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ORIGINAL ARTICLE

Evaluating the optimal serum ferritin level to identify hemophagocytic lymphohistiocytosis in the critical care setting

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Abstract Ferritin is known to be involved in numerous physiological roles, such as iron storage, as well as various pathological conditions and in generalized inflammatory states. Hyperferritinemia is also encountered in the setting of hemophagocytic lymphohistiocytosis (HLH). Current diagnostic criteria exist to define HLH based on several clinical and biochemical markers, including the serum ferritin level. In this study, we retrospectively evaluated the value of ferritin >500 ng/mL in diagnosing HLH in 344 consecutive patients admitted to the medical intensive care unit at our hospital. Nine cases of HLH were identified. Comparison of the HLH with the non-HLH group showed that their maximum median serum ferritin level was 25,652 (range 1977–100,727 ng/mL) versus 1180 (503–85,168 ng/mL) ($P < 0.001$), platelets were 30 (5–92 $\times$ $10^3/\mu L$) versus 113 (0–507 $\times$ $10^3/\mu L$) ($P < 0.001$), absolute neutrophil counts were 2.56 (0.02–23.7 $\times$ $10^3/\mu L$) versus 7.7 (0.01–82.7 $\times$ $10^3/\mu L$) ($P = 0.002$), and triglycerides were 255 (156–394 mg/dL) versus 127 (17–624 mg/dL) ($P = 0.002$), respectively. Using a receiver operating characteristic curve, the optimal maximum serum ferritin level for the diagnosis of HLH was 3951 ng/mL, exceeding the current diagnostic cutoff set forth in the HLH-2004 guidelines. These data suggest that a higher cutoff value of ferritin level may have improved utility in the diagnosis of secondary HLH in the critical care setting.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a unique disease state typically occurring as a provoked, uncontrolled proliferation and over activation of lymphocytes and macrophages. It can happen either secondary to a select group of genetic abnormalities or as a runaway inflammatory response [1, 2]. The condition was first described by Farquhar in 1952 [3]. Genetic HLH can be subdivided into those predisposed by a primary immune deficiency and familial hemophagocytic lymphohistiocytosis (FHL). Both are more commonly seen in children than adults. Indeed, most cases present in the first year of life [4], and are associated with abnormalities discovered in at least five genetic loci identified retrospectively in cases with known FHL [5]. In a study population of 2701 clinically suspected cases, 21 of 28 genes discovered demonstrated mutation in a perforin gene (*PRF1*), or genes involved in the degranulation pathway (including *UNC13D* or *STX11*), active in natural killer T cells [6]. The disease is devastating, with a mortality rate of >90 % if untreated. With treatment, long-term survival is reportedly around 30 % [7, 8].

Secondary HLH is usually secondary to viral septicemias, most notably disseminated Epstein-Barr virus (EBV) [9–12]. It can also occur with other systemic infections or disorders that generate an extreme and sustained level of soluble interleukin-2 receptor (sIL-2R), a receptor inextricably linked to lymphocyte activation [13–15].

Since ferritin is an acute phase reactant, it can have utility in diagnosing and determining the treatment response of patients with HLH [16]. However, given its generalized

presence in many disease states, ferritin itself may not be specific in secondary forms of HLH. Elevated ferritin levels will follow systemic inflammation, and elevations may be found regardless of the presence of HLH. This is especially troublesome with the current cutoff level of ferritin as originally defined by HLH-2004 (500 ng/mL), which is somewhat arbitrarily defined as the value for a positive result in the criteria. It was originally based on the assumption of low likelihood of having high ferritin level in a newborn child (except in cases when a child has the diagnosis of HIV) [17].

Prior to this study, our local experience with HLH raised the suspicion that this disease is often evident with ferritin levels markedly exceeding the 500 ng/ml threshold. Similar studies have been performed in children. In a retrospective study of 330 pediatric patients, Allen et al. [18] identified a cutoff of 10,000 ng/ml as generating a 96 % specificity while maintaining a 90 % sensitivity of FLH independent of other variables. To our knowledge, no such study has been performed in the adult population.

Methods

We retrospectively reviewed the medical records at our academic medical center for all consecutive patients admitted to the adult medical intensive care unit (MICU) from 2004 to 2014. Inclusion criteria were age >18 years, admission to MICU, and a ferritin level >500 ng/mL. The university's institutional review board approved this study.

We collected information about individual demographics (age, sex, race, date of admission, date of discharge, and date of death), clinical manifestations, laboratory findings, bone marrow biopsy, and treatment plans. When multiple values were available, the ones closer to the date of collection of the maximum ferritin level were used. All the laboratory values were collected prior to starting any specific therapy for HLH.

The hospital laboratory measured ferritin levels with immunoassay through the DXI Beckman system (Beckman Coulter, Brea, CA) until 2012, when the institution switched to the Cobas 8000 series (Roche, Germany). The normal ferritin levels found in our laboratory were 9–400 ng/mL. For patients who had their ferritin levels checked more than once during their hospital stay, the highest one was used in the analysis.

Diagnosis was based on the treating physician's documentation at the time of discharge or death. The diagnoses were categorized by clinical presentation and outcome, such as sepsis, pulmonary disease, hematological malignancy, solid tumors, transplant complication, cardiovascular disease, neurological disease, or HLH as a separate entity.

The data were fit with a receiver operating characteristic (ROC) curve using SigmaPlot version 12.3 (Systat software, Inc., San Jose, CA). The optimal sensitivity and specificity were calculated using a pretest probability of 50 % and cost ratio of 1. Mann–Whitney–Wilcoxon test was used to compare the medians for the HLH versus the non-HLH cohort.

Table 1 General characteristics

	Non-HLH	HLH	
Sex			
Male (%)	202 (60.3)	7 (77.8)	
Female (%)	133 (39.7)	2 (22.2)	
Race			
Caucasian (%)	277 (82.7)	6 (66.7)	
African American (%)	40 (11.9)	0 (0.0)	
Others or unknown (%)	18 (5.4)	3 (33.3)	
	Median (range)	Median (range)	*P*
Age (years)	58 (20–88)	49 (20–68)	
LOS (days)	17 (0–256)	22 (9–33)	0.43
Ferritin initial (ng/mL)	1091 (48–85,168)	25,652 (119–100,727)	<0.001
Ferritin max (ng/mL)	1180 (503–85,168)	25,652 (1977–100,727)	<0.001
Hemoglobin (g/dL)	8.8 (4.5–16.7)	8.7 (6.7–11.9)	0.7
Platelets ($\times 10^3/\mu L$)	113 (0–507)	30 (5–92)	<0.001
Absolute neutrophil count ($\times 10^3/\mu L$)	7.7 (0.01–82.7)	2.56 (0.02–23.7)	0.002
Triglyceride (mg/dL)	127 (17–624)	255 (156–394)	0.002
Fibrinogen (mg/dL)	334 (41–738)	258 (49–772)	0.46

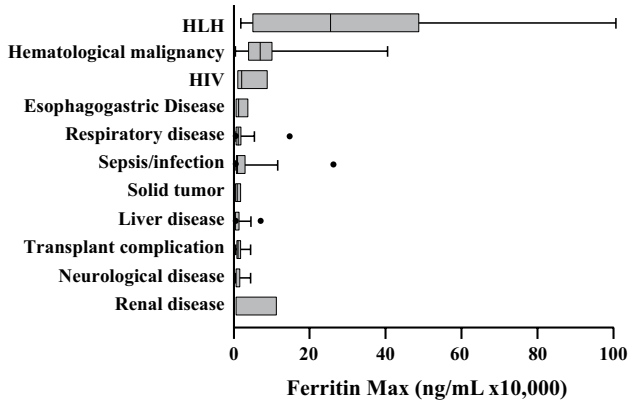

Fig. 1 Ferritin distributions over the major 11 disease categories. *Boxes* represent the 25–75 percentiles. *Dots* represent the 5/95 outliers

Results

We retrospectively evaluated 344 consecutive patients meeting the inclusion criteria; their demographic data are described in Table 1. Patients were divided into two groups based on their final diagnosis (i.e., those with HLH and all others). The second group included patients with sepsis (36 %), liver disease and respiratory failure (16 % each), hematological malignancies and neurological disorders (4 % each), transplant complications and solid tumors (3 % each), HIV and renal issues (2 %) each, and others (11 %). Patients with the final diagnosis of HLH constituted 3 % of the entire group, making it the seventh most common diagnosis in our study population.

Patients in the non-HLH and HLH groups had comparable characteristics. Men were predominant (60.3 % non-HLH group and 77.8 % HLH group). Caucasian was the most common ethnicity in our cohort (82.7 % non-HLH group and 66.7 % HLH group).

Of the 11 most common discharge categories, HLH had the highest maximum ferritin level with a median of 25,652 ng/mL (1977–100,727 ng/mL). Non-HLH patients had a median of 1180 ng/mL (503–85,168 ng/mL). The difference in median ferritin values between the HLH and non-HLH was statistically significant ($P < 0.001$). The hematological malignancy category had the second highest median ferritin, 7154 (561–60,774 ng/mL; $N = 15$, 4 %; Fig. 1).

All the patients in the HLH cohort met at least five of the diagnostic criteria according to HLH-2004. Their baseline characteristics are shown in Table 1. EBV infection was found in six patients, while two were thought to be having HLH secondary to malignancies (acute myeloid leukemia and chronic lymphocytic leukemia). HLH was secondary to unknown cause in only one patient, although he had disseminated *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* infection. sIL-2R was checked and found to be elevated in four patients (range 6400–74,034 units/mL). One patient had markedly decreased natural killer cells' activity, while the other three had inconclusive testing. Treatment was attempted for six patients with etoposide, cyclosporine, and/or thymoglobulin. None of the patients survived their MICU stay, and median survival was 23 days (range 10–33 days).

Patients with HLH also had statistically significant lower neutrophil and platelet counts ($P < 0.05$), while their triglyceride level was significantly higher than patients without HLH ($P = 0.002$). The sensitivity and specificity of different ferritin levels against the diagnosis of HLH are noted in Table 2. An optimal ferritin cutoff value of 3951 ng/mL would represent a sensitivity of 88 % [95 % confidence interval (CI) 51–99 %] and specificity of 82 % (95 % CI 78–86 %) in confirming HLH (Table 2). ROC curves plotting sensitivity versus 1-specificity of these data are demonstrated in Fig. 2. Ferritin at presentation was also evaluated; there were 31 subjects with an initial ferritin level lower than the maximum value during their hospital stay, of whom, three carried the HLH diagnosis. Running the ROC analysis using initial ferritin level yielded an area under the curve (AUC) of 0.75, which is lower than the AUC for maximum ferritin level of 0.92.

Discussion

Ferritin is a predominantly intracellular protein found in different cell types and is well studied for its role in iron metabolism, as well as its role as an acute phase reactant in times of cellular or organismal stress [19, 20]. Its release

Table 2 Sensitivity of HLH based on maximum ferritin level independent of other criteria with their prospective sensitivity, specificity, and likelihood ratios (LR)

Ferritin cutoff (ng/ml)	Sensitivity %	95 % CI (%)	Specificity %	95 % CI (%)	LR+	LR−
503.5	100.00	66.4–100	0.30	0.007–1.65	1.00	0.00
1004.0	100.00	66.4–100	41.19	35.9–46.7	1.70	0.00
2514.0	88.89	51.8–99.8	75.22	70.2–79.8	3.59	0.15
5020.0	77.78	40.0–97.1	85.67	81.5–89.2	5.43	0.26
10,053.0	55.56	21.2–86.3	92.84	89.5–95.4	7.76	0.48

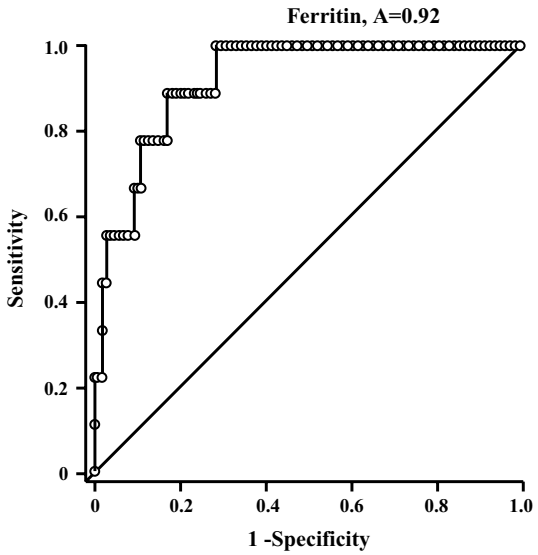

Fig. 2 Receiver operating characteristic (ROC) curve for maximum serum ferritin. (*A*) is area under the curve (AUC)

is induced by inflammatory cytokines in response to oxidative stress through a family of transcription factors related to the nuclear factor-kappa-B pathway [21]. Serum ferritin is derived mainly from T cells and macrophages, while iron overload and deficiency have strong influence on immunity [22, 23]. Hyperferritinemia is similarly described in many disease states, including those of infectious, autoimmune, iron overabundance and malignant varieties [24–26].

Genetic and acquired HLH disorders have different disease mechanisms with different fundamental pathophysiologies. The secondary form, found in the adult population and often without a genetic defect, results from a hyperinflammatory state with a massive cellular immune response. Hyperferritinemia, previously described in patients with multiple comorbidities such as iron overload, liver cirrhosis, autoimmune diseases, and as a consequence of acute inflammation [26–29], is frequently encountered in the critical care setting. This has confounded the evaluation of HLH in critically ill patients, given the relatively low ferritin cutoff threshold as described in HLH-2004 guidelines. Our data show that this cutoff value has poor specificity, which undermines the main diagnosis and might warrant unnecessary interventions. One could also theorize that secondary HLH, with normal baseline inflammatory "braking" mechanisms, may manifest with a different level of systemic inflammation compared with FLH. One step further, this may suggest that using a higher bar for levels of ferritin may be helpful to diagnose HLH while awaiting more invasive testing.

Platelets, absolute neutrophil counts, and triglycerides differed in the HLH group and as expected, go along with the diagnostic criteria of HLH-2004. This has also been reported in a previous case series of HLH [30–32]. Hemoglobin did not differ between two groups. The results could have been confounded by the known prevalence of anemia in critically ill patients, which is reported to be around 40 % [33], and in the recurrent transfusion that was not accounted for in our analysis.

On the other hand, fibrinogen did not differ significantly between the groups, although there was a trend toward lower values in the HLH group. That could have been confounded by the inflammatory nature of the fibrinogen and its changes due to severe illness [34, 35].

Limitations

This is a single-center study with nine total cases of HLH. The study used a preselection of all cases of hyperferritinemia, and control groups did not represent all patients admitted to the MICU. However, previous retrospective data showed that the prevalence of hyperferritinemia in HLH is >90 % (and approaches 100 %) [32, 36, 37]. Any conclusions regarding relative sensitivity and specificity must be drawn against this cohort selection.

An overabundance of shock in this clinical setting has the potential to confound these findings further. One would expect most cases with a significant degree of tissue necrosis to have elevated intracellular protein extravasation, and thus may not represent an accurate estimation of relative specificity of these values on the floor or in ambulatory patients. On the other hand, many cases of HLH would likely be diagnosed in the MICU. Our study is retrospective in design, and data generated should be considered hypothesis generating.

The clinical implications of changing the ferritin bar for diagnosis of HLH may be dramatic in this devastating disease. Ferritin is among the most readily obtained laboratory values in the HLH-2004 criteria. We may suppose that, with further research, setting a higher cutoff of ferritin by improving specificity may allow for relaxation of other clinical criteria (such as sIL-2R levels), which may aid a more prompt diagnosis.

Conflict of interest None to disclose.

References

1. Filipovich AH. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. Hematol Am Soc Hematol Educ Progr. 2009;2009(1):127–31.
2. Fisman DN. Hemophagocytic syndromes and infection. Emerg Infect Dis. 2000;6(6):601–8.
3. Farquhar JW, Claireaux AE. Familial haemophagocytic reticulosis. Arch Dis Child. 1952;27(136):519–25.
4. Arico M, Janka G, Fischer A, Henter JI, Blanche S, Elinder G, et al. Hemophagocytic lymphohistiocytosis. Report of 122

children from the International Registry. FHL Study Group of the Histiocyte Society. Leukemia. 1996;10(2):197–203.
5. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB. Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. Clin Exp Immunol. 2011;163(3):271–83.
6. Zhang K, Chandrakasan S, Chapman H, Valencia CA, Husami A, Kissell D, et al. Synergistic defects of different molecules in the cytotoxic pathway lead to clinical familial hemophagocytic lymphohistiocytosis. Blood. 2014;124(8):1331–4.
7. Niece JA, Rogers ZR, Ahmad N, Langevin AM, McClain KL. Hemophagocytic lymphohistiocytosis in Texas: observations on ethnicity and race. Pediatr Blood Cancer. 2010;54(3):424–8.
8. Ishii E, Ohga S, Tanimura M, Imashuku S, Sako M, Mizutani S, et al. Clinical and epidemiologic studies of familial hemophagocytic lymphohistiocytosis in Japan. Japan LCH Study Group. Med Pediatr Oncol. 1998;30(5):276–83.
9. Dandoy C, Grimley M. Secondary hemophagocytic lymphohistiocytosis (HLH) from a presumed brown recluse spider bite. J Clin Immunol. 2014;34(5):544–7.
10. Dinleyici EC, Bor O, Kiremitci A, Aksit MA. Secondary hemophagocytic lymphohistiocytosis associated with meningococcemia. Pediatr Hematol Oncol. 2006;23(8):683–6.
11. Pandey R, Kochar R, Kemp S, Rotaru D, Shah SV. Ehrlichiosis presenting with toxic shock-like syndrome and secondary hemophagocytic lymphohistiocytosis. J Ark Med Soc. 2013;109(13):280–2.
12. Kleynberg RL, Schiller GJ. Secondary hemophagocytic lymphohistiocytosis in adults: an update on diagnosis and therapy. Clin Adv Hematol Oncol. 2012;10(11):726–32.
13. Imashuku S, Hibi S, Sako M, Ishii T, Kohdera U, Kitazawa K, et al. Heterogeneity of immune markers in hemophagocytic lymphohistiocytosis: comparative study of 9 familial and 14 familial inheritance-unproved cases. J Pediatr Hematol Oncol. 1998;20(3):207–14.
14. Schneider EM, Lorenz I, Muller-Rosenberger M, Steinbach G, Kron M, Janka-Schaub GE. Hemophagocytic lymphohistiocytosis is associated with deficiencies of cellular cytolysis but normal expression of transcripts relevant to killer-cell-induced apoptosis. Blood. 2002;100(8):2891–8.
15. Rosado FG, Kim AS. Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis. Am J Clin Pathol. 2013;139(6):713–27.
16. Henter JI, Horne A, Arico 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.
17. Janka GE, Schneider EM. Modern management of children with haemophagocytic lymphohistiocytosis. Br J Haematol. 2004;124(1):4–14.
18. 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.
19. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340(6):448–54.
20. Hentze MW. Translational regulation of ferritin biosynthesis by iron: a review. Curr Stud Hematol Blood Transfus. 1991;58:115–26.
21. Kwak EL, Larochelle DA, Beaumont C, Torti SV, Torti FM. Role for NF-kappa B in the regulation of ferritin H by tumor necrosis factor-alpha. J Biol Chem. 1995;270(25):15285–93.
22. Porto G, De Sousa M. Iron overload and immunity. World J Gastroenterol. 2007;13(35):4707–15.
23. Ward RJ, Crichton RR, Taylor DL, Della Corte L, Srai SK, Dexter DT. Iron and the immune system. J Neural Transm. 2011;118(3):315–28.
24. Nemeth E, Ganz T. Anemia of inflammation. Hematol Oncol Clin North Am. 2014;28(4):671–81.
25. Zandman-Goddard G, Shoenfeld Y. Hyperferritinemia in autoimmunity. Isr Med Assoc J. 2008;10(1):83–4.
26. Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the clinician. Blood Rev. 2009;23(3):95–104.
27. Maiwall R, Kumar S, Chaudhary AK, Maras J, Wani Z, Kumar C, et al. Serum ferritin predicts early mortality in patients with decompensated cirrhosis. J Hepatol. 2014;61(1):43–50.
28. Agmon-Levin N, Rosario C, Katz BS, Zandman-Goddard G, Meroni P, Cervera R, et al. Ferritin in the antiphospholipid syndrome and its catastrophic variant (cAPS). Lupus. 2013;22(13):1327–35.
29. Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood. 2002;99(10):3505–16.
30. Switala JR, Hendricks M, Davidson A. Serum ferritin is a cost-effective laboratory marker for hemophagocytic lymphohistiocytosis in the developing world. J Pediatr Hematol Oncol. 2012;34(3):e89–92.
31. Li F, Li P, Zhang R, Yang G, Ji D, Huang X, et al. Identification of clinical features of lymphoma-associated hemophagocytic syndrome (LAHS): an analysis of 69 patients with hemophagocytic syndrome from a single-center in central region of China. Med Oncol. 2014;31(4):902.
32. Li J, Wang Q, Zheng W, Ma J, Zhang W, Wang W, et al. Hemophagocytic lymphohistiocytosis: clinical analysis of 103 adult patients. Medicine (Baltimore). 2014;93(2):100–5.
33. Walsh TS, Saleh EE. Anaemia during critical illness. Br J Anaesth. 2006;97(3):278–91.
34. Maung AA, Kaplan LJ. Role of fibrinogen in massive injury. Minerva Anestesiol. 2014;80(1):89–95.
35. Leung JM, Sin DD. Biomarkers in airway diseases. Can Respir J. 2013;20(3):180–2.
36. Riviere S, Galicier L, Coppo P, Marzac C, Aumont C, Lambotte O, et al. Reactive hemophagocytic syndrome in adults: a retrospective analysis of 162 patients. Am J Med. 2014;127(11):1118–25.
37. Rajajee S, Ashok I, Manwani N, Rajkumar J, Gowrishankar K, Subbiah E. Profile of hemophagocytic lymphohistiocytosis; efficacy of intravenous immunoglobulin therapy. Indian J Pediatr. 2014;81(12):1337–41.