Marrow Assessment for Hemophagocytic Lymphohistiocytosis Demonstrates Poor Correlation with Disease Probability

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ABSTRACT

The current guidelines for diagnosing non-familial hemophagocytic lymphohistiocytosis (HLH) requires meeting 5 of 8 criteria, including evidence of hemophagocytosis, a non-specific finding. We performed a retrospective study of 64 bone marrow core biopsies and aspirates from patients with clinical suspicion for secondary HLH, or with incidental findings of hemophagocytosis, and evaluated for amount of hemophagocytic histiocytes on aspirates and on biopsies with aid of CD68 immunostaining. Separate review of medical records assigned patients to low (18 cases) or high (46 cases) HLH probability, and the association with histologic findings were examined using exact test.

Neither the quantification of hemophagocytic histiocytes on aspirate nor the quantification aided by CD68 staining on core biopsy correlated with disease probability (p=0.11, p=0.25, respectively). Of the clinical/laboratory criteria assessed, variables with the most significant correlations with HLH were ferritin level ≥10,000 ug/L (p = 0.02), cytopenias (p = 0.002) and fever (p = 0.002). Furthermore, with every 1,000 ug/L increase in ferritin level, the likelihood of HLH increases by 9% (OR: 1.09, 95% CI: 1.01-1.17). Our findings indicate that marrow histologic findings alone do not predict probability of HLH, and isolated finding of hemophagocytosis appeared to be of little value in diagnosing HLH due to lack of specificity.

BACKGROUND

Hemophagocytic lymphohistiocytosis (HLH) is an often fatal syndrome of exaggerated but ineffective inflammatory responses, characterized by excessive macrophage and T cell activation, and impairment of the ability of NK and cytotoxic T cell to kill target cells[1,2,3,4]. Non-familial form of HLH is associated with infections, medications, autoimmune conditions, and malignancies[1,2,3,5].

According to HLH-2004, the updated diagnostic and therapeutic guidelines for HLH, non-familial HLH can be diagnosed by meeting 5 of 8 clinical and laboratory criteria, including: fever, splenomegaly, peripheral cytopenias affecting 2 cell lineages, hypertriglyceridemia or hypofibrinogenemia, microscopic evidence of hemophagocytosis, low/absent NK cell activity, elevated ferritin (≥500 ug/L), and elevated sCD25 (sIL-2 Receptor)[6]. In suspected HLH patients, bone marrow biopsies were often performed to check for evidence of hemophagocytosis. However, hemophagocytosis is a non-specific finding and can be seen in other conditions such as after blood transfusion, chemotherapy administration, sepsis and major surgeries[7,8,9,10], although the expected amount of hemophagocytic cells seen in these conditions has not been well-defined. There is so far no accepted interpretative threshold for positive findings, nor standardized reporting guidelines when such findings were present.

Very few studies to date have systematically looked at the amount of hemophagocytosis in the marrow and its correlation with eventual diagnosis of HLH, especially in the adult population[11]. A pediatric study found bone marrow hemophagocytosis in only 58% of patients with HLH[12], but there was no control group to assess for specificity. A small case-control study with six HLH cases from three patients found higher quantitation of marrow hemophagocytosis in HLH patients, with a sensitivity of 83% and a specificity of 60% in diagnosing HLH[7]. However, the control subjects were randomly selected and did not necessarily have clinical suspicion for HLH.

AIMS

- Assess correlation of the amount of hemophagocytosis in marrow aspirates and CD68+ hemophagocytic histiocytes in marrow core biopsies with the probability of a HLH diagnosis
- Examine the predictive value of other HLH-2004 criteria in diagnosing HLH

RESULTS

Bone Marrow Aspirate and Core Biopsy Evaluation:

- Total of 64 cases from 58 unique patients have bone marrow aspirates and/or core biopsies, and medical records available for review.
- No significant difference in amount of hemophagocytosis in marrow aspirates (p=0.11, Table 1) nor % of CD68+ hemophagocytic histiocytes in core biopsies (p=0.25, data not shown) between low and high HLH probability groups.
- Between two pathologists, kappa agreement for amount of hemophagocytic cells in the aspirates was 0.52 for simple kappa, and 0.67 for weighted kappa (Data from one pathologist shown).

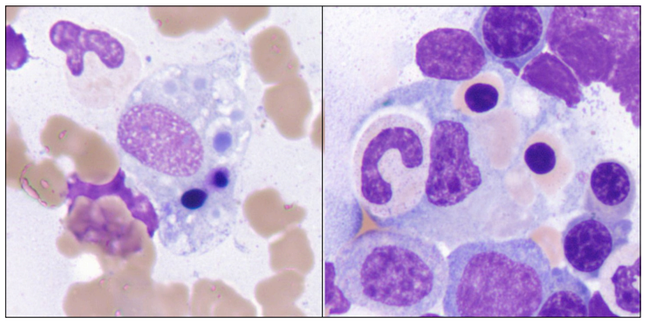


Figure 1: Hemophagocytic cells in bone marrow aspirates of selected cases.

	High Probability HLH	Low Probability HLH	p value
Number of Cases	18 (28.1%)	46 (71.9%)	
Median Age	48.5	51.5	**0.64**
Gender			**0.002**
Female	13 (72.2%)	14 (30.4%)	
Male	5 (27.8%)	32 (69.6%)	
Asp Cellularity			**0.01**
Decreased	10 (55.6%)	10 (21.7%)	
Adequate	6 (33.3%)	34 (73.9%)	
Increased	2 (11.1%)	2 (4.4%)	
Asp Hemophagocytosis			**0.11**
0 cell/slide	5 (27.8%)	4 (8.7%)	
1-5 cells/slide	10 (55.6%)	23 (50.0%)	
6-10 cells/slide	2 (11.1%)	9 (19.6%)	
>10 cells/slide	1 (5.6%)	10 (21.7%)	

Table 1: Correlation of marrow aspirate cellularity and number of hemophagocytic cells with the probability of a HLH diagnosis.

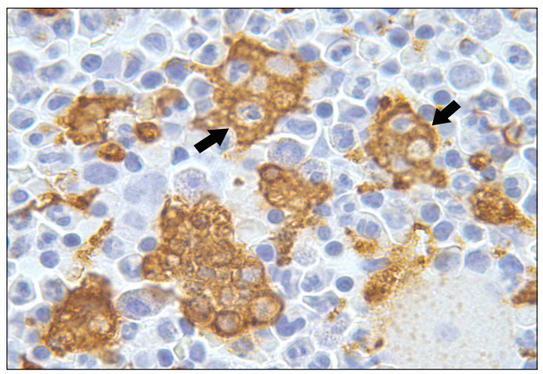
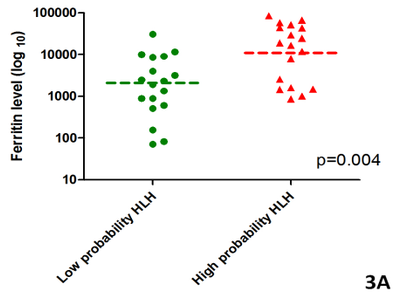


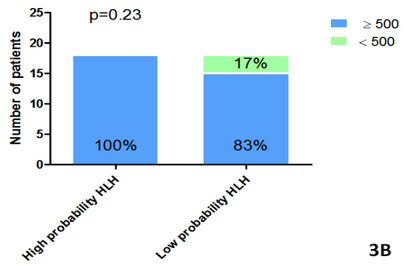
Figure 2: CD68 immunohistochemical stain highlights hemophagocytic histiocytes (arrows) in bone marrow biopsies of a selected case.

Clinical/Laboratory Criteria:

- Variables with significant correlation with HLH included high ferritin level (p=0.004), fever (p=0.002) and peripheral cytopenias (p=0.002).
- Ferritin level ≥500 ug/L has high sensitivity but low specificity for HLH, p=0.23 (Figure 3B).
- Ferritin level ≥10,000 ug/L is more specific for HLH, p=0.02 (Figure 3C), similar to findings in a previous study[13] .
- Using logistic regression analysis with continuous ferritin level, with every 1,000 ug/L increase in ferritin level, the likelihood of HLH increases by 9% (OR: 1.09, 95% CI: 1.01-1.17).

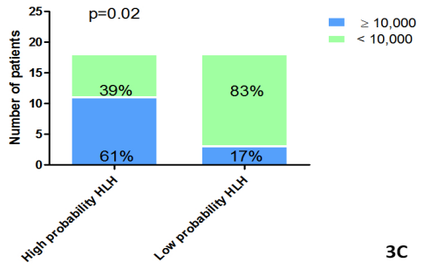


3A



3B

Ferritin Level (ug/L)	High Probability HLH	Low Probability HLH	Total
≥500	18	15	33
<500	0	3	3
Total	18	18	36



3C

Ferritin Level (ug/L)	High Probability HLH	Low Probability HLH	Total
≥10,000	11	3	14
<10,000	7	15	22
Total	18	18	36

Figure 3: Comparison between the high and low probability HLH groups, by absolute ferritin levels (A), by using a threshold of ≥500 ug/L (B) and a threshold of ≥10,000 ug/L ©.

MATERIALS & METHODS

Subjects: Study approval was obtained from the Yale University Human Investigation Committee. A natural language search was conducted on bone marrow aspirates and biopsies specimens in the surgical pathology database of the Yale-New Haven Hospital (YNHH), New Haven, CT, between January 1980 and May 2012. Cases with clinical suspicion for HLH, and cases with incidental marrow finding of hemophagocytosis were identified. Adult patients age 18 and over were included in the study.

Marrow Aspirate and Core Biopsy Review: All cases were reviewed blindly by two pathologists without knowledge of the original diagnoses. Number of hemophagocytic cells in aspirates were counted over the entire Wright-Giemsa stained slide, averaged over two slides. Percentage of nucleated marrow cells undergoing hemophagocytosis in core biopsies were estimated with the aid of CD68 immunohistochemical stains (clone PG-M1; DAKO)

Medical Record Review: Separately, the patients' records were reviewed by two clinical hematologists. Taking into consideration the clinical presentations, physical exams, laboratory findings, assessments of treating clinicians, and data for each of the HLH-2004 diagnostic criteria, each case was assigned to either the low or high probability HLH group after case discussion.

Statistical Analysis: Descriptive statistics such as frequency, percentage, and median were used to characterize the cases. Fisher's exact test or Wilcoxon rank sum test was used to compare high probability and low probability HLH groups as appropriate. Kappa coefficient was used to examine the agreement between two pathologists. Logistic regression analysis was performed for cases with complete ferritin data to identify the association between HLH and ferritin level. All the analyses were performed using software SAS 9.2 (Cary, NC). Statistical significance level was set as 0.05, two-sided.

CONCLUSIONS

- Neither amount of hemophagocytosis in marrow aspirate nor core biopsy correlate well with probability of HLH diagnoses.
- Bone marrow evidence of hemophagocytosis as a rule-in criteria for HLH deserves reconsideration.
- The value of a bone marrow biopsy in suspected cases of HLH may still be high given the importance of excluding other disease processes.
- Given the non-specificity of marrow hemophagocytosis, even when present in high amount, isolated finding of hemophagocytosis does not necessarily suggest HLH when the clinical presentation and lab findings are incompatible with the diagnosis.

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