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Cytological diagnosis of Langerhans cell histiocytosis. Apropos 2 cases

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SUMMARY

Langerhans cell histiocytosis (LCH) comprises a wide spectrum of clinical presentations in children and adults, ranging from favorable lesions to aggressive disseminated disease. We present 2 cases where cytology introduced the diagnosis of LCH in the appropriate clinicoradiological settings. Cytology can serve accurately in LCH interpretation based on the characteristic cytomorphology and selected immunocytochemistry, so unwarranted biopsy can be overruled and treatment can be adjusted largely where histopathology services are unavailable. Diagnosis may be difficult in cases with scant or insufficient cellular material.

Keywords: Langerhans cell histiocytosis, cytology, histopathology, langerin, CD1a, S-100.

INTRODUCTION

The diagnosis of LCH is based on clinical and radiological findings in combination with pathological studies identifying tissue infiltration by histiocytes with immunophenotypic characteristics of Langerhans cells (LCs). Pathologic histiocytes in LCH are mononucleated cells with coffee bean or kidney-shaped nuclei. Detection of LC markers is conclusive. In routine practice, detection of Birbeck granules by electron microscopy has been widely replaced by detection of CD1a and langerin expression, which can be performed on formalin-fixed samples, on Fine Needle Aspiration cytological samples or touch imprint cytological smears.

CASES REPORT

Case 1

A 14 aged male presented with swellings, and pain in the left temporomandibular joint, without prior injury, and uneventful medical record. Mouth opening was restricted. He felt discomfort on palpation of the joint with subsequent limited jaw movements. There was no occlusal contact on the first premolar area of the upper and lower dentition. Laboratory work up showed an elevated alkaline phosphatase, mirroring bone pathology.

Contrast enhanced MRI (Fig. 1) showed a mass 4.7 cm in the left infratemporal region, with increased, heterogeneous signal intensity and irregular poorly demarcated borders. It had obliterated the condyle and the coronoid process and invaded the masseter, the lateral and medial pterygoid muscles.

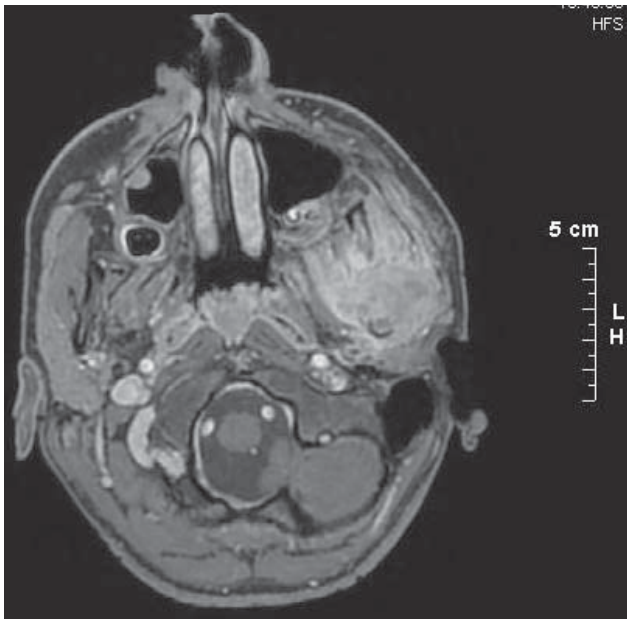

Fig. 1. Case 1: MRI showing a 4.7 cm mass in the left infratemporal region

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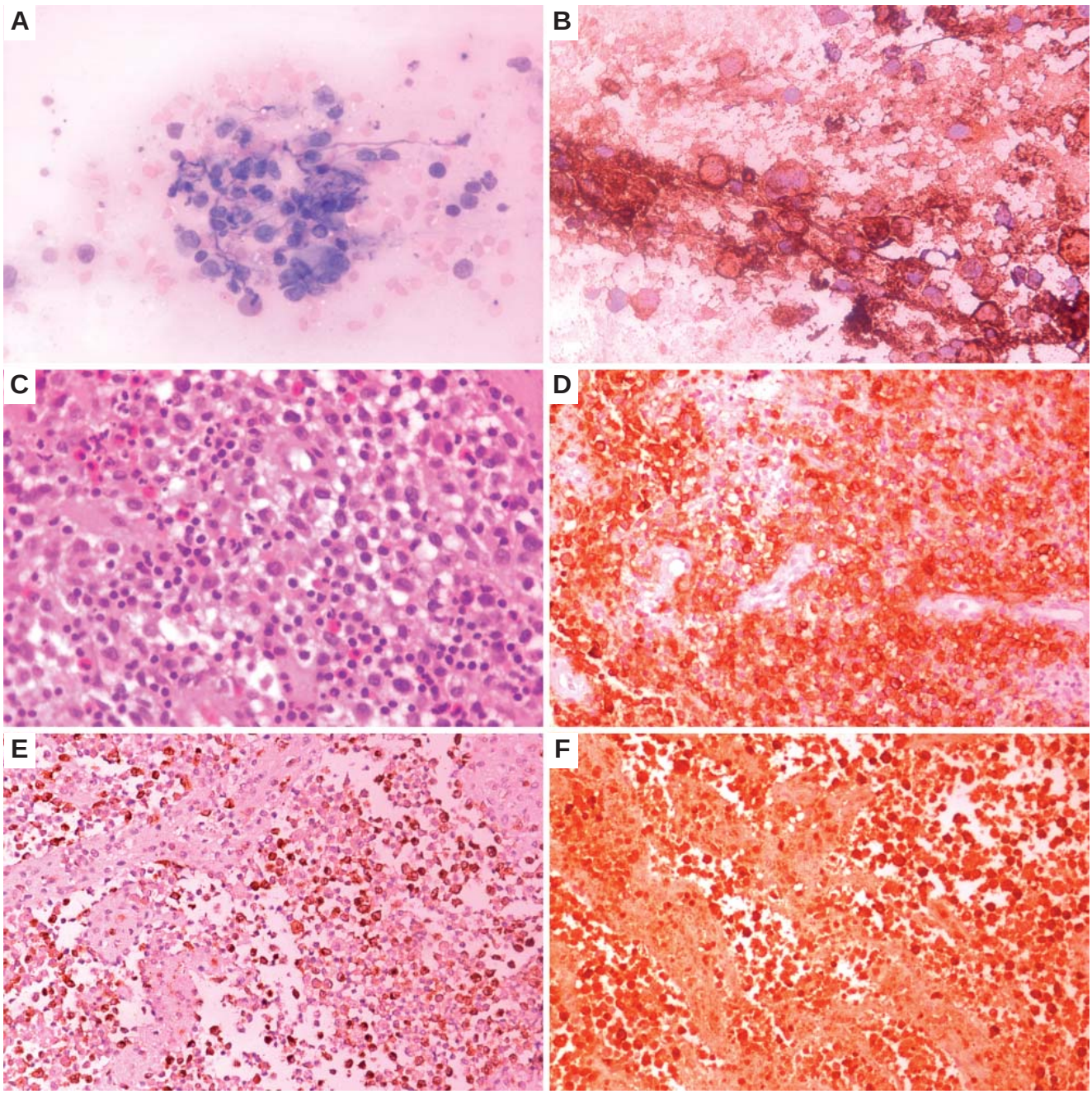

Fig. 2. Case 1 images: A – FNA smear; Langerhans cells; Giemsa stain ×400. B – FNA smear; Langerhans cells show membrane positivity by CD1a; CD1a immunostain ×400. C – tissue section; Hematoxylene-Eosin stain ×400; D – tissue section; Langerinimmunostain ×200; E – tissue section; CD1a immunostain ×400; F – tissue section; S-100 immunostain ×400.

Fine needle aspiration biopsy was performed and yielded 1ml hemorrhagic fluid. Smears were cellular and included a mixed population of lymphocytes, mature and transformed, plasma cells, neutrophils, rare eosinophils, histiocytes, in combination with and atypical polygonal cells. The latter harbored abundant cytoplasm, convoluted or grooved oval nuclei (with a coffee bean appearance), inconspicuous nucleoli and occassional intranuclear pseudoinclusions.(Fig. 2, A). These atypical cells were positive by showed membrane staining for CD1a (Fig. 2, B) and both cytoplasmic and nuclear staining for S-100. A diagnosis of LCH was rendered. An incisional biopsy was performed subsequently. Biopsy specimen was composed of a highly vascular interstitial connective tissue involved by a neoplasm with a diffuse growth pattern. Neoplastic cells (Fig. 2, C) were medium sized with ovoid nuclei with grooves, finely distributed chromatin, conspicuous nucleoli and abundant eosinophilic cytoplasm. Binucleated or multinucleated forms were also recognized surrounded by an inflammatory infiltrate composed of eosinophils, neutrophils and mature lymphocytes, plasmacytes

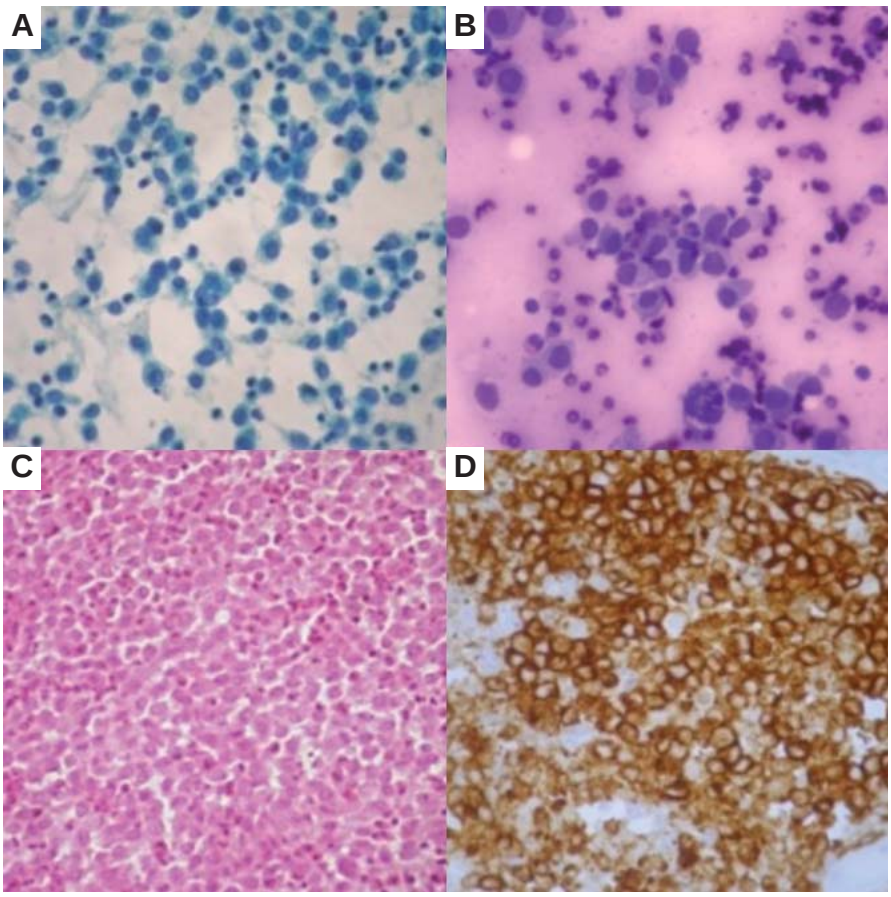

Fig. 3. Case 2 images: A – touch imprint preparation; Hemacolor rapid stain ×400. B – touch imprint preparation; Papanicolaou stain ×400. C – tissue permanent section; Hematoxylene-Eosin stain ×400. D – tissue permanent section; Langerin immunostain ×400.

and histiocytes. Areas of necrosis and eosinophilic microabscesses were identified. By immunochemistry neoplastic cells were strongly positive for langerin (Fig. 2, D), CD1a (fig 2, E),and S100 (Fig. 2, F). An LCH diagnosis was established. Because all of the examination results indicated only a single bone lesion, a trial of indomethacin was decided before administrating chemotherapy, (2.5 mg/kg/day, for 5 months, twice daily).

Case 2

A 26-year-old woman, presented with a painful mass on the chest wall that on MRI invaded the intercostal muscles caused osteolysis on the 7th rib and extended to underlying pleura. The clinical differential diagnosis included osteomyelitis and osteosarcoma. A 9 cm piece of rib bone with a central osteolytic lesion measuring 2.5 cm was sent for intraoperative frozen section. Touch imprints were obtained from the soft haemorhagic tissue of the osteolytic lesion before it was processed for frozen section. The frozen section showed mixed "inflammatory" cell population including eosinophils that could not be accurately further characterized due to artifacts. The cytological imprints stained with rapid Hemacolor stain (Fig. 3, A) and Papanicolaou stain (Fig. 3, B), showed a prominent neoplastic population with a Langerhans histiocyte morphology (polygonal cells with abundant cytoplasm, occasionally binucleated, with coffee bean shaped or nephroid nuclei with fine chromatin and indistinct nuclei. The neoplastic cells were admixed with many eosinophils, polymorphs and lymphocytes. The answer based mainly on cytology was lymphohematopoietic neoplasm mostly suggestive of Langerhans histiocytosis. The cytological diagnosis was confirmed on the paraffin sections (Fig. 3, C) further sustained with positive immunostains for S100 and Langerin (Fig. 3, D), cyclin D1 and negative for CD30 and cytokeratin.

DISCUSSION

The diagnosis of LCH is established by hematologic and histologic criteria (1).

Qualification has improved in accurate cytological diagnosis of LCH in various organs based on characteristic morphological features in the appropriate clinical setting (1) .

Diagnosis lies on the recognition of the Langerhans cell (LC) with nuclear grooves and pseudoinclusions (1, 3-5). LCs may be pleomorphic cells with mitotic figures. Dendritic cytoplasmic processes are unusual.

This cell combination is readily recognized even with Giemsa stain during rapid on site evaluation, as it was evident in our second case. In our case cytology imprints from a sample sent for frozen section suggested the correct diagnosis because histology section showed serious artifacts. In our first case diagnosis was based on FNA cytology showing LCs. In both cases cytology findings were correlated with the relevant radiology and clinical findings.

LCs are positive by S-100, PNA (peanut agglutinin), MHC class II, CD1a, and langerin (CD207) (1, 3-5). Langerin was not employed in our cases, but it was positive in the immunohistochemistry applied on histology,is their ultrastructural hallmark. The

Birbeck granule is the hallmark of LCs by electron microscopy though it is not considered essential for diagnosis (1).

It is necessary to be aware about cytological features of other differential diagnoses (2, 6)

An up-to-date categorization for LCH is grounded on a treatment protocol recommendation produced by the Histiocyte Society (7). Unifocal disease (eosinophilic granuloma) is a single-system (SS) disease that comprises a single site as well. The Hand–Schüller–Christian disease is a multifocal SS disease displaying multiple sites of involvement in a single organ system, while Letterer–Siwe disease is a multifocal multisystem (MS) disease with multiple involved sites in more than one organ system.

MS LCH is further subdivided into RO+ /RO– based on the involvement of a risk organ (RO) (e.g., hematopoietic system, liver, and/or spleen). Risk sites in the central nervous system (CNS) include the sphenoid, orbital, ethmoid, and temporal bones, representing an increased risk for the CNS (8).

The LCH pathogenesis stands vague, with recent studies suggesting that LCH is a monoclonal neoplasm due to the repeated appearance of a BRAF V600E mutation which regulates cell survival, proliferation, motility, and cell differentiation (9, 10).

In LCH lesions, neoplastic histiocytes constitute a small proportion, from less than 1% to more than 70% (median 8%), and are admixed with a variety of inflammatory cells, including eosinophils, macrophages, multinucleated giant cells, and lymphocytes (enriched with regulatory T cells) (11). Eosinophil infiltration ranges from poor to cellular in cytology specimens.

Developments in genomic sequencing techniques have aided in appreciating the pathophysiology of LCH. Activation of the mitogen-activated protein kinase (MAPK) pathway is a chief molecular mechanism involved in the development of LCH. Repeated BRAF and MAP2K1 mutations are the significant molecular alterations involved in the activation of the MAPK pathway. Recent studies have seconded the "misguided myeloid differentiation model" of LCH, where the range of the disease is determined by the differentiation stage of the cell in which the activating somatic MAPK mutation occurs, suggesting LCH (12, 13).

Due to the obliterative nature of LCH, it is commonly confused with osteomyelitis or malignancy, thus manifesting the benefit of a differential diagnosis based on patient age and radiologic features. Squamous cell carcinoma usually presents a solitary ill-defined radiolucency, and it may be related with a soft tissue mass. Sequestrum is a "hallmark" manifestation of osteomyelitis. Specifically, LCH must be differentiated from other dendritic cell diseases by cyto-histopathologic and immunophenotypic findings (CD1a, S-100 positive) (5, 14, 15).

CONCLUSION

In conclusion it is documented that LCH can be accurately diagnosed by cytology in consideration with the clinical and imaging findings, thus enabling a prompt and appropriate therapeutic management.

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