Small Cell Osteosarcoma

Cytopathologic Characteristics and Differential Diagnosis

Justin A. Bishop, MD,1 Chung H. Shum, MD, PhD,1 Sheila Sheth, MD,2 Paul E. Wakely Jr, MD,3 and Syed Z. Ali, MD1,2

Key Words: Small cell osteosarcoma; Bone; Cytopathology; Fine-needle aspiration

Abstract

Small cell osteosarcoma may present a challenging primary diagnosis on cytologic assessment owing to its rarity and its morphologic similarity to other small round blue cell tumors. Five cases of small cell osteosarcoma from our cytopathology archives were identified and reviewed and cytologic features elaborated. Three cases were fine-needle aspirations from bony lesions in the classic location for osteosarcoma (2 distal femur and 1 proximal tibia), and 2 aspirations were from metastases. Common cytomorphologic features included relatively small to intermediate cell size, high nuclear/cytoplasmic ratios, round nuclei, minimal anisocytosis, finely granular nuclear chromatin, fine cytoplasmic vacuoles, and only rare osteoid. Small cell osteosarcoma shares many of the well-described cytomorphologic features of classic osteosarcoma, but the relatively small cells, round hyperchromatic nuclei, and scant osteoid constitute the common denominator. Correlation with radiographic findings and ancillary tests can aid in definitive diagnosis.

The increasingly widespread use of preoperative fine-needle aspiration (FNA) of bone lesions necessitates the recognition of even rare primary osseous neoplasms. The appropriate classification of a primary bone neoplasm may drastically alter the planned therapy for the lesion. Small cell osteosarcoma is an exceedingly rare tumor, estimated to account for less than 1% of all cases of osteosarcoma.1 Small cell osteosarcoma was first described as a neoplasm having microscopic features of osteosarcoma and of Ewing sarcoma in that small cells cytomorphologically similar to those seen in Ewing sarcoma were found in a histologic background of osteoid.2 Owing to its unique histologic features, it can have a broad differential diagnosis. Only rare accounts of the cytopathologic findings on FNA exist in the literature.1,3

Materials and Methods

The cytopathology archives of The Johns Hopkins Hospital, Baltimore, MD, and Ohio State University Medical Center, Columbus, were searched, and all cases of small cell osteosarcoma were retrieved. FNA was performed using a 22- or 25-gauge needle under ultrasound or computed tomography scanning with on-site evaluation by a cytopathologist. The smears were air dried and wet fixed in 95% ethanol and stained with rapid Romanowsky and Papanicolaou stains, respectively. FNA smears were reviewed for cytomorphologic features, including presence or absence of osteoid, necrosis, pleomorphism, cytoplasmic processes, cytoplasmic vacuolization, mitoses/apoptosis, multinucleation, anisonucleosis, nuclear shape, chromatin characteristics, and nucleolar...
prominence. Available immunoperoxidase stains and flow cytometric analyses were reviewed as well, along with the clinical and radiologic findings in each case.

Results

Patient Demographics and Clinical and Radiologic Data

Five cases of small cell osteosarcoma were identified in a 19-year period (1990-2009). The ages of the patients ranged from 11 to 37 years. Three cases were FNAs from bony lesions in the classic location for osteosarcoma (2 distal femur and 1 proximal tibia) with a size range of 4.5 to 14 cm (mean, 10.2 cm). One case was a peripancreatic soft tissue metastasis from a primary paravertebral cervical mass, and another case was a metastasis to the L1 vertebral body from a primary femoral tumor. Clinically, all patients originally had pain ranging in duration from 1 to 20 months. Two of the patients initially noted the pain following a sports injury, and 2 of the patients complained of night pain.

Radiographically, the 3 primary bone cases were described as having prominent periosteal reaction. One case was heterogeneously contrast-enhancing, another case was permeative, and yet another case had a blastic appearance. One case displayed evidence of neoplastic bone formation. All 3 cases were radiographically “suspicious” for osteosarcoma.

Follow-up information was available for 4 patients. Three of the patients had metastatic disease, and the fourth patient had a local recurrence. All 4 patients were treated with resections and preoperative and postoperative chemotherapy. Of the 4 patients, 2 died of disease (4 and 3 years after diagnosis). Another patient continued to have progressive metastatic lung disease as of September 2009, and the fourth patient was free of disease as of July 2009.

Cytopathologic Features

Smears were generally cellular. Common cytomorphologic features observed included relatively small to intermediate-sized cells, high nuclear/cytoplasmic ratios, round nuclei, minimal anisonucleosis, hyperchromasia, finely granular nuclear chromatin, fine cytoplasmic vacuoles, and only rare osteoid. Osteoid had a variegated appearance in the smears and appeared mostly as faint purple or metachromatic material (rapid Romanowsky stain) surrounding individual cells or small cell groups in a lacy manner. In contrast with collagen, the osteoid had a more wispy or lacy quality with ill-defined borders lacking naked fibroblastic nuclei. Osteoid was difficult to identify on Papanicolaou stain, where it appeared as a pale green substance with embedded neoplastic cell nuclei.

All cases displayed numerous mitoses and abundant karyorrhectic nuclei reflecting high cell turnover and rapid tumor growth. Frank cellular necrosis was not observed. Features not commonly observed included prominent nuclei, necrosis, cytoplasmic processes, spindled nuclei, nuclear molding, and multiple small nucleoli. Scant focal osteoid was identified in 3 cases, as were occasional cells with moderate amounts of spindled to epithelioid cytoplasm in 2 cases, representing some heterogeneity in the tissue sampled.
Flow cytometry was performed in 2 cases, and immunoperoxidase studies were done in 3 cases using the standard method owing to morphologic similarities to lymphoid lesions and other small round blue cell tumors. Flow cytometry displayed the presence of a nonlymphoid phenotype (CD45–), and immunoperoxidase failed to stain the small malignant cells for CD99 (O13), bcl2, pancytokeratins, and neuroendocrine markers. The standard flow cytometric panel included CD45, CD71, CD33, HLA-DR, CD19, CD20, k, λ, CD3, CD56, and CD5.

Histopathologic

Image 2 Small cell osteosarcoma, fine-needle aspiration. The smear shows small to intermediate-sized malignant cells with scant blue cytoplasm, minimal anisonucleosis, high nuclear/cytoplasmic ratios, and mitosis (4 o’clock) (rapid Romanowsky, ×400).

Image 3 Small cell osteosarcoma, fine-needle aspiration. A loose fragment of relatively small malignant cells (compare with a leukocyte at 1 o’clock) with scant pale cytoplasm, finely granular chromatin, and inconspicuous nucleoli. Background is clean and devoid of osteoid (Papanicolaou, ×400).

Image 4 Small cell osteosarcoma, fine-needle aspiration. The smear shows tight clustering of small malignant cells with a high nuclear/cytoplasmic ratio and nuclear molding and occasional prominent nucleoli. Despite the tight molding, the cells have a lymphocyte-like appearance (rapid Romanowsky, ×600).

Image 5 Small cell osteosarcoma, fine-needle aspiration. The malignant osteoblasts have round nuclei, high nuclear/cytoplasmic ratios, finely granular chromatin, and conspicuous cytoplasmic vacuoles. The cytomorphologic features illustrated here are indistinguishable from Ewing sarcoma/primitive neuroectodermal tumor (rapid Romanowsky, ×400).
correlation was available for all cases as a follow-up excision (3 of 5) or an antecedent resection of a newly aspirated metastasis (2 of 5). Histologically, small cell osteosarcoma displayed sheets of uniform cells with round nuclei and minimal cytoplasm [Image 7] and [Image 8]. All resections showed at least focal osteoid production that was often lace-like (Image 6).

Discussion Small cell osteosarcoma is extremely rare; even at 2 highly specialized tertiary care academic hospitals only 5 cases were identified in a 19-year period. This rarity is similar to that seen in previously reported series.2,4-6 The demographics of small cell osteosarcoma are similar to those of conventional osteosarcoma.1,7 In our series, pain

[Image 6] Small cell osteosarcoma, fine-needle aspiration. A and B. The malignant osteoblasts are small and display nuclear monotony with inconspicuous nucleoli. The characteristic “lacy” osteoid is present, which appears as faintly metachromatic (with rapid Romanowsky stain) and pale green (with Papanicolaou stain) amorphous substance. When osteoid is present, its accurate identification and distinction from collagen and other matrix-type substances can be extremely helpful for the diagnosis of small cell osteosarcoma (rapid Romanowsky, x400).

[Image 7] Small cell osteosarcoma, fine-needle aspiration. Cell-block section shows loosely placed sheets of small, uniform cells with round nuclei, finely granular chromatin, and few mitotic figures. Osteoid is not seen (H&E, x200).

[Image 8] Small cell osteosarcoma, histologic section. A syncytium of small, round, and uniform malignant cells embedded in a loose, pale pink stroma and fine lacy osteoid (2 o’clock) (H&E, x100).
was the most common clinical manifestation (all cases), with pain at night seen in 2 cases. Three of the cases originated from the classic location for osteosarcoma (2 distal femur and 1 proximal tibia), whereas one was a paravertebral primary with peripancreatic soft tissue metastasis and another was a femur primary with a vertebral metastasis. The histories of small cell osteosarcoma were available in the 2 metastatic cases at the time of FNA evaluation. Because of the rarity of this lesion, details about the optimal treatment and prognosis of small cell osteosarcoma compared with conventional osteosarcoma are not clear.1

Radiologically, most small cell osteosarcomas show a mixed lytic and blastic pattern, often with soft tissue extension.1,5-7 In our series, a common feature was a periosteal reaction (all 3 primary cases). A helpful radiographic feature of small cell osteosarcoma previously described is the presence of new bone formation within the tumor; this helps to distinguish it from Ewing sarcoma, particularly if mineralization is seen in areas of soft tissue extension.1,5,8 The zonal mineralization pattern described in earlier reports of small cell osteosarcoma was not observed in the cases in this small study.

Cytologically, the most common features in our series were small to intermediate-sized cells, high nuclear/cytoplasmic ratios, focal pleomorphism, round nuclei, and dark finely granular chromatin, followed by cytoplasmic vacuoles, focal and minimal anisonucleosis, nuclear molding, and scant osteoid. Cytologic features that are typical of conventional osteosarcoma but were not observed include macronucleoli, prominent spindled morphologic features, plasmacytoid morphologic features, cellular necrosis, and relatively abundant osteoid.

Grossly, small cell osteosarcoma generally has a fleshy appearance that is seen in many highly cellular neoplasms.1 In tissue sections, 3 histologic patterns have been described: Ewing sarcoma—like (the most common), lymphoma-like, and small spindle cell.1,5-7 The presence of osteoid is required for the diagnosis of small cell osteosarcoma, but was not observed include macronucleoli, prominent spindled morphologic features, plasmacytoid morphologic features, cellular necrosis, and relatively abundant osteoid.

As alluded to earlier, the differential diagnosis of small cell osteosarcoma includes other small round blue cell tumors, including various non-Hodgkin lymphomas, small cell carcinoma, neuroblastoma, mesenchymal chondrosarcoma, and Ewing sarcoma/primitive neuroectodermal tumor (PNET).1,6,7 The cells of malignant lymphoma generally have larger nuclei than in small cell osteosarcoma, often with vesicular chromatin, irregular nuclear membranes, prominent nucleoli, a lack of cellular cohesion, and lymphoglandular bodies, features not generally seen in small cell osteosarcoma. In addition, immunoperoxidase staining for common leukocyte antigen and flow cytometry can effectively exclude a lymphoproliferative process. Small cell carcinoma manifesting as a bony mass, particularly in a young patient, would be extremely uncommon.6 In addition, a lack of immunoperoxidase staining for cytokeratins and neuroendocrine markers like synaptophysin and chromogranin would exclude this possibility.

Neuroblastoma metastatic to bone may also mimic small cell osteosarcoma with its small blue cell morphologic features and proclivity to young patients, particularly if the presence of a primary adrenal tumor is not known. The presence of Homer Wright rosettes or pseudorosettes supports the diagnosis of neuroblastoma over small cell osteosarcoma. In addition, neuroblastomas are immunoreactive for synaptophysin and chromogranin but are virtually never immunoreactive for CD99 (O13).

Although mesenchymal chondrosarcoma has areas of small round blue cell morphologic features, it characteristically has “staghorn,” hemangiopericytoma-like vessels and areas of differentiated cartilage, features not generally seen in small cell osteosarcoma.1,7 However, if these features are not present on the smears owing to sampling, mesenchymal chondrosarcoma can be very difficult to differentiate from small cell osteosarcoma.

The most difficult differential diagnosis is between small cell osteosarcoma and Ewing sarcoma/PNET. Indeed, the histologic appearance of Ewing sarcoma/PNET—sheets of monotonous, small, round cells with minimal cytoplasm—can be identical to that of small cell osteosarcoma. In addition, in the absence of mineralization, true osteoid of small cell osteosarcoma can be very difficult to differentiate from collagen or fibrin that can be seen in Ewing sarcoma/PNET.6 If present, any significant degree of cell spindling in the tumor effectively eliminates the diagnosis of Ewing sarcoma/PNET.6 The presence of Homer Wright rosettes and pseudorosettes supports the diagnosis of Ewing sarcoma/PNET over small cell osteosarcoma, although as mentioned, these features may also be seen in metastatic neuroblastoma.7 Ewing sarcoma/PNET (as well as mesenchymal chondrosarcoma) is usually strongly immunoreactive for CD99 (O13); unfortunately, small cell osteosarcoma can also be positive for CD99, so positive staining with this marker is not helpful. However, a negative result with this immunoperoxidase marker supports the diagnosis of small cell osteosarcoma over Ewing sarcoma/PNET.1,7 In addition, if molecular testing can be performed, the t(11;22) (q24;q12) translocation is diagnostic for Ewing sarcoma/PNET. However, in the absence of osteoid, it may not be possible to definitively differentiate small cell osteosarcoma and Ewing sarcoma/PNET in limited material obtained by FNA. Overall, in our series, flow cytometry (2 cases) and
immunoperoxidase staining (3 cases) were helpful in excluding potential mimics in some cases.

The most important reason to be able to recognize small cell osteosarcoma is the effect of this diagnosis on guiding appropriate therapy. Although the treatment of small cell osteosarcoma has not been optimized (owing to its rarity), small cell osteosarcoma is most often treated like conventional osteosarcoma, with neoadjuvant chemotherapy followed by surgical resection. Mesenchymal chondrosarcoma is not treated with chemotherapy, and the chemotherapeutic protocols for small cell carcinoma, non-Hodgkin lymphomas, Ewing sarcoma/PNET, and osteosarcoma are different. Specifically, osteosarcoma is most often treated with a regimen of cisplatin, doxorubicin, and high-dose methotrexate, whereas Ewing sarcoma/PNET is most often treated with doxorubicin, cyclophosphamide, vincristine, and dacarbazine. However, in cases in which a tumor cannot be definitively placed into the small cell osteosarcoma or Ewing sarcoma/PNET category, authorities recommend treating the tumor as a Ewing sarcoma/PNET.

Small cell osteosarcoma shares some of the previously described cytomorphologic features of classic osteosarcoma, but a common denominator is the predominance of small to intermediate-sized cells with scant osteoid. Clinical and radiographic parameters, in conjunction with cytomorphologic, flow cytometric, and immunoperoxidase studies, are helpful in the definitive diagnosis of small cell osteosarcoma.

References