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Hypofibrinogenemia is Associated with Poor Outcome and Secondary Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome in Pediatric Severe Sepsis

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Abstract

Objective: Some children with sepsis exhibit a sustained hyperinflammatory response that can trigger secondary HLH (sHLH)/macrophage activation syndrome (MAS). Although hypofibrinogenemia is a shared feature of sepsis and HLH, there are no data about fibrinogen as a biomarker to identify children with sepsis/sHLH/MAS overlap. We hypothesized that hypofibrinogenemia is associated with poor outcomes and sHLH/MAS and has utility as a screening biomarker for this sepsis phenotype.

Design: A retrospective cohort study of patients ≤21 years treated for severe sepsis from January 2012 to December 2014.

Setting: Emergency department and pediatric intensive care unit at a single academic children's hospital.

Patients: Consecutive patients with ≥1 episode of hypofibrinogenemia (serum fibrinogen <150mg/dL) within seven days of sepsis were compared to a random sample of patients without hypofibrinogenemia using an *a priori* sample size target of 190. Thirty-eight patients with hypofibrinogenemia were compared to 154 without hypofibrinogenemia.

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Interventions: None

Measurements and Main Results: The primary outcome was “complicated course” (composite of 28-day mortality or ≥2 organ failures at seven days). Secondary outcomes were 28-day mortality and fulfillment of diagnostic criteria for sHLH/MAS. We used Wilcoxon rank-sum, Fisher’s exact test, and multivariable logistic regression to compare patients with versus without hypofibrinogenemia. Patients with hypofibrinogenemia were more likely to have a complicated course (73.7% vs 29.2%, p<0.001), 28-day mortality (26.3% vs 7.1%, p=0.002), and meet diagnostic criteria for sHLH/MAS (21.1% vs 1.3%, p<0.001). After controlling for confounders, hypofibrinogenemia remained associated with complicated course (aOR 8.8, 95% CI 3.5, 22.4), mortality (aOR 6.0, 95% CI 2.0, 18.1), and sHLH/MAS (aOR 27.6, 95% CI 4.4, 173).

Conclusions: Hypofibrinogenemia was independently associated with poor outcome and sHLH/MAS in pediatric sepsis. Measurement of fibrinogen may provide a pragmatic biomarker to identify children with possible sepsis/sHLH/MAS overlap for whom further diagnostic testing and consideration of adjunctive immunomodulatory therapies should be considered.

Keywords

sepsis; pediatrics; hemophagocytic lymphohistiocytosis; reactive hemophagocytic syndrome

INTRODUCTION

Pediatric sepsis remains a significant cause of morbidity, mortality, and healthcare costs (1). Despite efforts to risk-stratify pediatric patients with severe sepsis, timely discrimination of those at highest risk for adverse outcomes who may benefit from adjunctive therapies remains limited (2,3). Clinically accessible biomarkers are needed to identify patient populations most likely to benefit from targeted therapies.

Increasing evidence supports that sepsis-associated multiple organ dysfunction syndrome (MODS) lays within a spectrum of a disordered host immuno-inflammatory response to infection that includes secondary hemophagocytic lymphohistiocytosis (sHLH) and macrophage activation syndrome (MAS) (4). Several prior reports demonstrate that sHLH is an under-recognized etiology of critical illness in children that portends a poor prognosis, especially when recognition and therapy are delayed (5,6,7). While primary HLH is caused by genetic defects in perforin and related cytotoxic granule dysfunction, sHLH and MAS are characterized by a failure of natural killer (NK) cell downregulation leading to a feed-forward progression of unchecked inflammation in response to infectious or rheumatologic triggers (8,9). Diagnostic criteria for sHLH and MAS overlap with clinical and laboratory features of sepsis-associated MODS, and the sepsis/sHLH/MAS phenotype may be a particularly high-risk group (10,11,12,13,14). While treatment for primary HLH requires intensive immunosuppression with possible bone marrow transplantation, sepsis/sHLH/MAS overlap has been successfully treated with plasma exchange, corticosteroids, intravenous immunoglobulin (IVIG), and anakinra (15,16,17,18,19,20). Such reports suggest that earlier identification of patients with sepsis/sHLH/MAS overlap may further optimize use of these adjunctive therapies.

The diagnosis of sHLH/MAS requires five of eight criteria, several of which usually involve consultation with reference laboratories than can delay diagnosis. However, routinely available laboratory tests in sepsis can raise suspicion for sepsis/sHLH/MAS overlap, including hyperferritinemia and hypofibrinogemia. Ferritin can be elevated in both HLH/MAS and sepsis (21), and as such may be less useful to raise clinical concern. Moreover, severe hyperferritinemia >10,000 μg/L that is typical in primary HLH is uncommon in sepsis/sHLH/MAS overlap (14). Hypofibrinogenemia, on the other hand, is a shared feature of sepsis and sHLH/MAS, and plasma fibrinogen ≤150 mg/dL has the additional benefit of association with poor prognosis in patients with sHLH/MAS (22,23). Unlike ferritin, serum fibrinogen is also a laboratory test that is widely and routinely measured in sepsis. Therefore, hypofibrinogenemia may have utility to raise suspicion for sepsis/sHLH/MAS overlap. To date, there are no studies specifically evaluating fibrinogen levels and outcome in pediatric sepsis, and no data on the utility of hypofibrinogenemia as a screening biomarker for sepsis/sHLH/MAS overlap. We therefore sought to test the hypothesis that hypofibrinogenemia is associated with poor clinical outcomes and fulfillment of criteria for sHLH/MAS in pediatric severe sepsis.

METHODS

Study Design and Population

We conducted a retrospective observational cohort study at a large university-affiliated pediatric hospital. The study was approved with waiver of consent by the Institutional Review Board (IRB) at the Children's Hospital of Philadelphia. All patients treated in the emergency department (ED) or pediatric intensive care unit (PICU) for severe sepsis or septic shock between January 1, 2012 and December 31, 2014 were eligible for inclusion. Severe sepsis and septic shock were defined using published consensus criteria (3). Severe sepsis was defined as 1) ≥ 2 age-based systemic inflammatory response syndrome criteria, 2) confirmed or suspected invasive infection, and 3) cardiovascular dysfunction, acute respiratory distress syndrome, or ≥ 2 organ system dysfunctions. Septic shock was defined as the subset of patients with cardiovascular dysfunction (24). Consecutive patients were identified through a systematic screening process in place in the ED and PICU during the study period. Three investigators (SLW, JCF, FB) reviewed each positive screen to ensure that patients met consensus criteria for severe sepsis or septic shock. Confirmed cases of severe sepsis were then entered into an IRB-approved institutional registry from which we queried for the following specific inclusion criteria for this study: 1) age ≤21 years on day of sepsis recognition and 2) at least one serum fibrinogen measured within two days before through seven days after sepsis recognition. Patients for whom serum fibrinogen was not measured at least once in this time window were excluded from the primary analysis but were retained for a secondary analysis of external validity. Patients with a known diagnosis of primary HLH were also excluded.

The hypofibrinogenemia group included all patients with at least one fibrinogen level ≤150mg/dl within two days before through seven days following sepsis recognition. The non-hypofibrinogenemia control group included patients randomly selected from the sepsis registry who had fibrinogen measured but without documented hypofibrinogenemia during

the same time window. In addition, patients were further divided into very low (50–100 mg/dL), low (101–150 mg/dL), normal (using laboratory reference range, 151–471 mg/dL), and high (>471 mg/dL) fibrinogen groups based on the lowest fibrinogen level during the specified time window.

Data Collection

Data were abstracted from our institutional sepsis registry and the electronic medical record for patient demographics, comorbid conditions, date and time of sepsis recognition, timing and severity of organ dysfunction, laboratory results, antimicrobial administration, requirement for use of invasive therapies including mechanical ventilation, vasoactive infusions, and extracorporeal membrane oxygenation, hospital and PICU length of stay, and vital status at hospital and PICU discharge. Sepsis recognition was defined as triage time for patients presenting to the ED or either PICU transfer time or first “sepsis-related” intervention for patients initially treated outside the ED. “Sepsis-related” intervention included a physician order for antibiotics, blood culture, or intravenous fluid bolus, whichever came first (25). Infectious etiology was abstracted using direct medical review of microbiologic studies including cultures, viral assays, and documentation of infection. To account for possible inaccuracies in timing of sepsis recognition using this algorithm, we included fibrinogen levels measured in the two days preceding the recorded time of sepsis recognition. The pediatric complex chronic conditions scheme was used to categorize comorbid conditions (26). Illness severity was quantified using the Pediatric Risk of Mortality (PRISM)-III, Pediatric Index of Mortality (PIM)-2, and the Pediatric Logistic Organ Dysfunction (PELOD) scores (27,28,29). Because fibrinogen is one criteria for disseminated intravascular coagulation (DIC), we also classified each patient using the International Society on Thrombosis and Hemostasis (ISTH) scoring system. Variables included in this score are platelet count, fibrin-related markers, prothrombin time, and fibrinogen, with overt DIC defined as score ≥ 5 (30).

To ascertain possible selection bias in the clinician decision to measure fibrinogen, we abstracted key demographic and clinical variables from patients who did not have fibrinogen levels recorded within 28 days of sepsis recognition, including age, sex, race, hospital and PICU length of stay, discharge vital status, comorbid conditions, and illness severity scores.

Outcomes

The primary outcome measure was all-cause hospital mortality at 28 days following sepsis recognition. Secondary outcomes were complicated course and fulfillment of criteria for sHLH/MAS. Complicated course is a composite endpoint defined as either 1) death within 28 days or 2) persistence of two or more new organ dysfunctions at seven days following recognition of severe sepsis/septic shock (31,32,33). Complicated course combines clinically relevant morbidity and mortality endpoints that account for prolonged resource utilization and has been utilized in several studies of pediatric sepsis. A patient was determined to meet diagnostic criteria for sHLH/MAS if at least five of eight criteria from the HLH-2004 consensus guidelines were present within 28 days of sepsis recognition: 1) fever, 2) splenomegaly (documented in physical exam or radiographically), 3) cytopenias affecting ≥2 of three hematopoietic lineages, 4) hypertriglyceridemia and/or hypofibrinogenemia, 5)

hemophagocytosis in bone marrow or spleen or lymph nodes, 6) low or absent NK-cell activity, 7) ferritin ≥500 ug/L, and 8) elevated soluble interleukin 2 (IL-2) receptor level. When data were not available for these eight criteria, we conservatively coded missing data as normal. Since hypofibrinogenemia was the primary independent variable studied and is included in the diagnostic criteria for sHLH/MAS, we also performed a sensitivity analysis in which fibrinogen was excluded in the outcome assessment for sHLH/MAS.

Sample Size

A total enrollment of 38 exposed patients with hypofibrinogenemia ≤150 mg/dL and 154 unexposed controls without hypofibrinogenemia was calculated to provide 80% power to detect an absolute difference in complicated course of at least 20% between patients with versus without hypofibrinogenemia with a standard type I error rate of 0.05.

Statistical Analysis

Statistical analyses were performed using STATA 14.0 (StataCorp, College Station, TX). Baseline and demographic characteristics were summarized using medians with interquartile range (IQR) for continuous variables and frequencies with percentages for categorical variables. Differences between groups were analyzed using the Wilcoxon rank sum, chi-square, and Fisher's exact tests, where appropriate. Clinical variables that were significantly different at the $p<0.05$ level between the hypofibrinogenemia and non-hypofibrinogenemia groups were entered into separate bivariable logistic regression models to identify potential confounders. Potential confounders were individually included as covariates in a model with hypofibrinogenemia as the independent variable and mortality as the outcome. Only those covariates that changed the base model odds ratio (OR) by 10% or greater were considered to be true confounders and retained within the final model (34). Both unadjusted and adjusted ORs with 95% confidence intervals (CI) are presented. The area under the receiver operating characteristic (AUROC) curve was calculated using minimum fibrinogen measured within two days before through seven days after sepsis recognition for each clinical outcome, along with the test characteristics for the optimal fibrinogen cutpoint. In order to differentiate the potential utility of hypofibrinogenemia as a biomarker of poor outcomes compared to coagulopathy in general, we compared the association of fibrinogen with International Normalized Ratio (INR) and platelet count using Spearman's rank correlation, as well as the relative strength of association of between hypofibrinogenemia and an elevated INR ≥1.5 and thrombocytopenia (defined as platelets <100 thous/μL) without outcomes. Statistical significance was defined as $p<0.05$.

RESULTS

Patient Characteristics:

One hundred ninety-two patients were included in the final analysis—including 38 with hypofibrinogenemia and 154 controls without hypofibrinogenemia. The 154 controls without hypofibrinogenemia were randomly selected from 311 potential patients who met all inclusion criteria but had no documented hypofibrinogenemia within the allotted time window. Patient characteristics are presented in Table 1. Notably, patients with hypofibrinogenemia were more likely to be Caucasian or Asian race and have a

gastrointestinal/hepatic comorbidity. PIM2 risk of mortality, but not PRISM-III score, was also slightly higher in the hypofibrinogenemia group. Overall, all-cause 28-day hospital mortality was 11%.

The primary infectious etiology was identified in 68% of patients, with the remainder having no identified microbial pathogen ("culture-negative sepsis"). The infectious etiology differed significantly between groups, with fungal infection and culture-negative sepsis more common in patients with versus without hypofibrinogenemia (8% vs 1% and 53% vs 36%, respectively; Table 2).

Twenty-two (58%) of the hypofibrinogenemia patients had their lowest fibrinogen level within two days of sepsis recognition, though a total of 26 (68%) met criteria for hypofibrinogenemia in this initial time window. Other laboratory parameters, including liver function tests, are shown in Table 3. Patients with hypofibrinogenemia were more likely to have alterations of liver function and coagulation studies. Sufficient laboratory data were available from 37 (19%) patients to calculate a DIC score. Overt DIC was more common the hypofibrinogenemia group (Table 3). Patients with hypofibrinogenemia were also more likely to have an elevated INR, with INR ≥1.5 on day of sepsis recognition in 58% (22/38) versus only 27% (41/154) in the non-hypofibrinogenemic controls ($p<0.001$). Results were similar when substituting elevated PTT for INR (data not shown). Initial and minimum fibrinogen level were also strongly correlated with INR (Spearman's rho= −0.36, $p<0.001$ and rho= −0.37, $p>0.001$, respectively). However, initial and minimum fibrinogen levels were not associated with platelet count on day of sepsis recognition (Spearman's rho=0.02, p=0.85 and rho=0.06, 0=0.54, respectively).

Outcomes:

Clinical outcomes by study group are shown in Figure 1. The hypofibrinogenemia group was more likely to have a complicated course (73.7% vs 29.2%, $p<0.001$), 28-day hospital mortality (26.3% vs 7.1%, p=0.002), and to meet diagnostic criteria for sHLH/MAS (21.1% vs 1.3%, $p<0.001$). When fibrinogen was excluded in the outcome assessment for sHLH/MAS, two patients no longer met criteria for sHLH/MAS. However, the proportion with sHLH/MAS continued to be higher in the hypofibrinogenemia group than controls (15.8% vs 1.3%, $p<0.001$). After controlling for age, race, gastrointestinal/hepatic comorbidity, and infectious etiology, hypofibrinogenemia remained associated with complicated course (aOR 8.8, 95% CI 3.5, 22.4), mortality (aOR 6.0, 95% CI 2.0, 18.1), and sHLH/MAS (aOR 27.6, 95% CI 4.4, 173). Outcomes by category of fibrinogen level are shown in Table 4 with no statistical differences between the two lowest groups or between the two highest groups supporting a clinically useful fibrinogen cut-point of 150 mg/dL. When analyzed as continuous variable, fibrinogen yielded an AUROC of 0.65 (95% CI 0.56, 0.74) for complicated course, 0.68 (0.54, 0.83) for 28-day hospital mortality, and 0.81 (CI 0.63, 0.99) to meet diagnostic criteria for sHLH/MAS. The optimal fibrinogen cutpoint for sHLH/MAS course was ≤130 mg/dL, yielding 80% sensitivity, 90% specificity, 31% positive predictive value, and 99% negative predictive value for sHLH/MAS (see supplemental Table for additional outcomes).

Clinical details of the ten patients who met criteria for sHLH/MAS can be found in Table 5. Two of the 10 patients did not have hypofibrinogenemia. Of the patients who met sHLH/MAS criteria, seven had clinical suspicion for sHLH/MAS and six received therapy for this diagnosis. These therapies included plasma exchange, intravenous immunoglobulin, high-dose systemic steroids (methylprednisolone or dexamethasone), and/or etoposide. Three expired, one of whom died before therapy could be initiated.

Although fibrinogen and INR were inversely correlated, there was only a non-significant trend toward an increase in complicated course in those with versus without an elevated INR ≥1.5 (46% versus 34%, p=0.12). In addition, when substituted for hypofibrinongemia, INR ≥1.5 was not associated with complicated course in an adjusted multivariable model (aOR 1.5, 95% CI 0.8, 2.9; p=0.22). For the 57 patients with discordance between fibrinogen and INR (i.e., fibrinogen ≤150 mg/dL but INR <1.5 or fibrinogen >150 mg/dL but INR ≥1.5), 88% (14/16) of those with hypofibrinogenemia but normal INR had a complicated course compared to 37% (15/41) of those with elevated INR but no hypofibrinogenemia (p=0.001). Finally, patients with hypofibrinogenemia but normal INR had an adjusted OR of 25.1 (95% CI 5.0–127; p<0.001) for complicated course compared to an adjusted OR of 1.5 (95% CI 0.7, 3.4; 0.31) for patients with elevated INR but no hypofibrinogenemia. Similarly, for the 74 patients with discordance between fibrinogen and platelet count (i.e., fibrinogen ≤150 mg/dL but platelets≥100 thous/μL or fibrinogen >150 mg/dL but platelets<100 thous/μL), 65% (13/20) of those with isolated hypofibrinogenemia had a complicated course compared to 41% (22/32) of those with thrombocytopenia but no hypofibrinogenemia (aOR 8.4, 95% CI 2.6, 26.9 versus aOR 2.4, 95% CI 1.1, 5.2). Similar results were found in comparing hypofibrinogenemia to INR ≥1.5 and platelets <100 thou/μL to sHLH/MAS (data not shown).

External Validation:

An additional 339 patients without any measured fibrinogen levels were available in the sepsis registry during the study time period. When compared to the 192 study patients in whom fibrinogen was measured and included in the primary analysis, patients without measured fibrinogen levels were younger (median age 4.6 vs 7.9 years, p = 0.003) and overall exhibited a less acutely ill phenotype (Supplemental Table 1).

DISCUSSION

In this retrospective study of pediatric patients with severe sepsis, hypofibrinogenemia ≤150mg/dL was significantly associated with complicated course, all-cause 28-day hospital mortality, and fulfillment of sHLH/MAS criteria. Moreover, two-thirds of patients exhibited hypofibrinogenemia within two days of sepsis recognition. These data suggest that fibrinogen may provide a timely and pragmatic biomarker to risk-stratify pediatric patients with severe sepsis. In particular, hypofibrinogenemia present within seven days following sepsis recognition should raise concern for possible sepsis/HLH/MAS overlap for whom further diagnostic testing could be considered. Although we had *a priori* selected a fibrinogen cutpoint of ≤150 mg/dL based on published values (8), our data suggest that

a slightly lower cutpoint of ≤130 mg/dL may be most useful to screen for sHLH/MAS overlap in pediatric sepsis.

Fibrinogen is a large soluble glycoprotein that is converted by thrombin into fibrin, the polymerization of which is essential to coagulation. Circulating fibrinogen can be consumed with systemic activation of the coagulation cascade, and patients with sepsis who develop disseminated intravascular coagulation (DIC) are known to have worse prognosis (35,36). However, while fibrinogen <100mg/dL remains a laboratory parameter on the ISTH scoring system for DIC (30), low fibrinogen is actually a rare finding in DIC (37). Hypofibrinogenemia was also indicative of adverse outcomes beyond coagulopathy as indicated by its persistent association with complicated course and sHLH/MAS in the subset of patients with normal INR and platelets >100 thou/μL. The observed higher rate of complicated course in patients with hypofibrinogenemia and normal INR (or normal PTT) versus normal fibrinogen but elevated INR (or elevated PTT) suggests that hypofibrinogenemia may be a better marker of risk for prolonged MODS or death than an elevated INR or aPTT alone. This may be because hypofibrinogenemia indicates a subgroup with, or on the pathway towards, a more severe sepsis phenotype that includes features of sHLH/MAS.

In HLH, hypofibrinogenemia may be attributable to concurrent DIC or alternative mechanisms. For example, in primary HLH, uptake of fibrin and/or fibrinogen by activated histiocytes can lead to hypofibrinogenemia (38). Thus, while hypofibrinogenemia does occur in sepsis-induced DIC, this finding may also provide early evidence for sepsis/sHLH/MAS overlap. Other laboratory features of DIC, including thrombocytopenia and elevated INR, were more common in patients with hypofibrinogenemia, but so too were criteria of possible sHLH/MAS overlap suggesting that, while sHLH/MAS can indeed co-occur, it may not always be appropriate to attribute low fibrinogen levels solely to DIC. For example, in a recent subgroup analysis of adult sepsis patients who met criteria for sHLH/MAS with hepatobiliary dysfunction plus DIC, treatment with anakinra improved survival compared to no effect for patients with either hepatobiliary dysfunction only, DIC only, or neither (39). Similar subgroup analyses of pediatric sepsis patients could help to identify the associations of hypofibrinogenemia, isolated DIC, sHLH/MAS and outcomes in pediatric sepsis. For example, Carcillo et al. found that 11% of pediatric sepsis cases met abbreviated criteria for MAS in whom mortality was 64% compared to 1% for those without MAS (14).

While implementation of consensus guidelines have improved overall mortality through early recognition and initial treatment of sepsis, there have been only modest advancements in our ability to stratify patients at high risk for morbidity and mortality or to select subgroups of patients who may benefit from targeted therapies. The identification of patients with sHLH/MAS overlap may allow for such prognostic and predictive enrichment in pediatric sepsis (40). The diagnostic criteria for sHLH/MAS are largely nonspecific, but also include invasive tests (hemophagocytosis in marrow or liver/spleen), or expensive, uncommon or slow-resulting tests (serum ferritin, soluble interleukin 2 receptor activity, NK cell activity). However, we show that fibrinogen may serve as a more pragmatic biomarker to identify those patients at-risk for sHLH/MAS overlap. Early identification

of these patients may allow for confirmatory testing in a timely manner and prompt earlier consideration of alternative therapeutic strategies, including immunotherapies.

We note several limitations of our study. First, due to the single center retrospective nature of the study at a large tertiary academic center, the results may not be generalizable to a broader pediatric intensive care population. Thirty percent of the patients treated for severe sepsis in our pediatric ICU came from referring institutions. This may also increase the overall acuity, and thus the proportion of patients with poor outcomes, including those with sHLH/MAS phenotypes of severe illness. However, PRISM-III and PIM-2 risk of mortality were similar to prior studies in pediatric sepsis. It is also not clear why PIM-2 was slightly higher in the hypofibrinogenemia group, while PRISM-III was not, though the inclusion of different variables in each score and timeframe for score measure may underlie this finding (e.g., PT and platelet included in PRISM-III but not PIM-2, and PRISM-III may reflect some early PICU therapies/interventions). Second, the lowest fibrinogen levels measured for each patient may not have captured the patients' true nadir which could have led to misclassification bias. Moreover, it also possible that some additional patients may have met criteria for sHLH/MAS had the diagnosis been considered and increased laboratory data been obtained. Third, as ferritin was not commonly measured in our cohort, we are not able to directly compare the utility of ferritin to fibrinogen (as we were for INR and platelets). However, because fibrinogen is more routinely measured in sepsis than ferritin, hypofibrinogenemia could prompt consideration of sHLH/MAS in patients for whom the clinicians may not have thought to consider this spectrum of disease. Finally, the external validation group exhibited a less critically ill phenotype with significantly fewer patients requiring ICU stay, with shorter durations of illness, decreased mortality, and lower severity of illness scores reflecting a relative selection bias in this retrospective study due to the common practice to obtain broader laboratory studies on patients who are more severely ill. However, less severely ill patients should be less likely to have hypofibrinogenemia or to have sepsis/sHLH/MAS overlap. Nonetheless, our data should be considered as "hypothesis-generating" and require validation in a prospective study in which fibrinogen is measured in all patients at similar timepoints.

CONCLUSIONS

Hypofibrinogenemia, often within the first two days of sepsis recognition, was independently associated with complicated course, 28-day all-cause hospital mortality, and sHLH/MAS in pediatric severe sepsis and septic shock. Fibrinogen levels ≤150 mg/dL in children with severe sepsis may help identify a high-risk subset of patients with a spectrum of disease including sHLH/MAS who may benefit from further diagnostic testing and, potentially, earlier immunomodulatory therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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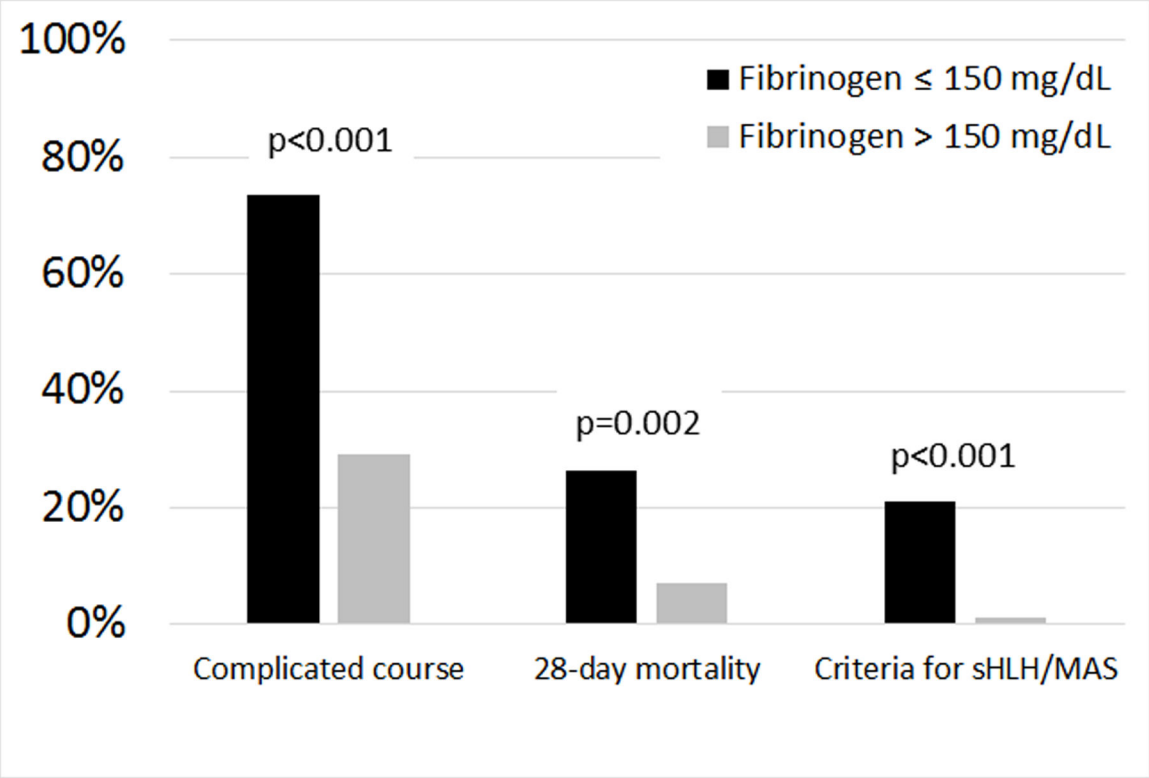

Figure 1:
Outcomes by minimum fibrinogen level. Patients with hypofibrinogenemia were more likely to have a complicated course (73.7% vs 29.2%, p<0.001), 28-day mortality (26.3% vs 7.1%, p=0.002), and meet diagnostic criteria for sHLH/MAS (21.1% vs 1.3%, p<0.001).

Table 1.

Patient Characteristics

Characteristic[a]	Fibrinogen ≤150 mg/dL	Fibrinogen >150 mg/dL	P-value
N	38	154	
Age, years	7.7 (1.2–10.5)	7.9 (2.9–14.3)	0.06
Male sex, n (%)	16 (42)	80 (52)	0.37
Race, n (%)			0.04
Caucasian	21 (55)	71 (46)	
African American	6 (16)	43 (28)	
Asian	4 (11)	3 (2)	
Unknown or Not reported	7 (18)	37 (24)	
PICU admission, n (%)	38 (100)	146 (94)	0.21
Comorbidity, n (%)			
Malignancy	10 (26)	24 (16)	0.15
Hematology-Immunology	0	13 (8)	0.05
Respiratory	0	3 (2)	0.99
Gastrointestinal/Hepatic	5 (13)	2 (1)	0.004
Metabolic	4 (11)	8 (5)	0.26
Neuromuscular	3 (8)	23 (15)	0.30
Cardiovascular	2 (5)	14 (9)	0.74
Renal	0	7 (5)	0.35
Severity of illness			
PRISM III score	10 (5–21)	10 (5–15)	0.33
PIM-2 risk of mortality (%)	4.3 (2.7–8.2)	3.05 (1.0–5.1)	0.009
Resuscitation goals			
Blood culture prior to antibiotics, n (%)	26 (68)	123 (80)	0.13
Antibiotics < 60 minutes, n (%)[b]	8 (21)	55 (36)	0.12
Invasive support requirement			
Vasoactive infusion, n (%)	29 (76)	99 (64)	0.18
Vasoactive days, when required	6 (4–11)	3 (2–6)	<0.001
Invasive mechanical ventilation, n (%)	29 (76)	85 (55)	0.03
Ventilator days, when required	10 (5–17)	8 (4–15)	0.21
ECMO, n (%)	5 (13)	2 (1)	0.004
Transfusions, n (%)			
pRBC, 1 or more transfusions	34 (89)	77 (50)	<0.001
FFP, 1 or more transfusions	32 (84)	32 (21)	<0.001
Cryoprecipitate, 1 or more transfusions	11 (29)	0	<0.001

[a] Data presented as median (IQR) unless otherwise noted

[b] Data exclude 50 patients who received initial therapy at a community hospital

PICU, pediatric intensive care unit; PRISM, pediatric risk of mortality; PIM; pediatric index of mortality; ECMO, extracorporeal membrane oxygenation; pRBC, packed red blood cells, FFP, fresh frozen plasma

Table 2.

Infectious Etiology

Primary Infectious Etiology, n (%)	Fibrinogen ≤150 mg/dL	Fibrinogen >150 mg/dL
Bacterial	11 (29)	64 (42)
Viral	4 (11)	32 (21)
Fungal	3 (8)	2 (1)
None identified	20 (53)	56 (36)

P-value 0.02 for distribution of infectious etiologies between groups

Table 3.

Laboratory Findings on Day of Sepsis Recognition

Characteristic	Fibrinogen ≤150 mg/dL		Fibrinogen >150 mg/dL		P-value
Laboratory values	**N**	**Median (IQR)**	**N**	**Median (IQR**	
Fibrinogen	38	121 (80–136)	154	325 (246–445)	
WBC count (thous/uL)	38	8.7 (3.4–20.6)	154	9.2 (31.−15.8)	0.51
Platelets (thous/uL)	38	120 (34–234)	154	165 (59–273)	0.19
C-reactive protein (mg/dL)	25	3.3 (1.5–5.3)	108	7.1 (2.3–18.6)	0.046
Total bilirubin (mg/dL)	35	1 (0.5–2.1)	145	0.6 (0.4–1.1)	0.01
Alanine aminotransferase (IU/L)	35	59 (38–142)	144	39 (27–67)	<0.001
International normalized ratio	36	1.6 (1.3–1.8)	144	1.3 (1.2–1.4)	<0.001
Prothrombin time (sec)	33	18.7 (15.7–21.3)	144	15.8 (14.6–17.4)	<0.001
Partial thromboplastin time (sec)	33	38.1 (33.0–42.4)	144	34.1 (29.6–40.1)	0.008
DIC categorization	**n (%)**		**n (%)**		
Overt DIC (Score ≥ 5)	12 (32)		11 (7)		0.001
Score < 5	2 (5)		12 (8)		
Unable to be determined	24 (63)		131 (85)		

IQR, interquartile range; WBC, white blood cell; DIC, disseminated intravascular coagulation

Table 4.

Outcomes by Fibrinogen Level

Outcome, n (%)	Minimum Fibrinogen (mg/dL)				
	≤100	101–150[a]	151–471	≥472[b]	P-value[c]
	(n=14)	(n=24)	(n=124)	(n=30)	
Complicated Course	12 (86)	16 (67)	35 (28)	10 (33)	< 0.001
28-day Mortality	4 (29)	6 (25)	8 (7)	3 (10)	0.007
sHLH/MAS criteria	3 (21)	5 (21)	1 (1)	1 (3)	< 0.001

sHLH – secondary hemophagocytic lymphohistiocytosis, MAS – macrophage activation syndrome

[a]For pair-wise comparison with fibrinogen ≤100 mg/dL group, p-values were >0.05 for all outcomes.

[b]For pair-wise comparison with fibrinogen 151–471 mg/dL group, p-values were >0.05 for all outcomes.

[c]P-value presented is comparison across all fibrinogen groups.

Table 5.

Clinical Details of Subjects Meeting HLH Criteria

ID	Fever	Max Ferritin[a] (ng/mL)	Max Triglyceride[a] (mg/dL)	Min Fibrinogen (mg/dL)	Splenomegaly	Cytopenias	↑sIL2r	↓NK Cell	Hemophago-cytosis	28-day mortality
6	+	45529	562	56	+	+	n/a	n/a	n/a	Alive
12	+	3613	276	106	+	+	+	+	n/a	Deceased
13	**+**	**1141**	**186**	**120**	**−**	**+**	**+**	**−**	**n/a**	**Alive**
14	+	84226	3813	114	+	+	+	n/a	+	Deceased
16	+	3137	248	130	+	+	n/a	n/a	+[b]	Deceased
23	**+**	**5720**	**452**	**76**	**−**	**+**	**n/a**	**n/a**	**n/a**	**Alive**
34	+	11400	785	113	+	+	+	–	n/a	Alive
36	+	59000	146	95	+	+	+	–	+	Alive
153	+	9056	362	658	–	+	+	–	+	Alive
230	+	4090	722	165	+	+	+	+	n/a	Alive

[a]2004 HLH Criteria include hypofibrinogenemia ≤ 150 mg/dL and/or triglyceride ≥ 265 mg/dL **Bold:** would not meet criteria if fibrinogen eliminated

Shaded: fibrinogen ≥ 150 mg/dL

[b]Hemophagocytosis in bone marrow at autopsy

sIL2r, soluble interleukin 2 receptor; NK, natural killer