Hyperferritinemia in Hemophagocytic Lymphohistiocytosis: A Single Institution Experience in Pediatric Patients

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Abstract Hemophagocytic lymphohistiocytosis (HLH) is an inflammatory condition that may run a rapid fatal course and calls for prompt diagnosis. Early intervention with steroids and other immunosuppressive drugs can contain the disease process and favours positive outcome. Ferritin ≥ 500 ng/ml is a HLH diagnostic criterion. We evaluated the diagnostic potential of admission ferritin, in children with HLH. Pediatric patients of a referral teaching hospital from Feb 2010–Oct 2013 having been investigated for ferritin on admission were included. HLH was confirmed when patients had clinical features and met 5/8 diagnostic criteria of the revised 2004 HLH guidelines. Ferritin was estimated on Cobas e411 by electrochemiluminescence, with a measuring range of 0.5–2000 ng/ml. Dilutions were made when linearity exceeded and absolute values were reported. 905 on-admission ferritin investigations were reviewed out of which 346 values ≥ 500 ng/ml. Hyperferritinemia was seen in HLH/MAS (macrophage activation syndrome) [HLH group, median age 4 year 4 month, 59% male] and in systemic lupus erythematosus, sepsis, juvenile idiopathic arthritis, impending HLH, haemolytic anemias and malignancy [non-HLH group, median age 4 year 6 - month, 60% male]. Of 346, 72 cases of hyperferritinemia were diagnosed with secondary HLH while one patient had primary HLH. 13/73 patients expired. The median ferritin

level of the HLH group was significantly higher [6556 (2402–11,734) ng/ml] compared to non-HLH group [median 1175 (943–2000) ng/ml] ($p < 0.0001$). Receiver operator characteristics curve analysis revealed optimal admission ferritin of 3120 ng/ml as the cut-off with sensitivity of 70% and specificity of 88.9% for HLH diagnosis, exceeding the currently prescribed cut-off of 500 ng/ml. Hyperferritinemia below 3120 ng/ml has higher negative predictive value to rule out secondary HLH on admission in the study population of children predominantly diagnosed with infection associated HLH than the prescribed cut-off as per the 2004 guidelines. This may prove to be beneficial to alert physicians for prompt intervention which considerably decreases mortality in this often fatal condition.

Keywords Hemophagocytic lymphohistiocytosis · Hyperferritinemia · Ferritin · Pediatric · Macrophage activation syndrome

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an immunological disorder characterized by hemophagocytosis of RBCs, neutrophils and platelets by activated macrophages (histiocytes) and proliferation of T cells and histiocytes in the bone marrow, spleen and infiltration into multiple organs. The activated macrophages secrete excessive amounts of cytokines, ultimately causing severe tissue damage that can lead to organ failure.

HLH is a life threatening syndrome—frequent in infants and babies, but is also encountered in children and adults of all ages. It can occur as a familial disorder (primary HLH due to genetic mutations like *PRF1*) or sporadic disorder

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(secondary acquired HLH) [1–3]. Macrophage activation syndrome (MAS) is a form of HLH that occurs primarily in patient with juvenile idiopathic arthritis or other rheumatologic diseases [4]. Various events that disrupt the immune homeostasis act as a trigger for the excessive macrophage activity and the resulting cytokine storm. Of these, infection is a common and frequent trigger [5, 6].

HLH is difficult to diagnose and treat. Prompt initiation of treatment with immune-suppressives and chemotherapeutic drugs is essential for survival in most cases [7]. The variable clinical presentation of HLH and the lack of specificity in laboratory findings pose the greatest hindrance to a successful outcome [8].

The HLH 2004 diagnostic criteria consists of a panel of clinical and laboratory parameters; fulfilling 5/8 of these is diagnostic of HLH [9]. Ferritin level of ≥ 500 ng/ml is an easily attainable criterion as serum ferritin analysis can be performed quickly and reliably in any moderate laboratory set up. Previous reports have claimed hyperferritinemia values of over 10,000 ng/ml to be specific and sensitive for the diagnosis of HLH [10], and others have shown declining serial ferritin estimations to be associated with favourable outcome [11]. In this study, we evaluated the diagnostic and prognostic potential of admission ferritin values in children predominantly afflicted with infection associated HLH (IAHLH).

Materials and Methods

Laboratory database and clinical charts of all patients having investigated for serum ferritin on admission were reviewed at Institute of Child Health (ICH), Kolkata from February 2010 through October 2013. Children with ferritin values ≥ 500 ng/ml were considered for further analysis. Diagnosis and final outcome of these patients were recorded. Of these, HLH/MAS was diagnosed by clinicians with the revised 2004 guidelines. Ferritin was re evaluated in some, at the discretion of the treating physicians. Approval for study was given by the Ethics Committee of ICH. Ferritin was estimated on Cobas e411 by electrochemiluminescence, with a measuring range of 0.5–2000 ng/ml. Recommended dilutions were made when linearity exceeded and absolute values were reported as routine practice. Quality controls (QC) from Bio-Rad India Pvt Ltd. were run as per laboratory quality system procedures; precision of the assay was measured as coefficient of variation of 9.7%.

Statistical Analysis

Descriptive statistics were calculated for age, gender, and first ferritin level (admission ferritin). Box whisker plots

for admission-ferritin by disease categories were generated. Mann–Whitney test was performed to compare the medians of continuous variables between HLH and non-HLH patients. The Fisher's Exact Test was used for categorical variables. Wilcoxon signed rank test was used to compare medians within each group. Statistical significance was defined as two-sided p value <0.05 . A non-parametric receiver operator characteristic (ROC) curve was derived from the admission ferritin values for HLH. The overall accuracy of the test is measured by the area under the curve (AUC); and the cut off value is the optimal value on the curve with the highest sensitivity and specificity. All statistical analyses were performed using the Medcalc software, Mariakerke, Belgium, version 8.1.

Results

The study was performed in a referral teaching pediatric hospital of Kolkata, India from February 2010 and October 2013. During this interval, there were 905 on admission ferritin investigations, of which 346 results were ≥ 500 ng/ml. These 346 investigations were ordered at the clinical discretion of independent pediatricians, and were classified on the basis of diagnosis as HLH ($n = 73$) or non-HLH etiology ($n = 203$). There were no significant differences in the age or gender of children with [4 year 4 month, 59% males] or without HLH [4 year 6 month, 60% males] (respective p values = 0.79, 0.99). The median ferritin levels of the two groups were significantly different ($p < 0.0001$), with *HLH group* ferritin having a higher median (IQR) of 6556 (2402–11,734) ng/ml compared to *non-HLH group* [median 1175 (943–2000) ng/ml]. The common conditions of non HLH hyperferritinemia according to discharge diagnosis were assigned into the following diagnostic groups with the median ferritin in parentheses: systemic lupus erythematosus (SLE) [3121 ng/ml], systemic onset juvenile idiopathic arthritis (SoJIA) [median ferritin 2500 ng/ml], ankylosing spondylitis [2100 ng/ml], impending HLH (or “potential HLH” when clinical criteria was suggestive of HLH, but not all criteria were met) [2008 ng/ml], sepsis [1160 ng/ml], haemolytic anemia [1147 ng/ml], and malignancy [818 ng/ml].

All the 73 patients with diagnosis of HLH met criteria for HLH according to the revised HLH-2004 treatment protocol. The maximum ferritin levels were seen in the HLH/MAS patients; of which one patient (*PRF1 mutation positive*) was diagnosed as primary HLH. Of the 72 cases of secondary HLH, majority were infection associated (37%), others were MAS (autoimmune disease associated, 27%), and one was a complication of Chediak Higashi syndrome, while the remaining 34% belonged to unknown etiologies where the initiating factor could not be

identified. The classification of the secondary HLH cases is presented in Table 1.

ROC curve using admission ferritin levels were generated (Fig. 1). The area under the curve (AUC) was 0.851 (95% confidence interval 0.804–0.891). The optimal cut off was 3120 ng/ml, with reported specificities and sensitivities, positive predictive value, and negative predictive value given in Table 2. Cut-off values of 500 ng/ml (diagnostic cut-off for HLH) and 10,000 ng/ml have also been reported in the table.

Thirteen (18%) of the 73 HLH patients did not survive. Repeat ferritin levels at or near discharge showed that the ferritin levels fell significantly ($p = 0.006$) (from admission values) in survivors but did not in non-survivors ($p = 0.8$) (Fig. 2). Repeat ferritin values in non-survivors were extremely high (median 14,667 ng/ml), values were over 10,000 ng/ml in five patients. Survivors also recorded high ferritin values on repeat estimation but fell significantly when patients responded to chemotherapy (See Fig. 3).

Discussion

Of the 905 initial ferritin investigations measured in the Department of Biochemistry of a children's teaching hospital over a three year period, 346 (38%) were ≥ 500 ng/ml, indicating that this is not a rare occurrence. Serum ferritin elevations were commonly associated with clinical syndromes such as HLH/MAS, probable or impending HLH, SLE, sepsis, hemolytic anemias including thalassemias, sickle cell syndromes; malignancies and sepsis.

Table 1 Classification of secondary HLH of the study population

	Median ferritin (ng/ml)	N
Chediak Higashi	1745	1
Infection associated HLH		
EBV	6535	11
Dengue	4793	8
Enteric	5459	5
CMV	6675	2
HIV	1672	1
MAS		
SoJIA	9000	15
SLE	22,000	3
KD	15,716	1
HLH unknown etiology	6562	25

EBV Epstein Barr virus, CMV cytomegalovirus, HIV human immunodeficiency virus, MAS macrophage activation syndrome, SLE systemic lupus erythematosus, SoJIA systemic onset juvenile idiopathic arthritis, KD kawasaki disease

Fig. 1 ROC curve analysis of admission ferritin for diagnosis of HLH (area under curve is 0.851, 95% confidence interval = 0.804–0.891)

Table 2 Statistical analysis of admission ferritin levels at selected cut-off values for the diagnosis of HLH

Cut-off (ng/ml)	Sensitivity (%)	Specificity (%)	PPV	NPV
3120	74.0	88.7	70.1	90.5
500	98.6	0.5	26.8	50.0
10,000	28.8	97	77.8	79.1

Fig. 2 Median ferritin values on admission and at discharge for survivors (blue line) and non-survivors (red line). Median ferritin levels fell significantly ($p = 0.006$) in survivors but showed an increase in survivors ($p = 0.8$)

Ferritin is a ubiquitous, 450-kDa, 24 subunit protein that is commonly measured to assess tissue iron stores. It is also a positive acute phase reactant that increases non-specifically in inflammatory conditions. In disorders not typically associated with iron overload, extreme elevations of serum

Fig. 3 Prognosis and response to therapy monitored by ferritin (red trend line, values on days 1, 7, 11, 15, 18 and 21 in boxes) in a 4 year old male child with EBV associated HLH over 21 days of ICU stay. (Dexa = Dexamethasone on day 3, CSa = cyclosporine on day 11 and MP = methylprednisolone on day 15)

ferritin may result from the activation of cytokines that mediate the acute phase response such as interleukin-1 (IL-1), IL-2, IL-6, IL-10, IL-12, interferon gamma and tumor necrosis factor-alpha. In fact, ferritin has been implicated not only to be induced by cytokines, but in turn to mediate the proinflammatory process by inducing the expression of pro-inflammatory cytokines [12].

In our study, the highest levels of ferritin (median 6556 ng/ml, range 605–61,155) were encountered in HLH/MAS patients. Hyperferritinemia is a hallmark of HLH. In the HLH 2004 criteria, a ferritin value ≥ 500 $\mu\text{g/l}$ is considered positive [9]. As hyperferritinemia is a not so rare occurrence, it may be prudent to investigate if higher cut-off values have increased sensitivity and specificity. As the higher ferritin threshold increases the probability that a patient identified will have HLH, therefore, a very high ferritin should raise the clinical suspicion of HLH especially when associated clinical and laboratory features are present. Several studies have shown evidence that this cut off may be higher with improved sensitivity and specificity in different clinical settings [13–15].

To determine an appropriate cut-off, an optimal value of 3120 ng/ml with a trade off between sensitivity (74%) and specificity (88.7%) was reached by the ROC curve analysis, exceeding the current diagnostic cut-off of 500 ng/ml put forth in the HLH-2004 guidelines. This suggests that a higher cut-off value of ferritin level may have improved utility in the diagnosis of secondary HLH in the pediatric setting, which in our study was predominantly infection associated HLH. Similar observations of a higher cut off have been proposed by Lehmborg et al. [13] and Saeed et al. [15] in the critically ill patients, and by. Considering the recommended cut-off of 500 ng/ml, all but one of the identified patients with HLH had admission ferritin levels

lower than this value. The sensitivity of this cut-off was quite high but suffered from poor specificity. The retrospective analysis performed by Allen et al. [10] at Texas Children's Hospital reported that maximum ferritin level higher than 10,000 ng/ml had a 90% sensitivity and 98% specificity for HLH. The same cut-off in our study using on admission ferritin showed a desirable specificity of 97% but a sensitivity of only 28.8%. Based on these findings, we advocate the use of a higher cut-off for ruling out HLH in the pediatric setting, which is predominantly infection associated, as the NPV is high. A very high value in the tune of 10,000 ng/ml may miss positive cases during screening but is beneficial to confirm HLH as the disease progresses. In the adult patients, hyperferritinemia of over 10,000 ng/ml are seen in several other conditions apart from HLH such as renal failure, hepatocellular injury, infections, and hematologic malignancies and hence does not predict very well for HLH [16]. In one study, 86% of pediatric patients with a ferritin $>10,000$ ng/ml had clinically defined or potential/possible HLH while only 60% of adult patients had clinically defined or potential/possible HLH [17].

The repeat ferritin estimations near or at discharge, clearly showed a non-declining trend in non-survivors. Lin et al. [11] reported that patients with less than a 50% drop in ferritin level as opposed to a 96% or greater drop had a 17-fold increased chance of dying. Ferritin has been shown to independently predict mortality in critically ill patients with HLH; a serum ferritin ≥ 2000 ng/ml predicted death with a sensitivity of 71% and specificity of 76% [18]. Changes in ferritin levels may aid the clinician in judging response to treatment as has been illustrated in the single case (Fig. 3). A noteworthy point in the use of serum ferritin levels in monitoring treatment is the requirement of the laboratory reporting protocol to report absolute values. Most automated analysers are capable of reliably generating extremely high values of ferritin following serum dilution and these are reported after validation of the clinical reportable range of the ferritin assay.

Conclusion

It is clear that diagnosis of HLH can lead to earlier recognition and prompt treatment of this often fatal condition. Our results suggest that marked hyperferritinemia can be seen in a variety of conditions in the pediatric setting and therefore a cut-off value of 3120 ng/ml, which is higher than the currently prescribed cut-off value of ≥ 500 ng/ml, can reliably rule out HLH in this population. Ferritin is also an excellent monitoring tool to follow up disease activity and treatment success. Laboratory should be informed to report absolute values of ferritin as values can exceed several times the analytical measurement range.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was waived by the ethics committee.

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