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CASE REPORT

Ewing sarcoma/primitive neuroectodermal tumor with chondroid differentiation: a potential diagnostic pitfall

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Abstract:

Ewing sarcoma/Primitive neuroectodermal tumor (ES/PNET) with chondroid differentiation and mesenchymal chondrosarcoma (MSC) have similar histopathological features. It may be difficult to distinguish both lesions without genetic or molecular analysis. The main aim of this case report is to highlight the difficulty in establishing the diagnosis of ES/PNET with areas of chondroid differentiation. A 14-year-old female patient with painful right mandibular swelling. Imaging exams showed an extensive osteolytic lesion with mineralized areas. Histomorphologic and immunohistochemistry studies were performed. In situ hybridization was necessary to confirm the diagnosis. Immunohistochemistry and H&E-stained sections did not provide sufficient evidence to differentiate ES/PNET from MCS. The genetic tool demonstrated EWS1 gene breakpoint. ES/PNET with chondroid differentiation is rare and new studies are necessary to standardize molecular biomarkers and/or the panel of antibodies required to differentiate ES/PNET from MCS.

Keywords: Ewing sarcoma; Primitive neuroectodermal tumor; Molecular Pathology

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INTRODUCTION

ES/PNET is an uncommon high-grade malignancy characterized by the EWSR1-ETS gene fusion. Generally, it affects young adults and children. Most ES/PNET occurs in bone specially pelvis, long bones diaphysis and chest wall[1]. Primary head and neck lesions are rare and mandible is more affected than maxilla[2]. Histologically, ES/PNET is characterized by the proliferation of small, round and blue neoplastic cells. Despite its monotonous microscopic appearance, some rare morphological variations have been described[3].

Cartilagenous differentiation in ES, although very rare, represents a diagnosis challenge and the distinction from primary chondroid neoplasms has important therapeutic relevance. We report a case of a mandibular ES/PNET with areas of chondroid differentiation in a 14-year-old girl.

CASE REPORT

A 14-year-old female patient presented with painful mandibular swelling with duration of 2 months. Imaging exams evidenced an extensive osteolytic lesion with mineralized areas in the right mandible, with buccal-lingual expansion and cortex perforation. The third molar was displaced to the region below the second molar roots, inducing external apical root resorption (Figure 1). As odontogenic tumor was the main clinical hypothesis, an incisional biopsy was performed.

Microscopically, sheets of small round blue cells were the main histological picture. The neoplastic cells presented round-to-oval cytoplasm with poorly distinct borders. The cell nucleus was also round and hyperchromatic with inconspicuous nucleoli. In frequent areas, the neoplastic cells assumed an immature chondroid aspect. Numerous mitotic figures, as well as apoptotic debris were identified (Figure 2).

The immunohistochemical staining revealed the following profile: positive expression for CD99, FLI-1 and synaptophysin, focal positivity for S-100 and enolase and negativity for desmin, myogenin and chromogranin (Figure 2). The demonstration of EWS1 gene breakpoint by in situ hybridization confirmed the ES/PNET diagnosis (Figure 3).

DISCUSSION

ES/PNET and MSC are included in the small-blue-round-cell tumors and both tumors affect children and young adults[4,5]. Only eleven cases of primary ES with genetic confirmation in gnathic region were described in international literature[6].

Morphologically, ES/PNET presents small round cells with well-defined nuclear limits and ill-defined cell margins. ES/PNET cells cytoplasm often presents

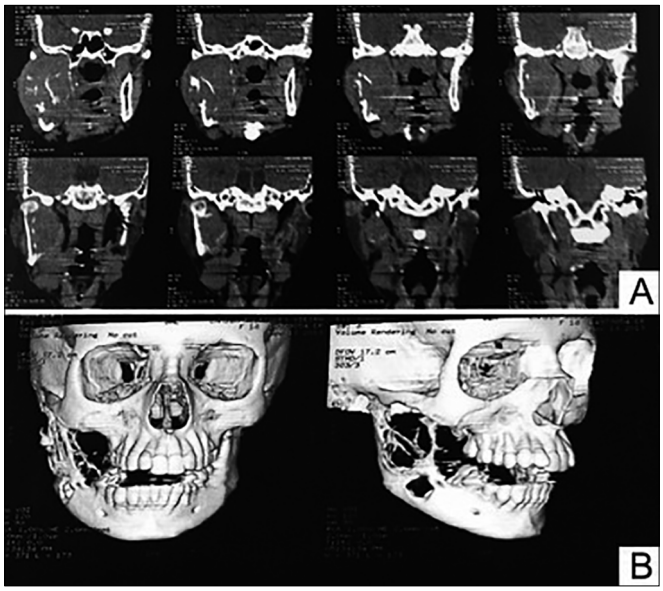

Figure 1. Coronal two-dimensional images and three-dimensional computerized tomography reconstruction demonstrated an extensive osteolytic lesion with mineralized areas and cortex perforation in the right mandible.

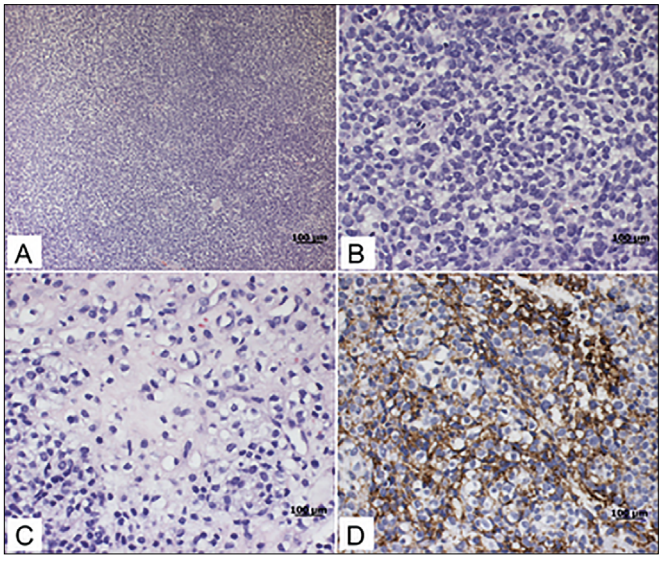

Figure 2. A- Sheets of small round blue cells with scant cytoplasm and hyperchromatic nucleus (H&E; ×100). B- Along the monotonous pattern of cell sheets, there were mitotic figures (black arrow) (H&E; ×400). C- Frequently, the neoplastic cells assumed an immature chondroid aspect with hyaline extracellular matrix (H&E; ×400). D- The immunohistochemical staining showed positive membranous expression for CD99 (×400).

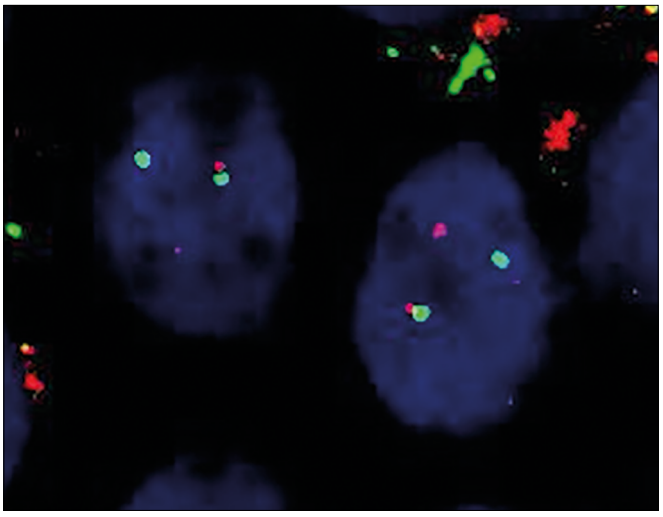

Figure 3. EWS1 gene breakpoint highlighted by in situ hybridization.

glycogen, but this is not an exclusive characteristic of this tumor, once other primary tumors, including chondrosarcoma, can also display this profile. Homer-Wright pseudorosettes, occasionally seen in ES/PNET, can support the neuroectodermal nature[7]. ES/PNET variants (such as large cells, spindle or vascular-like patterns) have been reported. Moreover, the presence of chondroid areas in ES/PNET is rare and can difficult its differentiation from MSC[8,9].

ES cells can differentiate through different pathways such as neural and mesenchymal, and ES cell lineage has been related to cartilage formation[10]. It was recently reported that Ewing sarcoma Ewsa protein, in a zebrafish research, was actively involved in the regulation of Meckel cartilage chondrogenesis through Sox9 modulation[11].

One of the differential diagnosis for ES/PNET is MSC. MSC presents two main components: highly cellular tissue composed by poorly differentiated small cells and the formation of cartilaginous tissue[12]. The MSC morphological pattern can be useful on the establishment of treatment protocol, once small undifferentiated cells tend to respond to adjuvant chemotherapy and radiotherapy. Despite more sensitive to drugs and radiotherapy, especially chemotherapy-sensitive, this tumor tends to be more aggressive and presents poorer outcome[4].

The other MSC type shows hemangiopericytomatoid component and, generally, treatment is similar to the one for osteosarcoma. Nevertheless, many clinicians choose not to direct the treatment by MSC morphology, since these patterns can be superimposed in the analyzed specimen[4]. Many MSC histological features are not exclusive and are found in other tumors, such as ES/PNET. Furthermore, the presence of cartilage cannot be a limiting factor in the MSC diagnosis because it is, frequently, a minor component and is present in many tumors (such as ES/PNET)[3].

The distinction between MSC and ES/PNET may be impossible in incisional biopsies that are not representative of the entire lesion and do not display evident cartilage differentiation[3,5,8]. There are several histologic similarities between these entities and several immunohistochemical markers have been used to differentiate them. CD- 99 and FLI-1 are prominent among these markers[5].

Other markers, such as Sox9 and NKX2-2, have been studied for differentiation of Ewing sarcoma and other neoplasms, such as MSC[13]. Despite the attempts, NKX2-2 has shown high affinity for Ewing sarcoma, but low specificity, making it difficult to differentiate between Ewing sarcoma and MSC[13]. The Sox9 expression also does not appear to be a determining factor in the Ewing sarcoma diagnosis since it has high affinity for MSC but can also stain EWS[14]. MSC can be differentiated from Ewing sarcoma due strong CD99 and desmin positivity in MSC blue cell[5]. From a molecular perspective, HEY1-NCOA2 fusion gene may be present in MSC[8].

ES/PNET exhibit wide histopathologic heterogeneity, so an accurate immunohistochemistry panel is essential to distinguish this tumor from other small-blue-round-cell tumors[7]. Many markers have been tested for the ES/PNET identification. Initially, CD99, a 32kDa glycoprotein, was designated as specific ES/PNET marker. Further studies showed that CD99 is present in other small- blue-round-cell tumors, such as mesenchymal chondrosarcoma, rhabdomyosarcoma and lymphoblastic lymphoma[3,5].

Despite attempts to find a marker for this tumor, it is known that the use of some markers simultaneously provides an accurate diagnosis establishment[7]. Bosh et al. (2009), evaluated 415 ES/PNET cases for CD99, FLI-1, HNK and CAV1. The authors described that these antibodies combination was capable of diagnosing ES/PNET cases. Besides, the authors reported CAV1 positivity in CD-99 negative cases, which may be useful for ES/PNET diagnosis.

Other markers; such as neuron-specific enolase, synaptophysin, and S-100 protein; can be helpful in ES/PNET diagnosis, based on the tumor neuroendocrine differentiation[15]. The difficulty of identifying the ES/PNET by immunohistochemical methods can be assisted by molecular genetic or cytogenetic analysis[5,8].

Ewing sarcoma family tumors is identified frequently by translocation between EWSR1 and ETS family member gene, commonly FLI-1 and ERG[1]. Around 90% of cases of ES/PNET undergo translocation t(11; 22)(q24; q12), which promotes the EWS-FLI-1 fusion gene(16). The ES/PNET cases, which do not present this mutation, can be genetically identified by EWS-ERG fusion due to translocation t(21; 12)(22; 12). A minority of cases present fusion of EWS and a transcription factor of ETS family[15].

Currently, the reverse transcriptase polymerase chain reaction (RT-PCR) can identify many of these genetic alterations in formalin-fixed paraffin-embedded samples. In many cases, RT-PCR analysis is not possible, either by problems with the samples, as small amount of tissue, or difficulty of efficient access to this tool, which is concentrated in academic centers[5]. There is still a difficulty in diagnosis due to the lack of markers, there are efforts to discover new marker panels for the differentiation of Ewing and other neoplasms, including MSC.

CONCLUSIONS

In our case, the immunohistochemical expression and histopathological features, including the cartilage presence in tumor tissue, associated to in situ hybridization allowed the differentiation between the ES/PNET and MSC. ES/PNET with chondroid presence is a rare entity which should be differentiated from MSC in such cases. In this context further studies are necessary to standardize easily accessible molecular biomarkers and/or the panel of antibodies required to differentiate ES/PNET from MSC.

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