Original Article

Spectrum of Hemophagocytosis in Bone Marrow Aspirates: Experience from a Tertiary Care Hospital in North India

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Abstract

Background: Hemophagocytosis refers to the engulfment of hematopoietic cells by histiocytes. It can be seen in various conditions but is usually reported in the setting of hemophagocytic lymphohistiocytosis (HLH). Optimal interpretation of hemophagocytosis in the bone marrow in relation to the underlying disease significantly contributes to correct patient management. **Aim:** The present study was done to identify the spectrum of conditions associated with hemophagocytosis in the bone marrow aspirates and grade the degree of hemophagocytosis. **Material and Methods:** This retrospective observational study included all the bone marrow aspirates showing hemophagocytosis, identified over a period of 5 years (January 2015 to January 2020). Two pathologists independently reviewed bone marrow slides. Hemophagocytosis was graded as mild, moderate, or severe by observing the number of histiocytes showing hemophagocytosis per 500 nucleated cells. **Results:** Eighty-eight patients showing hemophagocytosis in the bone marrow aspirate smear were included in the study. The most common cause of hemophagocytosis was infection (18%). There were 4 (5%) cases of HLH. Grade 1 (mild) hemophagocytosis was seen in 25 (29%) cases followed by Grade 2 (moderate) in 53 (60%) cases and Grade 3 (severe) in 10 (11%) cases. Fever was the most common clinical symptom present in 45 (51%) cases. **Conclusion:** Hemophagocytosis in bone marrow aspirates is a common and under-reported finding. It is not only seen in cases of HLH but also in infections and other conditions. Documenting hemophagocytosis, even in the absence of fulfilled HLH criteria, is vital to explain cytopenias.

Keywords: *Bone marrow hemophagocytosis, grading of hemophagocytosis, hemophagocytic lymphohistiocytosis*

Introduction

Hemophagocytosis refers to the engulfment of hematopoietic cells by histiocytes in the bone marrow, lymph node, spleen, or liver. It can be seen in a variety of conditions but is under-reported unless the diagnosis fulfills the criteria for hemophagocytic lymphohistiocytosis (HLH). Although hemophagocytosis is the only histomorphological evidence of HLH in the marrow, it is neither specific nor sensitive for HLH diagnosis. The presence of hemophagocytosis in the bone marrow only supports the diagnosis of HLH.[1,2] Some patients who do not fulfill the criteria for HLH may still show the presence of hemophagocytosis in the bone marrow. These conditions include infections, lymphoma, leukemia, myeloma, aplastic anemia, and nutritional anemias.[3,4] HLH is a life-threatening condition; hence, the treatment for HLH should be started in clinically suspected patients even if only some criteria for its diagnosis are present.[4] The present study identifies the spectrum of conditions associated with hemophagocytosis in bone marrow aspirates and grades the extent of hemophagocytosis.

Materials and Methods

This retrospective observational study was conducted in the Department of Pathology in a tertiary care hospital in North India. Of all the bone marrow aspirates performed over a period of 5 years, those showing hemophagocytosis were included in the study group. The relevant clinical information which included presenting symptoms and organomegaly was obtained from the medical records. Bone marrows with insufficient quantity or dry tap were excluded from the study. The study was approved by the institutional research and ethics committee. Consent for bone marrow aspiration/biopsy was taken prior to the procedure.

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May Grunwald-Giemsa-stained bone marrow aspirate slides (crush and wedge smears) of enrolled patients were retrieved from the records and reviewed independently by two experienced pathologists and findings were noted. To grade hemophagocytosis, 500 consecutive nucleated cells were counted independently by each pathologist and the number of histiocytes showing hemophagocytosis was noted. The overall grade was calculated by averaging 500 consecutive nucleated cell counts done by each observer. For the diagnosis of HLH, the Revised HLH 2004 diagnostic criteria were used as shown in Table 1.[5]

Hemophagocytosis was defined as the engulfment of erythrocytes, lymphocytes, or other hematopoietic precursors by histiocytes in the bone marrow.[4] Hemophagocytosis in the marrow aspirates was graded as Grade 0: absent, Grade 1 (mild) (<2 histiocytes with hemophagocytosis/500 nucleated cells), Grade 2 (moderate) (2–5 histiocytes with hemophagocytosis/500 nucleated cells), Grade 3 (severe) (>5 histiocytes with hemophagocytosis/500 nucleated cells).[4,6]

Trephine biopsies were also studied. Three out of eighty-eight patients (3.4%) showed hemophagocytosis in trephine biopsies.

The associated bone marrow findings such as cellularity and erythropoiesis, granulopoiesis, and megakaryopoiesis were noted.

Statistical analysis

Descriptive analysis was done with the measures of variance including mean, median, and standard deviation for continuous variables. Nominal variables were expressed as a percentage. Kruskal–Wallis test was used for the comparison of nonparametric data. The Chi-square test was used to compare the categorical variables. Box whisker plots were generated using Python matplotlib library ver 3.7.1 (Van Rossum G, Drake FL. Python3 Reference Manual. Scotts Valley CA:CreateSpace;2009).

Results

Eighty-eight patients showing hemophagocytosis in the bone marrow aspirate were included in the study. There were 54 males (61%) and 34 females (39%) with a male: female ratio of 1.6:1. The age ranged from 1 year to 88 years, with a mean age of 44.5 ± 20.8 years. The most common clinical feature was fever followed by hepatomegaly. The other clinical features are shown in Table 2. Various cytopenias in peripheral blood are shown in Table 3. Bone marrow was hypercellular in 51 cases (58%), normocellular in 20 cases (23%), and hypocellular in 13 cases (15%) cases.

The most common cause of hemophagocytosis was infection in 16 cases (18%) followed by acute leukemia in 10 cases (11%), as shown in Table 4. However, hematological malignancies taken together (including acute leukemia, myelodysplastic syndrome (MDS), non-Hodgkin lymphoma (NHL), myeloproliferative neoplasm, plasma cell dyscrasia, and beta-thalassemia) constituted 36% of cases of hemophagocytosis (32/88).

The 10 cases of acute leukemia in our study included 7 acute myeloid leukemia (AML) and 3 acute lymphoblastic leukemia. There were only 4 patients (5%) who fulfilled the clinical and laboratory criteria for HLH [Table 5]. Two of the HLH patients had Grade 3 hemophagocytosis while the other two showed Grade 2 hemophagocytosis.

MDS cases included 4 cases of MDS with multilineage dysplasia, 2 cases of MDS with excess blasts

Table 1: Revised hemophagocytic lymphohistiocytosis - 2004 diagnostic criteria[5]

The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled
1. A molecular diagnosis consistent with HLH is made
2. Diagnostic criteria for HLH are fulfilled (5 of the 8 criteria below)
Initial diagnostic criteria (to be evaluated in all patients with HLH)
Fever
Splenomegaly
Cytopenias (affecting >2–3 lineages in the peripheral blood)
Hb <90 g/L (in infants <4 weeks of age Hb <100 g/L
Platelets <100×10^9/L
Neutrophils <1.0×10^9/L
Hypertriglyceridemia and/or hypofibrinogenemia
Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dL)
Fibrinogen ≤1.5 g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
New diagnostic criteria
Low or absent NK-cell activity (according to local laboratory reference)
Ferritin ≥500 mg/L
Soluble CD25 (i.e., soluble IL-2 receptor) ≥2400 U/mL

HLH: Hemophagocytic lymphohistiocytosis, Hb: Hemoglobin

Table 2: Clinical features in patients with hemophagocytosis in bone marrow

Clinical features	Number of cases (%)
Fever	45 (51)
Hepatomegaly	32 (36)
Splenomegaly	29 (33)
Lymphadenopathy	12 (14)
Bleeding	7 (8)

Table 3: Peripheral blood cytopenias among various cases (*n*=88)

Cytopenias	Number of cases (%)
Unicytopenia	34 (39)
Bicytopenia	21 (24)
Pancytopenia	27 (31)
No cytopenia	6 (7)

2(MDS-EB-2), and 1 case each of MDS with unilineage dysplasia, MDS with excess blasts 1(MDS-EB-1) and MDS with monosomy 7.

Grade 1 hemophagocytosis was seen in 25 (29%) cases followed by Grade 2 in 53 (60%) cases and Grade 3 in 10 (11%) cases.

In our study, the association was explored between cytopenias and hemophagocytosis for all patients. No association was observed between hemophagocytosis grades and anemia (hemoglobin <10 g %; $P = 0.165$), nor between hemophagocytosis grades and leucopenia (cases with white blood cell (WBC) <4000 cu. mm) ($P = 0.168$).

Table 4: Clinical conditions showing hemophagocytosis in bone marrow (*n*=88)

Conditions	Number of cases (%)
Infection[#]	16 (18)
Acute leukemia	10 (11)
Myelodysplastic syndrome	9 (10)
NHL	6 (7)
Nutritional anemia	5 (6)
HLH	4 (5)
Aplastic anemia	4 (5)
Myeloproliferative neoplasm	4 (5)
Inflammation	4 (5)
Immune thrombocytopenia	3 (3)
Plasma cell dyscrasia	3 (3)
Beta-thalassemia	3 (3)
Hypersplenism	2 (2)
Others*	15 (17)

*2 cases of thrombocytopenia under evaluation and pancytopenia under evaluation, nonhematological malignancies and abdominal mass under evaluation each, 1 case each of anemia under evaluation, connective tissue disorder, Chediak-Higashi syndrome, liver abscess, amyloidosis, HL and uremic encephalitis, [#]11 bacterial, 2 protozoal, 1 fungal, 1 viral and 1 rickettsial infection. HL: Hodgkin's lymphoma, NHL: Non-HL, HLH: Hemophagocytic lymphohistiocytosis

A significant association was seen between the grade of hemophagocytosis and thrombocytopenia (platelet count <100,000 cu. mm; $P = 0.03$) with maximum cases of thrombocytopenia in grade 3 [Table 6].

Platelet counts showed statistically significant variation among the three grades of hemophagocytosis ($P = 0.01$). No statistically significant difference in the distribution of hemoglobin and WBC was seen among the three grades of hemophagocytosis [Table 7 and Figure 1].

Discussion

Hemophagocytosis is seen in the bone marrow in various conditions but is often overlooked unless the diagnosis meets the criteria for HLH. As hemophagocytosis can lead to severe cytopenia, its early recognition in bone marrow can be a guide for prompt workup of its cause and early initiation of treatment.[7] Our study provided important insights into the spectrum of hemophagocytosis in the marrow, which has implications for both the diagnosis and treatment of this condition. Our findings indicate that the severity of hemophagocytosis can vary widely, from scattered macrophages which contain engulfed blood cells to extensive hemophagocytosis with many macrophages containing engulfed cells [Figure 2]. This finding should be interpreted in the proper clinical setting.

HLH is a hyperinflammatory disease that is characterized by an abnormal ineffective immune response caused by an uncontrolled activation and proliferation of macrophages, natural-killer cells, and T-helper lymphocytes which lead to excessive production of cytokines. This may lead to amplification of pathologic immune stimulation, increased phagocytic activity, and tissue damage.[8]

HLH can be familial or acquired. Familial HLH is associated with mutations in the genes which are involved in the secretory lysosomes-dependent exocytosis pathway. Some genes which can get mutated

Table 5: Clinical features and investigations of patients showing hemophagocytic lymphohistiocytosis

Patient	Age/sex	Fever	Cytopenia	Splenomegaly	Hyper triglyceridemia (>265 mg/dL)	Ferritin (>500 mg/L)	Hypofibrinogenemia (<1.5 g/L)	Grade of hemophagocytosis in bone marrow
1	18/male	P	P	A	213	15,100	Not done	3
2	12/female	P	P	A	244	2000	0.6	2
3	13/female	A	P	P	85	6530	0.7	3
4	27/female	A	P	A	440	157,360	5.8	2

Table 6: Association of hemphagocytosis grades with various cytopenias

Grade	Grade 1 (*n*=25), *n* (%)	Grade 2 (*n*=53), *n* (%)	Grade 3 (*n*=10), *n* (%)	*P*
Anemia (Hb <10 g %)	14 (56)	40 (76)	8 (80)	0.165
Leucopenia (WBC <4000 cumm)	9 (36)	21 (40)	7 (70)	0.168
Thrombocytopenia (platelet count <1 lac)	11 (44)	34 (64)	9 (90)	0.033

Hb: Hemoglobin, WBC: White blood cell

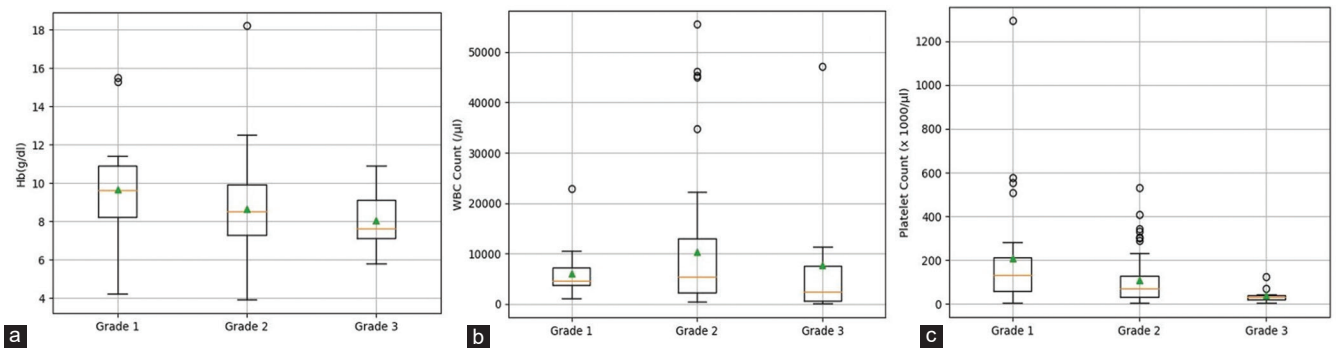

Figure 1: Box Whisker plot showing distribution of (a) hemoglobin with grade of hemophagocytosis. (b) White blood cell count with grade of hemophagocytosios. (c) Platelet count with grade of hemophagocytosios

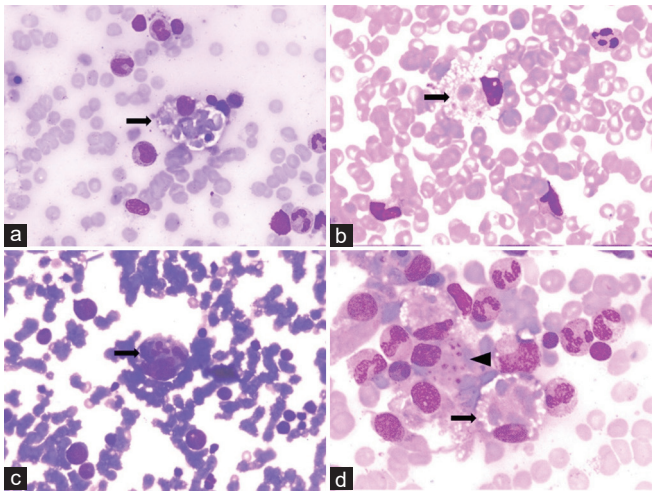

Figure 2: Bone marrow aspirate, May – Grunwald Giemsa (MGG) Stain, ×400. (a) Arrow shows macrophage engulfing red blood cells (RBCs), (b) Arrow shows macrophage engulfing RBCs and platelets. (c) Arrow shows macrophage engulfing neutrophil. (d) Bone marrow aspirate, MGG Stain, ×1000. Arrow shows macrophage engulfing RBCs, arrowhead shows macrophage engulfing RBCs and platelets

Table 7: Association of hemphagocytosis grades with hemoglobin, white blood cell, and platelet count

	Median (range)		
	Hb (g/dl)	**WBC (/ul)**	**Platelet count ($\times 10^3$/ul)**
Grade 1	9.6 (4.2–15.5)	4600 (110–22,900)	131 (5–1293)
Grade 2	8.5 (3.9–18.2)	5400 (500–55,500)	69 (4–531)
Grade 3	7.6 (5.8–10.9)	2450 (90–47,200)	32 (6–124)
P	0.053	0.20	0.01

Hb: Hemoglobin, WBC: White blood cell

in Familial forms of HLH are PRF1, UNC13D, STX11, and STXBP2.[9] Acquired HLH can be associated with hematological malignancies like lymphomas, myeloma, and leukemias, or non-hematological malignancies like prostate, lung, and hepatocellular carcinoma. Nonmalignant causes of acquired HLH include infections (viral, fungal, bacterial, and parasitic) and autoimmune diseases.[10,11] The patient presents with fever, cytopenias, and hepatosplenomegaly.[9]

HLH diagnosis by HLH-2004 Criteria requires 5 out of 8 clinicopathological criteria to be fulfilled, one of which is hemophagocytosis. Table 1[5,12] sometimes, the patient may not meet 5 out of 8 criteria of HLH but hemophagocytosis in bone marrow may be present. The presence of hemophagocytosis in bone marrow should always be documented as its existence in a particular clinical setting can help in initiation of proper treatment.[13] It is also suggested that as HLH is a life-threatening condition, even if 5 out of 8 HLH criteria are not fulfilled, treatment for HLH should be initiated.[4] This further emphasizes documenting the presence of hemophagocytosis in the bone marrow.

In our study, fever was the most common presenting symptom (51%) in most of the patients which was also observed by Chandra *et al*.[4] It was followed by hepatomegaly (36%) and splenomegaly (34%). Most of the patients had unicytopenia 31% (34/88) in the present study followed by pancytopenia in 31% (27/88). Chandra *et al*. observed pancytopenia in 15% of cases.[4]

In our study, infections were the most common cause of hemophagocytosis in bone marrow, seen in 18% of cases. A similar observation was reported by Chandra *et al*. who found infections in 12.5% of cases.[4] The presence of hemophagocytosis in bone marrow can be an indicator of infection in the appropriate clinical setting and it should guide the clinician to investigate the patient for source of infection based on clinical findings. It is also suggested that patients with HLH should undergo investigations to look for underlying infectious etiology.[14] In our study most patients had bacterial infections followed by protozoal, fungal, viral, and rickettsial infection. Rivière *et al*. found bacterial infection as the most common cause of HLH among various infections.[15]

After Infections, the second most common cause of hemophagocytosis in the present study was acute leukemia followed by MDS. However, hematological malignancies taken together constituted the maximum cases showing hemophagocytosis. Previous studies

have shown that patients of acute leukemia undergoing chemotherapy can have clinical features of HLH but may or may not fulfill the criteria for HLH. They can have HLH without hemophagocytosis in the marrow and vice versa.[16]

Hemophagocytosis has been observed in MDS patients by Sun *et al*.[17] In our study, nine cases of MDS showed hemophagocytosis in the bone marrow. MDS can be associated with HLH rarely. Hence, the presence of hemophagocytosis in bone marrow should be documented and it should be interpreted in terms of clinical setting.[18]

The present study also showed hemophagocytosis in the bone marrow of 6 patients of NHL. Rivière *et al*. found that among hematological malignancies, NHL was the most common cause of hemophagocytosis in the bone marrow.[15]

Hemophagocytosis in bone marrow was also observed in 5 patients with nutritional anemia. A similar finding was also observed by Chandra *et al*.[4] There may not be a direct link between hemophagocytosis and nutritional anemia and this may just be an incidental finding.

There were four cases that were treated as HLH based on the clinical findings and laboratory investigations. These four patients fulfilled 5 out of 8 criteria, and hence, they were diagnosed with HLH. All of them had secondary HLH. Two were secondary to AML while the other two were secondary to infection. Oliveira *et al*. in their study reported 4 cases of HLH, all of which were secondary to infection.[9]

Most of the bone marrows of the patients had grade 2 (60%) hemophagocytosis which was also observed by Chander *et al*. and Iqbal *et al*.[4,19] It was followed by grade 1 (28%) and grade 3 (11%).

We observed 60% (6/10) of patients with grade 3 hemophagocytosis had pancytopenia. We also noted that as the grade of hemophagocytosis increased, the mean hemoglobin level decreased however it was not statistically significant [Table 7]. Iqbal *et al*. in their study observed a decline in hemoglobin levels was much higher in grades 2 and 3 than in grades 1. Contrary to the findings by Iqbal *et al*. who observed that higher-grade patients had lower WBC counts, no association was seen in our study [Table 7].[19] We noted that as the grade of hemophagocytosis increased, platelet count decreased which was in concordance with the finding by Iqbal *et al*.[19]

Conclusion

Hemophagocytosis is seen in bone marrow in various conditions, even though they may not fulfill the criteria for HLH. However, documenting this in the report can provide the clinician with a valid reason for cytopenia and prewarn the severity of the disease process.

Ethical clearance

The study was approved by the institutional Ethics Committee of Christian Medical College and Hospital, Ludhiana(approval no. - Ref. CMC/2858).

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Conflicts of interest

There are no conflicts of interest.

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