COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Biopsy Specimens From Patients With Primary Tumors of Bone

Version: Bone Biopsy 4.0.1.0   **Protocol Posting Date:** February 2020

Accreditation Requirements
The use of this protocol is recommended for clinical care purposes but is <u>not</u> required for accreditation purposes.

For accreditation purposes, this protocol should be used for the following procedures and tumor types:

Procedure	Description
Biopsy	Includes specimens designated Core needle biopsy, curettage, excisional biopsy, and others
Tumor Type	**Description**
Primary malignant bone tumors	Includes chondrogenic tumors, osteogenic tumors, fibrogenic tumors, osteoclastic giant cell rich tumors, notochordal tumors, vascular tumors, myogenic tumors, and lipogenic tumors

The following should NOT be reported using this protocol:

Procedure
Resection (consider Bone Resection protocol)
Primary resection specimen with no residual cancer (eg, following neoadjuvant therapy)
Cytologic specimens
Tumor Type
Plasma cell neoplasms (consider the Plasma Cell Neoplasms protocol)
Lymphoma (consider the Hodgkin or non-Hodgkin Lymphoma protocols)
Pediatric Ewing sarcoma (consider the Ewing Sarcoma protocol)
Soft tissue sarcoma (consider the Soft Tissue protocol)

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Summary of Changes

4.0.1.0
Biopsy and resection procedures separated into individual protocols
Modified list of WHO Classification of Malignant Bone Tumors to remove non-malignant types

Surgical Pathology Cancer Case Summary

Protocol posting date: February 2020

BONE: Biopsy

Notes:
This case summary is recommended for reporting biopsy specimens 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.

Procedure (Note A)
___ Core needle biopsy
___ Curettage
___ Excisional biopsy
___ Other (specify): _____
___ Not specified

Tumor Site (Note B)
___ Appendicular skeleton (specify bone, if known): _____
___ Spine (specify bone, if known): _____
___ Pelvis (specify bone, if known): _____
___ Not specified

Tumor Location and Extent (select all that apply) (Note C)
___ Epiphysis or apophysis
___ Metaphysis
___ Diaphysis
___ Cortex
___ Medullary cavity
___ Surface
___ Tumor involves joint
___ Tumor extension into soft tissue
___ Cannot be determined

Histologic Type (World Health Organization [WHO] classification of malignant bone tumors) (Note D)
Specify: _____
___ Cannot be determined

Mitotic Rate (Note E)
Specify: ___ /10 high-power fields (HPF)
    (1 HPF x 400 = 0.1734 mm^2; X40 objective; most proliferative area)

Necrosis (Note A)
___ Not identified
___ Present
        Extent: ___%
___ Cannot be determined

Histologic Grade (Note F)
___ G1: Well differentiated, low grade
___ G2: Moderately differentiated, high grade
___ G3: Poorly differentiated, high grade
___ GX: Cannot be assessed

The routinely reported core data elements are bolded.   2

___ Not applicable

Lymphovascular Invasion (Note G)
___ Not identified
___ Present
___ Cannot be determined

Additional Pathologic Findings
Specify: _____

Ancillary Studies (if applicable)

Immunohistochemistry (specify): _____
___ Not performed

Cytogenetics (specify): _____
___ Not performed

Molecular Pathology (specify): _____
___ Not performed

Radiographic Findings (Note C)
Specify: _____
___ Not available

Comment(s)

Explanatory Notes

These recommendations are used for all primary malignant tumors of bone except hematopoietic neoplasms, ie, lymphoma and plasma cell neoplasms.

A. Procedure / Tissue Processing
The following is a list of guidelines to be used in defining what type of procedure has been performed. This is based on the surgeon's intent and not based on the pathologic assessment of the margins.

- **Intralesional Resection**: Leaving gross tumor behind. Partial debulking or curettage are examples.

- **Marginal Resection:** Removing the tumor and its pseudocapsule with a relatively small amount of adjacent tissue. There is no gross tumor at the margin; however, microscopic tumor may be present. Note that occasionally, a surgeon will perform an "excisional" biopsy, which effectively accomplishes the same thing as a marginal resection.

- **Segmental/Wide Resection**: An intracompartmental resection. A single piece of bone is resected, including the lesion and a cuff of normal bone.

- **Radical Resection:** The removal of an entire bone, or the excision of the adjacent muscle groups if the tumor is extracompartmental

Fixation
Tissue specimens from bone tumors optimally are received fresh/unfixed because of the importance of ancillary studies, such as cytogenetics, which require fresh tissue.

Tissue Submission for Histologic Evaluation
One section per centimeter of maximum dimension is usually recommended, although fewer sections are needed for very large tumors, especially if they are homogeneous. Tumors known to be high grade from a previous biopsy do not require as many sections as those that were previously diagnosed as low grade, as documentation of a high-grade component will change stage and prognosis in the latter case. Sections should be taken of grossly heterogeneous areas, and there is no need to submit more than 1 section of necrotic tumor (always with a transition to viable tumor), with the exception of chemotherapy effect on osteosarcomas and Ewing sarcoma.[1,2] Occasionally, gross pathology can be misleading, and areas that appear to be grossly necrotic may actually be myxoid or edematous. When this happens, additional sections of these areas should be submitted for histologic examination. When estimates of gross necrosis exceed those of histologic necrosis, the greater percentage of necrosis should be recorded on the surgical pathology report. In general, most tumors require 12 sections or fewer, excluding margins. Tumors with greater areas of heterogeneity may need to be sampled more thoroughly.

Fresh tissue for special studies should be submitted at the time the specimen is received. Note that classification of many subtypes of sarcoma is not dependent upon special studies, such as cytogenetics or molecular genetics, but frozen tissue may be needed to enter patients into treatment protocols. Discretion should be used in triaging tissue from sarcomas. Adequate tissue should be submitted for conventional light microscopy before tissue has been taken for cytogenetics, electron microscopy, or molecular analysis.

Molecular Studies
It is important to snap freeze a small portion of tissue whenever possible. This tissue can be used for a variety of molecular assays for tumor-specific molecular translocations (see Table 1) that help in classifying bone tumors.[3,4] In addition, treatment protocols increasingly require fresh tissue for correlative studies. Approximately 1 cm^3 of fresh tissue (less is acceptable for small specimens, including core biopsies) should be cut into small, 0.2-cm fragments, reserving sufficient tissue for histologic examination. This frozen tissue should ideally be stored at minus (-)70°C and can be shipped on dry ice to facilities that perform molecular analysis.

Table 1. Characteristic Cytogenetic and Molecular Events of Bone Tumors

Histologic Type	Cytogenetic Events	Molecular Events
Chondrosarcoma of bone	Complex	*IDH1* and *IDH2* mutations
Ewing sarcoma	t(11;22)(q24;q12)	*EWSR1-FLI1* fusion
	t(21;22)(q12;q12)	*EWSR1-ERG* fusion
	t(2;22)(q33;q12)	*EWSR1-FEV* fusion
	t(7;22)(p22;q12)	*EWSR1-ETV1* fusion
	t(17;22)(q12;q12)	*EWSR1-E1AF* fusion
	inv(22)(q12q12)	*EWSR1-ZSG*
	t(16;21)(p11;q22)	*FUS-ERG*
	t(2;16)(q35;p11)	*FUS-FEV*
Ewing-like sarcomas[#]		
	t(20;22)(q13;q12)	*EWSR1-NFATC2*
	t(6;22)(p21;q12)	*EWSR1-POU5F1*
	t(4;22)(q31;q12)	*EWSR1-SMARCA5*
	Submicroscopic inv(22)in	
	t(1;22)(p36.1;q12)	*EWSR1-PATZ*
	t(2;22)(q31;q12)	*EWSR1-SP3*
	t(4;19)(q35;q13)	*CIC-DUX4*
Osteosarcoma		
Low grade central	Simple	*MDM2* amplification
Parosteal	Ring chromosomes	12q13-15 amplification
High grade	Complex	

[#] Ewing-like sarcomas are similar both clinically and histologically to Ewing sarcoma, but it is not known at the present time whether they represent true Ewing sarcomas. They are treated the same as true Ewing sarcomas.

References
1. Amin MB, Edge SB, Greene FL, et al, eds. *AJCC Cancer Staging Manual*. 8th ed. New York, NY: Springer; 2017.
2. Pawel B, Bahrami A, Hicks MJ, Rudzinski E. Protocol for the Examination of Specimens From Pediatric Patients With Ewing Sarcoma (ES). 2016. Available at www.cap.org/cancerprotocols.
3. Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, Ladanyi M. Advances in sarcoma genomics and new therapeutic targets. *Nat Rev Cancer*. 2011;11(8):541-547.
4. Rubin BP, Lazar JF, Oliveira AM. Molecular pathology of bone and soft tissue tumors. In: Tubbs R, Stoler M. *Cell and Tissue Based Molecular Pathology*. Philadelphia, PA: Churchill Livingstone; 2009.

B. Tumor Site

Given the strong association between the primary anatomic site and outcome, the 8th edition of the *AJCC Cancer Staging Manual*[1] uses the following site groups for staging purposes:

- Appendicular skeleton, including trunk, skull, and facial bones
- Pelvis
- Spine

This site grouping is reflected by the provision of separate definitions for the primary tumor (T) for each anatomic site.

References
1. Amin MB, Edge SB, Greene FL, et al, eds. AJCC Cancer Staging Manual. 8th ed. New York, NY: Springer; 2017.

C. Tumor Location and Extent

Radiographic imaging plays an especially critical role in the diagnosis of bone tumors. Close collaboration with an experienced musculoskeletal radiologist and orthopedic surgeon is recommended.

Figure 1 is a diagrammatic representation of the "anatomic" regions of a long bone. These locations are very important in classifying bone tumors. For instance, chondroblastomas almost always arise in the epiphysis. Epiphyses and apophyses are secondary ossification centers and therefore are embryonic equivalents. The greater and lesser trochanters are apophyses, while the epiphyses are at the ends of long bones.
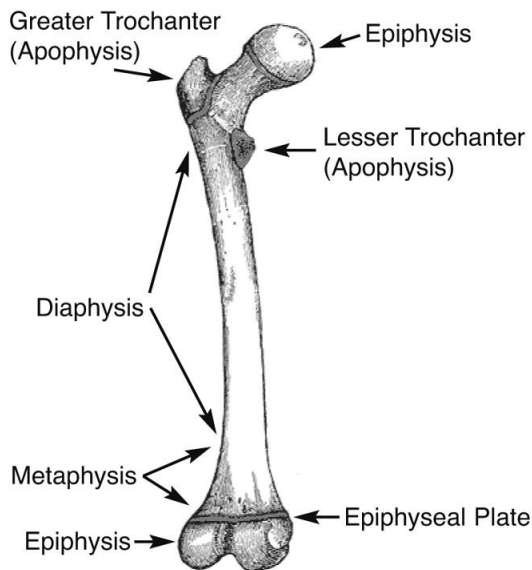

Figure 1. Important anatomic landmarks for tumor diagnosis in long bones. Adapted from *Gray's Anatomy.*[1]

References
1.   *Gray's Anatomy of the Human Body*. Philadelphia, PA: Lea & Febiger; 1918.

D. Classification of Bone Tumors
Intraoperative Consultation
Histologic classification of bone tumors is sufficiently complex that, in many cases, it is unreasonable to expect a precise classification of these tumors based on an intraoperative consultation. A complete understanding of the surgeon's treatment algorithm is recommended before rendering a frozen section diagnosis. In the case of primary bone tumors, an intraoperative diagnosis of benign versus malignant will generally guide the immediate decision to curette, excise, or wait for permanent sections, and certain therapeutic options may be lost if the wrong path is pursued. Intraoperative consultation is useful in assessing if "lesional" tissue is present and whether or not this tissue is necrotic, and in constructing a differential diagnosis that can direct the proper triage of tissue for flow cytometry (lymphoma), electron microscopy, and molecular studies/cytogenetics. Tissue triage optimally is performed at the time of frozen section. In many cases, it is important that a portion of tissue be submitted for ancillary studies, even from fine-needle aspiration (FNA) and core needle biopsy specimens, once sufficient tissue has been submitted for histologic evaluation.

Tumor Classification from Biopsies
It is not always possible to classify bone tumors precisely based on biopsy material, especially FNA and core needle biopsy specimens. Although pathologists should make every attempt to classify lesions in small biopsy specimens, on occasion, stratification into very basic diagnostic categories, such as lymphoma, carcinoma, melanoma, and sarcoma, is all that is possible. In some cases, precise classification is only possible in open biopsies or resection specimens.

Histologic Classification of Treated Lesions
Due to extensive treatment effects, such as necrosis, fibrosis, and chemotherapy-induced and radiation-induced pleomorphism, it may not be possible to classify some lesions that were either never biopsied or where the biopsy was insufficient for a precise diagnosis. In problematic cases, the grade of the pretreatment specimen (if available) should take precedence.

WHO Classification of Malignant Bone Tumors
Classification of tumors should be made according to the World Health Organization (WHO) classification of bone tumors listed below.[1]

WHO Classification of Malignant Bone Tumors

Chondrogenic Tumors
    Chondrosarcoma
    Dedifferentiated chondrosarcoma
    Clear cell chondrosarcoma
    Mesenchymal chondrosarcoma

Osteogenic Tumors
    Low-grade central osteosarcoma
    Conventional osteosarcoma
        Chondroblastic
        Fibroblastic
        Osteoblastic
    Telangiectatic osteosarcoma
    Small cell osteosarcoma
    Secondary osteosarcoma
    Parosteal osteosarcoma
    Periosteal osteosarcoma
    High grade surface osteosarcoma

Fibrogenic Tumors
    Fibrosarcoma of bone

Hematopoietic Tumors*
    Plasma cell myeloma*
    Solitary plasmacytoma of bone*
    Primary non-Hodgkin lymphoma, NOS*

Osteoclastic Giant Cell Rich Tumors
    Malignancy in giant cell tumor of bone

Notochordal Tumors
    Chordoma

Vascular Tumors
    Epithelioid hemangioendothelioma
    Angiosarcoma

Myogenic Tumors
    Leiomyosarcoma of bone

Lipogenic Tumors
    Liposarcoma of bone

Miscellaneous Tumors

Ewing sarcoma
Adamantinoma
Undifferentiated high-grade pleomorphic sarcoma

* Primary malignant lymphomas and plasma cell neoplasms are not staged using the AJCC system for malignant bone tumors.

References
1.   Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, eds. *WHO Classification of Tumors of Soft Tissue and Bone. 4th ed*. Geneva, Switzerland; WHO Press; 2013.

E. Mitotic Rate

Mitotic rate is determined by counting mitotic figures in 10 contiguous high-power fields (HPF) (40x objective), in the most mitotically active area of the tumor, away from areas of necrosis. The area of 1 HPF originally used measured 0.1734 mm^2. However, the area of 1 HPF using most modern microscopes with wider 40x lenses will most likely be higher. Pathologists are encouraged to determine the field area of their 40x lenses and divide 0.1734 by the obtained field area to obtain a conversion factor. The number of mitotic figures in 10 HPF multiplied by the obtained conversion factor and rounded to the nearest whole number should be used for reporting purposes.

F.   Grading

The grading of bone tumors is largely driven by the histologic diagnosis, and traditionally grading has been based on the system advocated by Broders, which assesses cellularity and nuclear features/degree of anaplasia.[1] The eighth edition of the *AJCC Cancer Staging Manual* recommends a 2-tiered system (low vs high grade) for recording grading.[2] Histologic grading uses a 3-tiered system: G1 is considered low grade, and G2 and G3 are grouped together as high grade for biological grading. However, we advocate a more pragmatic approach to grading aggressive and malignant primary tumors of bone.

Two bone tumors that are locally aggressive and metastasize infrequently, and thus are usually low grade, are low-grade central osteosarcoma and parosteal osteosarcoma. Periosteal osteosarcoma is generally regarded as a grade 2 osteosarcoma. Primary bone tumors that are generally high grade include malignant giant cell tumor, Ewing sarcoma, angiosarcoma, dedifferentiated chondrosarcoma, conventional osteosarcoma, telangiectactic osteosarcoma, small cell osteosarcoma, secondary osteosarcoma, and high-grade surface osteosarcoma.

Grading of conventional chondrosarcoma is based on cellularity, cytologic atypia, and mitotic figures. Grade 1 (low-grade) chondrosarcoma is hypocellular and similar histologically to enchondroma. Grade 2 (intermediate-grade) chondrosarcoma is more cellular than grade 1 chondrosarcoma; has more cytologic atypia, greater hyperchromasia and nuclear size; or has extensive myxoid stroma. Grade 3 (high-grade) chondrosarcoma is hypercellular, pleomorphic, and contains prominent mitotic activity.

Mesenchymal chondrosarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, undifferentiated high-grade pleomorphic sarcoma of bone and other "soft tissue-type" sarcomas that rarely occur in bone can be graded according to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system[3] (see College of American Pathologists protocol for soft tissue tumors[4]).

Chordomas are locally aggressive lesions with a propensity for metastasis late in their clinical course and are not graded. Adamantinomas tend to have a low-grade clinical course, but this is variable. Fortunately, they are very rare. According to the WHO classification of tumors of bone, adamantinomas are considered low grade.

Bone Tumor Grades (Summary)

Grade 1 (Low Grade)
Low-grade intramedullary (central) osteosarcoma
Parosteal osteosarcoma
Grade I chondrosarcoma
Clear cell chondrosarcoma

Grade 2
Periosteal osteosarcoma
Grade II chondrosarcoma
Classic adamantinoma
Chordoma

Grade 3 (High Grade)
Ewing sarcoma
Conventional osteosarcoma
Telangiectactic osteosarcoma
Mesenchymal chondrosarcoma
Small cell osteosarcoma
Secondary osteosarcoma
High-grade surface osteosarcoma
Dedifferentiated chondrosarcoma
Dedifferentiated chordoma
Malignancy in giant cell tumor
Grade III chondrosarcoma
Soft-tissue type sarcomas (eg, leiomyosarcoma)
Undifferentiated high-grade pleomorphic sarcoma

TNM Grading
The 8th edition of the American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) staging system for bone tumors includes a 3-grade system but effectively collapses into high grade and low grade.[2,5] Grading in the TNM grading system is based on differentiation only and does not generally apply to sarcomas.

GX   Grade cannot be assessed
G1   Well differentiated, low grade
G2   Moderately differentiated, high grade
G3   Poorly differentiated, high grade

For purposes of using the AJCC staging system, 3-grade systems can be converted to a 2-grade (TNM) system as follows: grade 1= low-grade; grade 2 and grade 3 = high-grade.

References
1. Inwards CY, Unni KK. Classification and grading of bone sarcomas. *Hematol Oncol Clin North Am.* 1995;9(3):545-569.
2. Amin MB, Edge SB, Greene FL, et al, eds. *AJCC Cancer Staging Manual. 8th ed.* New York, NY: Springer; 2017.
3. Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. J Clin Oncol. 1997;15(1):350-362.
4. Laurini JA, Cooper K, Fletcher CDM, et al. Protocol for the Examination of Specimens From Patients With Soft Tissue Tumors. 2017. Available at www.cap.org/cancerprotocols.
5. Brierley JD, Gospodarowicz MK, Wittekind C, et al, eds*. TNM Classification of Malignant Tumours. 8th ed.* Oxford, UK: Wiley; 2016.

G. Lymphovascular Invasion
Lymphovascular invasion (LVI) indicates whether microscopic lymphovascular invasion is identified. LVI includes lymphatic invasion, vascular invasion, or lymphovascular invasion. By AJCC/UICC convention, LVI does not affect the T category indicating local extent of tumor unless specifically included in the definition of a T category.