High serum ferritin alone as a predictor of mortality and hemophagocytic lymphohistiocytosis

INTRODUCTION

Serum ferritin levels are high in hemophagocytic lymphohistiocytosis (HLH), and higher the ferritin values more likely is the diagnosis of HLH [1]. The clinical symptoms of HLH are common and similar to other infections, autoimmune conditions immunodeficiencies, and malignancies and therefore it may be difficult to clinch the diagnosis [2]. Timely diagnosis is crucial as suggested by more than 90% fatality rate in patients before the advent and use of immunomodulating drugs [1]. Ferritin is thought to play a role in the rapid detoxification of iron and facilitates iron nucleation, mineralization, and long-term iron storage [3]. It is also a part of positive regulation of transcription in response to oxidative stress and proinflammatory cytokine signaling through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway [4]. These functions of ferritin suggest that it might serve as a cytoprotective protein, minimizing oxygen free radical formation [3]. Small quantities of ferritin are also present in human serum and are elevated in conditions of iron overload and inflammation [5]. Measurement of ferritin levels does not require a specialized laboratory and is cheaper, and the report can be availed on the same day. Interleukin-2 receptor alpha chain (IL-2Ra) levels, on the other hand, requires a focused laboratory and is expensive and cumbersome to perform even though it is a sensitive test for diagnosis of HLH [6,7]. In this retrospective study, we report here utility of high serum ferritin alone as a predictor of mortality and diagnosis of HLH.

METHODS

In this retrospective study, the Medanta hospital database was searched for all consecutive patients in whom serum ferritin levels had been done from January 2016 to December 2016. Patients with ferritin values above 500 ug/dL (as this has been used as a cutoff for diagnosis of HLH) were shortlisted. We looked for outcomes in terms of mortality in all these patients. Incidence of mortality was compared between patients with high serum ferritin levels > 5000 ng/mL and those with levels <5000 ng/mL.

All patients with Ferritin levels > 5000 ng/mL were analyzed further for presence of HLH. Patients on regular packed red cell transfusions were excluded from the study. Medical records were retrieved, and further information supporting the diagnosis of HLH based on the HLH 2004 criteria [8] (fever, splenomegaly, other laboratory tests like liver function tests, triglyceride levels, and fibrinogen levels) in these patients was collected as recorded by treating physicians based on clinical signs/symptoms.

In many patients all the criteria could not be fulfilled. Therefore, an alternative definition to recognize HLH was required. Thus, patients were assessed on the basis of presence of features of immune activation (fever, hepatomegaly/splenomegaly, elevated ferritin, elevated CD25, elevated CD163) or abnormal immunopathology (cytopenias, decreased fibrinogen/increased triglycerides, hepatitis, hemophagocytosis, CNS involvement) as discussed by Jordan et al to describe the clinical patterns and pathogenesis of HLH [9]. Liver function tests were assessed in addition to these criteria as supportive evidence. Mean, median values, and P-values were calculated wherever required.

RESULTS

3.1 Patients

During the study period there were a total of 128 patients with ferritin values >500 ng/mL, and 21 among these had ferritin levels >5000 ng/mL. The age range was 4-82 years. The mean age was 39.7 years, and median was 38 years. Of the 21 patients, 14 were male and 7 females. Patients were from department of internal medicine (43%), rheumatology (14%), gastroenterology (10%), pediatric hematology (14%), oncology (14%), and cardiology (5%).

3.2 Outcome

Mortality in patients with ferritin level >5000 ng/mL was 28.6% (6/21) versus 7.5% (8/107) in those with levels <5000 ng/mL (P-value .0048).

3.3 ICU admissions

Ten of the 21 (47.6%) patients required ICU admission and prolonged hospital stay, and six out of 10 (60%) died.
### TABLE 1  
HLH criteria/alternative criteria and outcome details in patients with high serum ferritin levels (>5000 ng/mL)

| Fever | Ferritin ng/dL | Hb g/dL | TLC /cumm | PLT /cumm | Bone marrow | Trig mg/dL | Fib mg/dL | Spleen | HLH score | LFT abnormal | Immune activation | Immune-pathology | ICU | Outcome |
|-------|----------------|---------|-----------|-----------|-------------|------------|-----------|---------|-----------|-------------|-----------------|-----------------|-----------------|------|---------|
| 1 Y   | 20000          | 6.6     | 40860     | 68000     | ND          | 134        | ND        | No      | 3         | Y           | Y               | Y               | Y               | Alive |
| 2 Y   | 23700          | 12.4    | 1860      | 18000     | ND          | 447        | 301       | No      | 3         | N           | Y               | Y               | N               | Alive |
| 3 Y   | 21800          | 7.6     | 17180     | 442000    | Y           | 169        | 156       | No      | 3         | Y           | Y               | Y               | N               | Alive |
| 4 Y   | 14100          | 6.3     | 160       | 20000     | ND          | 184        | ND        | 3       | Y         | Y           | Y               | Y               | Y               | Alive |
| 5 Y   | 67500          | 8       | 67000     | 18000     | ND          | 267        | ND        | 4       | N         | Y           | Y               | Y               | Y               | Alive |
| 6 Y   | 7990           | 13      | 16850     | 534000    | ND          | 397        | ND        | 3       | Y         | Y           | Y               | Y               | N               | Alive |
| 7 Y   | 5210           | 13      | 15400     | 391000    | ND          | ND         | 3         | Y       | N         | Y           | N               | N               | N               | Alive |
| 8 Y   | 6240           | 8.7     | 18050     | 40000     | ND          | ND         | ND        | 3       | N         | Y           | Y               | Y               | Y               | Alive |
| 9 Y   | 8710           | 12.2    | 21450     | 308000    | N           | 177        | ND        | 3       | N         | Y           | Y               | N               | N               | Alive |
| 10 Y  | 6380           | 7.9     | 1270      | 60000     | ND          | 236        | 72        | N       | 4         | Y           | Y               | Y               | N               | Alive |
| 11 Y  | 5200           | 10.8    | 12000     | 40000     | ND          | ND         | ND        | 2       | Y         | Y           | Y               | N               | N               | Alive |
| 12 Y  | 13500          | 10.8    | 14770     | 40000     | N           | ND         | ND        | Y       | 3         | N           | Y               | Y               | N               | Died  |
| 13 Y  | 11600          | 7.7     | 3590      | 22000     | Y           | 106        | 762       | Y       | 5         | Y           | Y               | Y               | Y               | Died  |
| 14 Y  | 5690           | 7.6     | 540       | 132000    | Y           | 371        | 172       | N       | 5         | N           | Y               | Y               | N               | Alive |
| 15 Y  | 18000          | 9       | 3290      | 80000     | ND          | ND         | Y         | 3       | Y         | Y           | Y               | Y               | N               | Alive |
| 16 Y  | 25300          | 9.4     | 7320      | 40000     | ND          | ND         | ND        | 3       | Y         | Y           | Y               | Y               | Y               | Died  |
| 17 Y  | 10700          | 7.5     | 8060      | 208000    | ND          | ND         | ND        | N       | 2         | Y           | Y               | Y               | N               | Alive |
| 18 Y  | 13000          | 6.1     | 59660     | 189000    | ND          | ND         | ND        | N       | 2         | N           | Y               | Y               | N               | Died  |
| 19 Y  | 5380           | 16.1    | 11300     | 236000    | ND          | ND         | ND        | N       | 2         | Y           | Y               | Y               | N               | Alive |
| 20 Y  | 171000         | 8.9     | 9640      | 12000     | ND          | 209        | 390       | N       | 3         | Y           | Y               | Y               | N               | Died  |
| 21 Y  | 5570           | 15.4    | 4680      | 130000    | ND          | ND         | ND        | N       | 2         | N           | Y               | N               | N               | Alive |

Abbreviations: Fib, fibrinogen; Hb, hemoglobin; HLH, hemophagocytic lymphohistiocytosis; ICU, intensive care unit; LFT, liver function test; N, no; ND, not done; PLT, platelets; TLC, total leucocyte count; Trig, triglycerides; Y, yes.

### 3.4  | HLH diagnostic criteria details

All patients with high serum ferritin (>5000 ng/mL) had fever (100%). Cytopenias (bi or pan) were present in 14 of 21 (66%) patients. Splenomegaly was present in six of 21 (28%) patients. Bone marrow aspiration showed presence of hemophagocytosis in three of five patients. Triglyceride levels were high in four of 12 patients. Hypofibrinogenemia was seen in one of six patients. Liver function tests had been done in all the patients. Deranged liver function (increase in enzyme level) was seen in 11 of 21 (52.3%) patients. Table 1 shows details of HLH criteria/alternative criteria and outcomes in patients with high serum ferritin >5000 ng/mL.

To confirm diagnosis of HLH in 21 patients with high ferritin >5000 ng/dL, we could use only six of eight diagnostic criteria [8] as soluble IL2R and NK cell activity tests were not available at our center. Two patients had a score of 5 of 6. Bone marrow examination was performed for only five patients (due to lack of consideration of diagnosis of HLH). Therefore, we had essentially five criteria to work with, in the remaining 16 patients. Another two patients had a HLH score of 4 of 5. The average HLH score was 3.04, and the median was 3.

Features of immune activation were present in all 21 patients, and abnormal immune-pathology was present in 16 of 21 (76.2%) patients. Deranged liver function was used as a supportive evidence of immunopathological involvement suggesting HLH in these patients. As per medical records none of the patients had documented central nervous system involvement, and no lumbar punctures were performed.

### 3.5  | Treatment for HLH

Only nine of 21 (42.8%) patients received treatment mostly with steroids except one patient who received additional treatment with cyclosporine and etoposide.

### 4  | DISCUSSION

Serum ferritin level has been included as a diagnostic criterion in HLH 2004 (>500 ng/mL). It is a simple and inexpensive test. In our patients only six criteria out of eight (as per HLH 2004 criteria [8]) were available to be tested as investigations like soluble CD25 and NK cell activity were not available. However, among the available tests also, not all were ordered due to lack of recognition of HLH as
an entity. Even though all criteria were not met among these patients, there was a higher rate of ICU admissions and mortality in patients with serum ferritin levels above 5000 ng/mL compared to those with ferritin levels below 5000 ng/mL. Despite the fact that our patients had clinical symptoms of HLH, due to lack of data we needed an alternative system to help in the diagnosis. In a study by Jordan et al., it had been discussed the diagnosis of HLH can be challenging, and the key is to consider underlying immune mechanisms (immune activation and immune pathology) along with the other diagnostic HLH criteria [9]. Also they further suggested that in sick patients even if less than five criteria are met, along with evidence of deranged liver functions, the diagnosis of HLH should be considered, and further evaluation and treatment should be initiated early [9]. In our study, >50% of patients with high serum ferritin levels (>5000 ng/mL) had evidence of deranged liver functions, and 47% patients required ICU admission, suggesting a high morbidity. Our study suggests that HLH may be underdiagnosed due to inadequate evaluation as a result of lack of awareness, cost constraints, and delayed results. This leads to increased mortality as HLH is a potentially fatal if left untreated. In our study we looked at outcomes in terms of ICU admission and mortality.

It has been suggested previously that a serum ferritin value of >3000 ng/mL is of concern, and a value >10000 ng/mL is highly suggestive of HLH [1]. A previous study by Hearnshaw et al suggested that such high ferritin levels as seen in HLH are not observed in other illnesses [10]. We used a cutoff of >5000 ng/mL in our study since we observed that in our cohort the incidence of mortality was higher in these patients, and as we had a small cohort keeping a cutoff value >10000 ug/dL could probably lead to missed diagnosis.

In our study only nine patients had received treatment (steroids) of which only one patient received further appropriate treatment with etoposide and cyclosporine. Of the treated patients, two succumbed to death (probably due to delay in diagnosis and initiation of treatment). Another four patients died among 12 patients not treated for HLH. If a protocol had been in place to look for HLH in those with high ferritin at diagnosis then all 21 patients would have been properly worked up and treated and mortality could have been reduced.

Our study suggests that high serum ferritin is highly predictive of mortality and morbidity and correlates well with the diagnosis of HLH. It can be used as a first line investigation to facilitate early recognition of the risk of mortality and aid in timely diagnosis of HLH and appropriate treatment. Performing serum ferritin level is a trouble free, low cost intervention which can be easily done in all critically ill patients. High ferritin level (>5000 ng/mL) can be used as a cautionary test to warrant further HLH work up and expedite management even before other cumbersome investigations can be done.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS
Study concept and design: Yadav. Drafting of the manuscript: Kohli and Yadav. Collection of data: Kohli and Chadha. Critical revision of the manuscript for important intellectual content: Rastogi.

Shruti Kohli
Ritu Chadha
Neha Rastogi
Satya Prakash Yadav

1 Pediatric Hematology Oncology and Bone Marrow Transplant Unit, Medanta - The Medicity Hospital, Cancer Institute, Gurgaon, India
2 Department of Hematopathology, Medanta - The Medicity Hospital, Gurgaon, India

Correspondence
Satya Prakash Yadav, Pediatric Hematology Oncology and Bone Marrow Transplant Unit, Cancer Institute, Medanta - The Medicity Hospital, Gurgaon, Haryana 122001, India.
Email: satya_1026@hotmail.com

KEYWORDS
ferritin, hemophagocytic lymphohistiocytosis, mortality

ORCID
Satya Prakash Yadav https://orcid.org/0000-0002-0507-1786

REFERENCES
1. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2008;50(6):1227-35.
2. Lin TF, Ferlic-Stark LL, Allen CE, Kozinetz CA, McClain KL. Rate of decline of ferritin in patients with hemophagocytic lymphohistiocytosis as a prognostic variable for mortality. Pediatr Blood Cancer. 2011;56(1):154-5.
3. Orino K, Lehman L, Tsuji Y, Ayaik H, Torti SV, Torti FM. Ferritin and the response to oxidative stress. Biochem J. 2001;357(Pt 1):241-7.
4. Wessling-Resnick M. Iron homeostasis and the inflammatory response. Annu Rev Nutr. 2010;30:105-22.
5. Torti SV, Torti FM. Iron and ferritin in inflammation and cancer. Adv Inorg Biochem. 1994;10:119-37.
6. Fall N, Barnes M, Thornton S, Luyrink L, Olson J, Ilowite NT, et al. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. Arthritis Rheum. 2007;56(11):3793-804.
7. Sumegi J, Barnes MG, Nestheide SV, Mollaran-Leen S, Villanueva J, Zhang K, et al. Gene expression profiling of peripheral blood mononuclear cells from children with active hemophagocytic lymphohistiocytosis. Blood. 2011;117(15):e151-60.
8. Henter J-I, Horne A, Arocio M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48:124-31.
9. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. Blood. 2011;118(15):4041-52.
10. Hearnshaw S, Thompson NP, McGill A. The epidemiology of hyperferritinemia. World J Gastroenterol. 2006;12:5866-69.