

Cytodiagnosis of Primary Ewing Sarcoma of the Skull: Diagnostic Clues and Difficulties

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ABSTRACT

Ewing sarcoma is a rare primary neoplasm of bone representing approximately 6-8% of all malignant bone tumours. Because of its aggressive clinical behaviour and rapid dissemination to other sites, an early accurate diagnosis is of utmost importance. It shares morphological features with other round cell tumours. Common differentials include lymphoblastic lymphoma, neuroblastoma, rhabdomyosarcoma and neuroendocrine tumours. Due to the morphological overlap, critical evaluation of the cellular details is essential. Fine needle aspiration cytology becomes a successful diagnostic tool when the subtle diagnostic clues and difficulties are considered during diagnosis. Judicious use of ancillary techniques will also aid in arriving at an accurate diagnosis. We present the case of a seven-year-old boy who presented with painful swelling of the scalp. Aspiration smears were cellular and showed atypical small round cells. Evaluation of morphological details along with special stains and immunohistochemistry in cell block preparation aided in rendering a diagnosis of Ewing sarcoma. The awareness of overlapping features in clinical presentation, morphology and immunohistochemical findings will help to arrive in the proper diagnosis. Early diagnosis on cytology samples can help in timely initiation of treatment, thus improve prognosis.

Keywords: Ewing Sarcoma, Fine Needle Aspiration Cytology, Scalp

Introduction

Malignant small round cell tumours include group of neoplasms characterised by small, round, relatively undifferentiated cells. They generally include Ewing sarcoma/primitive neuroectodermal tumour, rhabdomyosarcoma, neuroblastoma, non-Hodgkin lymphoma, retinoblastoma, nephroblastoma, hepatoblastoma, synovial sarcoma, small cell osteosarcoma and desmoplastic small round cell tumour. Due to their undifferentiated or primitive character, a definite diagnosis often requires ancillary studies like immunocytochemistry, flow cytometry or molecular studies.

Ewing Sarcoma is a small round cell tumour of neuroectodermal origin. It is the second most common primary malignant tumour of bone in young patients.^[1,2,3] The clinical symptoms can mimic osteomyelitis because of the pain and swelling. Ewing sarcoma is an aggressive tumour with potential for metastasis. The presence of metastatic disease indicates poor prognosis. Because of the aggressive nature and metastatic potential, it is very important to make an early diagnosis. Early diagnosis of the disease on cytology can help in timely initiation of treatment and thus improve prognosis. Fine needle aspiration material (both needle rinse and cell block) can provide sufficient material for diagnosis.

Overlapping morphological features and nonspecific nature of immunomarkers can lead to the diagnostic difficulty. The

awareness of subtle morphological details, the nonspecific nature of immunostains along with clinical correlation will help in arriving a definitive diagnosis.

Case Report

A seven-year-old male child presented with pain and swelling in the scalp of two-month duration. Radiological examination revealed expansile lytic lesion involving the frontal and temporal bones with soft tissue extension. With the differential diagnosis of metastatic neuroblastoma and osteosarcoma, Fine needle aspiration cytology (FNAC) was done and cell block was prepared.

Cellular smears showed atypical cells with scant to moderate amount of pale cytoplasm, ill-defined cell borders, punched out round nuclei, fine chromatin and 1-2 small nucleoli (Figure 1,2). Scattered small cells with scant cytoplasm and hyperchromatic irregular nuclei also noted. With a morphological diagnosis of Ewing Sarcoma, we proceeded with special stains and immunostains. The cytoplasm of larger cells showed Periodic Acid- Schiff (PAS) positive Diastase sensitive material (Figure 3a, 3b). The atypical cells showed diffuse strong membranous positivity for CD99 (Figure 3c). The cells were LCA negative, desmin negative and synaptophysin negative. Occasional cells showed faint positivity for cytokeratin (Figure 3d). Based on the morphology, special stains and immunostains, a diagnosis of Ewing Sarcoma was made.

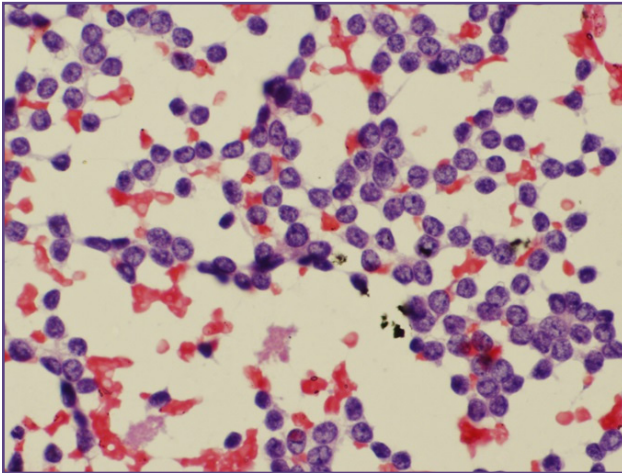


Fig. 1: Cellular smears showing atypical cells with ill-defined pale cytoplasm, punched out round nuclei, granular chromatin, small nucleoli. (Pap, X400).

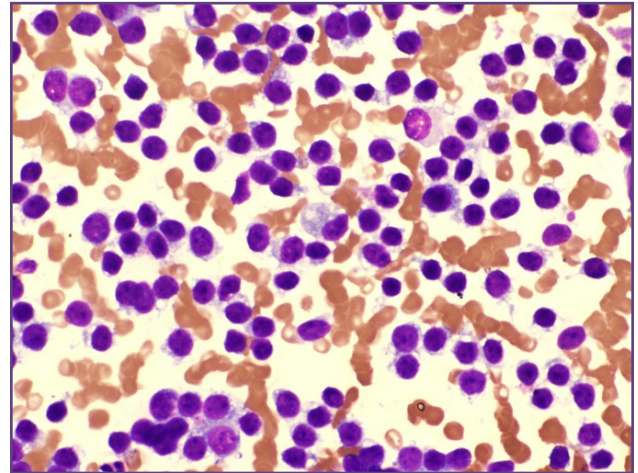


Fig. 2: Smears showing cells with vacuolated cytoplasm. (Giemsa, X400).

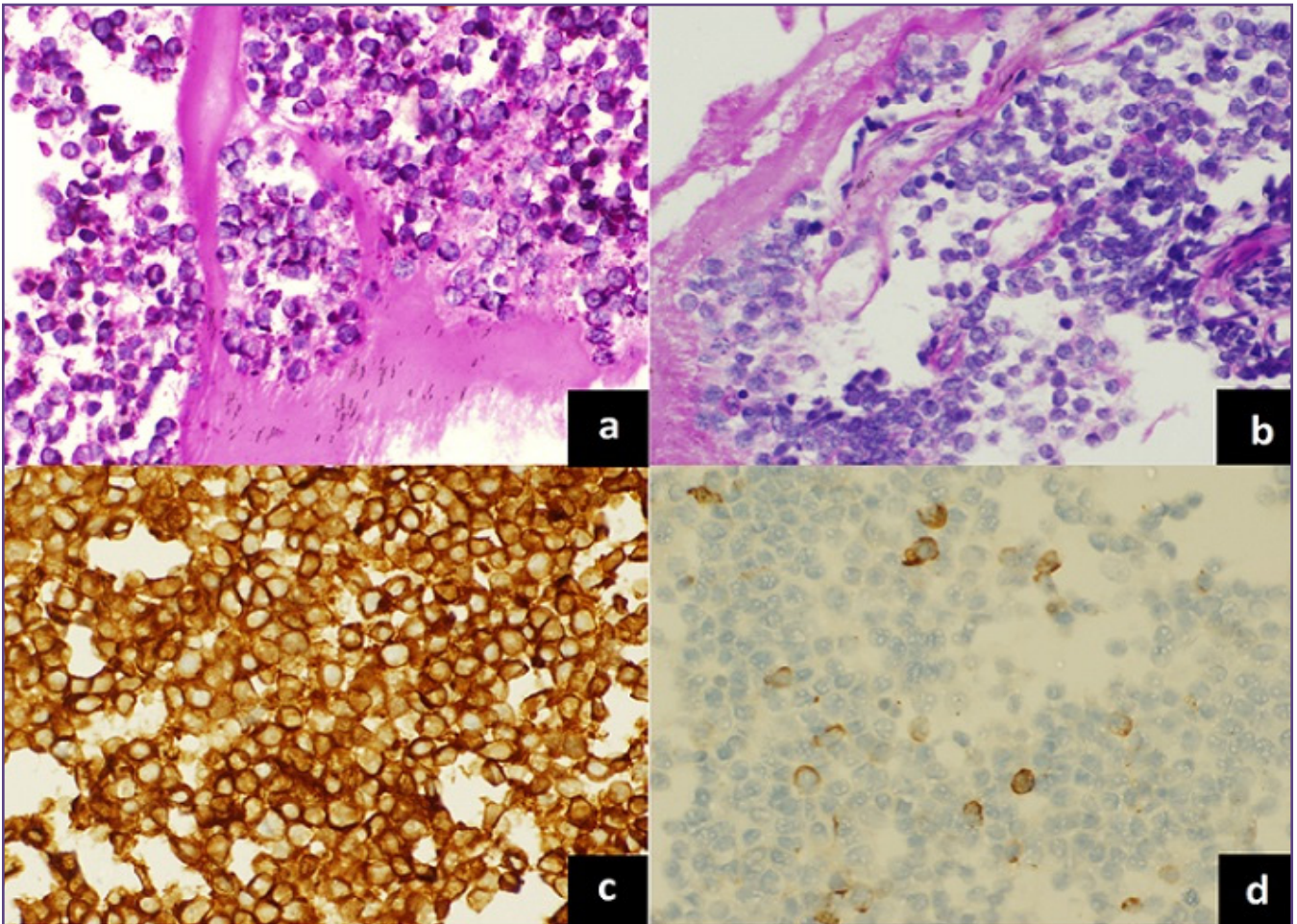


Fig. 3: a) PAS staining on cell block preparation showing PAS positive material in the cytoplasm, b) PAS with diastase showing the material is sensitive to diastase, c) Tumour cells showing diffuse strong membranous positivity for CD 99, d) Occasional tumour cells showing positivity for cytokeratin. (X200).

Discussion

Ewing sarcoma (ES) is an aggressive malignant tumour which involve skeletal and extra skeletal sites. Any portion of the skeleton may be involved, but more than half of the tumours involve the long bones, usually the diaphysis or meta-diaphysis. The flat bones also may be involved, especially the ilium and the ribs.

Primary Ewing sarcoma of the skull are rare and accounts for 1-9% of all Ewing sarcoma. ^[1,2] The frontal, temporal and parietal bones are relatively common sites. Sphenoid and ethmoid bones are less commonly involved. ^[2,3,4]

Clinically tumour may mimic osteomyelitis because of pain, fever and leucocytosis. ^[5] Ill-defined destructive intramedullary lesions involving diaphysis of the long tubular bones are seen in ES. "Moth eaten" pattern, accompanied by an onion skin type periosteal reaction is characteristic.

Ewing sarcoma exhibit aggressive clinical behaviour and rapid dissemination to other sites. Common sites of metastatic spread of Ewing sarcoma are to lung, pleura, other bones and lymph nodes.

Aspiration smears will be cellular with loosely cohesive and singly dispersed undifferentiated small round cells. Dimorphic population of light and dark cells is a feature of Ewing sarcoma. The lighter staining cells will be larger cells with moderate amount of pale vacuolated cytoplasm round nuclei with smooth nuclear membrane, fine chromatin, 1-2 small nucleoli. The darker staining cells will have scant cytoplasm, irregular nuclei, dense chromatin and inconspicuous nucleoli. Usually the two cell types will be intermingled with the lighter staining cells predominating. Rosette like structures with or without a fibrillar centre can be seen in some cases.

In atypical Ewing sarcoma the cellularity and nuclear atypia will be more. Distinction between the larger light staining cells and smaller dark staining cells will be less obvious. Rhabdomyoblast like cells and cells with thin cytoplasmic processes can be seen.

Nuclear moulding will not be present in Ewing sarcoma. Binucleation, multinucleation, multinucleate giant cells and stromal matrix formation are not features of Ewing sarcoma.

The important differential diagnoses include lymphoblastic lymphoma, neuroblastoma and alveolar rhabdomyosarcoma in children and neuroendocrine tumours in adults.

Singly dispersed cells without clustering, round/irregular /folded nuclei, immature chromatin, mitosis

and lymphoglandular bodies in the background point to the diagnosis of lymphoblastic lymphoma. In Burkitt lymphoma, there will be uniform cell population with thin rim of dark blue cytoplasm with small vacuoles. Nuclei are round with speckled chromatin and multiple small but prominent nucleoli. Brisk mitotic activity and starry sky macrophages are characteristic features of Burkitt lymphoma.

Metastatic neuroblastoma typically occurs in children younger than 2 years of age, a very uncommon age group for Ewing sarcoma, and clinicians are usually aware of the underlying disease. Round/ovoid cells with scant cytoplasm, nuclear moulding, granular chromatin favour the diagnosis of neuroblastoma. Presence of neuropil and ganglionic differentiation can be seen in primary and metastatic neuroblastoma. The presence of Homer-Wright rosettes is a diagnostic feature of neuroblastoma. Homer-Wright rosettes consists of primitive neuroblastic cells surrounding centrally located neuropil. Neuropil is the most helpful cytological feature for the diagnosis of neuroblastoma.

Cells of alveolar rhabdomyosarcoma will be more irregular. The presence of cells with dense eosinophilic cytoplasm and eccentrically placed nuclei favours the possibility of alveolar rhabdomyosarcoma. The chromatin is variably coarse, and nucleoli may be prominent. Multinucleate cells are also a clue for rhabdomyosarcoma.

Mesenchymal chondrosarcoma and small cell osteosarcoma may be confused with atypical Ewing sarcoma when biopsy tissue lacks matrix.

Special stains: The cytoplasm of the lighter staining cells of ES contain abundant glycogen. Glycogen is PAS positive and diastase sensitive. Demonstration of glycogen by special stains -PAS with and without diastase- either in aspiration material or in the cell block will help in the diagnosis of ES. But the presence of intracellular glycogen is not a specific feature of ES. Up to 35% of all Ewing sarcoma cases do not contain detectable glycogen and many other childhood tumours do contain detectable glycogen.

Immunostains: CD 99 is a sensitive marker of Ewing sarcoma and will be positive in 99% of cases. But this marker is not specific for Ewing sarcoma and can be positive in lymphoblastic lymphoma, small cell osteosarcoma, mesenchymal chondrosarcoma and synovial sarcoma. CD99 positivity is also described in rhabdomyosarcoma, desmoplastic small round cell tumour, small cell carcinoma and Merkel cell tumour. ^[6,7] FLI-1 has been reported to be positive in 75% of Ewing sarcomas. However, it is also often expressed in lymphoblastic lymphoma.

Leukocyte Common Antigen (LCA) positivity help to differentiate hematopoietic malignancies from Ewing sarcoma. But LCA negative hematopoietic malignancies like lymphoblastic lymphoma, myeloid sarcoma, anaplastic large cell lymphoma, plasma cell myeloma should also be kept in mind while dealing with malignant round cell tumours. Focal cytokeratin immunoreactivity has been reported in 20% to 40% of genetically confirmed Ewing sarcomas.^[8]

Ewing Sarcoma is characterised by the presence of recurrent chromosomal translocation. The most common is t (11;22) (q24; q12) which result in the formation of fusion gene ESWR1-FLI1. This fusion gene occurs in more than 90% of cases. Identification of EWS rearrangements by Florescence insitu hybridisation or Reverse transcriptase polymerase chain reaction will help to differentiate Ewing sarcoma from other small round cell tumors. The most common abnormality is the balanced translocation between chromosomes 11 and 22 resulting in fusion of portion of EWS gene on chromosome 22q12 with FLI1 gene on chromosome 11q24. Other less common translocations are t(21;22) resulting in fusion between EWS and ERG gene and t(7;22)resulting in fusion between EWS and ETV1.^[2,7,9]

Poor prognostic features include metastasis at the time of initial diagnosis, large tumour size, extension into soft tissue, extensive necrosis, overexpression of p53 and poor response to initial chemotherapy.^[10]

Conclusion

Early diagnosis and treatment prior to metastasis are essential for long-term survival in patient with Ewing sarcoma. Fine Needle Aspiration Cytology is a reliable approach for the diagnosis of Ewing sarcoma regardless of the site of clinical presentation. FNA of the lesion aided with cell block for IHC confirmation can serve as a rapid and inexpensive method for early diagnosis.

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Competing Interests

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Reference

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