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 not required for accreditation purposes.

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

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Includes specimens designated Core needle biopsy, curettage, excisional biopsy, and others</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type Description</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary malignant bone</td>
<td>Includes chondrogenic tumors, osteogenic tumors, fibrogenic tumors, osteoclastic giant cell rich tumors, notochordal tumors, vascular tumors, myogenic tumors, and lipogenic tumors</td>
</tr>
</tbody>
</table>

The following should NOT be reported using this protocol:

<table>
<thead>
<tr>
<th>Procedure</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection (consider Bone Resection protocol)</td>
<td></td>
</tr>
<tr>
<td>Primary resection specimen with no residual cancer (eg, following neoadjuvant therapy)</td>
<td></td>
</tr>
<tr>
<td>Cytologic specimens</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type Description</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cell neoplasms (consider the Plasma Cell Neoplasms protocol)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma (consider the Hodgkin or non-Hodgkin Lymphoma protocols)</td>
<td></td>
</tr>
<tr>
<td>Pediatric Ewing sarcoma (consider the Ewing Sarcoma protocol)</td>
<td></td>
</tr>
<tr>
<td>Soft tissue sarcoma (consider the Soft Tissue protocol)</td>
<td></td>
</tr>
</tbody>
</table>

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

* Denotes primary author. All other contributing authors are listed alphabetically.

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 mm²; 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.
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. 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. In addition, treatment protocols increasingly require fresh tissue for correlative studies. 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.
Table 1. Characteristic Cytogenetic and Molecular Events of Bone Tumors

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>Cytogenetic Events</th>
<th>Molecular Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondrosarcoma of bone</td>
<td>Complex</td>
<td>(IDH1) and (IDH2) mutations</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>t(11;22)(q24;q12)</td>
<td>(EWSR1-FLI1) fusion</td>
</tr>
<tr>
<td></td>
<td>t(21;22)(q12;q12)</td>
<td>(EWSR1-ERG) fusion</td>
</tr>
<tr>
<td></td>
<td>t(2;22)(q33;q12)</td>
<td>(EWSR1-FEV) fusion</td>
</tr>
<tr>
<td></td>
<td>t(7;22)(p22;q12)</td>
<td>(EWSR1-ETV1) fusion</td>
</tr>
<tr>
<td></td>
<td>t(17;22)(q12;q12)</td>
<td>(EWSR1-E1AF) fusion</td>
</tr>
<tr>
<td></td>
<td>inv(22)(q12q12)</td>
<td>(EWSR1-ZSG)</td>
</tr>
<tr>
<td></td>
<td>t(16;21)(p11;q22)</td>
<td>(FUS-ERG)</td>
</tr>
<tr>
<td></td>
<td>t(2;16)(q35;p11)</td>
<td>(FUS-FEV)</td>
</tr>
<tr>
<td>Ewing-like sarcomas*</td>
<td>t(20;22)(q13;q12)</td>
<td>(EWSR1-NFATC2)</td>
</tr>
<tr>
<td></td>
<td>t(6;22)(p21;q12)</td>
<td>(EWSR1-POUSF1)</td>
</tr>
<tr>
<td></td>
<td>t(4;22)(q31;q12)</td>
<td>(EWSR1-SMARCA5)</td>
</tr>
<tr>
<td>Submicroscopic inv(22) in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(1;22)(p36.1;q12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(2;22)(q31;q12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(4;19)(q35;q13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>Low grade central</td>
<td>Simple (MDM2) amplification</td>
</tr>
<tr>
<td></td>
<td>Parosteal</td>
<td>Ring chromosomes 12q13-15 amplification</td>
</tr>
<tr>
<td></td>
<td>High grade</td>
<td>Complex</td>
</tr>
</tbody>
</table>

*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

B. Tumor Site
Given the strong association between the primary anatomic site and outcome, the 8th edition of the *AJCC Cancer Staging Manual*7 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
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.

![Diagram of bone anatomy](image)

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

References

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/cyto genetics. 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.

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

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 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.

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, telangiectatic 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 system³ (see College of American Pathologists protocol for soft tissue tumors⁴).

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 1 chondrosarcoma
Clear cell chondrosarcoma
Grade 2
Periosteal osteosarcoma
Grade II chondrosarcoma
Classic adamantinoma
Chordoma

Grade 3 (High Grade)
Ewing sarcoma
Conventional osteosarcoma
Telangiectatic 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.\textsuperscript{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

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.