



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

Version: 4.2.0.0

Protocol Posting Date: June 2024

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, or incisional biopsy.
Tumor Type	Description
Primary malignant bone tumors	Includes tumors arising in bone for which pTNM staging on the resection is clinically relevant.

The following should NOT be reported using this protocol:

Procedure
Resection (consider Bone Resection protocol)
Cytologic fine needle aspiration (FNA) without cell block or biopsy
Tumor Type - Best reported using other protocols
Pediatric Ewing sarcoma (consider the Pediatric Ewing Sarcoma protocol)
Lymphoma / Leukemia (consider the Precursor and Mature Lymphoid Malignancies, Myeloid and Mixed / Ambiguous Lineage Neoplasms, or Plasma Cell Malignancies protocols)
Soft tissue primary sarcoma (consider the Soft Tissue protocol)

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

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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 4.2.0.0

- Cover page update
- Updates to content and explanatory notes, including WHO Histologic Types
- LVI question update from optional to required (core) and “Lymphovascular Invasion” to “Lymphatic and / or Vascular Invasion”
- “Mitotic Rate” answer update
- Addition of required (core) question “Tumor Laterality”
- Addition of conditional question “Multiple Sites (required only if applicable)”
- Addition of optional questions “Associated Syndrome”, “Other Clinical Findings”, “Decalcification Procedure”, and “Tumor Size (based on clinicoradiologic parameters)”
- SPECIAL STUDIES section update

Reporting Template

Protocol Posting Date: June 2024

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](#))

+Associated Syndrome

- Li-Fraumeni syndrome
- Mazabraud syndrome
- Ollier disease
- Maffucci syndrome
- Hereditary multiple exostoses
- Other (specify): _____
- Not specified

+Radiologic Findings (Notes [A](#),[B](#))

- Specify: _____
- Not available

+Other Clinical Findings

- Specify: _____
- Not available

SPECIMEN

Procedure (Note [C](#))

- Core needle biopsy
- FNA core needle biopsy
- Curettage
- Incisional biopsy
- Other (specify): _____
- Not specified

+Decalcification Procedure (Note [C](#))

- EDTA-decal or equivalent
- Harsh acid decalcification

TUMOR

Multiple Sites (required only if applicable)

- Not applicable
- Multifocal tumor / discontinuous tumor at primary bone site

___ Additional primary bone site(s) present (specify for synchronous malignant tumors or polyostotic aggressive tumors): _____

Tumor Site (Note D)

- ___ Appendicular skeleton, trunk, skull, facial bones (specify): _____
- ___ Spine (specify bone, if known): _____
- ___ Pelvis (specify bone, if known): _____
- ___ Not specified

Tumor Laterality

- ___ Left
- ___ Right
- ___ Central
- ___ Polyostotic ipsilateral
- ___ Polyostotic bilateral
- ___ Cannot be determined

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: _____

+Tumor Size (based on clinicoradiologic parameters)

- ___ Greatest dimension in Centimeters (cm): _____ cm
- ___ Not specified
- ___ Cannot be determined: _____

Histologic Type# (Note E)

The list is derived from the World Health Organization (WHO) classification of bone tumors, 5th edition, to include ONLY bone tumors of intermediate (locally aggressive and rarely metastasizing) potential and malignant bone tumors.

- ___ Chondrogenic tumors
 - ___ Synovial chondromatosis
 - ___ Atypical cartilaginous tumor
 - ___ Chondrosarcoma
 - ___ Chondrosarcoma secondary (specify): _____
 - ___ Dedifferentiated chondrosarcoma
 - ___ Periosteal chondrosarcoma
 - ___ Clear cell chondrosarcoma
 - ___ Mesenchymal chondrosarcoma
- ___ Osteogenic tumors

- Osteblastoma
- Low-grade central osteosarcoma
- Low-grade central osteosarcoma with high-grade transformation
- Parosteal osteosarcoma
- Parosteal osteosarcoma with high-grade transformation
- Conventional osteosarcoma
- Telangiectatic osteosarcoma
- Small cell 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 (specify, if known): _____
- CIC-rearranged sarcoma
- Sarcoma with BCOR genetic alterations
- Fibrogenic / fibrohistiocytic / histiocytic tumors
- Sclerosing epithelioid fibrosarcoma
- Primary malignant giant cell tumor of bone
- Secondary malignant giant cell tumor of bone
- Giant cell tumor of bone
- Langerhans cell histiocytosis
- +System Involvement**
- Single system (specify): _____
- Multisystem (specify): _____
- Other (leukemic, atypical, or other, specify): _____
- Desmoplastic fibroma
- Notochordal tumors
- Conventional chordoma
- Poorly differentiated chordoma
- Dedifferentiated chordoma
- Vascular tumors
- Epithelioid hemangioma
- Pseudomyogenic hemangioendothelioma
- Epithelioid hemangioendothelioma
- Angiosarcoma
- Epithelial tumors
- Adamantinoma of long bones
- Osteofibrous dysplasia-like adamantinoma
- Dedifferentiated adamantinoma
- Other mesenchymal or tumors of uncertain differentiation
- Leiomyosarcoma of bone
- Rhabdomyosarcoma of bone (specify fusion, if known): _____
- TK-fusion (NTRK, ALK, BRAF) tumor, primary intraosseous (specify fusion, if known):

Undifferentiated pleomorphic sarcoma
 Cannot be determined: _____
 Other histologic type not listed (specify): _____
+Histologic Type Comment: _____

Histologic Grade (Note E)

G1, well-differentiated, low-grade
 G2, moderately differentiated, high-grade
 G3, poorly differentiated, high-grade
 GX, cannot be assessed: _____
 Ungraded tumor / not applicable for this tumor type

+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

Not identified
 Present

Extent of Necrosis

Specify percentage: _____ %
 Other (specify): _____
 Cannot be determined (explain): _____
 Cannot be determined

Lymphatic and / or Vascular Invasion (Note H)

Not identified
 Present
 Cannot be determined

+Tumor Comment: _____

ADDITIONAL FINDINGS

+Additional Findings (specify): _____

SPECIAL STUDIES (Note E)

Immunohistochemistry

Specify results: _____
 Pending (specify): _____
 Not performed: _____
 Not applicable
 Other (specify): _____

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Cytogenetics

- Specify results: _____
- Pending (specify): _____
- Not performed: _____
- Not applicable
- Other (specify): _____

Molecular Studies

- Specify results: _____
- Pending (specify): _____
- Not performed: _____
- Not applicable
- Other (specify): _____

COMMENTS

Comment(s): _____

Explanatory Notes

A. Scope of Guidelines

This checklist may be used for malignant chondrogenic tumors, osteogenic tumors, fibrogenic tumors, osteoclastic giant cell-rich tumors, notochordal tumors, myogenic tumors, lipogenic tumors, undifferentiated small round cell sarcomas, and other mesenchymal tumors arising in bone. Locally aggressive entities such as synovial chondromatosis, osteoblastoma, giant cell tumor of bone, epithelioid hemangioma, pseudomyogenic hemangioma, and desmoplastic fibroma may be reported using this protocol but are not staged. Radiologic parameters include bone involved, size and extent (compartment) of tumor, location of tumor and extent, radiologic intrinsic characteristics including matrix or mineralization in bone-forming tumors, and differential diagnosis. Clinical parameters include patient age, sex, exact anatomic location, size, solitary or polyostotic, syndromes, and other pertinent medical and surgical history, if clinically relevant.

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 advised.

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, chondroblastoma almost always arises in the epiphysis. Epiphyses and apophyses are secondary ossification centers and therefore are embryonic equivalents; “epiphyses” are found within joints, whereas “apophyses”, the sites of tendonous and ligamentous attachments, are not found within joints. The greater and lesser trochanters are apophyses, while the epiphyses are at the ends of long bones.

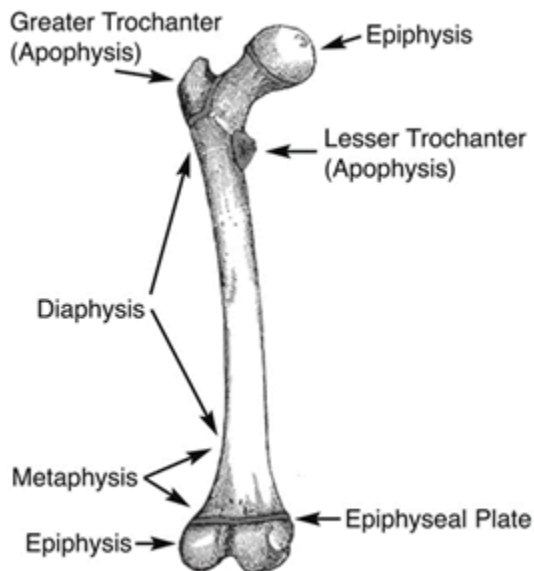


Figure 1. Important anatomic landmarks for tumor diagnosis in long bones. Adapted from *Gray's Anatomy*.¹

References

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

C. Biopsy/Tissue Processing/Tissue for Genetic-Molecular Studies

The following is a list of guidelines to be used in defining pathologic diagnosis for biopsy.

Biopsy

It is best to obtain enough cores for H&E, immunohistochemistry, and molecular genetic studies. Tissue for frozen, flow, and/or cytogenetics should be taken after enough is submitted for permanent assessment. For microbiology cultures, it is best to go directly from the Operating Room to the Microbiology Laboratory. It is optimal to have more than one block for a biopsy so that one can be for immunostains and the other for molecular genetics studies, as needed.

Fixation

Tissue specimens from bone tumors optimally are received fresh/unfixed in case fresh tissue for ancillary studies, such as cytogenetics, are needed. 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 over EDTA decalcification for molecular studies.

Tissue Submission for Histologic Evaluation and Genetic/Molecular Studies

While it has been helpful and often required for clinical trials to have snap frozen tissue, approximately 1 cm³ of fresh tissue stored at minus seventy (-70° C) that can be shipped on dry ice to facilities to perform molecular analysis, most full work up of sarcomas can be made on formalin-fixed and EDTA decalcified paraffin-embedded tissue and tissue should be entirely submitted, separated into at least two blocks.

ROSE or Intraoperative Assessment of Biopsy

Histologic classification of bone tumors is sufficiently complex that it is unreasonable to expect a precise classification of these tumors based on a rapid assessment or intraoperative consultation; best to assess viability of sampling and defer to permanent.

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).

D. Tumor Site

Given the strong association between the primary anatomic site and outcome, the 8th edition of the AJCC Cancer Staging Manual¹ 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

The list is derived from the World Health Organization (WHO) classification of soft tissue tumors, 5th edition,¹ edited to include ONLY bone tumors of intermediate (locally aggressive and rarely metastasizing) potential and malignant bone tumors.

Note on atypical cartilaginous tumor/grade 1 chondrosarcoma:

Atypical cartilaginous tumor (ACT) refers to cartilaginous neoplasms demonstrating features of a grade 1 chondrosarcoma and arising in the short and long tubular bones. This terminology should not be used when a pathologist cannot decide on the classification for the cartilaginous neoplasm.

Bone Primary Tyrosine Kinase Fusion Tumors:

While fusions involving the *RAS::MAPK* pathway are rare among bone tumors, these tumors have driver alterations in genes that encode tyrosine kinases and may respond to therapy targeting *NTRK*, *ALK*, *BRAF*, *RET*, *RAF*, *FGFR1*, or *ABL1*, etc. Notably, *NTRK* tumors fused with *KANK1* or *TPR* have been demonstrated to exhibit higher-grade appearance, including spindled and pleomorphic characteristics, accompanied by necrosis and mitoses, leading to unfavorable outcomes. Consequently, it is advisable to conduct comprehensive RNA-based Next-Generation Sequencing (NGS) for fusions, particularly in spindled pleomorphic tumors occurring in individuals under 50 years old, especially those in soft tissue or intraosseous locations. This recommendation is especially pertinent with tumors that have variable ovoid spindled to epithelioid morphology, variable collagenous to myxoid stroma, variable gaping to staghorn vasculature and specifically focal CD34 and/or focal S100 protein, without any staining for SOX10. In these tumors, BRAF, ALK, or panTrk or other immunostain may be identified.^{2,3,4,5,6,7,8,9,10,11}

Most Common Molecular/Genetic Findings:

The most common molecular/genetic findings in a subset of intermediate/malignant bone tumors are listed (Table 1).

Table 1: Subset of bone intermediate and malignant tumors with the most common diagnostic molecular/genetic findings.

<u>Diagnosis</u>	<u>Genes Involved</u>
Chondrosarcoma	<i>IDH1/IDH2</i> mutation
Intraosseous extraskeletal myxoid chondrosarcoma	<i>EWSR1/TAF15::NR4A3</i> fusion

Mesenchymal chondrosarcoma	<i>HEY1::NCOA2</i> fusion
Secondary chondrosarcoma arising in enchondroma	<i>IDH1/IDH2</i> mutation
Secondary chondrosarcoma arising in osteochondroma	<i>EXT1/EXT2</i> mutation
Sclerosing epithelioid fibrosarcoma of bone	<i>FUS::CREB3L2</i> fusion
Angiomatoid fibrous histiocytoma of bone/joint	<i>EWSR1::CREB1</i> or <i>EWSR1::ATF1</i> alternate
Primary malignant giant cell tumor of bone	<i>H3F3A</i> mutation
Leukemia/Multifocal atypical Langerhans cell histiocytosis	<i>BRAF</i> mutation
Poorly differentiated chordoma	<i>SMARCB1</i> deletion
Low-grade central osteosarcoma	<i>MDM2/CDK4</i> amplification
Parosteal osteosarcoma	<i>MDM2/CDK4</i> amplification
Rhabdomyosarcoma of bone (adult)	<i>FUS/EWSR1::TFCP2</i> , <i>MEIS1::NCOA2</i>
Ewing sarcoma	<i>EWSR1::FLI1</i> (85-90%), <i>EWSR1::ERG</i> (8-10%), others
<i>CIC</i> -rearranged sarcoma	<i>CIC::DUX4</i>
Round cell sarcoma with <i>EWSR1::non-ETS</i> fusion	<i>EWSR1::PATZ1</i> , <i>EWSR1::NFATC2</i> , <i>FUS::NFATC2</i>
Sarcoma with <i>BCOR</i> genetic alterations	<i>BCOR::CCNB3</i> fusion
Epithelioid hemangioendothelioma of bone	<i>WWTR1::CAMTA1</i> fusion
Angiosarcoma of bone	<i>MYC</i> amplification (post-irradiation)
Tyrosine-kinase fusion tumor	<i>NTRK1/2/3</i> , <i>ALK</i> , <i>BRAF</i> , etc. fusion (various fusion partners)

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).
2. Haller F, Knopf J, Ackermann A, et al. Paediatric and adult soft tissue sarcomas with *NTRK1* gene fusions: a subset of spindle cell sarcomas unified by a prominent myopericytic/haemangiopericytic pattern. *J Pathol.* 2016 Apr;238(5):700-10.
3. Hung YP, Fletcher CDM, Hornick JL. Evaluation of pan-TRK immunohistochemistry in infantile fibrosarcoma, lipofibromatosis-like neural tumour and histological mimics. *Histopathology.* 2018;73(4):634-644.

4. Agaram NP, Zhang L, Sung YS, et al. Recurrent NTRK1 Gene Fusions Define a Novel Subset of Locally Aggressive Lipofibromatosis-like Neural Tumors. *Am J Surg Pathol*. 2016 Oct;40(10):1407-16.
5. Helm M, Chang A, Fanburg-Smith JC, Zaenglein AL, Helm K. Cutaneous VCL::ALK fusion ovoid-spindle cell neoplasm. *J Cutan Pathol*. 2023;50(5):405-409. doi: 10.1111/cup.14420. Epub 2023 Mar 12. PMID: 36843055.
6. Fanburg-Smith JC, Smith JD, Flemming DJ. Bone and soft tissue tumors: clinoradiologic-pathologic molecular-genetic correlation of novel fusion spindled, targetable-ovoid, giant-cell-rich, and round cell sarcomas. *Skeletal Radiol*. 2023 Mar;52(3):517-540. doi: 10.1007/s00256-022-04244-w. Epub 2022 Dec 21. PMID: 36542130.
7. Wood ML, Fanburg-Smith JC, Brian JM, White JC, Powell JL, Freiberg AS. Successful Crizotinib-targeted Therapy of Pediatric Unresectable ERC1::ALK Fusion Sarcoma. *J Pediatr Hematol Oncol*. 2023. doi: 10.1097/MPH.0000000000002777. Epub ahead of print. PMID: 38099690.
8. Davis JL, Lockwood CM, Stohr B, et al. Expanding the Spectrum of Pediatric NTRK-rearranged Mesenchymal Tumors. *Am J Surg Pathol*. 2019 Apr;43(4):435-445.
9. Chen T, Wang Y, Goetz L, Corey Z, Dougher MC, Smith JD, Fox EJ, Freiberg AS, Flemming D, Fanburg-Smith JC. Novel fusion sarcomas including targetable NTRK and ALK. *Ann Diagn Pathol*. 2021;54:151800. PMID: 34464935.
10. Tan SY, Al-Ibraheemi A, Ahrens WA, Oesterheld JE, Fanburg-Smith JC, Liu YJ, Spunt SL, Rudzinski ER, Coffin C, Davis JL. ALK rearrangements in infantile fibrosarcoma-like spindle cell tumours of soft tissue and kidney. *Histopathology*. 2022 Mar;80(4):698-707. Epub 2022 Jan 2. PMID: 34843129.
11. Eyerer FIR, Bradshaw G, Vasalos P, Laser JS, Chang CC, Kim AS, Olson DR, Paler RJ, Rosenbaum JN, Walk EE, Willis JE, Yao J, Yohe SL. Getting Your Laboratory on Track with Neurotrophic Receptor Tyrosine Kinase. *Arch Pathol Lab Med*. 2023 Aug 1;147(8):872-884.

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 behavior 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, 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, 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

myxoid and more cellular/atypical than grade 1 chondrosarcoma. Grade 3 (high-grade) chondrosarcoma is hypercellular, pleomorphic, and contains observed mitotic activity.

Mesenchymal chondrosarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, undifferentiated 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.⁵

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, these are very rare. Other tumors such as periosteal chondrosarcoma (grading does not predict behavior) or bone angiosarcoma (always considered high-grade behavior) are also not graded. According to the 2020 WHO classification of tumors of bone, adamantinomas are not graded.^{2,3,6}

Bone Tumor Grades (Most Common)

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
Most round-cell sarcomas
Sarcoma with *BCOR* genetic alterations
CIC-rearranged sarcoma
Conventional osteosarcoma
Telangiectactic osteosarcoma
Mesenchymal chondrosarcoma
Small cell osteosarcoma
Secondary osteosarcoma
High-grade surface osteosarcoma
Dedifferentiated chondrosarcoma
Dedifferentiated chordoma
Poorly differentiated chordoma
Malignancy in giant cell tumor (primary and secondary malignant giant cell tumor of bone)
Grade III chondrosarcoma
Leiomyosarcoma
Rhabdomyosarcoma
Undifferentiated 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} Other grading systems in (TNM) are based on differentiation, yet this is not applicable to primary intraosseous 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. 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 1 mm², as an attempt to standardize the area used for mitotic count. Table 2 shows the approximate number of fields required to encompass 1 mm² based on the field diameter and its corresponding area.

Table 2. Approximate number of fields per 1 mm² based on field diameter

Formula to calculate the area of one high power field of a specific microscope = πr^2 /total magnification = $(\frac{1}{2} \text{ field diameter})^2 \times \pi$ /total magnification

Formula to calculate the field diameter = Objective Field Number/Objective Magnification

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. Lymphatic and/or Vascular Invasion

Lymphatic or vascular invasion (LVI) indicates whether microscopic lymphatic or vascular invasion is identified. LVI includes lymphatic invasion or vascular invasion or both. This may not be detectable on a” biopsy.