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Original research article

Identification and classification of hemophagocytic lymphohistiocytosis: clinical, laboratory, and prognostic insights from bone marrow aspiration analysis

[1]Swetha Kesarapu, [2]Pramod Kumar Pamu, [3]Dr. Tara Roshni Paul, [4]Dr. Sree Bhushan Raju, [5]YSN Raju, [6]Dr. Sadashivuudu Gundanti

[1]Assistant Professor, Department of Pathology, Prathima Institute of Medical Sciences, Karimnagar, Telangana, India

[2]Associate Professor, Department of Pathology, Nizam's Institute of Medical Sciences, Telangana, India

[3]Professor & Head of Department, Department of Pathology, Nizam's Institute of Medical Sciences, Telangana, India

[4]Professor, Department of Nephrology, Nizam's Institute of Medical Sciences, Telangana, India

[5]Professor, Department of Medicine, Nizam's Institute of Medical Sciences, Telangana, India

[6]Professor & Head, Department of Medical Oncology, Nizam's Institute of Medical Sciences, Telangana, India

Corresponding Author: Dr. Pramod Kumar Pamu

Abstract

Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening disorder characterized by excessive activation of the immune system, leading to uncontrolled proliferation of histiocytes and T cells. This study aims to identify and classify cases of hemophagocytosis observed in bone marrow aspiration samples and to provide clinical, laboratory, and prognostic insights.

Materials and Methods: A retrospective study was conducted in the Department of Pathology at Nizam's Institute of Medical Sciences, Hyderabad, from January 2009 to July 2018. Bone marrow studies were performed on cases with clinical suspicion of HLH, pancytopenia, prolonged fever, unexplained organomegaly, and lymphadenopathy. Seventy-two cases demonstrating hemophagocytosis in bone marrow aspiration were included. Clinical details and relevant investigations were recorded, and various diagnostic techniques were employed.

Results: Out of 72 cases, 40 fulfilled the criteria for HLH. The most common presenting symptoms were fever, bicytopenia, or pancytopenia, followed by organomegaly and lymphadenopathy. Infections, especially tuberculosis, were commonly associated with hemophagocytosis. Malignancy-associated HLH was observed in 16 cases. Drug-induced HLH was noted in 5 cases. The study found that endobutton reduced femoral tunnel widening more effectively than transfix and interference screws, with all groups showing similar clinical findings and knee laxity after one year.

Conclusion: Hemophagocytosis is a significant finding in bone marrow aspirations and warrants thorough investigation to identify HLH. Timely diagnosis and understanding of the pathophysiology are crucial for effective treatment and reducing mortality. Further large-scale controlled studies are needed to corroborate these findings.

Keywords: Hemophagocytic lymphohistiocytosis, hemophagocytosis, bone marrow aspiration, infections, malignancy, drug-induced HLH.

Introduction

Hemophagocytosis is a common phenomenon observed in bone marrow which is characterised by the engulfment of erythrocytes, leukocytes, platelets, and their precursors by the histiocytes either in the bone marrow or in other parts of the reticuloendothelial system [1]. This is observed in the bone marrow aspirates sent for examination with various clinical conditions and is usually identified in infections, inflammation, bone marrow hyperplasia or ineffective hematopoiesis. This is an important finding and in few cases, it may be the sole finding in bone marrow aspiration and prompts thorough search for the cause of hemophagocytosis as the treatment varies with the underlying cause of hemophagocytosis.

Hemophagocytic syndromes occur commonly secondary to bacterial or viral infections and commonly by Hemophagocytic lymphohistiocytosis (HLH).Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder of the mononuclear phagocytic system, characterized by excessive activation of the immune system due to infection, autoimmune diseases, or malignancy and associated with systemic proliferation of non-neoplastic histiocytes [2-4]. It is an aggressive and life threatening disorder which is often unrecognised. There is uncontrolled proliferation of histiocytes and T cells in the bone marrow, liver and

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spleen which secrete inflammatory cytokines and demonstrate phagocytosis of hemopoietic cells.
The hallmark of the disease is low or absent NK cell and CD8+ T lymphocyte cytotoxicity. It can be classified as familial (primary) or acquired (secondary). Primary is associated with certain genetic mutations and immunodeficiency syndromes. Secondary HLH includes Sporadic hemophagocytic lymphohistiocytosis, Infection associated hemophagocytic lymphohistiocytosis (Hemophagocytic syndrome) (HPS) and Macrophage activation syndrome (MAS), associated with some drugs and lymphomas [5].
Extensive investigation with biochemistry and biopsies of the bone marrow, lung or lymph nodes are often necessary. High index of suspicion and timely diagnosis is needed in order to reduce the mortality associated with this syndrome. There is no single feature that is pathognomonic for HLH, including hemophagocytosis.
This study was undertaken to identify the cases with hemophagocytosis in bone marrow aspiration and categorise them into HLH when they fulfil the criteria set up by the Histiocytic society and compare with other studies available in the literature and describe the clinical and laboratory presentation and prognosis of HLH due to various causes.

Materials and Methods
This is a retrospective study conducted in the Department of Pathology at NIMS, Hyderabad from January 2009 to July 2018. Bone marrow studies were done in cases with clinical suspicion of HLH, pancytopenia, prolonged fever, unexplained organomegaly and lymphadenopathy. 72 cases which demonstrated hemophagocytosis in the bone marrow Examination were included in the study. Clinical details and relevant investigations were noted for every case. BMA, trephine imprint smears, trephine biopsy were done in every case.
Cytochemistry, special stains, immunohistochemistry were done where ever possible. In every case, at least three BMA smears were examined and 500 nucleated cells were observed for evidence of hemophagocytosis. The smears showing even a single cell with hemophagocytosis were considered positive.

Inclusion criteria
Cases showing hemophagocytosis in bone marrow or spleen or lymph node aspiration or biopsy and fulfilling the criteria of HLH by Histiocyte society.

Exclusion criteria
Cases showing hemophagocytosis in bone marrow or spleen or lymph node aspiration or biopsy and not fulfilling the criteria of HLH by Histiocytic society.

According to the HLH Study Group of the Histiocyte Society, diagnosis of HLH is met if one of the following was fulfilled [27-29]:

- A molecular diagnosis consistent with HLH

Or

- Meeting five of the following eight criteria:

1. Fever
2. Splenomegaly
3. Cytopenia affecting ≥2–3 cell lines in peripheral blood
   - Hemoglobin <9 g/100 mL,
   - Platelets <100 × 109/L,
   - Neutrophils <1.0 × 109/L
4. Hypertriglyceridemia and/or hypofibrinogenemia (Fasting triglycerides ≥3.0 mmol/L, fibrinogen ≤1.5 g/L)
5. Hemophagocytosis in bone marrow, spleen, CSF, or lymph nodes. No sign of malignancy
6. Decreased or absent NK-cell activity
7. Serum Ferritin ≥500 μg/L
8. Elevated sCD25 (soluble IL-2–receptor) ≥2,400 U/mL

In the present study, molecular diagnosis was not done in any case. In all cases, only six of the above mentioned eight parameters were available. The cases were diagnosed as HLH only when any 5/8 of the above criteria were fulfilled.

Results
A total of 72 cases showed hemophagocytosis in BMA smears during the study period (Table 1). Not all cases fulfilled the criteria of HLH. In 32 cases, hemophagocytosis was merely an incidental finding in addition to the primary diagnosis and they fell short of the criteria to be designated as HLH (Table 2). Although, the presence of hemophagocytosis is an important observation, it is clinically insignificant in

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all these 32 cases without the evidence of HLH/MAS.
Of these 72 cases, 40 cases fulfilled the criteria of HLH as set by the histiocytic society (Table 3). There were no cases of primary HLH in this study. Among these 40 cases of HLH, there were 22 males and 18 females (Male: Female =1.2:1). The age of the patients ranged from 4 years to 75 years.
The most common presenting complaints were fever, and bicytopenia or pancytopenia followed by organomegaly and lymphadenopathy. Infections were commonly associated with hemophagocytosis in 9 cases. Tuberculosis was the most common infection associated with HLH in this study. Macrophage activation syndrome (MAS) was observed in 10 cases. Malignancy associated HLH was seen in 16 cases - 10 cases of leukemia and 6 cases of lymphoma (Table 4). Drug induced HLH was seen in 5 cases. (Table 3).

Table 1: Primary diagnosis in BMA smears with hemophagocytosis

Diagnosis	Number of Cases	Percentage
Nutritional anemia	10	13.8%
Leukemia	18	25%
Lymphoma	11	15.2%
MDS	3	4.16%
Myelofibrosis	2	2.77%
Malaria	6	8.33%
MAS	10	13.8%
Tuberculosis	7	9.72%
Parvo virus	2	2.77%
Drugs	3	4.16%
Total	72	100%

Table 2: Non HLH cases with only hemophagocytosis in BMA smears

Diagnosis	Number of Cases	Percentage
Nutritional anemia	10	31.25%
Leukemia	8	25%
Lymphoma	5	15.62%
MDS	3	9.37%
Myelofibrosis	2	6.25%
Malaria	3	9.37%
Tuberculosis	1	19.72%
Total	32	100%

Table 3: Bone marrow lesions showing HLH

Diagnosis	Number of Cases	Percentage
Leukemia	10	25%
Lymphoma	6	15%
Malaria	03	7.5%
Tuberculosis	06	15%
Parvo Virus	02	5%
Mas	10	25%
Drug Related	3	7.5%
Total	40	100%

Table 4: Malignancy associated HLH

Type of malignancy	Number of cases
Acute Myelocytic Leukemia	1
Acute Myelomonocytic Leukemia	2
Acute Monocytic Leukemia	4
Acute Lymphoblastic Leukemia	1
Chronic Lymphocytic Leukemia	2
Hodgkin Lymphoma	1
Nk Cell Lymphoma	1
Diffuse Large B Cell Lymphoma	2
T Cell Lymphoma	2
Total	16

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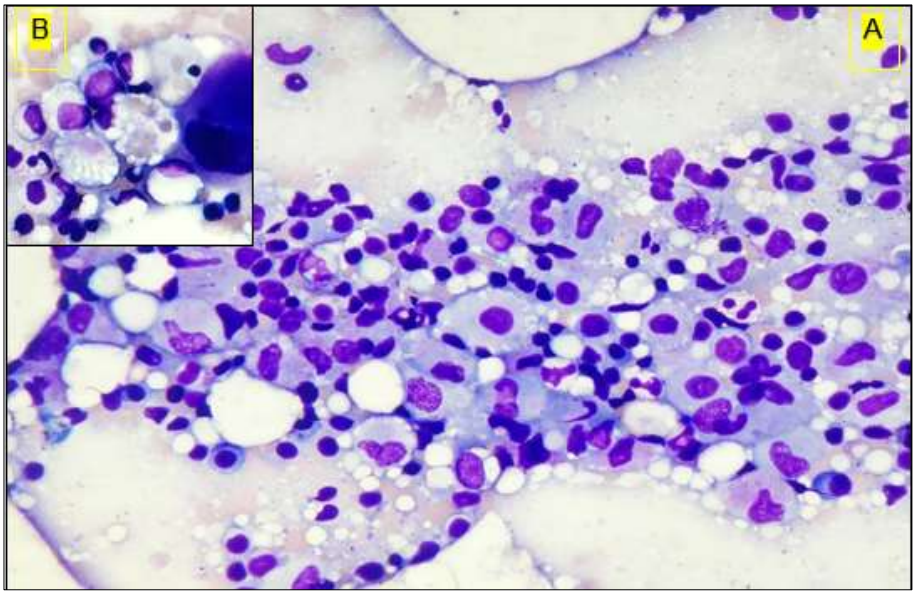


Fig 1 (A & B): Bone marrow aspiration: hemophagocytosis, with macrophage (Inset-B) ‘’Ingesting’’ other cells (B)

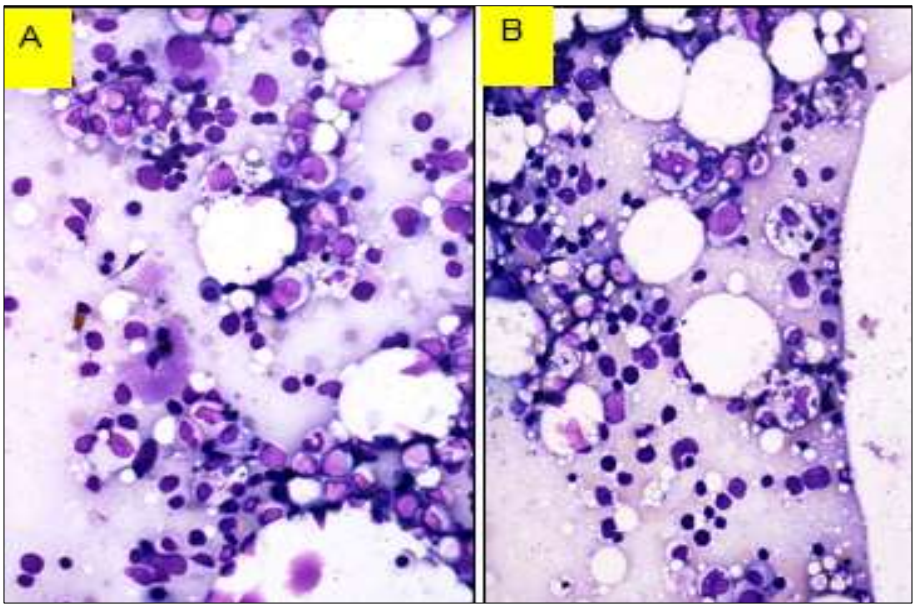
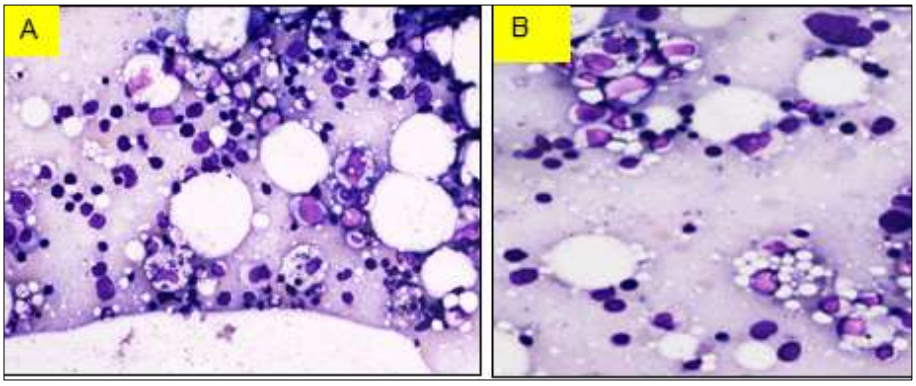


Fig 2 (A & B): Aspirate smears from case 2 with autoimmune disorder (29 years, female) with marked hemophagocytosis of all three elements (May Grunwald Giemsa stain, 400 _x0001_)



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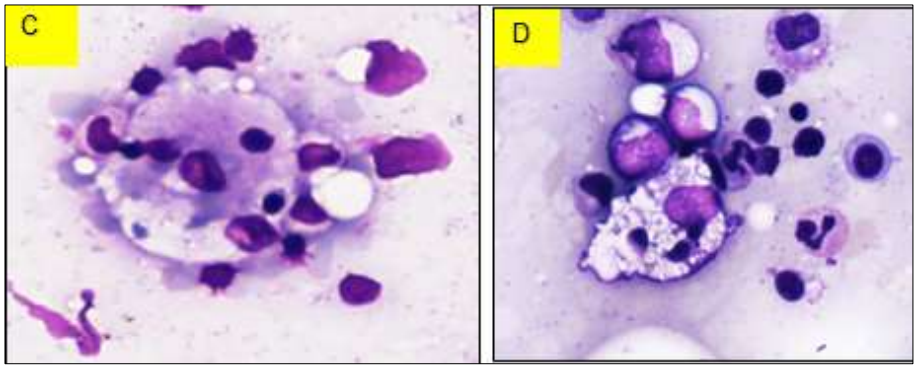

Fig 3 A-D: Bone marrow aspirate smears from a patient with miliary tuberculosis (case 7, 17 years, male) showing increased reticuloendothelial cells/histiocytes with engulfed debris and without evidence of hemophagocytosis (Black arrow) (May Grunwald Giemsa stain, 400 _x0001).

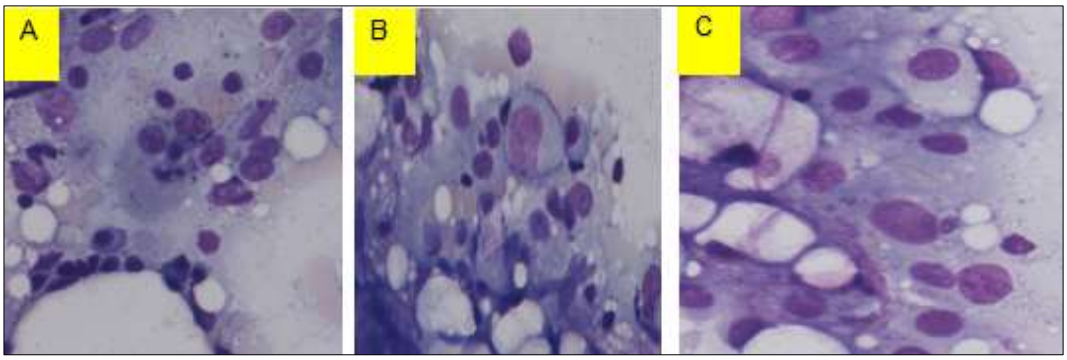

Fig 4 A: Light microscopic image of bone marrow showing stromal macrophages containing numerous red blood cells in their cytoplasm. B & C: Aspirate smears from another patient with infection - 19 years female - aspirate smear showing hemophagocytosis, macrophage engulfing nucleated RBC (BMA, 100X)

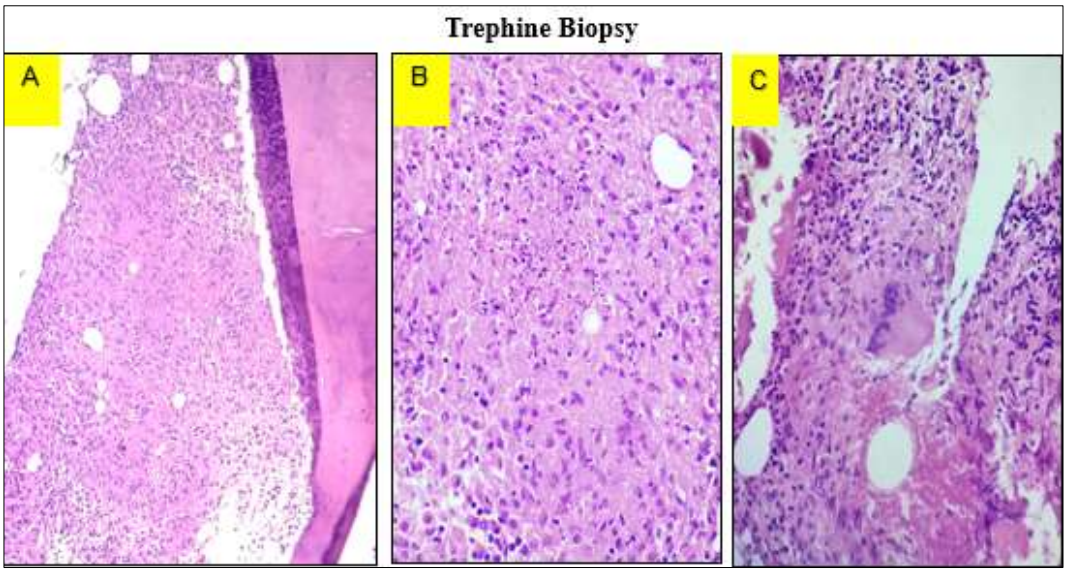

Fig 5 (A, B & C): Bone marrow biopsies sample with hematoxylin-eosin stain reveal Necrotizing granulomatous inflammation with multinucleated giant cells and increased number of activated macrophages with compatible with hemophagocytic lymphohistiocytosis

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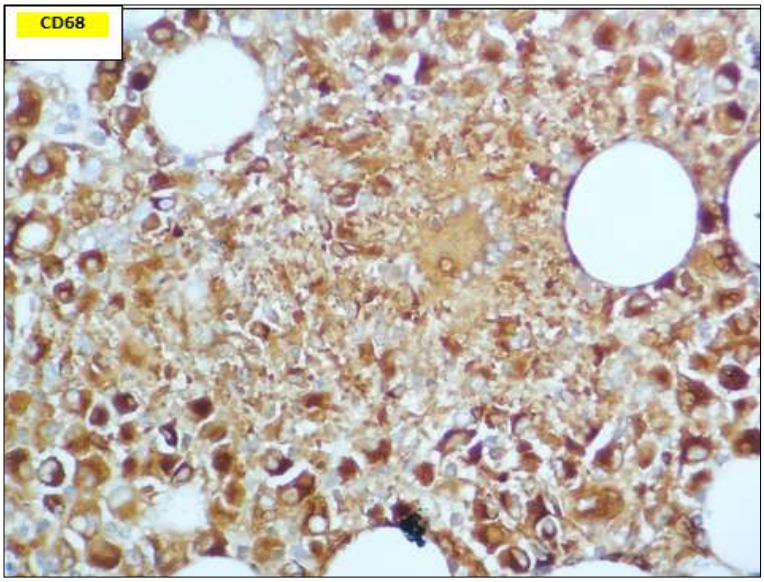

Fig 6: Bone marrow biopsy showing CD68 positive macrophages engulfing nucleated red blood cells (CD68, 100×)

Discussion

Hemophagocytosis is defined as a condition in which the phagocytosis of hemopoietic precursor cells by normal macrophages is observed in bone marrow, lymph nodes, spleen or liver. HLH is characterized by excessive activation of the immune system due to infection, autoimmune diseases, or malignancy and leads to uncontrolled hypercytokinemia and multi-organ dysfunction [6, 7]. The disease primarily affects pediatric age group but adults are also commonly involved [11]. The most common clinical features in this study were fever, pancytopenia hepatosplenomegaly, and lymphoadenopathy secondary to hemophagocytosis in bone marrow and peripheral lymphoid organs [8, 9]. The diagnosis of HLH needs to be considered in the differential diagnosis for any patient presenting with unexplained cytopenias, hepatosplenomegaly, and prolonged fevers. HLH can be rapidly progressive and potentially fatal if left undiagnosed.

The age range in our series varied from 4 yrs. to 65 years (mean age years). Male to female ratio in this study was 1.2:1 with a slight male predominance which is in contrast to Reddy *et al*. (M: F- 4:1) and Joshi *et al*. (M: F - 1:4) [12, 13]. The most common clinical features were fever with cytopenia and hepatosplenomegaly. This is similar to the observations in the studies by Joshi *et al.* [13] and Ramachandran *et al*. [14]. Infections were the most common cause of HLH (bacterial, viral-EBV, CMV, fungal and parasitic) in 14 cases followed by malignancy associated HLH in 16 cases. (Table 4). Malaria and tuberculosis were observed in 4 and 6 cases respectively.

Malignancy associated HLH can occur in the phase of diagnosis or chemotherapy, including induction, consolidation, and even maintenance. Patients with acute myeloid leukemia may be prone to develop HLH because of an impaired immune response and a high susceptibility to severe infections including Viruses, invasive fungi and bacteria after chemotherapy and result in Chemotherapy induced HLH. In this study, there were 16 cases of malignancy associated HLH- 10 cases of leukemia and 6 cases of Lymphoma. (Table 3). HLH was detected in 9 patients in the stage of remission; and in 4 patients in the stage of induction chemotherapy. 3 cases were found to have hemophagocytosis at the onset of leukemia which prompted thorough work up for establishing the diagnosis of HLH.

Persistent organomegaly, new onset of fever in leukemic patients undergoing chemotherapy should warrant a repeat bone marrow aspiration to identify hemophagocytosis. K.delavigne *et al.* [15] have utilised a set of criteria to identify HLH in cases of leukemia undergoing chemotherapy. The same criteria were also employed in the current study to suspect HLH in cases of leukemia.

Criteria for the diagnosis of HLH in AML patients

- AML patient undergoing intensive chemotherapy.
- Fever.
- Hepatosplenomegaly.
- Ferritin >5000microgram/L.
- Prolonged neutropenia and / thrombocytopenia (outside the expected range for Chemotherapy induced myelosuppression, on day 35).
- Liver abnormalities.

A study by Takahashi [16] reported the occurrence of refractory HLH in a case of acute myelocytic leukemia. Wang X *et al*. and Defeng Zhao *et al*. also reported incidence of HLH in AML [17, 18].

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Lehmberg *et al*. reported 21 cases of Malignancy associated HLH [19]. Malignancy associated HLH was also reported in 27 cases in a multicentre study from Turkey among which AML was the common neoplasm associated with HLH [20]. Unfortunately, malignancy and Chemotherapy associated HLH were known to be potentially fatal and early aggressive treatment is warranted [21].
The outcome of the malignancy associated HLH patients in this study was also very dismal.
Among infections, Tuberculosis was the most common infection causing HLH in this study. There were a total of 5 cases of pulmonary tuberculosis and 1 case of disseminated tuberculosis. Literature also showed several case reports of Tuberculosis and acute respiratory distress syndrome associated HLH [26]. Priscilla *et al*. reviewed 36 cases of tuberculosis associated with HLH. Follow up was possible in only 2 cases which showed clinical improvement at the end of four months of treatment with anti-tubercular drugs [26]. Hemophagocytosis was observed in 6 cases of malaria, out of which only 3 cases were found to be associated with HLH in the current study. The association of malaria with HLH is hard to predict as bone marrow examination is almost never warranted in a case of malaria. Persistent fever inspite of treatment in these three cases prompted the clinician to order bone marrow aspiration, along with other lab investigations, which led to the diagnosis of HLH. However, careful literature search had showed several case reports of malaria associated HLH [22, 23]. Srinivas *et al*. observed malaria in 15.6% of cases while current study showed malaria in 7.5% cases. Park *et al*. discussed four case reports of HLH secondary to plasmodium vivax, all of which resolved with anti-malarials. Similar results were obtained in this study also with excellent recovery of all 4 cases of malaria associated HLH. There were 2 cases of parvovirus induced HLH which presented with fever and pancytopenia [30].
Macrophage activation syndrome, yet another significant cause of secondary HLH was noticed in 10 cases. 6 (out of 10) cases presented with clinical suspicion of MAS and diagnosis was confirmed accordingly with relevant lab investigations. However, in the remaining cases, clinical diagnosis of MAS was not obvious at the onset of disease and was subsequently diagnosed as MAS only after thorough work up. MAS was associated with rheumatoid arthritis and Systemic lupus erythematosus (SLE) in our study. A study by Behrens EM *et al*. [24] reported MAS in 8 patients with systemic juvenile idiopathic arthritis. A study by Alessandro parody *et al*. reported MAS in 38 cases of Juvenile SLE.
Although the suspicion of HLH increases with detection of hemophagocytosis in BMA smears, mere finding of hemophagocytosis doesn't warrant a diagnosis of HLH and extensive work up is essential. Fatality is commonly associated with HLH due to delay in diagnosis and lack of suscpicion for this possible entity.
Hence, all patients with prolonged fever and unexplained cytopenias must be evaluated for HLH by obtaining a detailed history and meticulous physical examination and by using the appropriate laboratory investigations. The prognosis of genetic HLH is dismal. The prognosis of acquired HLH is variable depending on the underlying cause, with malignancy associated cases having the worst outcome [25].

Conclusion

Hemophagocytosis is a common phenomenon observed in bone marrow aspiration and is known to occur in a variety of conditions. The clinical features and lab investigations assisted the clinicians in the early diagnosis of MAS and HLH in few cases. HLH is a clinically aggressive condition with evidence of hemophagocytosis in bone marrow, which is rapidly progressive and fatal if not diagnosed early. Application of the criteria set up by the Histiocytic society aids in the diagnosis of HLH.The study of the pathophysiology of this syndrome will allow a better understanding and treatment of the same.

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