Pathology lab investigations in bone tumors

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Dr Zahraa al Najjar
• Primary bone tumors are fairly rare.
• Conditions that may simulate primary bone tumors, such as metastasis and non-neoplastic conditions such as inflammatory processes, bone cysts, fibrous dysplasia, non-ossifying fibroma, Paget’s disease of bone, etc., by far outnumber the cases of true bone tumors.
• The three most common genuine primary bone malignancies (osteosarcoma, chondrosarcoma, and Ewing’s sarcoma) account for only 0.2% of all malignancies in the UK and USA;
• however, in children (< 15 years) malignant bone tumors account for approximately 5% of all malignancies.
• Due to the rarity of primary bone tumors, few physicians accumulate enough experience in the diagnosis and treatment of these neoplasias.

• The diagnostic algorithm of a primary tumor of the bone is, and always has been, a collaborative effort in which clinical, radiologic, and pathologic features have to be considered.

• In the majority of cases, the pathologist can rely exclusively on histopathologic examination to provide an accurate diagnosis.

• In some cases, however, a variety of ancillary studies have to be employed to distinguish entities that share morphologic characteristics.
• Morphology and classification
• Cancer response criteria
• Frozen section
• Fine needle aspiration
• Ancillary tests
Bone Tumors can be divided into primary and secondary.
Primary tumors are subdivided according to the last WHO Classification system (2013) into:

- Chondrogenic tumours
- Osteogenic tumours
- Fibrogenic tumours
- Fibrohistiocytic tumours
- Haemtopoetic neoplasms
- Osteoclastic giant cell tumours
- Notochordal tumours
- Vascular tumours
- Myogenic tumours
- Lipogenic tumours
- Tumours of undefined neoplastic nature
- Miscellaneous tumours
Secondary tumors can be further subdivided into:
- Metastatic tumors
- Tumors resulting from contiguous spread of adjacent soft tissue neoplasms
- Tumors representing malignant transformation of the pre-existing benign lesions.
Multiple grading and staging systems are recognized
WHO grading and staging system

<table>
<thead>
<tr>
<th>T: primary tumor</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Tx</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor ≤5 cm</td>
</tr>
<tr>
<td>T1a</td>
<td>Superficial tumor (above the non-invaded fascia)</td>
</tr>
<tr>
<td>T1b</td>
<td>Deep tumor (under the fascia or with invasion of the fascia)</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor &gt; 5 cm</td>
</tr>
<tr>
<td>T2a</td>
<td>Superficial tumor</td>
</tr>
<tr>
<td>T2b</td>
<td>Deep tumor (retroperitoneum = always deep)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>N: regional lymph nodes</th>
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<tbody>
<tr>
<td>Nx</td>
<td>Lymph node status unknown</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph nodes</td>
</tr>
<tr>
<td>N1</td>
<td>Regional lymph node metastasis</td>
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<table>
<thead>
<tr>
<th>M: distant metastasis</th>
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<tbody>
<tr>
<td>Mx</td>
<td>Distant metastasis unknown</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G: histopathological grading</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>TNM-two grade system</td>
<td>Four grade systems</td>
</tr>
<tr>
<td>Low grade</td>
<td>Grade cannot be assessed</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Gx</td>
</tr>
<tr>
<td>Grade 2</td>
<td>G1</td>
</tr>
<tr>
<td>Grade 3</td>
<td>G2</td>
</tr>
<tr>
<td>High grade</td>
<td>G3</td>
</tr>
<tr>
<td>Stage grouping</td>
<td>Grade cannot be assessed</td>
</tr>
<tr>
<td>Stage Ia</td>
<td>G4</td>
</tr>
<tr>
<td>Stage Ib</td>
<td>Undifferentiated</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>Undifferentiated</td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>Undifferentiated</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Undifferentiated</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage grouping</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage Ia</td>
<td>T1a</td>
</tr>
<tr>
<td>Stage Ib</td>
<td>T2a</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>T2b</td>
</tr>
<tr>
<td>Stage III</td>
<td>T1b</td>
</tr>
<tr>
<td>Stage IV</td>
<td>T2b</td>
</tr>
<tr>
<td>Any T</td>
<td>N0</td>
</tr>
<tr>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>

Remark: for bone and soft tissue sarcoma, preference is given to a 2-tier instead of 3- or 4-tier system: low versus high grade.
# STAGING SYSTEMS

## Musculo Skeletal Tumor Society Classification

<table>
<thead>
<tr>
<th>Stage</th>
<th>Grade</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>Low (G1)</td>
<td>Intracompartmental (T1)</td>
</tr>
<tr>
<td>IB</td>
<td>Low (G1)</td>
<td>Extracompartmental (T2)</td>
</tr>
<tr>
<td>IIA</td>
<td>High (G2)</td>
<td>Intracompartmental (T1)</td>
</tr>
<tr>
<td>IIB</td>
<td>High (G2)</td>
<td>Extracompartmental (T2)</td>
</tr>
<tr>
<td>III</td>
<td>Any G Regional or distant metastasis (M1)</td>
<td>Any (T)</td>
</tr>
</tbody>
</table>

## Enneking et al

- **Stage IA (G1, T1, M0):** Low-grade intracompartmental lesion, without metastasis
- **Stage IB (G1, T2, M0):** Low-grade extracompartmental lesion, without metastasis
- **Stage IIA (G2, T1, M0):** High-grade intracompartmental lesion, without metastasis
- **Stage IIB (G2, T2, M0):** High-grade extracompartmental lesion, without metastasis
- **Stage IIIA (G1 or G2, T1, M1):** Intracompartmental lesion, any grade, with metastasis
- **Stage IIIB (G1 or G2, T2, M1):** Extracompartmental lesion, any grade, with metastasis
Bone sarcoma grading by the college of American pathologists

- **Grade 1**
  - Low-grade central osteosarcoma
  - Parosteal osteosarcoma
  - Low-grade chondrosarcoma
  - Clear cell chondrosarcoma
  - Osteofibrous dysplasia-like adamantinoma

- **Grade 2**
  - Periosteal osteosarcoma
  - Intermediate - grade chondrosarcoma
  - Classic adamantinoma
  - Chordoma

- **Grade 3**
  - Osteosarcoma (conventional, telangiectatic, small cell, secondary, high-grade surface)
  - Ewing’s sarcoma
  - High-grade chondrosarcoma
  - Dedifferentiated chondrosarcoma
  - Mesenchymal chondrosarcoma
  - Dedifferentiated chordoma
  - Malignant giant cell tumour
**French Federation of Cancer Centres Sarcoma Group (FNCLCC) grading system**

**Tumor differentiation**
- Score 1: Sarcomas that closely resemble normal adult mesenchymal tissues
- Score 2: Sarcomas for which histologic typing is certain
- Score 3: Embryonal and undifferentiated sarcomas, synovial sarcoma, and sarcomas of uncertain differentiation

**Mitotic count**
- Score 1: 0–9 mitoses/10 hpf
- Score 2: 10–19 mitoses/10 hpf
- Score 3: ≥20 mitoses/10 hpf

**Tumor necrosis**
- Score 0: No necrosis
- Score 1: <50% tumor necrosis
- Score 2: ≥50% tumor necrosis

**Histologic grade (tumor differentiation + mitotic count + tumor necrosis)**
- Grade 1 (low grade): Total score: 2 or 3
- Grade 2 (intermediate grade): Total score: 4 or 5
- Grade 3 (high grade): Total score: 6, 7, or 8

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**FNCLCC, Fédération Nationale des Centres de Lutte Contre le Cancer; hpf, high-power field.**

*Data from Ref. (52).*
Clinical & radiological information required for the pathological diagnosis of bone tumours
Origine du tissu

enseignements cliniques / Procédure chirurgicale

9. Soft tissue - Right leg
9. Bone tissue - Right leg

Date: 29/3/2016

Signature:

L.B. Les Blocs de Paraffine sont la propriété de L'I.N.P.
Prière de remplir adéquatement les demandes pour un résultat optimal
Prière d'utiliser le fixateur adéquat

Reservé au Laboratoire:

Récupération numérotée: non étiqueté

0 = 19 gr adipeux, p: 39
m: 3.5 x 9.7 cm
Aspect adipeux difficile à la coupe
BONE TUMOUR DIAGNOSIS: CLINICAL FEATURES

• Age (date of birth) and sex of the patient.

• Racial background

• A record of the anatomical bone involved by tumour.

• Clinical features associated with the tumour, such as nature and duration of signs and symptoms, including the presence or absence of pain, swelling, deformity, and relation to a previous traumatic episode.

• The presence or absence of a pre-existing or concomitant skeletal disease, history of familial syndrome or other relevant disease predisposing to tumour development.

• Occupational or treatment (eg chemotherapy, radiation therapy) history that may predispose to bone malignancy.

• The presence or absence of systemic features of disease.

• Results of relevant laboratory investigations.
BONE TUMOUR DIAGNOSIS: RADIOLOGICAL FEATURES

- The precise anatomical location of the lesion in the affected bone (i.e., epiphyseal, metaphyseal, diaphyseal, medullary, cortical, periosteal or extraosseous in location).
- The size of the lesion
- The matrix composition of the lesion
- The nature of the zone of transition or interface between the lesion and surrounding bone.
- The pattern of bone destruction
- The presence or absence of infiltration of medullary bone.
- The presence or absence of cortical destruction and soft tissue involvement
- The nature of the periosteal reaction
- The presence of multiple lesions within bone
Cancer response criteria
Histologic Grading of the Effect of Preoperative Chemotherapy on Primary Osteosarcoma

<table>
<thead>
<tr>
<th>Grade</th>
<th>Effect of Preoperative Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no effect identified</td>
</tr>
<tr>
<td>2</td>
<td>Areas of acellular tumor osteoid, necrotic and/or fibrotic material attributable to the effect of chemotherapy, with other areas of histologically viable tumor (&gt;10%)</td>
</tr>
<tr>
<td>3</td>
<td>Predominant areas of acellular tumor osteoid, necrotic and/or fibrotic material attributable to the effect of chemotherapy, with only scattered foci of histopathologically viable tumor cells identified :&lt;10% viable tumor</td>
</tr>
<tr>
<td>4</td>
<td>No histologic evidence of viable tumor identified within entire specimen.</td>
</tr>
</tbody>
</table>
Frozen section of bone tumours
Frozen section of bone tumours

• Frozen section examination of bone tumours should only be carried out by pathologists who have good experience of osteoarticular pathology and who have knowledge of the clinical background and radiological appearances of the lesion.

• The usefulness of frozen section histological examination is predicated on close cooperation between the surgeon, radiologist and pathologist.

A definitive diagnosis of bone tumour, however, should not be based on the examination of frozen sections alone
Frozen section histology provides information on:-

- Adequacy of the biopsy specimen.
- The nature of the lesion.
- Ancillary investigations which may be required for diagnosis.
- Adequacy of resection margins.
• Frozen section analysis is particularly useful in determining whether the sampled tissue is adequate and representative of the biopsied lesion
• It’s also useful intraoperatively for the examination of resection margins to determine the level of excision or amputation of a bone tumour.
• It has a diagnostic role, often indicating to an experienced pathologist the nature of the lesion (It is particularly useful in this regard in indicating whether a lesion is likely to be inflammatory or neoplastic).
• In some cases, the appearances of the lesion are sufficiently characteristic to permit a definitive diagnosis; in the appropriate clinical and radiological context, this may permit immediate surgical treatment of the lesion
Fine needle aspiration
Fine needle aspiration

- When sampling is adequate and the clinicoradiologic findings are available, FNAC of bone lesions is a highly accurate and diagnostic technique.
- Inflammatory conditions, non-fibrotic bone lesions, benign tumors as well as primary and metastatic malignant tumors can be correctly diagnosed by FNAC.
- Considering the overall advantages and cost-analysis, FNAC may be suggested as the initial method of choice for evaluation of bone lesions in most clinical settings.
Metastatic bone tumor. (a) X-Ray of the left humerus shows osteolytic bone lesion of the shaft with ‘moth eaten’ appearance. In view of the patient history of primary carcinoma, metastatic deposit was diagnosed (b) metastatic colonic adenocarcinoma (c) tumor cells positive for CDX2 (d) metastatic breast ductal carcinoma (e) Tumor cells positive for GCDFP-15 (Papanicolaou stain, a. X400; d. X200; immunostain, c. X200, e. X400). FNAC proves metastasis.
Radiologically; the picture is consistent with Ewing sarcoma. FNAC confirms the diagnosis (d) small round cells dispersed singly and forming loose clusters with frequent rosettes (e) Cell blocks section of the same case (f) The cells show positive immunohistochemical reaction to CD99 (d. Papanicolaou stainX200; e. H&E X200; f. immunostainX400).
Ancillary investigations
Ancillary investigations

**Immunohistochemistry:**

- **Immunohistochemistry (IHC)** refers to the process of detecting **antigens** (e.g. proteins) in cells of a tissue section by exploiting the principle of **antibodies** binding specifically to antigens in **biological tissues**
- It is useful in identifying or confirming the nature of cells in a bone tumour and particularly valuable in the differential diagnosis of primary and secondary malignant tumours of bone.
- Expression of epithelial markers, such as epithelial membrane antigen and cytokeratin, may point to a diagnosis of metastatic carcinoma; tumour cell expression of specific antigens (eg PSA, TTF-1) may indicate the likely primary origin.
• Epithelial membrane antigen and cytokeratin → Metastatic carcinoma
• NB84a, synaptophysin and chromogranin → metastatic neuroblastoma
• CD31, CD34 → Vascular tumors
• S100, type II and type X collagen → Clear cell chondrosarcoma
Figure 2 - (A) Ewing’s sarcoma/primitive neuroectodermal tumour (ES/PNET) as a small round cell neoplasm, exhibits relatively uniform oval-shaped nuclei, finely dispersed chromatin and pale eosinophilic to clear cytoplasms. The cytoplasmic borders were indistinct (H&E staining; magnification, ×400). (B) FLI-1 immunoreactivity in ES cell nuclei (magnification, ×400). (C) Positivity for CD99 on cell membranes (magnification, ×400). (D) Fluorescence in situ hybridization with the LSI EWSR1 (22q12) Break Apart Probe demonstrated chromosomal rearrangement in the EWSR1 gene region. Cells with t(22q12) revealed one fusion, one orange and one green signal pattern.
- **Molecular biology (cytogenetic and molecular analysis)**
  - Cytogenetic analysis shows changes in the number and/or structure of chromosomes in a tumour.
  - Some of these chromosomal abnormalities are tumour-specific and are useful in bone tumour diagnosis.
  - It is especially useful in the detection of the 11;22 translocation which is commonly found in Ewing's sarcoma.
  - It’s also used in other tumours including osteochondroma, osteofibrous dysplasia, fibrous dysplasia, giant cell tumour, osteosarcoma, chondrosarcoma and chordoma.
- Molecular genetic analysis is used to characterise changes in gene and gene expression in tumour cells, particularly translocations and mutations in oncogenes and tumour suppressor genes.
- The most common of these translocations is t [11; 22] (q24; q12), which is present in nearly 85% of cases of Ewing's sarcoma.
**Other ancillary investigations**

- Flow cytometry is not routinely employed for tumour diagnosis as it does not accurately distinguish between benign and malignant bone tumours.

- Electron microscopy is also not employed routinely for tumour diagnosis; it can in some cases help in establishing the diagnosis of some bone tumours; for example identifying Birbeck granules in Langerhan's cell histiocytosis or cytoplasmic glycogen in Ewing's sarcoma.

- Radio-immuno assay (RIA) and ELISA are useful for the detection of serum biomarkers of bone remodeling especially in metastatic bone lesions.
Conclusion

- Close cooperation is needed between the histopathologist radiologist, surgeon, oncologist and other clinical colleagues in the diagnosis and treatment of bone tumours.
- In many cases, the radiographic appearance of the lesion provides clues to its clinical behavior.
- A definitive diagnosis of bone tumour, should not be based on the examination of frozen sections alone.
- In the majority of cases, the pathologist can rely exclusively on histopathologic examination to provide an accurate diagnosis.
- In some cases, however, a variety of ancillary studies have to be employed to distinguish entities that share morphologic characteristics.
- Immunohistochemistry remains the technique of choice for the distinction of primary tumors vs metastases of non-osseous origin and for the characterization of a small subset of neoplasias, such as those with small round-cell morphology.
Thank you