Small Round Cell Tumors of Bone

Meera Hameed, MD

Context.—Primary small round cell tumors of the bone are a heterogeneous group of malignant neoplasms presenting predominantly in children and adolescents. They include Ewing sarcoma/peripheral neuroectodermal tumor or Ewing family tumors, lymphoma, mesenchymal chondrosarcoma, and small cell osteosarcoma. Even though they share many morphological similarities, their unique biological and genetic characteristics have provided substantial insights into the pathology of these diverse neoplasms.

Objective.—To provide an overview of the clinical, radiologic, pathologic, and genetic characteristics of these tumors along with a pertinent review of the literature.

EWING SARCOMA FAMILY OF TUMORS

Since the original descriptions of tumors of the Ewing sarcoma family in 1918 by Arthur Purdy Stout and in 1921 by James Ewing, who called the tumor diffusely endothelium of bone, several nomenclatures have been assigned to these tumors, albeit with much skepticism in the literature. A possible relationship between Ewing sarcoma (ES) and peripheral neuroectodermal tumor (PNET) was established after the description of extracellular ES by Angerwalt and Enzinger and PNET of the bone by Jaffe. At the present time, ES and PNET, otherwise called Ewing family tumors (EFTs), form a single neoplastic entity sharing common histologic and molecular features, and differing only in their extent of cellular differentiation. Askin’s tumor of the thoracopulmonary region is also currently grouped under EFT. Nonrandom translocation involving the EWS gene with one of the ETS family of transcription factors is the hallmark of their oncogene

Incidence and Location

Ewing family tumor commonly occurs in the adolescent age group between 10 and 20 years, but is extremely rare in African American children. The annual incidence is about 3 per million, and there is a slight male predominance. These neoplasms constitute the second most common pediatric malignancy occurring in the skeletal system. Adult patients (older than 40 years) with EFT are rare, but isolated cases in patients up to 81 years old have been reported.

Ewing family tumor involves both the axial and appendicular skeleton, including the pelvis. Among long tubular bones, the femur is most commonly affected, followed by tibia, fibula, and humerus. However, virtually any bone can be affected, including the acral skeleton, craniofacial bones, and clavicle. Neoplasms in the long bones are often diaphyseal.

Clinical Presentation

Local tenderness or mass is the most common clinical presentation. In about 10% to 15% of the cases, there is a pathological fracture when the femur is involved. It is not uncommon to encounter children with systemic symptoms such as fever, fatigue, leukocytosis, and raised erythrocyte sedimentation rate mimicking signs and symptoms of acute osteomyelitis. This is an important clinical and radiologic differential diagnosis.

Radiographic Features

Radiographic correlation is a prerequisite to all orthopedic pathology and more so in tumors such as EFT, where clinical findings may point to benign processes such as osteomyelitis. The radiographic features are that of a diaphyseal or metadiaphyseal lesion in the long bones. Typically, the lesion is intramedullary (symmetric or eccentric) and is associated with cortical thickening and cyclical periosteal reaction, giving rise to the characteristic onion-skin appearance (Figure 1). The rapid growth of the tumor produces a continuous lifting of the periosteum leading to perpendicular striations. Within the marrow, the lesion is poorly margined, with a permeative pattern of osteolysis (without a beginning and an end) (Figure 1). Magnetic resonance imaging reveals an accompanying soft tissue mass in about 90% of cases (Figures 2 and 3). Computed tomographic scan will demonstrate that the lesion itself does not produce any matrix (bone or cartilage). Occasionally there is prominent diffuse thickening of the bone and cloudy opacities mimicking osteosarcoma (OS). Radiologic differential diagnoses include osteomyelitis, Langerhans histiocytosis, lymphoma, and OS. Imaging workup of these patients involves skeletal scintigraphy (bone scan) and computed tomography of the chest to rule out metastatic disease at the time of presentation.

Data Sources.—A literature search using PubMed and Ovid MEDLINE was performed, and data were obtained from various articles pertaining to clinicopathologic, biological, and genetic findings in these tumors. Additionally, findings from rare cases have been included from author’s subspecialty experience.

Conclusion.—The diagnosis of small round cell tumors can be made accurately by applying clinicopathologic criteria, as well as a panel of immunohistochemical and genetic studies in appropriate cases. Molecular genetic studies may provide further insight into the biology, histogenesis, and prognosis of these tumors.

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Pathology

An open biopsy is usually performed to obtain adequate material for pathological examination and ancillary studies. Multiple core biopsies may be performed in patients with large, accessible soft tissue component of the tumors, keeping in mind that additional studies such as cytogenetics will be needed. Fine-needle aspiration can be used in an appropriate setting where adequate material can be obtained. grossly, the biopsied material is often hemorrhagic and necrotic and usually has a tan-gray appearance. Touch imprints of the submitted pieces allow one not only to examine morphology, but also to select viable material for cytogenetic analysis. Molecular studies (reverse transcriptase polymerase chain reaction [RT-PCR] and fluorescence in situ hybridization [FISH]) can be done using frozen and paraffin-embedded tissue. If tissue is snap-frozen for molecular studies, care should be taken to freeze tumor material in a timely manner to avoid RNA degradation. Whenever possible, bony spicules should be separated from soft tissue to avoid unnecessary decalcification of the soft tissue component.

Microscopic examination of classic EFT shows a highly cellular neoplasm consisting of a monotonous population of uniform round blue cells (diameter, about 14 μm) arranged in a nested, sheetlike, or solid pattern. The nuclei are round with fine chromatin, scant clear or eosinophilic cytoplasm, indistinct cytoplasmic membranes, and 1 to 3 small to medium-sized nucleoli. The cytoplasm contains abundant glycogen, which can be confirmed with periodic acid–Schiff stain. Necrosis and apoptosis are common. The presence of apoptotic cells sometimes gives the biphasic appearance of dark and light cells (Figure 4). Homer-Wright rosettes and pseudorosettes can be present (Figure 5). Stromal elements are minimal, and the tumor generally fills the medullary cavity and replaces bone marrow elements. Interspersed collagen bundles are usually seen in the soft tissue component. A vascular component is not prominent and is usually composed of slitlike capillaries. Thick-walled vessels are seen in areas where there are prominent stroma and collagen bundles. A minimal degree of spindle cell change can be present. The tumor cells permeate the cortex and elicit periosteal new bone formation with reactive osteoblasts, osteoclasts, and sometimes cartilaginous metaplasia. The tumor generally does not induce an inflammatory response.

Morphological Variants

A number of variants have been described in EFT. These include atypical ES (large cell EFT),18 adamantinoma-like ES,19,20 sclerosing ES, and spindle cell sarcoma-like EFT.21 The tumor cells of the atypical or large cell EFT are heterogeneous compared with classic EFT. Their size ranges between 20 and 24 μm, and they have irregular nuclear membranes and prominent nucleoli (Figure 6). The cytoplasm is vacuolated, and cytoplasmic glycogen is less...
Figure 3. Axial T2-weighted magnetic resonance imaging without contrast showing the lesion occupies the entire marrow cavity encircling the entire cortex with soft tissue extension.

Figure 4. Photomicrograph of Ewing sarcoma showing diffuse monotonous round cells with dark and light pattern (hematoxylin-eosin, original magnification ×400)

Figure 5. Rosettelike pattern in Ewing sarcoma (hematoxylin-eosin, original magnification ×400).

Figure 6. Photomicrograph of large cell Ewing family tumor with irregular nuclear contours and prominent nucleoli (hematoxylin-eosin, original magnification ×400).

Figure 7. Pelvic atypical Ewing sarcoma showing nests of tumor cells in a desmoplastic stroma (hematoxylin-eosin, original magnification ×200).

Figure 8. Interphase fluorescence in situ hybridization section showing split signal with EWS probes (yellow signal normal and green and red signal representing split EWS).

Table 1. Immunohistochemical Profile*

<table>
<thead>
<tr>
<th>Tumor</th>
<th>CD99</th>
<th>FLI-1</th>
<th>LCA</th>
<th>B Cell</th>
<th>T Cell</th>
<th>TdT</th>
<th>CK</th>
<th>Chr</th>
<th>S100</th>
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<tbody>
<tr>
<td>EFT</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
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<tr>
<td>LBL/ALL</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>NHL-other</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Mesenchymal chondrosarcoma</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Small cell OS</td>
<td>+/−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>DSCRT</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
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<td>−</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
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<tr>
<td>Neuroblastoma</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
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*LCA indicates leukocyte common antigen; TdT, terminal deoxynucleotidyltransferase; CK, pan-cytokeratin; Chr, chromogranin; EFT, Ewing family tumors; LBL/ALL, lymphoblastic lymphoma/acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; OS, osteosarcoma; DSCRT, desmoplastic small round cell tumor; and ?, not known.
plasmic glycogen and scarce organelles. Occasionally, desmosome-like junctions are seen. Evidence of neural differentiation such as dense core neurosecretory granules, dendritic processes, and microtubules can be present.

**Immunohistochemistry**

It is advisable to use a panel of immunohistochemical markers in small round cell tumors of the bone which include vimentin, CD99, FLI-1, leucocyte common antigen, terminal deoxynucleotidyl transferase (TdT), B-cell, T-cell markers, pan-cytokeratin, desmin, MYOD1/myogenin, chromogranin, and S100 protein. These markers assist in differentiating EFT from other tumors such as lymphoblastic lymphoma/acute lymphoblastic leukemia, rhabdomyosarcoma, desmoplastic small round cell tumor, and neuroblastoma. Other differential diagnoses to consider would be mesenchymal chondrosarcoma, non-Hodgkin lymphoma (NHL) (other), and small cell OS. The most useful, though nonspecific, marker in the diagnosis of EFT is CD99, which often produces a diffuse membranous staining pattern in these tumors (Figure 9). This product is a 32-kd transmembrane protein whose function is not well established; it is encoded by the MIC2 gene located in the pseudoautosomal region of X and Y chromosomes.24–26 Apart from EFT, this protein is also highly expressed in T and B lymphocytes, hence also expressed by lymphoblastic lymphoma/acute lymphoblastic leukemia. In addition, positive staining has been observed in some rhabdomyosarcomas, desmoplastic small round cell tumors, synovial sarcomas, and mesenchymal chondrosarcomas.27–30 Another useful immunomarker is the FLI-1 protein, which is expressed in about 84% of EFTs (Figure 10).31 This transcription factor is also expressed by lymphoblastic lymphomas and other NHLs.31 Other variably expressed markers in EFT include vimentin, cytokeratin (up to 25%), desmin (very rare), and neural markers such as synaptophysin, CD57, S100, and chromogranin.21,23,32–34 The presence of neural markers in an EFT indicates neuroectodermal differentiation (PNET). High-molecular cytokeratin (34E12) is positive in adamantinoma-like ES and not in classic and other variants of EFT.21 Table 1 summarizes the immunohistochemical profile of these tumors.

**Cytogenetics and Molecular Genetics**

The pathogenetic relationship of ES and PNET was defined by the discovery of the unique and specific nonrandom chromosomal translocation, seen in about 85% to 95% of the tumors, involving chromosome 11q24 and chromosome 22q12, t(11;22)(q24;q12) (Figure 11).35–37 This leads to an in-frame fusion of the EWS gene at chromosome 22q12 to the FLI-1 gene at chromosome 11q24. The EWS-FLI-1 chimeric protein on the (der)22, contains the 5’ end of the EWS gene and the 3’ end of the FLI-1 gene.38 The second most common translocation in EFT is t(12;22)(q22;q12), seen in about 5% to 10% of the cases, resulting in the fusion of EWS to ERG at chromosome 12q22.39 Other variant translocations reported in bone EFT accounting for less than 1% of cases include t(7;22)(p22;q12), t(17;22)(q12;q12), and t(2;22)(q23;q12), involving fusion of EWS to ETV1, E1AE, and FEV genes, respectively (Table 2).40–43

The EWS gene has 17 exons, of which the first 7 exons encode the N-terminal region responsible for regulating its RNA binding capacity. It belongs to the TET family of RNA binding proteins (EWS, TLS, TAFII68) whose amino terminal (NTD) has the capacity to bind to DNA-binding domains of various transcription factors, thus providing a strong transcriptional activating domain to the resulting chimeric protein.44,45 EWS itself is ubiquitously expressed in all tissues.46 The partner genes of EWS in the EFT of bone, such as FLI-1, ERG, ETV1, E1AE, and FEV, belong to the ETS family of transcription factors possessing a conserved ETS domain recognizing a core DNA motif of GGAA/T.47 FLI-1 expression is seen in high levels in he-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Extended</th>
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<tbody>
<tr>
<td>Desmin</td>
<td>Myogenin/MyOD1</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
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<td>+/-</td>
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<td>+</td>
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matopoietic cells, endothelial cells, and mesenchyme derived from neural crest cells.\textsuperscript{46,47} The product of EWS-ETS translocation results in fusion proteins containing the C-terminal region of the FLI-1 and N-terminal region of EWS, thus bringing the ETS component under the powerful transactivating domain of EWS.\textsuperscript{48} These fusion products modulate multiple target genes, resulting in malignant transformation.

The most commonly used molecular assays to identify the EWS-ETS fusion are FISH and RT-PCR. Multiple chimeric transcripts are possible, all of which involve exons 1 through 7 of EWS and exon 9 of the ETS gene. The genomic break points are intronic, and occur at 1 of 4 EWS introns and, in the case of EWS-FLI-1, 1 of 6 FLI-1 introns (Figure 12).\textsuperscript{51} Type 1 (exon 7 of EWS to exon 6 of FLI-1) and type II (exon 7 of EWS to exon 5 of FLI-1) are the most common (>85%) fusion transcripts.\textsuperscript{52} Commonly used RT-PCR assays use EWS exon 7 forward primer and FLI-1 exon 9 reverse primer, which amplifies all fusion products, or EWS exon 7 forward primer and FLI-1 exon 6 to detect type 1 or type II fusion products, which would identify 85% of the cases (Figure 13). In addition, RT-PCR for EWS-ERG fusion product can be performed, which would identify the 10% of cases with this fusion. A multiplex real-time PCR has also been used to identify EWS-ETS fusion products.\textsuperscript{53} RT-PCR has also been applied to formalin-fixed, paraffin-embedded tissues, and a combination of FISH and RT-PCR may yield increased sensitiv-

Table 2. Translocation and Fusion Genes in Ewing Family Tumors of Bone

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Fusion Genes</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;22)(q24;q12)</td>
<td>EWS-FLI-1</td>
<td>85%–95%</td>
</tr>
<tr>
<td>t(21;22)(q22;q12)</td>
<td>EWS-ERG</td>
<td>5%–10%</td>
</tr>
<tr>
<td>t(7;22)(p22;q12)</td>
<td>EWS-ETV1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(17;22)(q12;q12)</td>
<td>EWS-E1AF</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>t(2;22)(q23;q12)</td>
<td>EWS-FEV</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>
ity and accuracy due to limitations of formalin-fixed, paraffin-embedded tissue material with suboptimal preservation of nucleic acids. Fluorescence in situ hybridization assay with the commercially available (such as Vysis) dual-color break-apart DNA probes flanking the EWS break point region on chromosome 22 detects the translocation of EWS and can be used in fresh and formalin-fixed, paraffin-embedded tissues. In a metaphase spread, the split signal enables one to identify the chromosome involved in the translocation; however, the partner gene is not identified by this methodology (Figure 14).

Additional reported secondary karyotypic changes in EFT include gains of chromosomes 8, 12, and 18, deletion of the short arm of chromosome 1, unbalanced translocation t(1;16)(q12;q11), and other rare structural abnormalities.

### Treatment and Treatment Response

The management of EFTs includes systemic chemotherapy followed by surgery, with or without radiation and continuation chemotherapy. Induction chemotherapy before definitive surgery allows us to assess the chemoresponse in resected specimens, which has some prognostic significance and has a role in selection of agents for continuation chemotherapy. A sagittal section of the resected bone, with mapping and examining the entire central face of the tumor and adjacent tissue after careful decalcification, allows one to assess the chemotherapeutic response (Figure 15). The grading system used to assess chemoresponse is similar to that used in OS (Table 3).

### Prognosis and Outcome

Up to one third of patients with EFT have metastatic disease at the time of presentation, and their outcome remains poor in spite of aggressive chemotherapy. The most common sites of metastases are lung, bone, and bone marrow. Patients who present only with lung metastases have better survival compared with those who present with bone metastases. One third of the patients with localized disease are also prone to distant relapse, especially if there are residual viable tumor cells at definitive resection. Multiple biological factors, such as type of fusion transcript, mutations in INK4a gene, mutations in p53, deletion of p16, telomerase, IGF-1, and aneuploidy, have been implicated to play a role in the prognosis of EFT. In patients with localized disease, EWS-FLI-1 type 1 fusion transcript has been reported as being associated with improved outcome compared to other fusion transcript types, and it appears that type 1 fusion transcript encodes a less active chimeric protein. Reports on the detection of minimal residual disease by RT-PCR for the chimeric proteins in peripheral blood and bone marrow remain controversial with respect to disease progression. Current chemotherapeutic regimens with selective surgery

### Table 3. Chemotherapy Response—Pediatric Oncology Group Study

<table>
<thead>
<tr>
<th>Grade</th>
<th>Tumor Response</th>
<th>3-y Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No chemotherapy effect</td>
<td>30</td>
</tr>
<tr>
<td>II A</td>
<td>1%–10% necrosis</td>
<td>30</td>
</tr>
<tr>
<td>II B</td>
<td>11%–90% necrosis</td>
<td>49</td>
</tr>
<tr>
<td>III</td>
<td>91%–99% necrosis</td>
<td>73</td>
</tr>
<tr>
<td>IV</td>
<td>100% necrosis</td>
<td>100</td>
</tr>
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have significantly improved the overall survival up to 60% to 80% in nonmetastatic EFT. Radiation-induced sarcomas and chemotherapy-related acute leukemias are the most frequent secondary malignancies.

Conclusion

Even though the cell of origin is still unknown in EFT, considerable strides have been made since the original description by Drs Stout and Ewing. The combination of morphology, immunohistochemistry, and molecular genetics has opened many doors, including some novel approaches to therapeutics, such as the use of CD99 as a target, inhibition of IGF-1 receptor with antisense oligonucleotides, and small interfering RNA (siRNA) all of which have shown promising results in cell lines and animal models.

NON-HODGKIN LYMPHOMA

Primary NHL of bone was described as a series, first by Jackson and Parker in 1939. Before this, Oberling in 1928 had reported a case of reticuloendothelial sarcoma histologically indistinguishable from ES. The description of this rare entity is restricted to lymphoma involving a single skeletal site with or without regional lymph node involvement or multiple skeletal sites without visceral or lymph node involvement.

Incidence and Location

Approximately 3% to 7% of extranodal lymphomas present as primary bone neoplasms, and lymphomas constitute about 7% of all bone malignancies. The disease tends to involve older adults (older than 40 years), and there is a male predominance, with male-female ratio ranging from 1.2:1 to 1.6:1. The femur, pelvis, vertebra, and humerus are the most common sites. In the long bones, the preferred site is the metaphyseal region. Involvement of the small bones of the hand and feet is extremely unusual. It is not uncommon to encounter multifocality, and soft tissue extension may be present.

Clinical Presentation

Most patients present with pain, and depending on location, such as spine, neurological symptoms may be present. Systemic symptoms such as fever (as seen in ES) and B symptoms of nodal lymphomas are unusual. Occasionally patients can present with hypercalcemia, lethargy, constipation, etc. For a neoplasm to be classified as a primary bone lymphoma, an interval of 4 to 6 months between the skeletal manifestation and extraskeletal disease is required. Thus appropriate staging procedures, such as skeletal imaging, computed tomographic scans, bone scan/positron emission tomography, and bone marrow biopsy, are an integral part of the patient workup.

Radiographic Features

On conventional x-rays, primary lymphomas are lytic radiolucent lesions with a moth-eaten or permeative destructive pattern. This may be associated with sclerotic areas. These changes are nonspecific and overlap with other round cell tumors. Cortical erosion and destruction with soft tissue extension is frequently seen. Periosteal reaction (interrupted or solid single-layer) can be present, but less so than in ES. Generally the disease tends to involve the long bones extensively, sometimes occupying the entire length. In some instances, the tumor may evoke a sclerotic response and present as a blastic lesion, which can sometimes be mistaken for Paget disease. Computed tomography and magnetic resonance imaging are useful in assessing extent of disease, and positron emission tomography and bone scans are used for staging and assessment of remission after therapy. Of importance is the fact that occasionally plain radiographs can be normal, and persistence of clinical symptoms would warrant further investigation such as magnetic resonance imaging, which will demonstrate the signal abnormalities of the marrow. Pathologic fracture can be present in 22% of cases. Due to the nonspecificity of the radiographic findings, the differential diagnoses include OS, eosinophilic granuloma, metastases of solid tumors, round cell tumors (ES, neuroblastoma, rhabdomyosarcoma), and chronic osteomyelitis.

Pathology

Grossly, bone lymphomas tend to be fleshy gray-white or hemorrhagic, and the gross appearance is also related to the response of the host bone, leading to sometimes very sclerotic and bony biopsy specimens requiring decalcification. Histologically, the most common finding is a diffuse pattern of growth with tumor cells permeating between bony trabeculae and invading Haversian channels of the cortex. There is often a sclerotic or collagenized stroma, and the tumor cells can invade in cords, nests, or solid sheets. As is often evidenced in the x-ray, the host bony trabeculae can be thickened and irregular with extensive sclerosis. The most frequent type (92%) of primary NHL of bone is diffuse large B-cell lymphoma (DLBCL). Unlike nodal lymphomas, DLBCL of bone characteristically shows a polymorphous appearance (Figures 18 and 19). The tumor cells themselves show marked variation in size and shape, often with multinodulation, and are accompanied by a mixture of small and medium-sized lymphocytes that are reactive B and T cells. The large tumor cells can have prominent nucleoli, and the cytoplasm is not usually abundant. Occasionally, the fibrotic stromal reaction elicited by the tumor cells can result in tumor cell spindling with storiform features, mimicking a sarcoma. The polymorphous nature of the infiltrate can lead to a mistaken diagnosis of osteomyelitis, which is also a radiologic differential diagnosis. Crush artifact is fairly common due to the delicate nature of the tumor cells and can be a vexing problem in pathologic diagnosis. Apart from DLBCL, other NHLs, which can occasionally present as primary neoplasms of bone, include anaplastic large cell lymphoma, lymphoblastic lymphoma, Burkitt lymphoma, and T-cell lymphomas. Of these, lymphoblastic lymphoma and small lymphocytic lymphomas such as chronic lymphocytic lymphoma generally do not present as primary destructive bone lesions.

Immunohistochemistry and Genetics

In a suspected case of NHL, a panel of markers is used to delineate the lineage and type of the neoplasm. They include leucocyte common antigen, B-cell markers (CD20), and T-cell markers (CD2, CD3, CD5, CD7), and additional markers such as CD30, epithelial membrane antigen, and ALK-1 to rule out anaplastic large cell lymphoma. In a case with a monomorphic morphology such as lymphoblastic
lymphoma, the panel should include all of the lymphoma and other round cell tumor markers (CD45, CD20, CD2, CD3, CD5, CD7, TdT, vimentin, CD99, FLI-1, keratin, desmin, chromogranin). In addition, other lymphoid antigens such as CD79a and CD43 may be required, as lymphoblastic lymphomas are often negative for CD20 and positive for CD99 and FLI-1. They can also be negative for leucocyte common antigen, and in some instances show focal cytoplasmic keratin positivity, a major pitfall that can lead to an erroneous diagnosis of ES. In pediatric cases of small round cell tumors, TdT appears to be a sensitive and specific marker for precursor B or T lymphoblastic lymphomas and should be included in the immunopanel (Table 1). Other special stains such as κ and λ can be used to ensure monoclonality, but the results are often variable due to technical limitations. Primary T-cell lymphomas of the bone are extremely rare, and the diagnosis is established based on morphology of the polymorphous population with large tumor cells and loss of pan–T-cell antigens such as CD7. Two cases of primary adult T-cell leukemia of the bone have been reported. Additional studies such as flow cytometry can be used in appropriate cases. B-cell and T-cell rearrangement studies can be performed in fresh and paraffin-embedded tissues to confirm clonality in doubtful cases. Specific cytogenetic studies of primary bone lymphomas are sparse. There is a single report of a pediatric DLBCL of the spine with t(3;22)(q27;q11). Recently we encountered a case of an atypical Burkitt lymphoma arising in the calcaneus of an elderly male patient histologically mimicking DLBCL (Figure 20); however, cytogenetic analysis showed t(8;14)(q24.1;q32), characteristic of a Burkitt lymphoma.

**Treatment and Prognosis**

The primary modality of therapy for primary bone lymphomas is chemotherapy and radiotherapy, depending on the stage of disease and the grade and type of the lymphoma. The prognosis is dependent upon the stage of the disease. Patients with stages I and II have a very good prognosis compared with patients with stages III and IV. The 5-year overall survival ranges between 56% and 61%.82,95
Small Round Cell Tumors of Bone—Hameed

Figure 21. Plain x-ray of proximal femur showing a permeative pattern in the intertrochanteric and subtrochanteric region with endosteal scalloping across the distalmost aspect of the neoplasm. There are questionable foci of matrix calcification proximally. (Courtesy of Dr Joseph Benevenia, Department of Orthopedics, University of Medicine and Dentistry of New Jersey, Newark.)

Conclusion

Primary NHL of bone is a rare but well-defined entity with radiologic and morphologic heterogeneity. The histologic and immunohistochemical similarities, ranging from benign conditions such as osteomyelitis to monotonous round cell sarcomas, should be borne in mind when assessing these neoplasms to avoid misdiagnosis and inappropriate treatment.

MESENCHYMAL CHONDROSARCOMA

This rare neoplasm was first described by Lichtenstein and Bernstein96 in 1959 as an unusual variant of chondrosarcoma affecting bone and soft tissues. This was followed by recognition of 9 more cases from the files of the Mayo Clinic by Dahlin and Henderson.97 Subsequently it has been established as a separate entity distinct from conventional and dedifferentiated chondrosarcoma.

Incidence and Location

Mesenchymal chondrosarcomas constitute less than 2% of all chondrosarcomas.98,99 The tumor most commonly affects young adults and teenagers, with a peak incidence during the third decade.100 Males and females are equally affected.98 Craniofacial bones, especially the jaw, are most frequently affected.101 Other commonly involved sites include vertebrae, ribs, pelvis, and humerus. About one third of these tumors are extraskeletal and arise in soft tissues, and meninges are a favored extraskeletal location for these tumors.98,99 Skeletal neoplasms can be multifocal.99

Radiographic Features

Plain x-ray reveals a radiolucent lesion, usually eccentric with stippled calcifications denoting its chondroid nature (Figure 21). The lesion is often destructive, with expansion of bone, cortical destruction, and extrasosseous extension (Figure 22). Sometimes the calcifications can be large and present as discrete opacities or heavily calcified masses. Rarely, a sharply demarcated sclerotic margin is present. Magnetic resonance imaging appearance can be variable and can include a heterogeneous, low attenuation on T1-weighted images (Figure 23) and high signal intensity on T2-weighted images.104,105

Pathology

Biopsy of the lesion generally shows a grossly grey to pink tissue with foci of mineralization. Microscopically, the tumor shows a biphasic pattern made up of solid areas of round or spindle mesenchymal cells interspersed with islands of well-differentiated cartilage (Figure 24). The mesenchymal component often exhibits a hemangiopericytic pattern with multiple vascular spaces. The cartilage component is variable and can show endochondral ossification with bone formation, coarse calcifications, or loosely arranged mature hyaline cartilage. There can be distinct demarcation or gradual blending between the mesenchymal component and the chondroid component (Figure 25).98,99 The mesenchymal cells when they are round can simulate ES or small cell OS in biopsy specimens. On the other hand, the cartilage component alone in a biopsy can lead to the erroneous diagnosis of a benign cartilaginous neoplasm.

Ultrastructure

The primitive mesenchymal cells show round to oval cells with little intercellular matrix, and the cartilaginous foci reveal mature cells containing glycogen, rough endoplasmic reticulum, and numerous mitochondria.106

Immunohistochemistry

The chondroid foci stain for S100 protein, and the primitive mesenchymal cells are positive for CD99 (Figure 26).107 Both components are vimentin positive. It has been proposed that immunohistochemical positivity for collagen II and IIA, which are considered to be markers of chondroprogenitor cells, could be used to differentiate mesenchymal chondrosarcoma from other small round cell malignancies, such as small cell OS and ES.108 Another study109 has shown that the transcription factor Sox9, a master regulator of chondrogenesis,110,111 differentiates...
mesenchymal chondrosarcoma from various small cell malignancies, including small cell OS, lymphoma, and ES, by immunohistochemical analysis using a rabbit polyclonal antibody.

Cytogenetics and Molecular Genetics

There are a few reported cytogenetic alterations in mesenchymal chondrosarcoma, and so far no specific or recurrent translocations have been identified. Case reports of near-tetraploid and other diverse structural abnormalities, translocation der(13;21)(q10;q10) with loss of chromosomes 8 and 20 material and gain of chromosome 12 material in 2 cases, and 1 case with t(11;22)(q24;q12) similar to ES have been reported. Molecular genetic analyses for mutational alteration of p53 and p16 tumor suppressor genes have shown a low incidence (<25%) in these tumors. Biologic pathways involving PKC-α, PDGFR-α, and Bcl-2 expression seem to play a role in pathogenesis.

Treatment and Prognosis

Mesenchymal chondrosarcomas are primarily treated with surgery. They are aggressive lesions with a high propensity for metastases; however, some patients can have a protracted clinical course, so the behavior can be unpredictable. The overall 10-year survival rate is approximately 25%. Tumors of the jawbones tend to have a more indolent course. Negative correlation between survival times and proliferation rates using Ki-67 in 10 patients has been recently reported. Distant metastases which can occur after many years can involve other bones, lung, etc. Hence long-term follow-up of these patients is advocated.

SMALL CELL OSTEOSARCOMA

This tumor is discussed here in the context of the differential diagnosis of the above-mentioned small round cell tumors of the bone. This is a distinctive microscopic variant of OS, originally described in 1979, as a tumor simulating ES. This rare tumor accounts for about 1% to 1.5% of OSs and has similar age, sex, and skeletal distribution as conventional OS. Radiologically the tumor can resemble ES or conventional OS. The majority of the lesions show a mixed lytic and blastic pattern, but they can be purely lytic or permeative; they usually involve metaphysis and may extend into epiphysis. A soft tissue component is present in most of the cases. Three histologic patterns have been described: ES-like, lymphoma-like, and small spindle cell. The cells can be round and uniform, similar to ES, or show variation in size. Nucleoli can be inconspicuous to prominent. The chromatin can be fine to hyperchromatic. The nuclei can be very small to medium (6.7–15 μm), thus showing a spectrum of sizes between ES and large cell lymphoma. Hemangiopericytic pattern, myxoid change, cordlike arrangement, and epithelioid features can be seen. The presence of malignant cartilage in some tumors can simulate mesenchymal chondrosarcoma. The presence of osteoid is a prerequisite for diagnosis. The new osteoid matrix is usually lacelike and can be difficult to find in small biopsies (Figure 27). The presence of mineralized matrix in imaging studies supports the diagnosis of OS and is helpful in differentiating other small blue cell tumors of bone. ES may show a blastic component in the medullary cavity, but production of matrix outside the bone is seen only in OS. Cytoplasmic glycogen can be seen in both neoplasms. Immunohistochemical features do not distinguish small cell OS from sarcomas, as CD99 can be pos-
itive in small cell OS. Ewing family tumor–specific translocations such as t(11;22) have not been reported in small cell OS. Lymphomas can be distinguished using lymphoma-specific markers. The treatment of small cell OS is similar to conventional OS, which includes neoadjuvant chemotherapy followed by surgery, usually a limb salvage procedure. According to some authors, when there is questionable matrix formation in a small round cell tumor, it is best to err on the diagnosis of ES rather than small cell OS.

**OTHER TUMORS**

Rhabdomyosarcoma and desmoplastic round cell tumors are primarily soft tissue neoplasms; rare primary bone tumors are cited in the literature as case reports. Neuroblastomas can present as metastases in the bone and can be distinguished from other primary round cell tumors of bone with immunohistochemical positivity for neuron-specific enolase, synaptophysin, and chromogranin and absent staining for CD99, lymphoid markers, desmin, myogenin, and MyoD1.

**SUMMARY**

Small round cell tumors of bone are a heterogeneous group of neoplasms with overlapping clinical, radiologic, morphologic, and immunohistochemical features. Even though questions remain regarding cell of origin and histogenesis, cytogenetic and molecular genetic studies have enhanced our understanding in bringing together some of these tumors into single entities. In addition, cDNA microarray analysis and the availability of techniques to unravel the various signaling pathways will open many doors toward targeted chemotherapy in these aggressive, highly lethal tumors.

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