



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

Version: 4.1.0.1

Protocol Posting Date: November 2021

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

This protocol may 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, lipogenic tumors, undifferentiated small round cell sarcomas and other mesenchymal tumors arising in bone.

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

* Denotes primary author.

Accreditation Requirements

The use of this biopsy case summary is recommended for clinical care purposes, but is not required for accreditation purposes. The core and conditional data elements are routinely reported for biopsy specimens. Non-core data elements are included to allow for reporting information that may be of clinical value.

Summary of Changes

v 4.1.0.1

- The CAP made no changes to Cancer Protocol content. We updated metadata only for the electronic Cancer Checklists (eCC), requiring a version number change for the Word and PDF Cancer Protocols.

Reporting Template

Protocol Posting Date: November 2021

Select a single response unless otherwise indicated.

CASE SUMMARY: (BONE: Biopsy)

Standard(s): AJCC-UICC 8

This case summary is recommended for reporting biopsy specimens, but is not required for accreditation purposes.

CLINICAL (Note [A](#))

+Radiographic Findings (Note [B](#))

Specify: _____

Not available

SPECIMEN

Procedure (Note [C](#))

Core needle biopsy

Curettage

Excisional biopsy

Other (specify): _____

Not specified

TUMOR

Tumor Site (Note [D](#))

Appendicular skeleton (specify bone, if known): _____

Spine (specify bone, if known): _____

Pelvis (specify bone, if known): _____

Not specified

Tumor Location and Extent (Note [B](#)) (select all that apply)

Epiphysis or apophysis

Metaphysis

Diaphysis

Cortex

Medullary cavity

Surface

Involves joint

Extends into soft tissue

Cannot be determined: _____

Histologic Type (World Health Organization [WHO] Classification of Malignant Bone Tumors) (Note [E](#))

Chondrogenic Tumors

Chondrosarcoma

Dedifferentiated chondrosarcoma

- Periosteal chondrosarcoma
- Clear cell chondrosarcoma
- Mesenchymal chondrosarcoma
- Osteogenic Tumors
 - Low grade central osteosarcoma
 - Osteosarcoma NOS
 - Conventional osteosarcoma
 - Telangiectatic osteosarcoma
 - Small cell osteosarcoma
 - Parosteal osteosarcoma
 - Periosteal osteosarcoma
 - High grade surface osteosarcoma
 - Secondary osteosarcoma
- +Precipitating Factor for Secondary Osteosarcoma:** _____
- Undifferentiated Small Round Cell Sarcomas
 - Ewing sarcoma
 - Round cell sarcoma with EWSR1-non ETS fusions
 - CIC-rearranged sarcoma
 - Sarcoma with BCOR genetic alterations
- Fibrogenic Tumors - Fibrosarcoma of bone
- Malignancy in giant cell tumor of bone
- Notochordal Tumors
 - Chordoma NOS
 - Chondroid chordoma
 - Poorly differentiated chordoma
 - Dedifferentiated chordoma
- Vascular Tumors
 - Epithelioid hemangioendothelioma
 - Angiosarcoma
- Other Mesenchymal Tumors
 - Leiomyosarcoma of bone
 - Adamantinoma
 - Dedifferentiated adamantinoma
 - Undifferentiated high-grade pleomorphic sarcoma
- Cannot be determined: _____
- Other histologic type not listed (specify): _____
- +Histologic Type Comment:** _____

Histologic Grade (Note F)

- G1, well differentiated, low grade
- G2, moderately differentiated, high grade
- G3, poorly differentiated, high grade
- GX, cannot be assessed: _____
- Not applicable

+Mitotic Rate (Note G)

- Specify mitotic rate per mm²: _____ mitoses per mm²
 Specify mitotic rate per 10 high-power fields (HPF): _____ mitoses per 10 high-power fields (HPF)
 Cannot be determined (explain): _____

Necrosis (Note C)

- Not identified
 Present

Extent of Necrosis

- Specify percentage: _____ %
 Cannot be determined (explain): _____
 Cannot be determined

+Lymphovascular Invasion (Note H)

- Not identified
 Present
 Cannot be determined: _____

+Tumor Comment: _____

ADDITIONAL FINDINGS

+Additional Findings (specify): _____

SPECIAL STUDIES

Immunohistochemistry

- Specify: _____
 Not performed: _____
 Not applicable

Cytogenetics

- Specify: _____
 Not performed: _____
 Not applicable

Molecular Pathology

- Specify: _____
 Not performed: _____
 Not applicable

COMMENTS

Comment(s): _____

Explanatory Notes

A. Scope of Guidelines

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

B. 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.

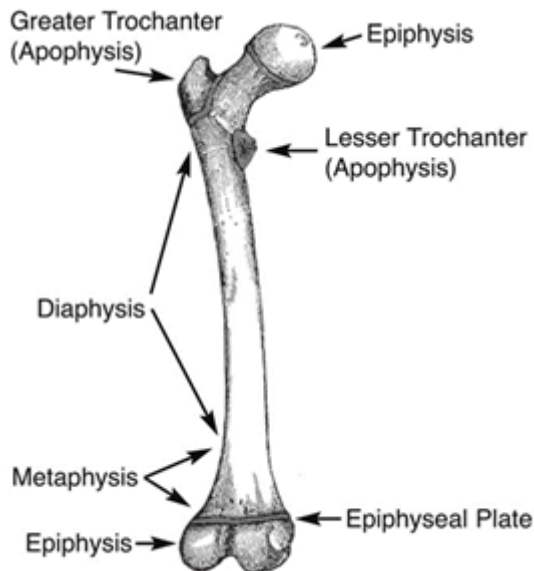


Figure 1. Important anatomic landmarks for tumor diagnosis in long bones. Adapted from *Gray's Anatomy*.¹

References

1. Gray H, Lewis WH. *Gray's Anatomy of the Human Body*. 20th ed. Philadelphia, PA: Lea & Febiger; 1918.

C. 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 in case fresh tissue for ancillary studies, such as cytogenetics or molecular studies, needs to be collected. All tissue should be processed in a manner that would allow molecular studies to be undertaken successfully. ^{1,2,3} Decalcification using harsh acid reagents may be detrimental for nucleic acid-based molecular studies and therefore utilization of EDTA as decalcifying agent has been recommended. Freezing a portion of the sample and/or fixing soft portions of the lesion in buffered formalin is encouraged.

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 specimens obtained to assess chemotherapy effect on osteosarcomas and Ewing sarcoma. ^{4,5} 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.

Molecular Studies

Additionally, it may be important to snap freeze a small portion of tissue as availability of frozen tissue may be a requirement for patient enrollment in clinical trials. Approximately 1 cm³ 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. Discretion should be used in triaging tissue from bone sarcomas. Adequate tissue should be submitted for conventional light microscopy before tissue has been taken for cytogenetics, electron microscopy, or molecular analysis.

References

1. 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.
2. 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.

3. WHO Classification of Tumours Editorial Board. *Soft Tissue and Bone Tumors*. Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed.; vol. 3).
4. Amin MB, Edge SB, Greene FL, et al., eds. *AJCC Cancer Staging Manual*. 8th ed. New York, NY: Springer; 2017.
5. 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.

D. 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.

E. 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 2020 World Health Organization (WHO) classification of bone tumors.¹ As part of the WHO classification system, soft tissue tumors are divided into 4 categories: benign, intermediate (locally aggressive), intermediate (rarely metastasizing), and malignant. Primary malignant lymphomas and plasma cell neoplasms are **not** staged using the AJCC system for malignant bone tumors.

References

1. WHO Classification of Tumours Editorial Board. *Soft Tissue and Bone Tumors*. Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed.; vol. 3).

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: Grade 1 is considered low grade, and Grade 2 and Grade 3 are grouped together as high grade for biological grading. In bone sarcomas, the histologic subtype often determines the clinical behaviour and grade. Therefore, a more pragmatic approach to grading aggressive and malignant primary tumors of bone can be used.³

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, following the grading system proposed by Evans et al.⁴ 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 system⁵ (see College of American Pathologists protocol for soft tissue tumors⁶).

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 2020 WHO classification of tumors of bone, adamantinomas are not graded.³

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

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.^{2,7} 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. WHO Classification of Tumours Editorial Board. *Soft Tissue and Bone Tumors.* Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed.; vol. 3)
4. Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone: a clinicopathologic analysis with emphasis on histologic grading. *Cancer.* 1977 Aug;40(2):818-31.

5. 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.
6. Laurini JA. Protocol for the Examination of Specimens From Patients With Soft Tissue Tumors. 2021. Available at www.cap.org/cancerprotocols.
7. Brierley JD, Gospodarowicz MK, Wittekind C, et al., eds. *TNM Classification of Malignant Tumours*. 8th ed. Oxford, UK: Wiley; 2016

G. Mitotic Rate

Mitotic rate is determined by counting mitotic figures in the most mitotically active area, away from areas of necrosis, in either 10 consecutive high-power fields (HPF) (use the X40 objective) (1 HPF x 400 = 0.1734 mm²) or in the appropriate number of HPF to encompass 1 mm² based on each individual microscope.

The area of 1 HPF originally used measured 0.1734 mm². 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.

An important change in the 5th Edition of the WHO Classification of Tumours series¹ is the conversion of mitotic count from the traditional denominator of 10 HPFs to a defined area expressed in mm², as an attempt to standardize the area used for mitotic count. Table 1 shows the approximate number of fields required to encompass 1 mm² based on the field diameter and its corresponding area.

Table 1. Approximate number of fields per 1 mm² based on field diameter and its corresponding area

Field diameter (mm)	Area (mm ²)	Approximate number of fields per 1 mm ²
0.40	0.126	8
0.41	0.132	8
0.42	0.138	7
0.43	0.145	7
0.44	0.152	7
0.45	0.159	6
0.46	0.166	6
0.47	0.173	6
0.48	0.181	6
0.49	0.188	5
0.50	0.196	5
0.51	0.204	5
0.52	0.212	5
0.53	0.221	5
0.54	0.229	4
0.55	0.237	4
0.56	0.246	4
0.57	0.255	4
0.58	0.264	4
0.59	0.273	4
0.60	0.283	4
0.61	0.292	3
0.62	0.302	3
0.63	0.312	3
0.64	0.322	3
0.65	0.332	3

0.66	0.342	3
0.67	0.352	3
0.68	0.363	3
0.69	0.374	3

References

1. WHO Classification of Tumours Editorial Board. Soft Tissue and Bone Tumors. Lyon (France): International Agency for Research on Cancer; 2020. (WHO classification of tumours series, 5th ed.; vol. 3)

H. 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.