The prognostic role of plasma fibrinogen in adult secondary hemophagocytic lymphohistiocytosis

Guangli Yin  
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital

Changfeng Man  
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital

Shengen Liao  
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital

Hon'gxia Qiu  (qhx9805@126.com)  
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital

https://orcid.org/0000-0002-6348-4888

Research

Keywords: hemophagocytic lymphohistiocytosis; fibrinogen; prognostic; nonlinear; mortality

DOI: https://doi.org/10.21203/rs.3.rs-40436/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Background:** Coagulation abnormality is common in hemophagocytic lymphohistiocytosis (HLH), but the more frequently reported anomaly is an isolated decrease in fibrinogen (FIB). Nonetheless, the prognostic value of FIB in adult secondary HLH (sHLH) remains uncertain.

**Methods:** Circulating plasma levels of FIB were measured at diagnosis in 293 cases of adult sHLH. We categorized FIB levels into tertiles. Multivariable Cox proportional hazards models were conducted to evaluate the relationship between FIB and mortality. Restricted cubic spline models and two-piecewise Cox proportional hazards models were used to address the nonlinear association between FIB and mortality.

**Results:** During a median follow-up of 52 (interquartile ranges, 18-221) days, 208 deaths occurred, with 137 deaths in MHLH and 71 deaths in non-MHLH. After multivariable adjustment, compared to the highest tertile of FIB, the hazard ratios (HRs) with 95% confidence intervals (CIs) of mortality for tertile 2 and tertile 1 were 0.88 (0.61-1.26) and 1.51 (1.05-2.18), respectively. The restricted cubic spline curve displayed a nonlinear and inverse relationship between FIB and mortality. Furthermore, threshold effect analysis demonstrated that the inflection point for the curve was at a FIB level of 1.76 g/L. The HRs (95% CIs) for mortality were 2.47 (1.52-4.04) and 0.83 (0.64 -1.09) on the left and right side of the inflection point, respectively.

**Conclusions:** These results suggest that plasma fibrinogen is nonlinearly and inversely associated with the risk of mortality in adult secondary hemophagocytic lymphohistiocytosis.

Background

Adult secondary hemophagocytic lymphohistiocytosis (sHLH) without a family history or known genetic predisposition is a condition of pathologic immune dysregulation characterized by uncontrolled activation of cytotoxic T lymphocytes and macrophages producing abnormal hypercytokinemia and inducing severe, sometimes fatal, immune-mediated injury of multiple organ systems [1]. These abnormal cytokines are responsible for variable clinical symptoms and signs such as fever, profound pancytopenia, organomegaly (including lymphadenopathy, hepatomegaly, and splenomegaly), liver dysfunction, hypertriglyceridemia, hyperferritinemia, central nervous system (CNS) symptoms, dermatologic abnormalities, and coagulation disorders that may result in serious morbidity and mortality [2]. Among the previous studies focusing on outcomes, mortality ranged from 20.4–88% depending on the underlying disease and triggering condition, including malignancies, infectious, and autoimmune disorders [3]. Early identification of the severity of adult HLH disease and prognosis would be helpful to enhance the patients’ chances of survival; therefore, improved prognostic indicators are needed.

Coagulation disorders are common in sHLH patients and are reported in up to 60% of patients with sHLH [4]. Fibrinogen (FIB) less than or equal to 1.50 g/L, one of the most frequently reported coagulopathies and the hallmark of HLH, is also a diagnostic criterion in in HLH [5]. Several studies have also
demonstrated that 50–80% of sHLH patients have hypofibrinogenemia [6, 7]. Recently, one pediatric HLH study reported that plasma FIB levels (< 150 mg/dL) at diagnosis may be prognostic measures of inferior survival [8]. Several retrospective studies have reported the prognostic role of plasma fibrinogen in adult in adult sHLH [4, 9]. However, it is important to emphasize that these studies were limited by a relatively small sample size (< 120 patients), reported an association between FIB and in-hospital mortality with no follow-up [4] or no adjusted key confounders factors influencing prognosis for sHLH patients, and varied FIB cut-off points [9, 10]. In addition, no quantitative analyses were performed to address the dose-response relationship between FIB and the risk of mortality, and whether there is a linear or nonlinear relationship between FIB and the risk of mortality is still unknown.

Therefore, it remains currently unclear whether reduced FIB levels are associated with poor survival in adult sHLH, independent of conventional risk factors. Thus, we aimed to explore whether reduced FIB levels independently affected long-term survival in a retrospective cohort study with sHLH.

Methods

Study patients

A total of 293 consecutive patients with a primary diagnosis of adult secondary hemophagocytic lymphohistiocytosis (sHLH) between January 1, 2014, and January 1, 2020, were enrolled. The HLH-2004 criteria developed by Henter and colleagues [11] and the HScore by Fardet L [12] were routinely used to help with the diagnosis of HLH. The inclusion of patients was based on 1) aged ≥ 18 years and 2) patients who fulfilled at least 5 of the 8 criteria proposed by the Histiocyte Society in 2004. Exclusion criteria included previous history of anticoagulant therapy, cirrhosis or other serious liver diseases and lack of follow-up data. Our study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (Clinical Trial: ChiCTR2000032421). The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained to review patient medical records.

Clinical data

Patients’ histories and clinical characteristics, including age, gender, fever, complete blood cell counts (CBC), hepatosplenomegaly, blood biochemical tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), lactate dehydrogenase (LDH), fibrinogen (FIB), ferritin, serum soluble interleukin-2 receptor (sIL-2R, sCD25), and β2-microglobulin (β2-MG), from their medical records were reviewed on admission. Epstein-Barr virus (EBV) was evaluated by both serology and EBV DNA real-time quantitative polymerase chain reaction (RQ-PCR) analysis. The NK-cell cytotoxicity assay is not available at our facility. Bone marrow aspiration and biopsy samples were reviewed at the first diagnosis. Tumor or lymph node biopsy and PET/CT were identified as malignancy-associated hemophagocytic lymphohistiocytosis (MHLH).

Measurement of FIB levels was performed in the clinical laboratory of our hospital following regular procedures and was assayed by the Clauss method with an automatic coagulometer (Sysmex CS5100,
Japan). FIB levels were categorized in tertiles according to the distributions of the study population: tertile 1 (T1) ≤ 1.20, tertile 2 (T2) ≤ 1.97 and tertile 3 (T3) > 1.97.

Follow-up and endpoints

The primary outcome of the current study was overall survival (OS). It was calculated as the time in days from sHLH diagnosis to the date of death from any cause or the last follow-up. The survival status of all participants was confirmed with death records or a telephone call to the patient’s relatives and themselves and was ascertained by checking government records of death.

Treatments according to causes of sHLH

Two hundred and seventy-eight patients (95.2%) received a specific treatment for sHLH. In our 169 MHLH patients, 120 patients had received systemic combination chemotherapy as first-line therapy for sHLH; 33 patients were treated according to HLH-94 as initial therapy; 10 patients with progressive multiple organ dysfunction were treated with only intravenous immunoglobulins (IVIg) and glucocorticoid (GC) chemotherapy. In 124 non-MHLH patients, first-line treatment using HLH-94 was administered in 14 patients; GC in 11 patients; GC + IVIg in 46 patients; GC + etoposide in 26 patients; GC + IVIg + cyclosporine in 15 patients; and GS + IVIg + cyclophosphamide in 4 patients. There was no difference in treatment regimens across FIB groups.

Statistical analysis

Continuous variables were presented as the mean with standard deviations (SDs) for normally distributed variables or median with interquartile range (IQR) for non-normally distributed variables, and categorical variables were described as numbers and proportions (percentages). Differences across FIB tertiles were analyzed using one-way ANOVA and the Kruskal-Wallis and chi-square tests, where appropriate. We used univariate and multivariable Cox proportional hazards models to estimate hazard ratios (HRs) and 95% CIs to examine the relationship of fibrinogen concentrations to mortality (variables with P < 0.05 in univariate Cox regression were subsequently entered into the multivariable model). Fibrinogen of T3 was used as a reference to estimate HR and 95% CI. Kaplan-Meier curves for tertiles of fibrinogen were plotted to show mortality rates. Survival curves were adjusted for covariates derived from the final model according to multivariable Cox proportional hazards models.

A restricted cubic spline (with 3 knots located at the 10th, 50th, and 90th percentiles; the number of knots was selected according to Akaike information criterion) was conducted to address the nonlinear relationship between fibrinogen and the risk of mortality after adjusting for confounding factors. If nonlinearity was detected, we calculated the inflection point using a recursive algorithm and then constructed a two-piecewise Cox proportional hazards model on both sides of the inflection point. The threshold level was calculated according to the inflection point with maximum model likelihood using the trial and error method. A log likelihood ratio test comparing the one-line Cox proportional hazards model with a two-piecewise Cox proportional hazards model was performed to examine the statistical significance.
Stratified analyses were conducted according to gender (male, female), age (≤ 60 years, > 60 years), ferritin (≤ 10000ug/L, > 10000ug/L), sCD25 (≤ 20000 ng/L, > 20000 ng/L), EBV status (no EBV infection, EBV infection), and etiologies (MHLH, non-MHLH). We tested for potential effect modification by these stratification variables by including interaction terms between fibrinogen and a potential effect modifier (overall survival) in the multivariate adjusted model and by conducting a likelihood ratio test (LRT) comparing the models with and without interaction terms. Tests for trends were calculated by including the median value for each corresponding tertile as a continuous variable in the models [13]. All statistical analyses were conducted with R software (version 3.6.0; The R Foundation for Statistical Computing) and STATA statistical software (version 14.0; StataCorp, TX, USA). A two-sided $P<0.05$ was considered statistically significant for all analyses.

**Results**

**Distribution of plasma fibrinogen and patient characteristics**

Baseline characteristics for patients stratified by FIB tertiles are listed in Table 1. The mean age of the total study population was 53 (41–64) years and 62.5% were male. The median FIB level in the overall population was 1.50 g/L (IQR 1.02–2.18 g/L). Participants with lower FIB had lower platelet levels, had higher ALT, AST, LDH, TG, $\beta_2$-MG, serum ferritin, sCD25 and total Hscore points and were more likely to be infected with EBV (all $P<0.05$).
|                        | Total (N = 293) | Tertile 1 (N = 99) | Tertile 2 (N = 97) | Tertile 3 (N = 97) | P     |
|------------------------|-----------------|-------------------|-------------------|-------------------|-------|
| FIB, g/L               | 1.50 (1.02–2.18)| 0.86 (0.70–1.03)  | 1.51 (1.38–1.72)  | 2.59 (2.18–3.33)  | < 0.001|
| Male, n (%)            | 183 (62.5)      | 62 (62.6)         | 60 (61.9)         | 61 (62.9)         | 0.988 |
| Age, years             | 53 (41–64)      | 51 (33–64)        | 56 (39–62)        | 55 (46–66)        | 0.118 |
| ANC, × 10⁹/L           | 1.32 (0.69–2.35)| 1.04 (0.57–2.11)  | 1.36 (0.77–2.24)  | 1.55 (0.81–2.71)  | 0.057 |
| HB, g/L                | 86 (71–100)     | 84 (77–95)        | 90 (73–107)       | 82 (68–97)        | 0.111 |
| PLT, × 10⁹/L           | 43 (24–72)      | 31 (18–52)        | 45 (25–80)        | 54 (32–97)        | < 0.001|
| ALT, U/L               | 67.1 (36.3–149.1)| 98.6 (50.6–235.4) | 77.6 (32.4–143.2) | 50.1 (28.4–82.3)  | < 0.001|
| AST, U/L               | 91 (46.1–203.8) | 178.5 (78.1–411.0)| 90.2 (45.1–198.1) | 61.0 (39.5–121.65)| < 0.001|
| LDH, U/L               | 676 (394.0–1308.5)| 1004 (580.0–1793.0)| 646 (354.5–1101.0)| 540 (379.5–934.0) | < 0.001|
| TG, mmol/L             | 2.47 (1.71–3.70)| 2.78 (1.77–4.04)  | 2.52 (1.89–3.67)  | 2.01 (1.58–3.18)  | 0.015 |
| Ferritin, ug/L         | 4739 (1522–12798)| 8133 (2000–20000)| 3639 (1613–9558)  | 2841 (1494–7095)  | < 0.001|
| sCD25, ng/L            | 33480 (17442–51668)| 40042 (22660–55919)| 26920 (14266–48289)| 32939 (16018–47762)| 0.024 |
| β₂-MG, mg/L            | 5.97 (4.41–8.53)| 5.91 (4.19–8.53)  | 7.63 (4.85–9.28)  | 5.69 (3.88–8.04)  | 0.030 |
| Fever, °C              | 39.5 (39.0–40.0)| 39.6 (39.0–40.0)  | 39.4 (39.0–40.0)  | 39.3 (39.0–40.0)  | 0.143 |

FIB, fibrinogen; ANC, absolute neutrophil count; HB, hemoglobin; PLT, platelet; ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; ALB, albumin; TG, triglyceride; sCD25, soluble interleukin-2 receptor; β₂-MG, beta₂-microglobulin; EBV, Epstein-Barr virus; MHLH, malignancy-associated haemophagocytic lymphohistiocytosis; Non-MHLH, non-malignancy associated haemophagocytic lymphohistiocytosis; GC, glucocorticoid; IVIg, intravenous immunoglobulins; CsA, cyclosporine; VP16, etoposide.
|                       | Total (N = 293) | Tertile 1 (N = 99) | Tertile 2 (N = 97) | Tertile 3 (N = 97) | \(P\) |
|-----------------------|-----------------|-------------------|-------------------|-------------------|------|
| Splenomegaly (%)      | 260 (88.7)      | 90 (90.9)         | 88 (90.7)         | 82 (84.5)         | 0.278|
| Lymphadenopathy (%)   | 152 (51.9)      | 45 (45.5)         | 52 (53.6)         | 55 (56.7)         | 0.265|
| Hemophagocytic (%)    | 260 (88.7)      | 88 (88.9)         | 85 (87.6)         | 87 (89.7)         | 0.900|
| HScore, points        | 234 (205–269)   | 255 (224–283)     | 235 (210–274)     | 219 (179–244)     | <0.001|
| EBV infection (%)     | 148 (50.5)      | 65 (65.7)         | 44 (45.4)         | 39 (40.2)         | 0.001|
| Etiology              |                 |                   |                   |                   | 0.120|
| MHLH (%)              | 169 (57.7)      | 65 (65.7)         | 50 (51.5)         | 54 (55.7)         |      |
| Non-MHLH (%)          | 124 (42.3)      | 34 (34.3)         | 47 (48.5)         | 43 (44.3)         |      |
| Treatment             |                 |                   |                   |                   | 0.960|
| Chem ± HLH-94 (%)     | 167 (57.0)      | 58 (58.6)         | 53 (54.6)         | 56 (57.7)         |      |
| GC ± IVIG ± CsA ± VP16 (%) | 112 (38.2) | 36 (36.4)         | 40 (41.2)         | 36 (37.1)         |      |
| Support (%)           | 14 (4.8)        | 5 (5.1)           | 4 (4.1)           | 5 (5.2)           |      |

**Associations of the plasma fibrinogen with overall survival**

The median follow-up duration was 52 (IQR, 18–221) days. During follow-up, there were 208 deaths, including 137 deaths in MHLH and 71 deaths in non-MHLH. Figure 1 shows the survival curves with regard to different categories of FIB in total patients for cumulative overall survival. Patients with T3 and T2 had a similar risk of death \( (P = 0.726) \), while patients with T1 had a significantly greater risk of death \( (HR = 1.89, 95\% CI: 1.36–2.62; P = 0.001) \) than T3. After adjusting for confounding factors, including age, PLT, ferritin, EBV infection, MHLH, and treatment remedies, the HRs with 95% CIs of mortality for T2 and T1 were 0.88 (0.61–1.26) and 1.52 (1.05–2.18), respectively \( (P\) for trend: 0.04) (Table 2 and Fig. 1).
| characteristics                  | Unadjusted          | Adjusted          |
|---------------------------------|---------------------|-------------------|
|                                 | HR (95%CI)          | P                 | HR (95%CI)          | P                 |
| Male                            | 1.47 (1.10–1.96)    | 0.010             | 0.83 (0.50–1.38)    | 0.462             |
| Age, years                      | 1.01 (1.00-1.02)    | 0.009             | 1.01 (1.00-1.02)    | 0.017             |
| ANC > 1.0 × 10⁹/L               | 1.15 (0.87–1.51)    | 0.331             | 1.62 (1.02–2.58)    | 0.043             |
| HB < 90 g/L                     | 1.38 (1.04–1.84)    | 0.027             | 1.62 (1.02–2.58)    | 0.043             |
| PLT < 100 × 10⁹/L               | 1.91 (1.23–2.98)    | 0.004             | 1.62 (1.02–2.58)    | 0.043             |
| LDH ≥ 2.5 × ULN                 | 0.74 (0.47–1.18)    | 0.205             | 0.83 (0.50–1.38)    | 0.462             |
| TG ≥ 3.0 mmol/L                 | 1.18 (0.89–1.56)    | 0.245             | 1.01 (0.99–1.03)    | 0.851             |
| Fever, ≥ 39.1°C                 | 0.96 (0.73–1.27)    | 0.775             | 1.01 (0.99–1.03)    | 0.851             |
| Splenomegaly                    | 1.12 (0.71–1.75)    | 0.636             | 1.01 (0.99–1.03)    | 0.851             |
| Hemophagocytosis                | 0.84 (0.56–1.27)    | 0.403             | 1.01 (0.99–1.03)    | 0.851             |
| Log₁₀(Ferritin)                 | 1.96 (1.47–2.62)    | < 0.001           | 1.75 (1.27–2.41)    | 0.001             |
| Log₁₀(sCD25)                    | 1.83 (1.22–2.73)    | 0.003             | 1.51 (1.05–2.18)    | 0.025             |
| EBV infection                   | 1.78 (1.35–2.34)    | < 0.001           | 1.47 (1.09–1.98)    | 0.011             |
| LHLH                            | 1.79 (1.34–2.38)    | < 0.001           | 2.85 (2.00-4.05)    | < 0.001           |

The associations between FIB (continuously) and the risk of mortality are shown in Fig. 2. The fully adjusted cubic curve fitting showed that FIB was nonlinearly and inversely associated with the risk of death, and tests for nonlinearity were significant (P for nonlinearity < 0.001). We further conducted a
threshold effect analysis of FIB on the risk of mortality. When we fitted the association between FIB and death using a Cox proportional hazards model and a two-piecewise Cox proportional hazards model, the \( P \) value for the log likelihood ratio test was less than 0.001, indicating that the two-piecewise Cox proportional hazards model was more suitable for fitting the association between FIB and the risk of death. The inflection point that we identified for FIB was 1.76 g/L. When FIB was \( \leq 1.76 \) g/L, decreased FIB was significantly associated with an increased risk of poor survival (HR = 2.47, 95% CI, 1.52–4.04; \( P < 0.001 \)). Conversely, when FIB was > 1.76 g/L, the risk of death did not change significantly with increased FIB (HR = 0.83, 95% CI, 0.64–1.09; \( P = 0.187 \)) (Table 3).

| Inflection point | group | HR (95%CI)       | \( P \)-value | \( P \) for log likelihood ratio test |
|------------------|-------|------------------|---------------|-------------------------------------|
| 1.76             | \( \leq 1.76 \) | 2.47 (1.52–4.04) | 0.001         | 0.001                               |
|                  | \( > 1.76 \)    | 0.83 (0.64–1.09) | 0.187         |                                     |

Analyses were adjusted for age, PLT (platelet), EBV, log10-transform ferritin, MHLH and treatment strategies.

Subgroup analysis

The subgroup analyses on inferior survival according to multiple confounding variables are shown in Table 4. Although no significant interactions for the associations between FIB and the risk of death were observed across strata by age, sex, ferritin, sCD25, EBV infection and etiologies, participants who were younger, male, who had ferritin \( \leq 10000 \) µg/L, who had a high level of sCD25, who had EBV infection, and who were non-MHLH were more likely to have a higher risk of death with a decrease in FIB.
Table 4

Stratified associations between fibrinogen and the risk of mortality

| Subgroups                  | Tertile 3 HR (95% CI) | Tertile 2 HR (95% CI) | Tertile 1 HR (95% CI) | P for trend | P for interaction |
|----------------------------|-----------------------|-----------------------|-----------------------|-------------|-------------------|
| Gender                     |                       |                       |                       | 0.800       |                   |
| Male                       | 1.00(Ref)             | 0.95(0.60–1.50)       | 1.67(1.04–2.70)*      | 0.068       |                   |
| Female                     | 1.00(Ref)             | 0.74(0.39–1.41)       | 1.41(0.74–2.71)       | 0.458       |                   |
| Age ≤ 60 years             |                       |                       |                       | 0.478       |                   |
| Yes                        | 1.00(Ref)             | 0.99(0.57–1.71)       | 1.56(0.91–2.70)       | 0.172       |                   |
| No                         | 1.00(Ref)             | 0.76(0.47–1.25)       | 1.26(0.77–2.06)       | 0.480       |                   |
| Ferritin ≥ 10000ug/L       |                       |                       |                       | 0.855       |                   |
| Yes                        | 1.00(Ref)             | 1.25(0.56–2.81)       | 1.30(0.61–2.75)       | 0.499       |                   |
| No                         | 1.00(Ref)             | 0.77(0.51–1.16)       | 1.66(1.08–2.53)*      | 0.140       |                   |
| sCD25 ≥ 20000ug/L          |                       |                       |                       | 0.510       |                   |
| Yes                        | 1.00(Ref)             | 1.18(0.77–1.82)       | 1.64(1.07–2.52)*      | 0.030       |                   |
| No                         | 1.00(Ref)             | 0.40(0.19–0.82)*      | 1.07(0.51–2.23)       | 0.604       |                   |
| EBV infection              |                       |                       |                       | 0.905       |                   |
| Yes                        | 1.00(Ref)             | 1.08(0.65–1.79)       | 1.70(1.03–2.83)*      | 0.057       |                   |
| No                         | 1.00(Ref)             | 0.66(0.39–1.13)       | 1.43(0.83–2.48)       | 0.531       |                   |
| MHLH                       |                       |                       |                       | 0.171       |                   |
| Yes                        | 1.00(Ref)             | 0.88(0.56–1.39)       | 1.26(0.81–1.96)       | 0.378       |                   |
| No                         | 1.00(Ref)             | 0.87(0.47–1.59)       | 2.10(1.11–3.99)*      | 0.080       |                   |

Analyses were adjusted for age, PLT (platelet), EBV, log_{10}-transform ferritin, MHLH and treatment strategies when they were not stratified variables. *P<0.05, **P<0.001
Discussion

To our knowledge, this is the largest cohort study assessing the prognostic role of fibrinogen in adult sHLH. In addition, our study first indicated that plasma fibrinogen level is nonlinearly and inversely associated with poor survival in adult sHLH patients regardless of conventional confounding factors, including age, PLT, EBV infection, ferritin, etiologies and treatment strategies. Further threshold effect analysis showed that the inflection point was 1.76 g/L, and the risk of mortality was inconsistent on the left and right sides of the inflection point.

A few previous studies regarding the association of FIB and its threshold with the risk of mortality defined plasma FIB levels using different categories or cut-off values. In pediatric patients with HLH [8, 14], Signoff demonstrated that hypofibrinogenemia (FIB ≤ 1.5 g/L) was independently associated with higher mortality rates (adjusted odds ratio, 6.0; 95% CI, 2.0–18.1), while in adult sHLH studies, Sandrine et al. used 117 ICU patients and identified that coagulation disorders were associated with higher mortality, especially FIB < 2 g/L (adjusted OR = 2.42, 95% CI 1.08–5.41; \( P = 0.04 \)) [4]. The large sample size of this study, combined with the application of RCS, afforded us greater statistical power and model flexibility to characterize the associations of plasma FIB concentrations with mortality.

The increased risk of mortality associated with low fibrinogen might be ascribed to the activation of lymphocyte and macrophage immune cells, creating an uncontrolled loop of inflammation that is responsible for liver dysfunction, DIC and fibrinolysis. Stimulated macrophages secrete proinflammatory cytokines, including TNF-\( \alpha \), IL-1\( \beta \), and IL-6, which in turn, can release tissue plasminogen activator in excess; correspondingly, an increase in plasmin responsible for fibrinolysis leads to low fibrinogen levels [15, 16]. In addition, high IFN-\( \gamma \), presented in sHLH, can induce the production of tissue factor expression in activated macrophages, and activated histiocytes can activate factor X through Mac-1 receptors, eventually initiating blood coagulation and aberrantly provoking an overconsumption of fibrinogen [17, 18]. Another mechanism could be caused by diffuse liver infiltration with activated T lymphocytes and macrophages and hypercytokinemia [19]. All of the above mechanisms, alone or together, may be attributed to low fibrinogen levels, which reflect low in vivo disease activity. Hypofibrinogenemia was also indicative of adverse outcomes beyond coagulopathy, as indicated by its persistent association with complicated course and sHLH/MAS with hyperinflammation.

Our study explored the nonlinear association between plasma FIB levels and the risk of mortality and indicated that FIB displayed an L-shaped relationship with the risk of mortality. One hypothesis is that the lower fibrinogen level (≤ 1.76 g/L) is only the reflection of a more severe form of HLH (with more intense cytokine storm and intense hemophagocytic activity). These lower fibrinogen patients who have higher mortality may reach a certain threshold of excessive activated immune cells and an overwhelming systemic inflammation. Early identification of these patients may prompt earlier consideration of alternative therapeutic strategies, including intensive immunotherapies.

Our study has several strengths. This is the first study that addressed the nonlinearity between FIB levels at the diagnosis of adult sHLH and the risk of mortality and further explained this nonlinearity with a
threshold effect analysis. However, several limitations of our study were noted. First, due to the single-center retrospective study, the results may not be generalizable to a broader population. Second, although we had fully adjusted a broad set of covariates available to influence prognosis, we could not rule out the possibility that our findings were biased by unmeasured or unrecognized confounders. Finally, the observational study could only demonstrate the association between FIB levels and the prognosis of patients with sHLH but could not provide conclusions for causality.

Conclusion

Our study demonstrated that plasma FIB levels are nonlinearly and inversely associated with poor survival in adult sHLH patients. The threshold effect of FIB on the risk of mortality was 1.76 g/L. Each 1 unit decrease in FIB exhibited a 1.47-fold increase in the risk of mortality when the serum FIB levels were ≤1.76 g/L.

Abbreviations

sHLH, secondary hemophagocytic lymphohistiocytosis; FIB, fibrinogen; ANC, absolute neutrophil count; HB, hemoglobin; PLT, platelet; ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; TG, triglyceride; sCD25, soluble interleukin-2 receptor; β2-MG, beta2-microglobulin; EBV, Epstein-Barr virus; MHLH, malignancy-associated haemophagocytic lymphohistiocytosis; Non-MHLH, non-malignancy associated haemophagocytic lymphohistiocytosis; GC, glucocorticoid; IVIg, intravenous immunoglobulins; CsA, cyclosporine; VP16, etoposide. HR, hazards ratio; 95% CI, 95% confidence interval.

Declarations

Acknowledgements

Authors expressed their gratitude for all persons who were involved in reporting patients and gathering data.

Consent for publication

Consent for publication was obtained from all participants.

Availability of data and material

The data of our patients is available in the department of medical records at Jiangsu Province Hospital and the First Affiliated Hospital of Nanjing Medical University. This data can be released with the agreement of the patients and is available from the corresponding author upon reasonable request.

Author contributions
GLY and HXQ designed the experiment. GLY performed the experiments. GLY, CFM and HXQ organized the clinical materials. GLY and SGL performed the data analysis. GLY wrote the paper. All authors contributed to the final approval of the manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (grant number 81570175).

Ethics approval and consent to participate

Our study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University and registered on the Chinese Clinical Trial Registry (ChiCTR2000032421).

Competing interests

The authors declare that they have no competing interests.

References

1. Al-Samkari H, Berliner N. Hemophagocytic Lymphohistiocytosis. Annu Rev Pathol. 2018,13:27-49.
2. La Rosee P, Horne A, Hines M, von Bahr Greenwood T, Machowicz R, Berliner N, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. Blood. 2019,133:2465-77.
3. Hayden A, Park S, Giustini D, Lee AY, Chen LY. Hemophagocytic syndromes (HPSs) including hemophagocytic lymphohistiocytosis (HLH) in adults: A systematic scoping review. Blood Rev. 2016,30:411-20.
4. Valade S, Azoulay E, Galicier L, Boutboul D, Zafrani L, Stepanian A, et al. Coagulation Disorders and Bleedings in Critically Ill Patients With Hemophagocytic Lymphohistiocytosis. Medicine (Baltimore). 2015,94:e1692.
5. Jordan MB, Allen CE, Greenberg J, Henry M, Hermiston ML, Kumar A, et al. Challenges in the diagnosis of hemophagocytic lymphohistiocytosis: Recommendations from the North American Consortium for Histiocytosis (NACHO). Pediatr Blood Cancer. 2019,66:e27929.
6. Creput C, Galicier L, Buyse S, Azoulay E. Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. Intensive Care Med. 2008,34:1177-87.
7. Ramos-Casals M, Brito-Zeron P, Lopez-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. Lancet. 2014,383:1503-16.
8. Signoff JK, Fitzgerald JC, Teachey DT, Balamuth F, Weiss SL. Hypofibrinogenemia Is Associated With Poor Outcome and Secondary Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome in Pediatric Severe Sepsis. Pediatr Crit Care Med. 2018,19:397-405.
9. Li F, Yang Y, Jin F, Dehoedt C, Rao J, Zhou Y, et al. Clinical characteristics and prognostic factors of adult hemophagocytic syndrome patients: a retrospective study of increasing awareness of a disease from a single-center in China. Orphanet J Rare Dis. 2015,10:20.

10. Park HS, Kim DY, Lee JH, Lee JH, Kim SD, Park YH, et al. Clinical features of adult patients with secondary hemophagocytic lymphohistiocytosis from causes other than lymphoma: an analysis of treatment outcome and prognostic factors. Ann Hematol. 2012,91:897-904.

11. 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. 2007,48:124-31.

12. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol. 2014,66:2613-20.

13. Liao S, Yao W, Cheang I, Tang X, Yin T, Lu X, et al. Association between perfluoroalkyl acids and the prevalence of hypertension among US adults. Ecotoxicol Environ Saf. 2020,196:110589.

14. Lee JY, Kim JH, Lee JS, Ryu JM, Seo JJ, Im HJ. Initial Characteristics and Clinical Severity of Hemophagocytic Lymphohistiocytosis in Pediatric Patients Admitted in the Emergency Department. Pediatr Emerg Care. 2018;10.1097/PEC.0000000000001518.

15. Friesecke S, Stecher SS, Greinacher A. Tranexamic acid for treatment of bleeding in hemophagocytic lymphohistiocytosis. Thromb Res. 2015,135:1037-9.

16. Unkeless JC, Gordon S, Reich E. Secretion of plasminogen activator by stimulated macrophages. J Exp Med. 1974,139:834-50.

17. Kato J, Okamoto T, Motoyama H, Uchiyama R, Kirchhofer D, Van Rooijen N, et al. Interferon-gamma-mediated tissue factor expression contributes to T-cell-mediated hepatitis through induction of hypercoagulation in mice. Hepatology. 2013,57:362-72.

18. Ooe K. Pathogenesis of hypofibrinogenemia in familial hemophagocytic lymphohistiocytosis. Pediatr Pathol. 1991,11:657-61.

19. de Kerguenec C, Hillaire S, Molinie V, Gardin C, Degott C, Erlinger S, et al. Hepatic manifestations of hemophagocytic syndrome: a study of 30 cases. Am J Gastroenterol. 2001,96:852-7.

Figures
Figure 1

Survival curves in all sHLH patients according to fibrinogen tertiles. A, Kaplan-Meier survival curves. B, Adjusted survival curves: curves were adjusted for age, PLT (platelet), EBV, ferritin, malignancy - associated HLH (MHLH) and treatment strategies.
Figure 2

Cubic spline plot of the association between plasma fibrinogen and the risk of mortality among adult onset HLH. The solid line and dashed line represent the estimated hazard ratios and their corresponding 95% CIs. Analyses were adjusted for age, PLT (platelet), EBV, ferritin, malignancy - associated HLH (MHLH) and treatment strategies.