Original Article

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Pathology Section

Quantification of Haemophagocytes on Bone Marrow Aspirate Smears and its Correlation with the Clinical Outcomes- A Prospective Study

MAMTA SONI[1], SUPRAJA SUNDARAM[2], SINDHUJA KESAVAN[3]

ABSTRACT

Introduction: Haemophagocytic Lymphohistiocytosis (HLH) is a rapidly progressive life threatening condition in which activated macrophages engulf haematopoietic cells. HLH is often overlooked because of its rarity and lack of awareness of this prevailing condition. Though previously attempts have been made to quantify hemophagocytes in marrow aspirates, to the best of present knowledge, none of the studies have correlated the number of haemophagocytes with the clinical outcomes of the patients.

Aim: To study the role of laboratory parameters and number of haemophagocytes detected in bone marrow aspirates in determining the clinical outcome of the patients.

Materials and Methods: This was a prospective observational study conducted in Apollo Hospitals, Chennai between November 2013 to November 2015 on 41 patients who had haemophagocytosis in bone marrow aspirates. The laboratory parameters and clinical findings of these patients were obtained and were correlated with the outcome of these patients. The role of quantification of haemophagocytes in determining the outcome of the patients was also studied. The continuous variables were expressed either as mean±Standard deviation (SD) or median, as appropriate. All the categorical variables were expressed either as percentage or in proportions. The p-value was derived using Chi-square test or Fischer's exact test and value of <0.05 was considered significant.

Results: The mean age was 49±16.1 years and was constituted by adults in majority. The occurrence was common in males (75.6%). The most common underlying cause of HLH was found to be infection [78.9%]. Mortality rate was 17.1%. It was found that laboratory parameters and number of haemophagocytes in the bone marrow aspirates had no significant association with the clinical outcome of the patients.

Conclusion: The number of haemophygocytes detected do not have any impact on the clinical outcome of the patients. Also there is no significant association between abnormality of laboratory parameters and clinical outcome. An early detection of haemophagocytosis is important to aid in an early diagnosis of HLH but quantifying it has no clinical significance. Timely therapeutic interventions based on prompt reporting of haemophagocytes significantly reduces mortality, irrespective of abnormalities in other test parameters. Careful examination of the bone marrow aspirate is also important to determine the underlying condition causing HLH.

Keywords: Haemophagocytic lymphohistiocytosis, Infection, Laboratory parameters, Macrophages

INTRODUCTION

Haemophagocytic Lymphohistiocytosis is a severe end spectrum of hyperinflammatory disorders when the immune system starts to damage the host tissues by releasing cytokines [1]. The most common triggering factors for this fatal immune response are infections, malignancy or a combination of both. Patients with autoimmune dysregulation or autoinflammatory disorders and in long term immunosuppressants are at increased risk of developing the dreadful syndrome [2]. HLH is classified into primary (familial) or secondary (acquired). Primary HLH occurs as a result of gene mutations responsible for an underlying immunodeficiency syndrome. Secondary HLH is seen in patients without a known genetic mutation responsible for predisposing to HLH but have associated underlying triggering factors which would lead to the development of HLH [3]. A high chance of overlap between primary and secondary HLH can occur as any illness capable of triggering secondary HLH can precipitate the condition in a person with a known genetic mutation [3]. As HLH shares similar clinical presentation as of sepsis, septic shock or other systemic inflammatory condition, the diagnosis of HLH becomes challenging from the clinical front. According to HLH-2004, the Histiocyte Society's updated diagnostic and therapeutic guidelines for HLH, a familial HLH can be diagnosed by a molecular diagnosis with specific gene mutations associated with HLH and a non-familial HLH can be diagnosed by meeting five of eight clinical and laboratory diagnostic criteria [4,5]. These criteria include fever; splenomegaly; peripheral blood cytopenias affecting at least two of three cell lineages; hypertriglyceridemia or hypofibrinogenemia, microscopic evidence of haemophagocytosis in bone marrow, spleen, or lymph nodes; low or absent Natural Killer (NK)- cell activity; hyperferritinemia; and elevated soluble Cluster of Differentiation (CD) 25 {sCD25; i.e., Interleukin 2 (IL-2) receptor} [4,5]. In HLH, due to excessive immune response, macrophages non selectively phagocytise haematopoietic cell elements, presumably leading to the cytopenias and microscopic finding of haemophagocytosis. Haemophagocytosis is not specific to HLH and can be seen in other conditions as well, such as after blood transfusion, chemotherapy, sepsis, and major surgeries [6-9].

A diagnosis of HLH needs a high index of clinical suspicion and other laboratory parameters including demonstration of haemophagocytosis in the bone marrow to support the diagnosis which would aid in early and effective management of the patient. Detection of haemophagocytes increases clinician's suspicion and confidence towards a diagnosis of HLH. Moreover, genetic mutation analyses, NK-cell activity and CD25 levels are expensive, not so readily available tests, which is not helpful in acute settings when an early diagnosis and prompt treatment decisions are crucial [6]. Though previously attempts have been made to quantify haemophagocytes in marrow aspirate

smears [6,10], to the best of our knowledge, none of the studies have correlated number of haemophagocytes with the clinical outcomes of the patients. In present study, we aimed at studying the significance of number of haemophagocytes in bone marrow aspirates with clinical outcome of the patients. In addition, we studied relation between laboratory parameters with the clinical outcome of the patients.

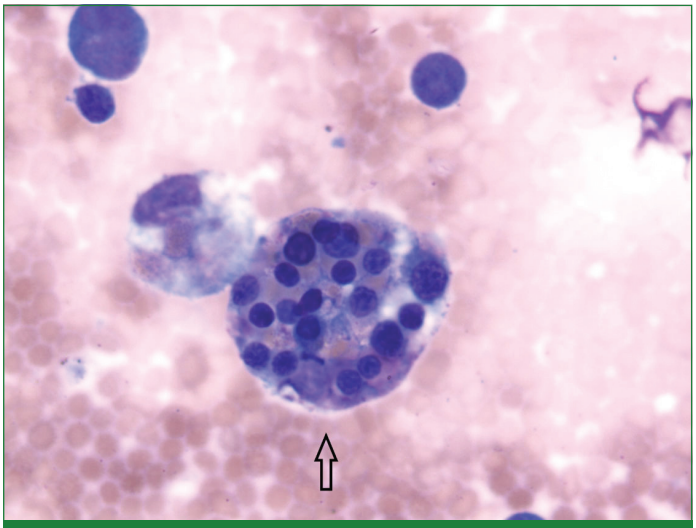
MATERIALS AND METHODS

This was a prospective observational study conducted in Apollo Hospitals, Chennai between November 2013 to November 2015, on 41 patients who had haemophagocytosis in bone marrow aspirates. Approval for the study was obtained from the Ethics Committee of the institution (Ethical number No. ECR/37/Inst/TN/2013/RR-19).

Inclusion criteria: A total of 41cases (1 paediatric and 40 adult patients) with bone marrow aspirates showing haemophagocytosis and clinically suspected of HLH according to HLH diagnostic criteria [4,5] during the study time period were included in the study after obtaining informed consent. Surface Cluster of Differentiation (CD) CD25 and Natural Killer (NK) -cell activity was not done for any of the patients.

Exclusion criteria: Cases proven positive for haemophagocytosis in bone marrow aspirates with non availability of laboratory parameters were excluded from the study.

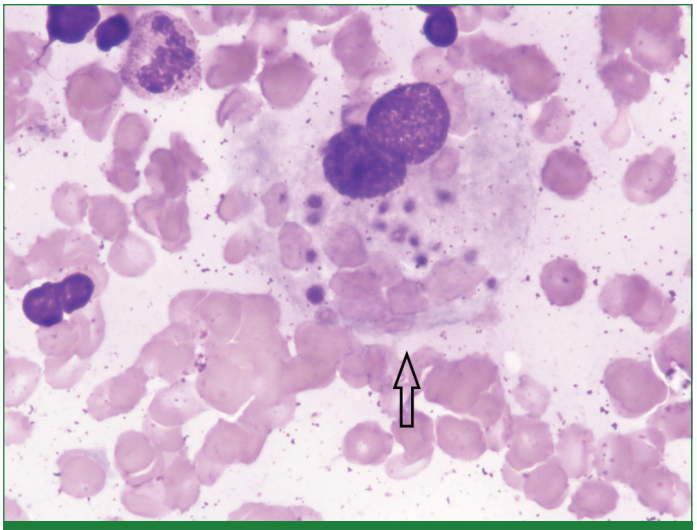
Bone marrow aspirate smear review: The bone marrow aspirates were stained by May-Grunwald-Giemsa stain and were studied in 4X, 10X, 40X, 100X magnification for the cellularity, number and maturation of erythroid, myeloid and megakaryocytic lineages, plasma cells, presence of atypical cells, granulomas, parasites and for haemophagocytosis. For quantification of haemophagocytes, two bone marrow aspirate slides were examined and number of macrophages showing haemophagocytosis were counted at 10x power and ten fields of each slide were examined. In total, 20 low power fields were examined per case. The number of macrophages showing haemophagocytosis per 10 low power fields was derived. The haemophagocytosed elements were mainly erythroid, some myeloid and few platelets [Table/Fig-1-3].



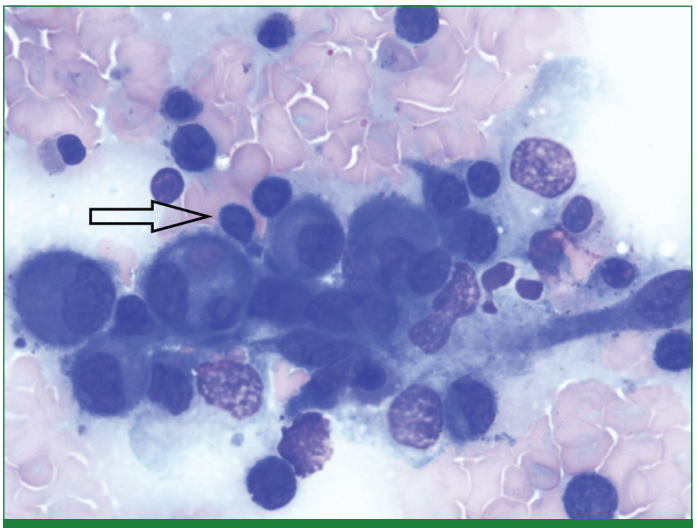
[Table/Fig-1]: Haemophagocyte in bone marrow aspirate 100X.

STATISTICAL ANALYSIS

All the continuous variables were expressed either as mean±standard deviation or median, as appropriate. All the categorical variables were expressed either as percentage or proportions. Comparison of categorical variables was done by Chi-Square's or Fischer's-exact test. Data analysis was done using Statistical Package for Social Sciences (SPSS) software version 23 (IBM Corporation). A p-value of less than 0.05 was considered as statistically significant.



[Table/Fig-2]: Activated macrophage phagocytising platelets in bone marrow aspirate 100X.



[Table/Fig-3]: Reticuloendothelial cell cluster with few showing haemophagocytosis in bone marrow aspirate100X.

RESULTS

Out of 41 cases showing haemophagocytosis in bone marrow aspirates, one case was in the paediatric age group and rest were adults. The mean age of the patients was 49±16.1 years ranging from 2-74 years. There were 31 (75.6%) males and 10 (24.4%) females. A total of 34 (82.9%) survived while 7 (17.1%)hospital. About 93% of the study population satisfied five of the diagnostic criteria while 7% satisfied 6 of the diagnostic criteria [Table/Fig-4].

Characteistics of patients	Frequency {n/N(%)}
Hemphagocytosis	41/41 (100%)
Fever (t ≥38.5°C)	40/41 (97.6%)
Splenomegaly	35/41 (85.4%)
Pancytopenia	11/41 (26.8%)
Hyperferritinemia	35/37 (94.6%)
Hypertriglyceridemia	9/32 (28%)
Hypofibrinogenemia	9/32 (28%)

[Table/Fig-4]: Clinical and laboratory findings according to HLH-2004 criteria [4,5].

Clinical and laboratory findings: The major presenting clinical symptom was fever which was present in 97.6% of cases. Weight loss, loose stools, cough, pain abdomen, joint pain and dyspnoea, altered sensorium and rash were other common symptoms noted.

Among the clinical signs, splenomegaly was detected to be the most frequent finding seen in 85.4% of the patients. Pallor, lymphadenopathy, hepatomegaly, pedal oedema, icterus and ascites were other clinical signs noted among patients.

38 out of the 41 patients demonstrated an underlying condition whereas three patients did not have a definite triggering factor. The most common underlying factor associated with patients was infection (30/38) which included bacterial (scrub typhus, tuberculosis, meliodosis) and viral (Human immunodeficiency virus, Cytomegalovirus, Epstein-Barr virus, Haemagglutinin Type 1 and Neuraminidase Type 1 (H1N1) virus) aetiology. Malignancy (acute leukaemia, myelodysplastic syndrome, lymphoma) was associated in five out of the 38 patients, while autoimmune aetiology (systemic lupus erythematosus, myasthenia gravis) was found to be associated in seven out of the 38 patients.

Among the laboratory findings, mean haemoglobin level was 7.7 g/dL. Haemoglobin level of <9 g/dL was observed in 90.2% of the patients. Mean absolute neutrophil count was 1.51×10^3/µL. About 58.5% of the patients had an Absolute Neutrophil Count of $<1\times10^3$/µL. Median platelet count was 79×10^3/µL. In 78.04% of the patients, platelet count of $<100\times10^3$/µL was observed. Median ferritin levels were 1619.5 µg/L. 85.36% of the patients had ferritin values ≥500 µg/L. Median triglyceride levels were 167.5 mg/dL. Triglyceride levels of ≥265 mg/dL were observed in 21.9% of the patients. Mean fibrinogen values were 262.6±130.5 mg/dL. In 21.9% of the patients, fibrinogen levels of <150 mg/dL was noted. Ferritin, triglycerides and fibrinogen levels were not available for few patients. These patients satisfied five criteria for the diagnosis of HLH, hence additional investigations were not ordered.

Association between laboratory parameters, number of haemophagocytes and clinical outcome of the patients

There was no significant association between the laboratory parameters and clinical outcome of the patients [Table/Fig-5]. Quantification of haemophagocytes did not have significance in determining the clinical outcome of the patients [Table/Fig-6].

Patient details	Survivors (n=34)	Non survivors (n=7)	p-value
Age (years)	47.27±16.34	58.29±11.94	0.100
Sex			
Males, (n=31)	26 (83.9%)	5 (16.1%)	0.77
Females, (n=10)	8 (80%)	2 (20%)	
Laboratory parameters			
ANC ($<1.0\times10^3$/µL, n=24)	20 (83.3%)	4 (16.7%)	1.000
Ferritin (>500 µg/L, n=35)	29 (82.9%)	6 (17.1%)	1.000
Fibrinogen (<150 mg/dL, n=9)	7 (77.8%)	2 (22.2%)	0.604
Haemoglobin (<9 g/dL, n=37)	30 (81.1%)	7 (18.9%)	1.000
Platelet count ($<100\times10^3$/µL, n=32)	27 (84.4%)	5 (15.6%)	0.637
Triglycerides (>265 mg/dL, n=9)	6 (66.7%)	3 (33.3%)	0.314

[Table/Fig-5]: Comparison of laboratory parameters among survivors and nonsurvivors.
ANC: Absolute neutrophil count; Age expressed as mean±SD; p-value derived by Chi-square test or Fischer's exact test as appropriate

No. of haemophagocytosis/ 10 low power field	Survivors (n) N=34	Non survivors (n) N=7	p-value
1-3.5	21	3	0.422
4-6.5	12	3	0.421
>7	1	1	0.157

[Table/Fig-6]: Haemophagocytosis quantification among survivors and nonsurvivors.
p-value derived using Pearson's Chi-square test

DISCUSSION

Haemophagocytic Lymphohistiocytosis (HLH) is a life-threatening condition which requires high index of clinical suspicion and prompt intervention to prevent morbidity and mortality. As this condition has several overlapping clinical features and presentation similar to other conditions including sepsis, haematological malignancies, strong clinical and laboratory evidence helps in making the timely diagnosis and proper management of the patients. Importantly, these disorders can trigger HLH, further complicating the diagnosis. Often, the largest obstacle is considering HLH in the differential diagnosis. Studies on adult HLH are very minimal. Secondary HLH is most commonly associated with infection by fungi, viruses, bacteria or parasites or in association with lymphoma, autoimmune diseases or metabolic diseases. Studies have reported that clinical outcome of patients with HLH depends on the underlying triggering factor [11,12].

Most of the patients in the present study were adults and infection was the most common underlying condition to cause HLH (78.9%). In these patients, 34 were survivors and seven were non-survivors. This is similar to finding detected by Jumic S and Nand S, and Chandra H et al., [3,10]. Production of high levels of activating cytokines such as IFN-γ, IL-6, IL-10, IL-12, IL-18, TNF-α, macrophage inflammatory protein and CD 25 by host lymphocytes and monocytes observed in infection associated HLH leads to accumulation of lymphohistiocytic infiltrates into organs and causes organ damage [11].

Malignancy associated HLH has been well documented and is associated with poor outcomes [12-15]. In present study, five (13.2%) patients had HLH in association with malignancy and all of them were discharged, though long term survival data was not available. In current study, HLH was associated with autoimmune disorder in seven (18.4%) patients among which six patients survived and one died. When HLH arises in association with rheumatologic disease, it is termed Macrophage Activation Syndrome (MAS). MAS are most commonly seen in association with adult -onset Still's disease, systemic juvenile idiopathic arthritis and systemic lupus erythematosus but have also been described in other rheumatologic conditions [12].

Thrombocytopenia as an important adverse prognostic factor has been suggested in various studies [16-18]. We also observed thrombocytopenia in five out of the seven non survivors. Thrombocytopenia occurs as a result of decreased production by insufficient bone marrow or over consumption as a result of disseminated intravascular coagulation or hypersplenism. Studies reveal that platelets express Toll-like receptors via which they bind directly to leukocyte-like microbial pathogens [19]. They bind to Von Willebrand factor attached to endothelial cells; as a result, the leukocytes accumulate on the endothelial surface. Also, the reciprocal relationship that platelets share with complementary system aids in eliminating the infection [20]. Thus, thrombocytopenia causes increased risk of bleeding, impairs immune response and causes bone marrow failure proving to be a poor prognostic factor [16].

Hyperferritinemia has been reported as an independent prognostic variable of mortality in HPS [16,21,22]. In present study, hyperferritinemia was observed in all the non survivors but significant association of hyperferritinemia with mortality could not be ascertained. Hypertriglyceridemia which has been reported as a predictor of poor clinical outcome was not found to be significantly associated with outcome of patients in present study [23].

Present study revealed that there was no significant correlation of number of haemophagocytes detected in the aspirate smears with the clinical outcomes of the patients. So prompt detection of haemophagocytes is important but quantifying it has no clinical significance. Literature review reveals that increasing age, hyperferritinemia, thrombocytopenia, hypertriglyceridemia, elevated LDH levels are predictors of poor survival outcomes [21-23]. In the present study, the authors did not find any significant association of these parameters with the clinical outcomes of the patients.

Though HLH is a life threatening condition, timely therapeutic interventions, based on prompt laboratory reporting of haemophagocytes in bone marrow and other biochemical parameters can significantly reduce mortality. In the present study study, none of the test parameters considered for the diagnosis of HLH was found to

have an influence on the mortality which could be explained by prompt diagnosis and therapeutic interventions.

Limitation(s)

All the laboratory parameters mentioned in the diagnostic criteria were not performed. Once the diagnosis was made, due to limited resources, the clinicians did not order any further investigations.

Genetic testing to confirm familial HLH was not performed.

CONCLUSION(S)

The number of haemophagocytes detected do not have any impact on the clinical outcome of the patients. Also, there was no significant association between abnormality of laboratory parameters and clinical outcome. An early detection of haemophagocytosis is important to aid in an early diagnosis of HLH but quantifying it has no clinical significance. Timely therapeutic interventions based on prompt reporting of haemophagocytes significantly reduces mortality, irrespective of abnormalities in other test parameters. Careful examination of the bone marrow aspirate is also important to determine the underlying condition causing HLH.

REFERENCES

[1] Filipovich A, McClain K, Grom A. Histiocytic disorders: recent insights into pathophysiology and practical guidelines. Biol Blood Marrow Transplant. 2010;16(1):S82-89. Available from: http://dx.doi.org/10.1016/j.bbmt.2009.11.014.

[2] Birndt S, Schenk T, Heinevetter B, Brunkhorst FM, Maschmeyer G, Rothmann F, et al. Hemophagocytic lymphohistiocytosis in adults: Collaborative analysis of 137 cases of a nation wide German registry. J Cancer Res Clin Oncol. 2020;146(4):1065-77. Available from: https://doi.org/10.1007/s00432-020-03139-4.

[3] Jumic S, Nand S. Hemophagocytic lymphohistiocytosis in adults: Associated diagnoses and outcomes. A ten-year experience at a single institution. J Hematol. 2019;8(4):149-54.

[4] Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-31. Doi: 10.1002/pbc.21039. PMID: 16937360.

[5] Bergsten E, Horne AC, Aricó M, Astigarraga I, Egeler RM, Filipovich AH, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: Long-Term results of the cooperative HLH-2004 study. Blood. 2017;130(25):2728-38.

[6] Ho C, Yao X, Tian L, Li FY, Podoltsev N, Xu ML. Marrow assessment for hemophagocytic lymphohistiocytosis demonstrates poor correlation with disease probability. Am J Clin Pathol. 2014;141(1):62-71.

[7] Goel S, Polski JM, Imran H. Sensitivity and specificity of bone marrow hemophagocytosis in hemophagocytic lymphohistiocytosis. Ann Clin Lab Sci. 2012;42(1):21-25.

[8] Nesbit ME, Balfour H. Virus-associated hemophagocytic syndrome. Cancer. 1979;44(3):993-1002

[9] Favara BE. Hemophagocytic lymphohistiocytosis: A hemophagocytic syndrome. Semin Diagn Pathol. 1992;9(1):63-74. PMID: 1561489.

[10] Chandra H, Chandra S, Kaushik R, Bhat N, Shrivastava V. Hemophagocytosis on bone marrow aspirate cytology: single center experience in north himalayan region of India. Ann Med Health Sci Res. 2014;4(5):692-96.

[11] Fisman DN. Hemophagocytic syndromes and infection. Emerg Infect Dis. 2000;6(6):601-08.

[12] Schram AM, Berliner N. How I treat hemophagocytic lymphohistiocytosis in the adult patient. Blood. 2015;125(19):2908-14.

[13] Parikh SA, Kapoor P, Letendre L, Kumar S, Wolanskyj AP. Prognostic factors and outcomes of adults with hemophagocytic lymphohistiocytosis. Mayo Clin Proc. 2014;89(4):484-92. Available from: http://dx.doi.org/10.1016/j.mayocp.2013.12.012.

[14] Arca M, Fardet L, Galicier L, Rivière S, Marsac C, Aumont C, et al. Prognostic factors of early death in a cohort of 162 adult haemophagocytic syndrome: Impact of triggering disease and early treatment with etoposide. Br J Haematol. 2015;168(1):63-68.

[15] Takahashi N, Chubachi A, Kume M, Hatano Y, Komatsuda A, Kawabata Y, et al. A clinical analysis of 52 adult patients with hemophagocytic syndrome: The prognostic significance of the underlying diseases. Int J Hematol. 2001;74(2):209-13.

[16] César Antonio Egües D, Jaime CA, Lisbeth Patricia CM, Andrea De Diego S, José Ramon Furundarena S, Nerea Alcorta L, et al. Clinical characteristics and prognostic factors in patients with hemophagocytic syndrome. Int J Rare Dis Disord. 2020;3(2). Doi: 10.23937/2643-4571/1710020.

[17] Li J, Wang Q, Sheng W, Ma J, Shang W, Wang W, et al. Hemophagocytic lymphohistiocytosis: Clinical analysis of 103 adult patients. Med [United States]. 2014;93(2):100-05.

[18] Shou M, Li L, Shang Q, Ma S, Sun J, Shu L, et al. Clinical features and outcomes in secondary adult hemophagocytic lymphohistiocytosis. QJM An Int J Med. 2017;111(1):23-31. Available from: https://doi.org/10.1093/qjmed/hcx183.

[19] Bernardo A, Ball C, Nolasco L, Choi H, Moake JL, Dong JF. Platelets adhered to endothelial cell-bound ultra-large von Willebrand factor strings support leukocyte tethering and rolling under high shear stress. J Thromb Haemost. 2005;3(3):562-70.

[20] Eriksson O, Mohlin C, Nilsson B, Ekdahl KN. The human platelet as an innate immune cell: Interactions between activated platelets and the complement system. Front Immunol. 2019;10:01-16. https://doi.org/10.3389/fimmu.2019.01590.

[21] Otrock SK, Eby CS. Clinical characteristics, prognostic factors, and outcomes of adult patients with hemophagocytic lymphohistiocytosis. Am J Hematol. 2015;90(3):220-24.

[22] Yoon JH, Park SS, Jeon YW, Lee SE, Cho BS, Eom KS, et al. Treatment outcomes and prognostic factors in adult patients with secondary hemophagocytic lymphohistiocytosis not associated with malignancy. Haematologica. 2019;104(2):269-76.

[23] Shao Y, Lu D, Ma S, Li L, Shu J, Shou D, et al. Risk factors of early death in adult patients with secondary hemophagocytic lymphohistiocytosis: a single-institution study of 171 Chinese patients. Hematol [United Kingdom]. 2019;24(1):606-12.

PARTICULARS OF CONTRIBUTORS:

1. Senior Consultant and Head, Department of Haematology and Clinical Pathology, Apollo Hospitals, Greams Lane, Chennai, Tamil Nadu, India.
2. Junior Consultant, Department of Haematology and Clinical Pathology, Apollo Hospitals, Greams Lane, Chennai, Tamil Nadu, India.
3. Senior Registrar, Department of Haematology and Clinical Pathology, Apollo Hospitals, Trichy, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Mamta Soni,
Apollo Hospitals, 21, Greams Lane, Off Greams Road, Chennai, Tamil Nadu, India.
E-mail: drmamtasoni@gmail.com

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