Case Report

The Challenges of Bone Marrow Biopsy in Diagnosing Multisystem Langerhans Cell Histiocytosis

Rana Naous*, Mary K. Allen, Sylva Bem

Department of Pathology, SUNY Upstate Medical University, USA

*Corresponding author: Rana Naous, Department of Pathology, SUNY Upstate Medical University, USA. Tel: +2163135942; Email: naousr@upstate.edu

Abstract

Langerhans Cell Histiocytosis (LCH) is a disease characterized by infiltration of Langerhans-type histiocytes of one or more organ systems, most commonly bone, skin, lymph nodes, liver, or lung. While localized disease tends to follow a more indolent course, multiorgan involvement is usually common in infants and portends a worse prognosis, with dysfunction of the involved organs and a mortality rate nearing twenty percent. Bone marrow involvement often presents clinically with cytopenias, however in such cases the presence of bone marrow infiltration by Langerhans cells is infrequent. We are presenting a case of a 10-month-old male child with significant pancytopenias, marked hepatosplenomegaly and scalp lesions on initial presentation. Bone marrow biopsies showed aggregates of histiocytes and evidence of hematophagocytosis without clinically meeting the diagnostic criteria for Hemophagocytic Lymphohistiocytosis (HLH). The child further developed, during his second cycle of chemotherapy, secondary HLH. Ultimately, a definitive diagnosis of multisystem Langerhans cell histiocytosis was only made following biopsy of an occipital mass, which initially was clinically considered to be a hemangioma. This case report highlights the bone marrow findings and the challenges in diagnosing multisystem Langerhans cell histiocytosis with high-risk organ involvement. It also illustrates the relationship between multisystem LCH and macrophage activation/secondary HLH.

Introduction

Langerhans Cell Histiocytosis (LCH) is a disease characterized by infiltration of Langerhans-type histiocytes into one or more organ systems, most commonly bone, skin, lymph nodes, liver, or lung [1]. The clinical presentation varies widely and is dependent on the organ systems involved. The disease can be localized to a single site, multiple sites within a single system, usually bone, or more disseminated and multisystem. The dominant sites of involvement in the solitary form are bone and adjacent soft tissue (skull, femur, vertebra, pelvic bones and ribs), and less commonly, lymph node, skin and lung. The multifocal lesions are largely confined to bone and adjacent soft tissue. In multisystem disease, the skin, bone, liver, spleen and bone marrow (BM) are the preferential sites of involvement [2]. Symptoms may include skin rash, bone pain, dyspnea, lymphadenopathy, or systemic symptoms such as fever and weight loss. While localized disease tends to follow a more indolent course, multiorgan involvement portends a worse prognosis, with dysfunction of the involved organs and a mortality rate nearing twenty percent [3]. The group with the worst prognosis and survival throughout these clinical trials has continued to consist of children with disease involving high risk organs including bone marrow, liver, or spleen who did not respond to routine induction therapy or had a reactivation of their disease involving one of these high-risk organs. This group of patients has an overall 2-year survival rate of 30% [4]. There have been a small series of reports suggesting that more aggressive therapy mimicking acute myeloid leukemia therapy may have an impact on this disease [5]. Bone marrow involvement often presents clinically with cytopenias [6], however in such cases the presence of bone marrow infiltration by CD1a-positive Langerhans cells is infrequent. More often such bone marrow biopsy specimens show aggregates of CD68 positive histiocytic cells that are negative for CD1a, and occasionally show

evidence of hematophagocytosis [7].

Case Report

We are presenting a case of a 10-month-old male child with persistent fever, significant pancytopenias including anemia and thrombocytopenia, marked hepatosplenomegaly and scalp lesions on initial presentation. Bone marrow biopsies showed aggregates of histiocytes and evidence of hematophagocytosis without clinically meeting the diagnostic criteria for HLH. The child further developed during his second cycle of chemotherapy (Cladribine/cytarabine/etoposide) secondary HLH, which was diagnosed based on his high serum ferritin level of 19353 ng/ml (serum ferritin of >10,000 is 90% sensitive and 96% specific for HLH), and markedly elevated serum levels of soluble interleukin 2-receptor alpha (8460 U/ml; reference range 223-710 U/ml). Additionally, genetic testing to evaluate for familial HLH was performed and was negative. Ultimately, a definitive diagnosis of multisystem Langerhans cell histiocytosis with high-risk organ involvement was only made following biopsy of an occipital soft tissue mass, which initially was clinically considered to be a hemangioma. Microscopically, the first bone marrow biopsy showed extensive CD163-positive histiocytic infiltrate with hemophagocytosis and extensive erythroid hyperplasia (Figure 1).
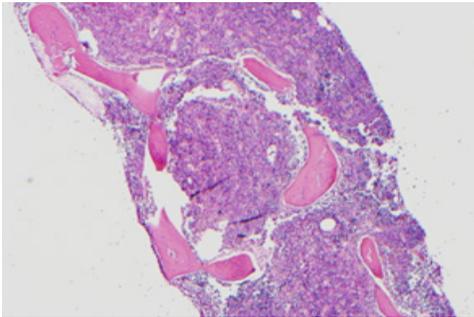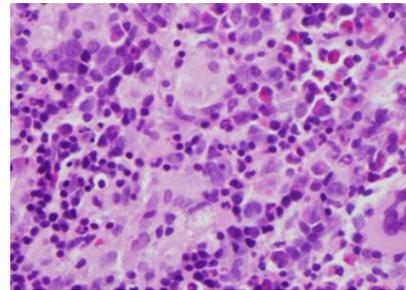

**Figure 1a:** Bone marrow biopsy shows a normocellular bone marrow relative to age (H&E, x40).
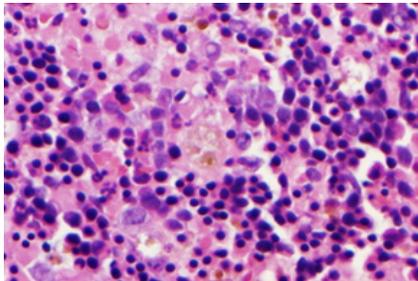
Figure 1b: High power magnification of bone marrow biopsy reveals erythroid hyperplasia (H&E, x500).
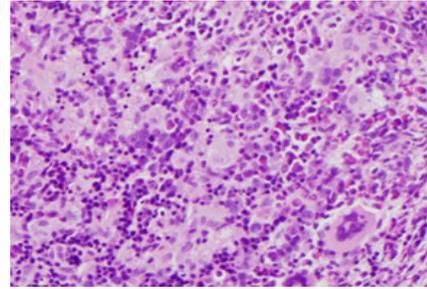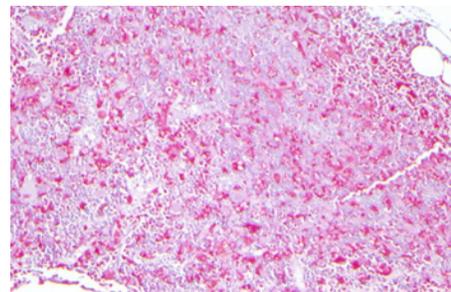

Figure 1c: Bone marrow biopsy shows abundant macrophages in a background of eosinophils, lymphocytes, megakaryocytes, erythroid and myeloid precursors (H&E, x200).
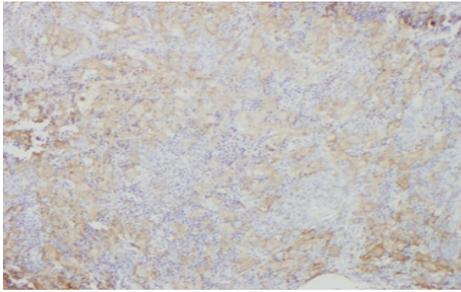

Figure 1d: High power magnification highlights the macrophages with associated hemophagocytosis (H&E, x400).
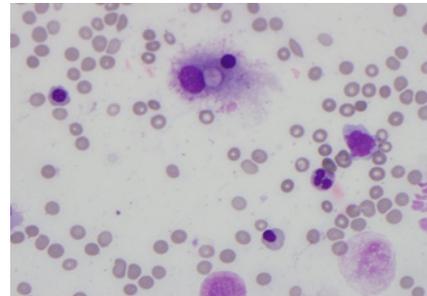
**Figure 1e:** Bone marrow aspirate highlighting a macrophage with hemophagocytosis (Giemsa, x400).

Figure 1f: CD68 immunostain highlights the extensive bone marrow involvement by macrophages (x100).
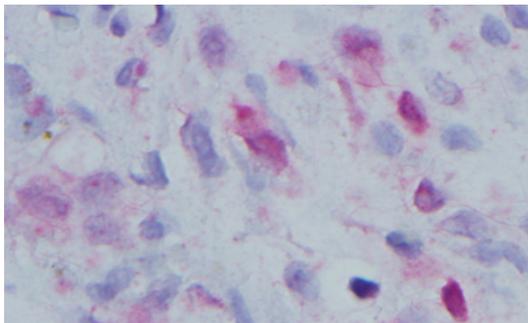
Figure 1g: CD163 immunostain shows abundant macrophages in clusters within the bone marrow biopsy (x100).

Very few singly scattered S100-positve cells with morphology consistent with Langerhans cells were evident (Figure 2).
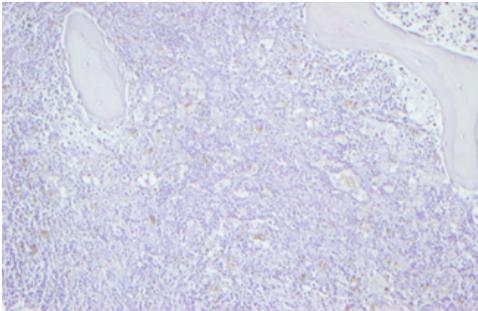

Figure 2 a: S100 immunostain shows a non-specific pattern of staining with no evidence of langerhans cells (x100).


**Figure 2b:** S100 immunostain highlights rare langerhans cells (x1000).

Meanwhile, a biopsy of the scalp lesion (Figure 3a-3c) was performed and showed sheets and individual histiocytic cells intermingled with eosinophils, neutrophils and lymphocytes and characterized by oval shaped nuclei, grooves and invaginations with positivity for CD1a, S100, CD68 and BRAF immunostains (Figure 4a-4d); consistent with the diagnosis of Langerhans cell histiocytosis. A second bone marrow biopsy performed two months later showed a more extensive CD163-positive histiocytic infiltrate with no morphologic or immunophenotypic evidence of CD1a-positive Langerhans cells. Currently, the patient's LCH has remained controlled while HLH treatment is being given, and is scheduled for a bone marrow transplant.
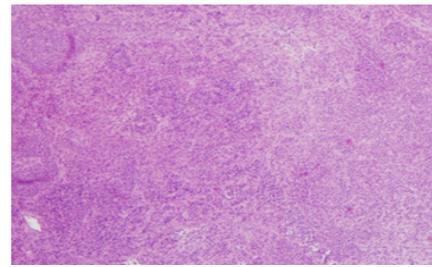
Figure 3a: Scalp biopsy shows sheets of dyscohesive cells in a minimally fibrotic background (H&E, x100).
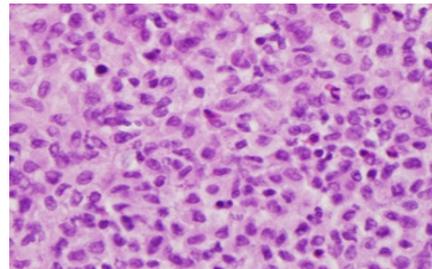
Figure 3b: High power magnification of the scalp biopsy shows oval shaped histiocytes with nuclear grooves intermingled with eosinophils, neutrophils and lymphocytes (H&E, x400).
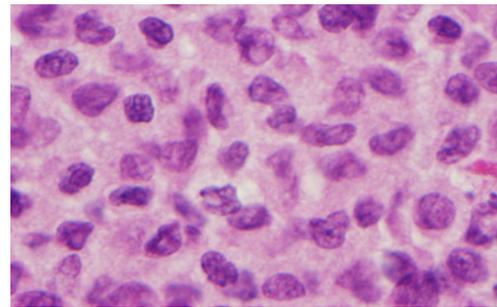
Figure 3c: Bean shaped nuclei and nuclear grooves characteristic of Langerhans cells (H&E, x1000).
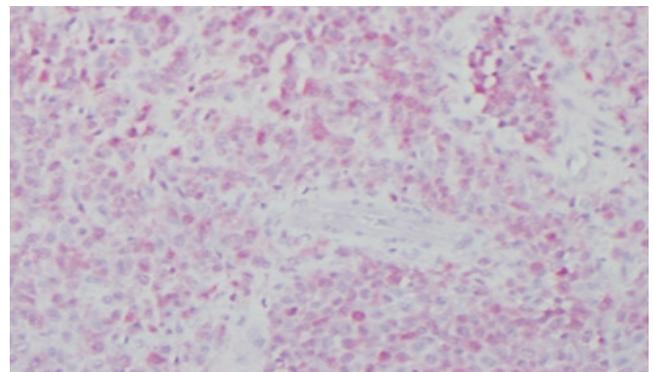
Figure 4a: S100 immunostain shows nuclear and cytoplasmic positivity in Langerhans cells (x200).

Figure 4b: CD1a immunostain is highlighted in Langerhans cells with cytoplasmic and membranous positivity (x100).

Figure 4c: CD68 immunostain is diffusely positive in both Langerhans cells and admixed macrophages (x100).

Figure 4d: BRAF immunostain shows cytoplasmic positivity in Langerhans cells (x200).

Discussion

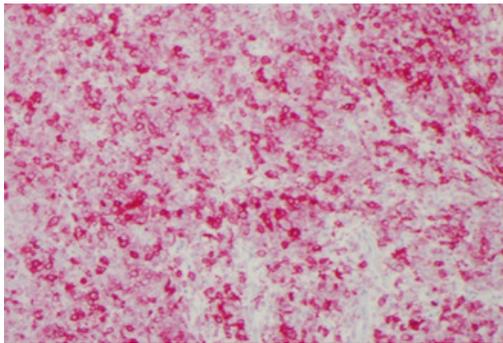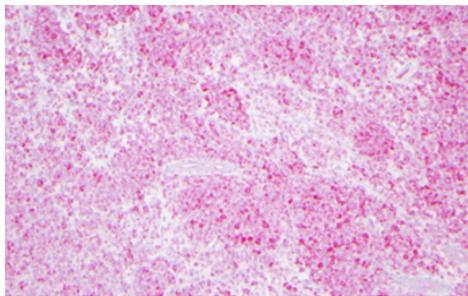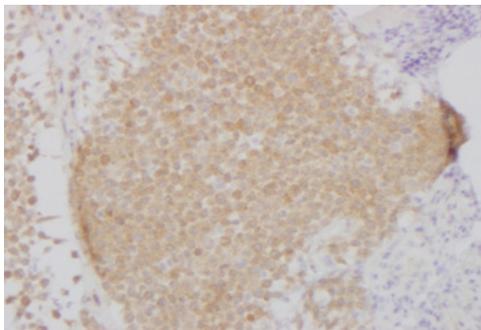LCH is a form of 'functional neoplasia', that is a neoplastic disease that retains functions of a normal cellular counterpart [8]. LCH is caused by activating mutations of the RAS-activated factor/ MAPK ERK kinase/ERK pathway that result in accumulation of Langerhans-like cells with an immature phenotype in ectopic sites [8,9].

These mutations have been documented in all cases of LCH. In two thirds of cases, pathway activation is secondary to somatic mutation in BRAF V600E [9], which appears to be associated with high-risk features, as seen in our case, and poor short-term response to chemotherapy [10]. In other cases, mutations in MAP2K1 or other members of the pathway such as ARAF have been described, which appear to be mutually exclusive with BRAF mutations [9,11]. About one-quarter of cases have no genomic abnormality. According to a study by Galluzzo et al. [7] the presence of LCH cells is rarely seen in the bone marrow of multisystem LCH patients with risk-organ involvement at diagnosis (14%). These findings are concordant with a study by Minkov [6] and colleagues who reported the prevalence of 14.3% (2/14) of CD1a-positive histiocytes by immunocytochemistry in severe multisystem LCH. On the other hand, Mcklain [12] et al. reported a prevalence of 18% bone marrow involvement; defined as clusters of 5-10 histiocytes with characteristic morphologic features of LCH replacing normal hematopoietic elements in a focal or multifocal manner, in patients diagnosed with multisystem Langerhans cell histiocytosis. They also noted that LCH of the marrow was more easily diagnosed from trephine biopsy sections than aspiration smears. In addition, the presence of LCH cells was negatively associated with hemophagocytosis [7]. On the other hand, increased histiocytes and hemophagocytosis are a more common finding (41%) in the bone marrow of multisystem LCH patients, and hemophagocytosis is positively associated with severe cytopenias [7]. LCH diagnosis should always be based on histological and immunophenotypic examination of lesional tissue that should be taken from the most easily accessible, yet representative lesion. The characteristic appearance of the LCH cells, in addition to positive CD1a and/or CD207 (Langerin) staining of the lesional cells is required for a definitive diagnosis [13].

In our case there was no clear evidence of LCH in the bone marrow biopsies after thorough histological and immunophenotypic inspection for characteristic Langerhans cells that would stain positively for CD1a and S100 immunostains. On the other hand, the biopsies showed aggregates of histiocytes and evidence of hematophagocytosis. One plausible explanation for our findings would be that the LCH cells have undergone regression similar to the previously described LCH findings in the liver whereby LCH cells regress after causing sclerosing cholangitis and cirrhosis [14]. However, Onishi [15] and colleagues demonstrated that the expression of M-CSF in Langerhans Cells (LC) significantly correlated with the density of CD163-positive infiltrated macrophages; and because M-CSF also promotes macrophage migration, tumor derived M-CSF may also facilitate

macrophage infiltration into tumor tissue. They also showed that the density of infiltrated macrophages was inversely correlated with the number of Ki-67-positive proliferating LC. Based on that, it is possible that tumor derived M-CSF promotes macrophage migration and infiltration into other non-tumor organs, including the bone marrow in this case, and induces their activation that explains the hemophagocytosis noted in our bone marrow biopsies and the subsequent diagnosis of secondary HLH.

The association between LCH and HLH has been previously investigated by Favara [16] and colleagues. In their article they note that high levels of GM-CSF have been found in the blood of patients with disseminated LCH but not in patients with localized disease, and serum levels of soluble IL-2 receptor have been proposed as an indicator of disease activity in LCH patients. Interferon γ (IFN-γ) and Tumor Necrosis Factor α (TNF-α) are closely associated with enhanced phagocytosis by macrophages in vitro and appear to be elevated in the blood of patients with HLH, but they have not been reported to be elevated in LCH patients (Lay). In their opinion, the presence of multiple operating stimulatory loops in LCH leads to a "cytokine storm" which perpetuates the disorder until the burden of LCH reaches a level sufficient to produce enough cytokines to provoke systemic macrophage activation and subsequent hemophagocytic syndrome. In other words, widespread LCH in multisystem or disseminated disease results in the release of sufficient systemic cytokines to induce macrophage activation, the extent of which is determined by the patient's burden of disease. In our case, the patient had multisystem disease with evidence of macrophage activation manifested by hemophacytosis noted in both of his bone marrow biopsies. There was no clear evidence of LCH in his bone marrow. Initially, the patient didn't meet the clinical criteria of HLH; however, after his second cycle of intensive chemotherapy he developed the disorder. This supports the argument by Jorge Braier [17] who questioned the role of chemotherapy agents in LCH patients and whether they induce cytotoxicity against the clonal infiltration of multisystem LCH in organs, or promote immunosuppression that modulates the cytokine storm; which, in our opinion, might explain the gradual emergence of HLH in our patient.

Conclusion

In conclusion, LCH is a complex neoplastic disorder with an immunologic dysregulatory nature characterized by composite interactions of cellular and chemical/cytokine-related events leading to varying degrees of macrophage activation. LCH can be diagnostically challenging, especially if it is initially presented to the pathologist in a bone marrow biopsy.

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