

WHO has an update on the Histiocytoses? ... Check your *Blood*:

A brief update on the pathogenesis and histopathology of histiocytic neoplasms

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The pathology of histiocytic neoplasms and disorders of the macrophage-dendritic cell lineages is heterogeneous and may have overlapping features. The morphologic features together with immunophenotype and pattern of involvement should always be taken together with the clinical and radiographic findings to make a unifying diagnosis(1). The field has witnessed a molecular enlightenment over the past few years which has helped further unravel the pathogenesis and biologic potential for some of these lesions, namely in Langerhans cell histiocytosis (LCH) and Erdheim-Chester Disease (ECD) ((2-7). Having a myeloid cell of origin along with molecular alterations in the mitogen-activated protein kinase (MAPK) pathway has led to the proposal that LCH and ECD better fit into a group of inflammatory myeloid neoplasms. The histopathologic clues for the diagnosis of LCH, ECD, Juvenile xanthogranuloma (JXG) family of lesions, Rosai-Dorfman Disease (RDD), and Malignant histiocytic lesions, including Histiocytic Sarcoma (HS) and Langerhans Cell Sarcoma (LCS), will each be briefly discussed in the presentation (Table 1) with molecular integration relevant to diagnosis and therapeutic prognostics.

Classifications

The hematopoietic derived cells that compose both the monocyte-macrophage and the dendritic cell families have long been grouped under the colloquial term “histiocytes” with pathologic tissue proliferations of such cells termed “the histiocytoses.” Our understanding of histiocytic disorders has evolved from the first classification published by the Histiocyte Society Working group in 1987 that included disorders of Langerhans cells (LC), non-Langerhans cell related,

and the malignant histiocytoses (MH)(8). A more contemporary classification was laid out in 1997 by the World Health Organization (WHO) Committee on Histiocytic/Reticulum Cell Proliferations and the Histiocyte Society Reclassification Working Group.(9). These classifications were based on biologic behavior and histopathology, including “dendritic cell” related (e.g. LCH, JXG family), “macrophage” related (e.g. “hemophagocytic syndromes”, RDD), and malignant disorders (typically grouped by their most common morphologic/immunophenotypic counterpart, which at that time also included monocyte leukemias). The most recently proposed 2016 WHO classification has now separated ECD from disseminated JXG based on increasing data that this is a distinct disease (10). While the separation and recognition of ECD as a distinct histiocytic disease is an important one, others have proposed that the emerging molecular data would support the theory that LCH and ECD (and possibly also systemic JXG lesions with gain of function mutations) along with indeterminate cell histiocytosis are best classified together as inflammatory myeloid neoplasms of an “L group.” (11) Recent discussion lead by Emile et al (11) on behalf of the Histiocyte Society is now focused on a revised classification scheme in which the molecular signature of these disorders is more strongly emphasized and proposes to lump seemingly disparate groups (ie. LCH and ECD) based on common molecular alterations and overlapping clinical presentations, as outlined in the recent *Blood* 2016 classification (11). While in adults the overlap between LCH and ECD has been reported with some frequency (12, 13) and the shared molecular MAPK alterations between the LCH and non-LCH groups is notable, newer transcriptional data with RNA seq analyses in LCH and non-LCH histiocytoses may still support two separable groups (6), as originally supported by their divergent immunophenotype and distinct clinical presentations.

Pathogenesis

The discovery that LCH has a distinct gene expression profile from epidermal Langerhans cells (LCs) in which the LCH cells more closely overlap with circulating dendritic cells (cDCs) and late-stage myeloid progenitor cells(3) was a paradigm shift in the field, since it was long thought that LCH was derived from LCs by virtue of their shared phenotype and ultrastructure(14). Allen et al, based on in vivo animal models and human studies have shown that LCH is derived from immature myeloid dendritic cells of the bone marrow and that *BRAF-V600E* mutations in precursor versus differentiated dendritic cells define clinically distinct LCH groups(4).. Dr. Carl Allen will expound upon this work in more detail during his presentation, but briefly, activating mutations of the ERK signalling pathway at critical stages in myeloid differentiation appear to be an essential and universal driver of LCH pathology and clinical phenotypes (4, 5). Thus the genetic and molecular data from expression profiling of LCH, together with the timing of ERK activation, help support LCH as an inflammatory myeloid neoplasm (3-5, 15).

In contrast, non-LCH lesions (i.e. systemic JXG, ECD) may share gene expression profiles more similar to monocytes and earlier hematopoietic stem and progenitor cells in preliminary transcriptional data with RNAseq analysis (6, 16). Thus, while sharing similar MAPK pathway mutations, LCH and non-LCH histiocytoses may still support two divergent groups. Recent work presented at the 2016 Histiocyte Society has further elaborated that while *BRAF-V600E* is localized to hematopoietic stem cells (HSC) in multi-system (MS) LCH, ECD, and hairy cell leukemia (HCL), the mutant was only enriched in the myeloid progenitors of MS-LCH and ECD; also, circulating monocytes and myeloid DCs carried the mutant *BRAF* in MS-LCH and ECD, as opposed to B cells and NK lymphocytes in HCL (16). Furthermore, *in vitro* experiments and gene expression studies suggest that LCH arises from DCs and ECD from monocytes(16). Thus while there is still strong support that ECD should also be classified as an inflammatory myeloid neoplasm based on recurrent mutations in the MAPK pathway and shared cases of

ECD/LCH occurring in same patient (7, 11), there is emerging transcriptional profiling data that suggests a distinct cell of origin in these “L group” lesions.

Updates on histologic diagnosis with help from the molecular microscope

The diagnostic challenges in histiocytic lesions are multifaceted and require not only an understanding of varied presentations of these rare diseases but also an understanding that a tissue diagnosis is not always feasible with our current histopathologic tools (e.g. false negative). Furthermore, application of immunostains without recognition of the correct pattern of tissue involvement and clinical/radiographic correlates may lead to an erroneous diagnosis (e.g. false positive).

Specific molecular alterations have better defined certain histiocytic subgroups including indeterminate cell histiocytosis with an unique *ETV-NCOA2* fusion (17), and systemic JXG-like *ALK*-positive histiocytosis seen in early infancy (18). Furthermore, demonstration of *BRAF*-V600E mutations in the bone marrow, liver, or brain biopsies of LCH patients where it is often difficult to demonstrate distinct CD1a/Langerin positive cells, within a macrophage rich inflammatory background, has helped direct a diagnosis towards LCH or at least an “L group” lesion in some of these difficult cases within the correct clinicoradiographic context (4, 19, 20).

The same could be said for ECD which is not a pathologic diagnosis per se but rather requires strict integration of histopathology, clinical, and radiographic findings. The mere presence of CD68+ xanthomatous cells is not enough for a confident diagnosis of ECD and the diagnosis may also be missed as a non-specific inflammatory or fibrosing process if integration of clinicoradiographic finding is not stringent. The epithelioid histiocytes should display a JXG-like immunophenotype within the correct clinicoradiographic setting. In both LCH and ECD, sensitive methods of *BRAF* mutant detection can further aid in the diagnosis, which includes clinically validated VE1 immunohistochemistry (21-23) and highly sensitive molecular assays (i.e.

quantitative real-time PCR (qPCR) or allele specific PCR/amplification refractory mutation system (ARMS)) with a limit of detection down to 1% or less for mutated alleles for accurate *BRAF*-V600E status (4, 24-26).

Furthermore, it is still important to recognize that despite our molecular advances, the diagnosis of many histiocytic neoplasms rests on congruent integration of the pathologic features of cell morphology, immunophenotype, and correct pattern of involvement. In LCH a false positive diagnosis can be made in cases of chronic inflammatory dermatoses, including chronic scabies and pseudo-lymphomatous folliculitis in which application of immunohistochemistry will reveal increased numbers of spindled perivascular dendritic cells (high S100+, CD1a+, low Langerin+) within a mostly superficial perivascular/perifollicular mononuclear inflammatory infiltrate. This pattern should not be diagnosed as LCH, which is a sheet like infiltration of the dermis with epidermotropism, and may ulcerate the surface (1). Reactive lymphadenopathies with a dermatopathic lymphadenopathy pattern will also reveal an expanded population of CD1a+/Langerin+ spindled/dendritic cells, in addition to a rich population of S100+/fascin+ interdigitating dendritic cells within the pale staining interfollicular nodular areas. This pattern should also not be diagnosed as LCH which is a disease primarily of the sinuses (1) Of note, in normal lymph nodes a sinus pattern of Langerin staining has been appreciated, typically staining a fixed tissue component rather than circulating cells and should not be used as a sole criterion of nodal LCH disease(27). Similarly a false positive ECD diagnosis should be avoided with foamy, xanthomatous CD68+ histiocytes only, which is a finding in a number of conditions including xanthogranulomatous inflammation (e.g. pyelonephritis, ruptured appendix) or dyslipidemia-associated xanthelasmas.

Impact of molecular findings on prognosis and treatment

For the first time, the French Histiocyte group lead in part by J. Donadieu and colleagues have shown in their cohort of 315 French LCH patients that the *BRAF-V600E* mutation in tissue LCH appears to be a prognostic marker associated with high-risk LCH, increased resistance to first-line chemotherapy, a higher reactivation rate, and more permanent, long-term consequences from disease or treatment (28). Others have shown that *BRAF-V600E* mutation correlated with increased risk of recurrence but that it did not specifically define clinical risk groups based on lesional BRAF status alone (4). However, Berres et al was the first to show that patients with *BRAF-V600E* high-risk LCH (e.g. involvement of the spleen, liver, and bone marrow) also had circulating peripheral blood CD11c+ and CD14+ fractions and bone marrow (BM) CD34+ hematopoietic cell progenitors with mutant *BRAF* during active disease, as opposed to active low-risk, single system (SS) LCH patients, which harbored the mutation only in lesional tissue (4). Others have found similar results with circulating *BRAF-V600E* mutant myeloid and monocytic cells (16) or *BRAF-V600E* allele detection in cell free DNA of the blood in active MS-LCH as opposed to single system LCH(29, 30). While still at the nascent, “experimental” stages, a highly sensitive, clinically-validated assay to quantitate *BRAF-V600E* mutations in blood and bone marrow samples is available that may one day better monitor active disease in MS-LCH patients(31) Similarly ECD patients may be monitored with detection of circulating BRAF-V600E mutation in blood (e.g. peripheral circulating cells, cell free DNA), bone marrow and/or urine (16, 26, 32, 33).

Targeted therapy with MEK/BRAF inhibitors has been under active investigation with case reports highlighting improved outcomes in refractory disease. Larger studies, primarily out of Europe are beginning to show promising results with vemurafenib (BRAF inhibitor) in pediatric LCH and vemurafenib and cobimetinib (MEK inhibitor) in the adult ECD population; however, long term treatment duration is still an open discussion as many patients experience relapse shortly after inhibitor interruption.

Conclusions

The pathology of histiocytoses and neoplasms of the macrophage-dendritic cell lineages can be a minefield at times, given their varied and sometimes overlapping features with one another and other primary inflammatory conditions. The sustained progress that has been made in the field will continue as we further explore the diverse histopathology, now with a strong emphasis on the molecular underpinnings that drive these disorders in order to better describe, classify, and ultimately treat these rare disorders/neoplasms. The latest attempt to revise the classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages has proposed grouping this diverse group of over 100 clinical entities into five main groups based on clinical, histologic, and molecular relevance (11). The clinical/pathologic relevance of this revised classification will ultimately determine if this new grouping is further adopted into practice as a framework for further study. Through dedicated international collaboration of pathologists, clinicians, and scientists, it is the hope that we can continue to make sustained progress in the pathogenesis and best-practice treatments regimens for these rare diseases.

Table 1. Clues to diagnosis for the most common histiocytic neoplasm and disorders

Diagnosis	Immunostain panel	Pearls and Pitfalls to be aware
Langerhans cells histiocytosis	CD1a (membranous) CD207/Langerin (cytoplasmic) S100 (nuclear & cytoplasmic)	CD207 replaced need for EM and more sensitive than CD1a; Correct pattern of involvement for given site is needed
Indeterminate cell histiocytosis	CD1a (membranous) Negative for CD207 S100 (nuclear & cytoplasmic).	<i>ETV-NCOA2</i> fusion
Erdheim-Chester Disease	CD163 (surface to cytoplasmic) CD14 (surface) CD68 (granular cytoplasmic) Factor XIIIa (cytoplasmic) Fascin (cytoplasmic) S100 (typically negative)	The morphology and phenotype of “juvenile xanthogranuloma family” should be correlated with clinical and radiographic findings for ECD diagnosis. Factor XIIIa can be lost in heavily xanthomatous cells
Juvenile xanthogranuloma family of lesions	Similar to ECD above	While cutaneous lesions with typical morphologic patterns do not require extensive immunophenotyping, deep and visceral lesions without classic morphology can be aided by IHC
Rosai Dorfman Disease	S100 and fascin positive. CD1a and CD207 negative	Large pale histiocytes with a hypochromatic nucleus expanding the sinuses is diagnostic. Emperipolesis is variable. Exclusion of metastatic malignant melanoma in an adult, and LCH in a child should be made with a S100+ lesion in lymph node
Histiocytic sarcoma	CD163, CD14, CD4 and CD11c. Lysozyme (Golgi dot), CD45 and HLA-DR positive S100 (+ in dendritic type), CD56 (rare), and variable JXG phenotype, Ki-67 proliferation rate >10%.	CD163 in a surface and or cytoplasmic pattern has high specificity, more so than CD68 (present in a variety of cell types). Cytologic pleomorphism, increased mitoses, including atypical forms.
Langerhans cell sarcoma	CD1a (membranous) CD207/Langerin (cytoplasmic) S100 (nuclear and cytoplasmic), Ki-67 proliferation rate >30%.	Cytologic pleomorphism, increased mitoses, including atypical forms.

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