marrow examination, particularly for patients in whom the bone marrow is the first identified site of involvement. While the list of the entities provided in the case summary covers the majority of primary bone marrow neoplasms, it is not exhaustive and omits rare and provisional entities in the 2017 WHO classification.

D. Cytochemical/Special Stains
A number of cytochemical stains may be utilized in the evaluation of hematopoietic neoplasms involving the bone marrow. An iron (Prussian blue) stain is paramount in the evaluation of myelodysplastic syndromes and some myeloproliferative disorders. A reticulin stain is necessary for the assessment of myelofibrosis, a primary or secondary feature of many bone marrow neoplasms, most importantly myeloproliferative disorders. Cytochemical staining for myeloperoxidase is rapid, convenient, and helpful for the assessment of myeloid neoplasms. Other cytochemical stains for leukocyte alkaline phosphatase, and naphthol-ASD chloroacetate esterase may also be useful in certain conditions. Notwithstanding, cytochemical stains are no longer required for the diagnosis of most disorders.

E. Immunophenotyping by Flow Cytometry and/or Immunohistochemistry
Immunophenotyping of bone marrow specimens can be performed by flow cytometry or immunohistochemistry. Each 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 a major tool for minimal residual disease detection in patients with acute leukemia. Antigen reactivity, however, cannot be correlated with architecture or cytologic features. In patients from whom a dry tap is obtained, an additional bone marrow core biopsy submitted fresh in transport medium may be disaggregated and utilized for flow cytometry immunophenotyping. Immunohistochemistry allows correlation of antigen expression with architecture and cytology. Not all antibodies are available for immunohistochemistry, particularly in fixed tissues, but one of its advantages is that it can be performed on archival tissue. Both techniques can provide diagnostic, prognostic, and therapy-guiding information.

F. Cytogenetic and Molecular Genetic 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 required viable cells. FISH may be performed on metaphase spreads from karyotyping studies or on air-dried, fresh unfixed aspirate or touch preparation slides. The use of 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 use of molecular diagnostics is another central tool in the evaluation of many hematolymphoid malignancies. In addition to PCR-based techniques, molecular profiling using next-generation sequencing-based panels is increasingly available and provides valuable diagnostic, prognostic, and therapy-guiding data.

References