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ORIGINAL ARTICLE

# Prognostic factors in patients with secondary hemophagocytic lymphohistioc ytosis in a Chinese cohort

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## Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory syndrome with high mortality mediated by an unbridled and persistent activation of cytotoxic T lymphocytes and natural killer cells. However, the influence factors of early death in adult sHLH patients are still not fully elucidated, which need further investigating. We have conducted an observational study of adult HLH patients between January 2016 and December 2022. All patients are enrolled according to HLH-2004 criteria. Clinical manifestations, laboratory data, treatments, and outcomes have been recorded. Influence factors associated with prognosis are calculated by using logistic regression models. Overall, 220 patients enrolled in this study. The etiologies of HLH were divided into five groups including autoimmune-associated hemophagocytic syndrome (AAHS) ($n$ = 90, 40.9%), malignancies ($n$ = 73, 33.2%), EBV-HLH ($n$ = 18, 8.2%), infection excluded EBV ($n$ = 24, 10.9%), and other triggers ($n$ = 15, 6.8%). Among them, EBV-HLH had the highest mortality (77.8%), and AAHS had the lowest mortality (14.4%). Multivariate analysis indicated that age (≥ 38 years old), cytopenia ≥ 2 lines, platelets (≤ 50 × 109/L), aspartate aminotransferase (≥ 135U/L), prothrombin time (≥ 14.9 s) and activated partial thromboplastin time (≥ 38.5s), EBV, and fungal infection are independent risk factors for poor prognosis of HLH. Adult HLH patients with elder age, cytopenia ≥ 2 lines, levels of decreased platelets, increased AST, prolonged PT and APTT, EBV, and fungal infection tend to have a poor prognosis.

**Keywords** Hemophagocytic lymphohistiocytosis · Prognostic factors · Cytopenia · Coagulation dysfunction · EBV

## Introduction

Hemophagocytic lymphohistiocytosis (HLH), which is also known as hemophagocytic syndrome (HPS), first reported by pediatricians Scott and Robb-Smith in 1939, is a rare and fatal hyperinflammatory syndrome mediated by an unbridled and persistent activation of cytotoxic T lymphocytes and natural killer (NK) cells [1, 2]. Clinical and laboratory manifestations are characterized as fever, cytopenia, organom egaly (including splenomegaly, lymphadenopathy, hepatomegaly, and pancreas), liver and coagulation dysfunction, hypertriglyceridemia, elevations of acute phase reactants (notably serum ferritin), hemophagocytosis, increased soluble interleukin-2 receptor (sIL2R/sCD25) levels, and absent/decreased NK cell activity [2, 3].

Traditionally, HLH is classified as primary or familial HLH (occurring in the presence of an underlying predisposing genetic defect in immune function) and secondary HLH (sHLH) [2–4]. Primary HLH is mainly reported in children with specific autosomal-recessive mutations about granule-dependent lymphocyte toxicity [2–4]. sHLH is prevailingly observed in adults and can be caused by a variety of triggers, typically malignancies, autoimmune diseases, and infections. Among them, autoimmune-associated hemophagocytic syndrome (AAHS) is regularly denoted as macrophage activation syndrome (MAS) [5, 6]. Recently, mutations related familial HLH genes have been found in about 15% adult HLH patients [2, 7]. Therefore, it is not always possible to make a clear clinical separation between the two types.

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In the past few decades, there has been an increasing awareness to recognize HLH for clinicians due to its high fatality. However, the diagnostic criteria and treatment regimen for HLH are based on research evidence in pediatrics. Only few small research projects provide clinical features and prognostic data on adult HLH patients. Therefore, we conducted a bidirectional cohort study on adult sHLH to investigate the epidemiologic data, underlying triggers, clinical characteristics, and initial therapies, and identify the possible influence factors associated with in-hospital prognosis, in order to facilitate early clinical detection of high-risk critical adult sHLH patients and give prompt treatment.

## Methods

### Study population

This bidirectional observational cohort study was conducted in the Peking University People's Hospital, Beijing, China, and included 230 consecutive patients diagnosed with HLH between January 2016 and December 2023 through the electronic medical record system, and 220 patients enrolled in the end. The following patients were excluded: (1) patients under the age of 18 (2 cases); (2) those clinical data were incomplete (4 cases); (3) after the inquiry of clinical data, patients who were not enough to diagnose HLH (4 cases); and (4) primary HLH (0 case). Our study has been approved by the Peking University People's Hospital ethics committee (No. 2022PH B258-001). The total process of this study has followed the Declaration of Helsinki.

### Definition

Secondary HLH was diagnosed according to HLH-2004 criteria defined by the International Organization Cell Association [4]. Patients who meet at least five of eight following criteria could be diagnosed as HLH: (1) fever; (2) splenomegaly; (3) bicytopenia or pancytopenia (hemoglobin < 90 g/L, platelets < $100 \times 10^9$/L, neutrophil < $1.0 \times 10^9$/L); (4) triglyceride(TG) > 3.0 mmol/L and/or fibrinogen (FI B) < 1.5 g/L; (5) hemophagocytosis in the bone marrow, spleen, or lymph nodes; (6) low or absent activity of NK cells; (7) ferritin ≥ 500 μg/L; and (8) sCD25 ≥ 2400 U/mL.

In this study, cytopenia is defined as bicytopenia or/and pancytopenia; transaminase ≥ 3 fold the normal upper limit is defined as acute liver injury (ALI), and pancreatic involvement includes acute pancreatitis and pancreatic enlargement. Acute kidney injury (AKI) is characterized by an increase in serum creatinine of 0.3 mg/dL within 48 h, an elevation to 1.5-fold the baseline level within the first 7 days, or a decline in urine output to not more than 0.5 mL/kg/h for at least 6 h [8]. Gastrointestinal involvement includes bleeding and/or perforation. The central nervous system involvement of HLH (CNS-HLH) is characterized by neurological and/or psychiatric symptoms (e.g., irritability, meningeal irritation, epilepsy, altered consciousness, convulsions, etc.), CNS imaging abnormalities, and cerebrospinal fluid (CSF) abnormalities [9]. When one or more manifestations are developed, CNS-HLH should be considered.

Patients are divided into the non-early death group and early death group. The non-early death group was the patients with improved clinical condition who could be discharged. The early death group was defined as death in hospital and/or 1 month after discharge.

### Clinical data collection

The medical records were reviewed to obtain comprehensive data on the baseline characteristics using a standardized spreadsheet. The following items were recorded: (1) the etiological distribution of HLH; (2) demographic data (gender, age); (3) clinical symptoms including temperature, arthrodynia, rash, myodynia, and organomegaly; (4) complications mainly including ALI, AKI, digestive tract involvement (bleeding or perforation), hemocytopenia, pancreatic, gastrointestinal and CNC involvement, diffuse alveolar hemorrhage(DAH), and thrombotic microangiopathy (TMA); (5) laboratory data including white blood cell (WBC), hemoglobin, platelet, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, procalcitonin (PCT), liver and kidney function, coagulation function, TG, lactic dehydrogenase (LDH), serum complement levels, hemophagocytosis, sCD25 levels, and NK cell activity. Meanwhile, infection-related indicators included cytomegalovirus (CMV), Epstein-Barr virus (EBV), fungal and blood culture, (6) treatments, and in-hospital outcomes.

### Statistical analysis

The primary analysis compared the non-early death group with the early death group. All variables are tested for a normal distribution through the Kolmogorov-Smirnov test. All descriptive statistics are summarized and displayed as the mean ± standard deviation or the median (25~75%). Continuous variables and normal distribution data are compared using independent sample *t* tests. And continuous variables that are not normally distributed are compared by using the Mann-Whitney *U* test. Categorical data are tested by the chi-square test or Fisher's exact test. $P < 0.05$ is considered to be statistically significant. A logistic regression model is generated using the Enter mode, and the association measures are calculated (adjusted odds ratio) with a confidence interval (CI) of 95%. For development of the logistic regression model, continuous variables were categorized according to the cut-off point on the receiver operating characteristic

curve (ROC curve). Variables determined by logistic regression underwent probit regression to calculate the weight of each variable in the predicting score based on the probit coefficient of each variable. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the agreement; $P > 0.05$ would indicate a good fit for the model. All analyses are performed with SPSS 25.0 software.

## Results

### Baseline characteristics of sHLH patients

Overall, 230 consecutive patients have been screened. Of these, 10 patients are later excluded according to the exclusion criteria and 220 patients enrolled in total. Among these patients, 97 (44.1%) are male and 123 (55.9%) are female, with a median age of 41 (37, 60) years. The clinical characteristics of patients are presented in Tables 1 and 3. Based on 2004 HLH criteria, 214 patients (99.2%) had hyperferritinemia with a median serum ferritin of 8507 ng/mL (median, 2765~29,626 ng/mL). One hundred seventy-nine patients (95.7%) have increased sCD25 levels in the tested 187 patients, with a median 16,009 U/mL (median, 9014~33,190U/mL), and 203 (92.3%) presented a persistent fever of ≥ 38.5 °C. One hundred eighty-six patients (84.6%) developed cytopenia. And splenomegaly presented in 123 patients (55.9%). Decreased NK cell activity is identified in 55.2% patients. At diagnosis, median serum triglyceride was 3.02 mmol/L (range, 2.11~4.21 mmol/L), and median plasma fibrinogen was 141 mg/dL (range, 113~188 mg/dL), Meanwhile, it is worth pointing that only 47.9~56.8% patients meet the diagnostic criteria of triglyceride/fibrinogen at diagnosis, as well as less than 20% of patients meet the diagnostic criteria at the time of admission. Bone marrow biopsy was performed in 193 patients, on 97 (50.3%) of whom hemophagocytosis were observed.

In addition to the diagnostic criteria findings, 34 (15.7%) had hepatomegaly and 95 (43.8%) had lymphadenectasis. Concerning complications, 120 patients (55.3%) developed ALI; AKI occurred to 38 patients (17.5%); 24 patients (11.1%) presented CNS involvement; 17 patients (7.8%) complicated with gastrointestinal involvement; 11 (5.1%) had acute interstitial pneumonia, and 8 (3.7%) patients accompanied with pancreatic involvement, respectively.

In terms of treatment, 145 (67.3%) patients were treated with intravenous immunoglobulin; 83 (38.2%) received methylprednisolone pulse. Seventy-eight (36.1%) patients were treated with cyclosporine A, as well as 73 (33.6%) treated with etoposide. In addition, 34 (15.7%) received JAK inhibitor and 27 (12.4%) received IL-2 therapy.

**Table 1** Clinical characteristics in 220 adult sHLH patients

| Variables | Median or no. of total patients (%) |
|---|---|
| Demographic data | |
| Age (years) | 41 (37, 60) |
| Male | 97 (44.1) |
| Clinical findings according to 2004 criteria | |
| $T \geq 38.5$ °C | 203 (92.3) |
| Splenomegaly | 123 (55.9) |
| Bicytopenia or pancytopenia at admission | 117 (53.2) |
| Bicytopenia or pancytopenia | 186 (84.6) |
| TG ≥ 3 mmol/L on admission | 40/214 (18.7) |
| TG ≥ 3 mmol/L | 102/214 (47.7) |
| FIB ≤ 150 mg/dL on admission | 36 (16.4) |
| FIB ≤ 150 mg/dL | 125 (56.8) |
| Hyperferritinemia ≥ 500 ng/mL | 214/216 (99.2) |
| Hemophagocytosis | 97/193 (50.3) |
| sCD25 ≥ 2400 U/mL | 179/187 (95.7) |
| Absent or decreased NK cell activity | 95/172 (55.2) |

### Etiological distribution and respective mortality of sHLH

As shown in Table 2, underlying trigger factors of HLH were divided into four groups, which were 90 MAS (40.9%), 73 malignancies (33.2%), 42 infection (20.1%), and 15 other triggers (6.8%), respectively. We divided the infection group into two subgroups, including 18 EBV infection (8.2%) and 24 other infection (10.9%). We further analyze the mortality of HLH caused by different triggers and find that EBV infected inpatients has the highest (77.8%) fatality and the lowest mortality is in MAS (14.4%). The overall fatality in this cohort was 32.3%.

### Comparison of clinical and laboratory findings between different outcome groups

Compared with the early death group, the non-early death group tends to be younger and includes more female. In

**Table 2** Underlying triggers and mortality in adult HLH patients

| Underlying triggers | Total | Non-early death group | Early death group | Mortality (%) |
|---|---|---|---|---|
| Malignancy | 73 | 42 | 31 | 42.5 |
| Autoimmune disease | 90 | 77 | 13 | 14.4 |
| Infection | 42 | 18 | 21 | 50.0 |
| EBV infection | 18 | 4 | 14 | 77.8 |
| Other infection | 24 | 17 | 7 | 29.2 |
| Other triggers | 15 | 9 | 6 | 40.0 |

**Table 3** Baseline characteristics of the patients in different outcomes groups

| Variables | Total ($n = 220$) | Non-early death group ($n = 149$) | Early death group ($n = 71$) | $P$ value |
|---|---|---|---|---|
| Demographic data | | | | |
| Age (years) | 41 (37, 60) | 41 (29, 59) | 51 (38, 64) | 0.010 |
| Male (%) | 97 (44.1) | 53 (35.6) | 44 (62.0) | 0.000 |
| Clinical symptoms | | | | |
| Tmax (°C) | 39.5 (39.0, 40.0) | 39.7 (39.0, 40.0) | 39.5 (38.9, 39.8) | 0.039 |
| Arthrodynia (%) | 62 (28.6) | 51 (34.9) | 11 (15.5) | 0.002 |
| Emaciation (%) | 81 (37.3) | 54 (37.0) | 27 (38.0) | 0.882 |
| Rash (%) | 103 (47.5) | 77 (52.7) | 26 (36.6) | 0.018 |
| Myodynia (%) | 36 (16.6) | 30 (20.5) | 6 (8.5) | 0.017 |
| Hepatomegaly (%) | 34 (15.7) | 17 (11.6) | 17 (23.9) | 0.016 |
| Splenomegaly (%) | 120 (55.3 | 80 (54.8) | 40 (56.3) | 0.772 |
| Lymphadenectasis (%) | 95 (43.8) | 69 (47.3) | 26 (36.6) | 0.188 |
| Complications (%) | | | | |
| Pancreatic involvement | 8 (3.7) | 5 (3.4) | 3 (4.2) | 0.332 |
| ALI | 120 (55.3) | 74 (50.7) | 46 (64.8) | 0.034 |
| AKI | 38 (17.5) | 17 (11.6) | 21 (29.6) | 0.001 |
| Gastrointestinal involvement | 17 (7.8) | 5 (3.4) | 12 (16.9) | 0.001 |
| Cytopenia | 183 (84.3) | 114 (78.1) | 69 (97.2) | 0.001 |
| CNS involvement | 24 (11.1) | 9 (6.2) | 14 (21.1) | 0.001 |
| Acute interstitial pneumonia | 11 (5.1) | 6 (4.1) | 5 (7.0) | 0.345 |
| DAH | 3 (1.4) | 1 (0.7) | 2 (2.8) | 0.250 |
| TMA | 4 (1.8) | 1 (0.7) | 3 (4.2) | 0.104 |
| Laboratory data | | | | |
| WBC at admission ($\times 10^9$/L) | 3.72 (2.13, 7.26) | 3.85 (2.40, 8.30) | 3.50 (1.80, 5.72) | 0.051 |
| Peak WBC ($\times 10^9$/L) | 8.50 (5.10, 14.50) | 9.65 (6.18, 15.23) | 5.60 (3.07, 11.22) | 0.000 |
| Valley WBC ($\times 10^9$/L) | 1.90 (0.70, 3.49) | 2.45 (0.94, 3.75) | 1.19 (0.40, 2.30) | 0.000 |
| Valley Hgb (g/L) | 68 (55, 89) | 71 (57, 92) | 61 (49, 76) | 0.001 |
| PLT at admission ($\times 10^9$/L) | 70 (29, 134) | 97 (44, 167) | 42 (16, 112) | 0.000 |
| Valley PLT ($\times 10^9$/L) | 35 (12, 74) | 46 (19, 86) | 15 (7, 41) | 0.000 |
| CRP at admission (mg/L) | 35.4 (9.5, 84.5) | 39.3 (8.0, 83.5) | 29.6 (10.0, 85.7) | 0.963 |
| Peak CRP (mg/L) | 75.2 (26.9, 149.0) | 70.3 (24.1, 136.8) | 112.0 (47.1, 189.3) | 0.022 |
| ESR (mm/h) | 44 (16, 87) | 48 (17, 89) | 29 (13, 82) | 0.232 |
| Ferritin at admission (ng/mL) | 3465 (1267, 10,786) | 4044 (1282, 13,560) | 3192 (1246, 8271) | 0.218 |
| Peak ferritin (ng/mL) | 8507 (2765, 29,626) | 7695 (2765, 27,503) | 8590 (2645, 35,051) | 0.507 |
| PCT (ng/mL) | 0.510 (0.190, 2.025) | 0.364 (0.166, 1.128) | 1.135 (0.425, 4.153) | 0.000 |
| AST (U/L) | 126 (55, 296) | 103 (50, 256) | 180 (76, 375) | 0.027 |
| ALT (U/L) | 114 (50, 275) | 108 (45, 248) | 153 (64, 306) | 0.237 |
| Tbil (mmol/L) | 21.9 (12.9, 45.4) | 17.4 (10.7, 34.1) | 41.4 (17.9, 155.6) | 0.000 |
| Dbil (mmol/L) | 10.4 (5.0, 31.3) | 7.8 (4.2, 18.6) | 26.9 (8.9, 132.8) | 0.000 |
| LDH (U/L) | 845 (509, 1416) | 813 (489, 1293) | 931 (510, 2338) | 0.121 |
| TG at admission (mmol/L) | 1.93 (1.31, 2.70) | 1.89 (1.26, 2.61) | 2.05 (1.36, 3.06) | 0.277 |
| Peak triglyceride (mmol/L) | 3.02 (2.11, 4.21) | 2.81 (2.06, 3.98) | 3.31 (2.50, 4.80) | 0.044 |
| Albumin | 26.4 ± 4.6 | 26.9 ± 4.4 | 25.1 ± 4.8 | 0.012 |
| BUN (mmol/L) | 7.74 (5.5, 11.9) | 7.2 (5.2, 10.1) | 11.4 (6.6, 20.7) | 0.000 |
| SCr (μmol/L) | 59 (44, 89) | 56 (43, 76) | 71 (52, 117) | 0.001 |
| eGFR (mL/min ×1.73 $m^2$) | 103.67 (79.66, 126.53) | 109.23 (87.28, 130.23) | 88.98 (49.76, 109.29) | 0.000 |
| FIB at admission (mg/dL) | 284 (186, 375) | 293 (199, 374) | 273 (169, 398) | 0.568 |
| Valley FIB (mg/dL) | 141 (113, 188) | 145 (118, 198) | 134 (99, 180) | 0.081 |
| PT (s) | 14.4 (12.3, 17.6) | 13.7 (11.8, 16.8) | 16.3 (14.1, 18.6) | 0.000 |
| APTT (s) | 35.0 (30.3, 41.8) | 33.5 (29.6, 38.1) | 40.3 (33.1, 47.9) | 0.000 |

**Table 3** (continued)

| Variables | Total ($n = 220$) | Non-early death group ($n = 149$) | Early death group ($n = 71$) | $P$ value |
|---|---|---|---|---|
| D-dimer (ng/mL) | 3612 (1396, 9144) | 3143 (1295, 8949) | 4576 (2055, 9267) | 0.370 |
| FDP (ug/mL) | 22.0 (11.6, 71.7) | 19.3 (10.9, 68.2) | 30.5 (14.1, 80.4) | 0.156 |
| C3 (g/L) | 0.72 (0.55, 1.03) | 0.74 (0.55, 1.08) | 0.69 (0.49, 1.02) | 0.253 |
| C4 (g/L) | 0.20 (0.13, 0.30) | 0.20 (0.13, 0.29) | 0.21 (0.12, 0.31) | 0.681 |
| sCD25 (U/mL) | 16,009 (9014, 33,190) | 14771 (8192, 28,525) | 21,745 (13,149, 40,136) | 0.003 |
| NK cell activity (%) | 14.38 (12.14, 16.79) | 14.14 (12.18, 16.69) | 14.43 (11.95, 17.17) | 0.626 |
| CMV positive (%) | 44 (20.4) | 27 (18.5) | 17 (23.9) | 0.471 |
| EBV positive (%) | 48 (22.1) | 22 (15.1) | 26 (36.6) | 0.000 |
| Hemoculture positive (%) | 24 (16.4) | 12 (8.2) | 7 (16.9) | 0.065 |
| Fungal infection (%) | 51 (23.5%) | 21 (14.4) | 30 (42.3) | 0.000 |
| Hemophagocytosis (%) | 97 (50.3) | 60 (45.8%) | 37 (59.7%) | 0.090 |

*ALI* acute liver injury, *AKI* acute kidney injury, *CNS* central nervous system, *DAH* diffuse alveolar hemorrhage, *TMA* thrombotic microangiopathy, *PLT* platelet, *TG* triglyceride, *BUN* blood urea nitrogen, *SCr* serum creatinine, *eGFR* estimated glomerular filtration rate, *FIB* fibrinogen, *FDP* fibrinogen degradation product

$P < 0.05$ is considered significant

terms of clinical symptoms, arthrodynia, emaciation, rash, and myodynia are more often observed in the non-early death group. Notably, the proportion of hepatomegaly is increased significantly in patients with poor prognosis. As for complications, the proportion of ALI, AKI, cypopenia, gastrointestinal, and CNS involvement is obviously elevated in the early death group, which tends to have more severe panhematopenia, liver and kidney function injury (elevated AST, bilirubin, BUN and serum creatinine levels, decreased eGFR levels), increased inflammatory markers including CRP and PCT, prolonged PT and APTT, increased sCD25 levels, and more EBV and fungal active infections. It is worth noting that HLH-related diagnostic indicators such as splenomegaly, ferritin and FIB levels, NK cell activity, and hemophagocytosis showed no statistical difference between the two groups as shown in Table 3. In treatment, the proportion of cyclosporine A was significantly increased in the non-early death group. Meanwhile, early death patients tend to have more plasma replacement and anti-infection and virus therapies, as well as more supportive treatment, which are displayed in Table 4.

**Table 4** Treatments of 220 patients in different outcome groups

| Therapies | Group | Non-early death | | Early death | | $P$ value |
|---|---|---|---|---|---|---|
| | *N* | 149 | % | 71 | % | |
| MP pulse | | 62 | 42.5 | 21 | 29.6 | 0.075 |
| Immunoglobulin | | 96 | 65.8 | 49 | 69.0 | 0.649 |
| CsA | | 63 | 43.2 | 15 | 21.1 | 0.001 |
| Etoposide | | 46 | 31.5 | 27 | 38.0 | 0.363 |
| Anti-infection therapy | | 130 | 89.0 | 69 | 97.2 | 0.031 |
| Lituximab | | 15 | 10.3 | 8 | 11.3 | 0.817 |
| Tocilizumab | | 6 | 4.1 | 0 | 0.0 | 0.099 |
| IL-2 | | 19 | 13.0 | 8 | 11.3 | 0.826 |
| JAK inhibitor | | 21 | 14.4 | 13 | 18.3 | 0.551 |
| Anti-virus therapy | | 15 | 9.4 | 19 | 26.8 | 0.002 |
| Plasma replacement | | 3 | 2.1 | 6 | 8.5 | 0.036 |
| CRRT | | 2 | 1.4 | 2 | 2.8 | 0.599 |
| Noninvasive or invasive ventilation | | 4 | 2.7 | 7 | 9.9 | 0.031 |
| Chemotherapy | | 29 | 19.9 | 9 | 12.7 | 0.253 |

*MP pulse* methylprednisolone pulse, *CsA* cyclosporine A

$P < 0.05$ is considered significant

### Influencing factors on the sHLH inpatient prognosis

We have created a regression model to elucidate the influence factors associated with in-hospital prognosis of adult sHLH patients. The regression model is displayed as follows, $P = 1/\{1 + \exp[-(-12.222 + 0.838 \times \text{age} + 1.764 \times \text{cytopenia} + 1.131 \times \text{PLT} + 0.966 \times \text{AST} + 1.200 \times \text{PT} + 1.017 \times \text{APTT} + 0.984 \times \text{EBV infection} + 0.955 \times \text{fungal infection})]\}$. The major risk factors for the prognosis of HLH are age (≥ 38 years old) (odds ratio (OR) = 2.313, 95% CI 1.019–5.248, $P = 0.045$), cytopenia ≥ 2 lines (OR = 5.833, 95% CI 1.046–32.544, $P = 0.044$), PLT ≤ 50 × $10^9$/L at admission (OR = 3.099, 95% CI 1.381–6.953, $P = 0.006$), AST ≥ 135 U/L (OR = 2.629, 95% CI 1.176–5.875, $P = 0.018$), EBV infection (OR = 2.675, 95% CI 1.167–6.133, $P = 0.020$), fungal infection (OR = 2.599, 95% CI 1.158–5.836, $P = 0.021$), PT ≥ 14.9 s (OR = 3.319, 95% CI 1.538–7.165, $P = 0.002$), and APTT ≥ 38.5 s (OR = 2.765, 95% CI 1.227–6.229, $P = 0.014$). The derived model (Table 5) has a good correlation when tested with the Hosmer-Lemeshow method ($x^2 = 10.126$, $P = 0.256$) and discrimination capacity (AUROC) of 0.857 (95% CI 0.801–0.914, $P < 0.001$), of which the sensitivity and specificity are 0.716 and 0.866, respectively, which is displayed in Fig. 1.

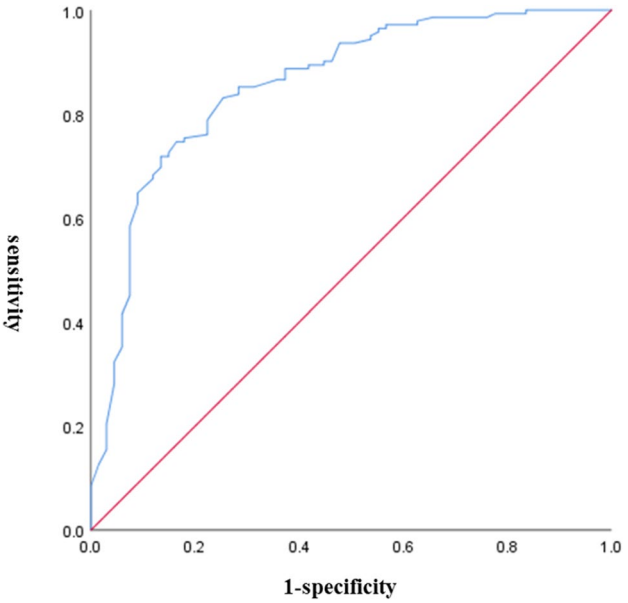


**Fig. 1** The discrimination performance of logistics regression model regarding the in-hospital prognosis of adult sHLH patients. The AUROC is 0.857 (0.801–0.914, $P<0.01$)

## Discussion

Whether primary or secondary, HLH is characterized by losing control of an initial immune response progressing to uncontrolled and persistent macrophage activation, with exaggerated secretion of inflammatory cytokines, causing systemic inflammatory symptoms and signs, which is known as "cytokine storm" [2, 3]. The prognosis of HLH is generally poor, with an estimated median survival of less than 2 months if untreated, warranting early recognition, rapid diagnosis, and prompt management [10, 11]. Clinically, diagnosis of HLH is often delayed due to lack of pathognomonic clinical manifestations and laboratory findings. In this study, by reporting on the experience with adult HLH in the National-Level Rheumatology and Hematology Center, we aim to observe the etiological distribution and clinical characteristics of adult sHLH patients and to further explore the influence factors for early poor prognosis.

Different from previous studies that have reported infection or malignancy as the most common cause of sHLH [11–14], MAS is the primary cause in this study, accounting up to 40.6%, followed by malignancy (33.6%) and infection (19.4%). In terms of fatal rate, the EBV-HLH has the highest mortality (77.8%) and MAS-HLH has the lowest mortality (14.8%), which is broadly consistent with previous studies [15–17]. The mortality of other infection-related HLH (excluded EBV-HLH) is 29.2%, while the malignancy group is similar to other triggers group (42.5% and 42.9%, respectively).

Due to the high fatality of HLH, it is necessary to explore the influence factors related to its poor prognosis, which is helpful to identify high-risk critical patients earlier and take timely treatments. Therefore, we further establish a regression model and find that elder age, cytopenia ≥ 2 lines, platelets ≤ 50 × $10^9$/L at admission, AST ≥ 135 U/L,

**Table 5** The predictive factors for prognosis of adult HLH patients in-hospital

| Risk factor | *B* | S.E. | Wald | OR(95% CI) | *P* |
|---|---|---|---|---|---|
| Age (≥ 38 years) | 0.838 | 0.418 | 4.022 | 2.313 (1.019–5.248) | 0.045 |
| Cytopenia ≥ 2 lines | 1.764 | 0.877 | 4.043 | 5.833 (1.046–32.544) | 0.044 |
| PLT (≤ 50 × $10^9$/L) at admission | 1.131 | 0.412 | 7.525 | 3.099 (1.381–6.953) | 0.006 |
| AST (≥ 135 U/L) | 0.966 | 0.410 | 5.548 | 2.629 (1.176–5.875) | 0.018 |
| PT (≥ 14.9 s) | 1.200 | 0.393 | 9.337 | 3.319 (1.538–7.165) | 0.002 |
| APTT (≥ 38.5 s) | 1.017 | 0.877 | 4.043 | 2.765 (1.227–6.229) | 0.014 |
| EBV infection | 0.984 | 0.423 | 5.400 | 2.675 (1.167–6.133) | 0.020 |
| Fungal infection | 0.955 | 0.413 | 5.361 | 2.599 (1.158–5.836) | 0.021 |

prolonged PT and APTT, EBV infection, and fungal infection are the risk factors for early death in sHLH patients.

Consistent with previous studies, older age onset is the independent risk factor for poor prognosis of HLH [12, 14, 18]. It may be related to the etiology of HLH, as the overall onset age of the MAS group is relatively younger and has a favorable prognosis. Otherwise, older age patients are more likely to develop complications such as infection and severer organ dysfunction.

Infections are both the trigger and cause of HLH, and we have found that HLH patients with EBV infection or fungal infection have a higher in-hospital mortality. EBV was shown to be associated with adverse outcomes of HLH patients in many retrospective studies [15, 16, 19]. Without appropriate therapy, patients with EBV-HLH have a mortality up to 20~95.7% [15, 16]. Although haemopoietic stem cell transplantation has been found as an effective method to treat EBV-HLH, about two-thirds of patients will die during induction therapy [15]. Recently, Liu et al. [20] reveals anticipated preliminary data on the potential role of immune checkpoint inhibition for the treatment of adult EBV-HLH. They treated seven adults with relapsed or refractory EBV-HLH through nivolumab monotherapy, resulting in clinical complete remission in five patients with a median follow-up of 16 months. This study provides a potential possibility that anti PD-1–targeted therapy may recover immune function against diseases mediated by EBV infection. HLH triggered by fungal infection is mainly reported by case reports [21, 22]. In this study, there were HLH patients caused by *Cryptococcus* and *Pneumocystis carinii*, as well as HLH patients combined with fungal infections during the treatment. The common denominator is that inferior immune function is accompanied by a hyperinflammatory state. Under the premise of anti-infection therapies, how to find the balance point of regulation in immune function, like walking on a tightrope, has always been the focus and difficulty of clinical practice.

In the early death group, up to 97.2% of patients have observed bicytopenia/pancytopenia and showed significant thrombocytopenia (median platelets $42 \times 10^9$/L) on admission in our study. When the majority of previous studies focused on the diagnostic value of cytopenia ≥ 2 lines, we discover that it is strongly correlated with poor prognosis (OR 5.833, 95% CI 1.046–32.544) in HLH patients. Bin Q et al. [23] have found that severe neutropenia (neutrophils $< 0.5 \times 10^9$/L) is an independent risk factor for the 30-day poor prognosis in pediatric HLH patients. Patients with leukopenia or agranulocytopenia are more prone to have opportunistic infections such as CMV and/or fungal infection, which are common causes of death in the total HLH patients. Recently, Huang et al. [24] reported that the routine CBC parameters (a low lymphocyte-to-monocyte ratio and high red blood cell distribution width-to-platelet ratio) could be regarded as independent risk factors for the prognosis of sHLH in 2020. These results all suggest that cytopenia is closely related to prognosis. Meanwhile, thrombocytopenia is frequently observed in patients with HLH; platelets $< 50 \times 10^9$/L at admission can predict poor prognosis in this study, in consonance with results of other studies [12, 14, 25, 26]. The potential mechanisms of thrombocytopenia in HLH may be due to severe cytokine-mediated inflammation, excessive depletion and destruction of bone marrow regeneration, DIC, and hypersplenism. And platelet reflects the function and reserve of bone marrow, so thrombocytopenia may indicate bone marrow failure to some extent. In addition, platelets have been found to be linked with inflammation recently [27, 28]. Patients with IL-10 ≥ 800 pg/mL have exhibited decreased platelet counts in one study [27]. Severe thrombocytopenia is often associated with abnormal coagulation function and related to adverse bleeding complications such as cerebral hemorrhage and gastrointestinal bleeding, which are often fatal.

Recently, liver involvement has been reported to be one of the most common complications of HLH, manifested as the elevation of aminotransferase and bilirubin, liver enlargement, coagulation disorders, and even acute hepatic failure [29, 30]. In this study, we have found that the proportion of hepatomegaly and the incidence of acute liver injury are significantly increased in the early death group, as well as the obvious increases of AST and bilirubin, decreased albumin, and prolonged PT and APTT. Further multivariate analysis has showed that the mortality of HLH patients with AST ≥ 135 U/L increased by 3.541 times when compared with the early death group, which may be related to the increased incidence of hepatic haemophagocytosis. Researchers have found that hepatic haemophagocytosis was detected in 56% of liver biopsies in patients with hematological malignancies, when patients' hepatic dysfunction remained unresolved after standard examination, and was obviously associated with a poor prognosis [30]. Meanwhile, Zhao YC et al. [14] have also observed a more significant increase in AST in the early death group. Conversely, Wang DG et al. [12] have reported that patients with lower AST (< 119 IU/L) tend to have a worse long-term outcome. However, there is growing evidence that liver is not only a target organ injured by the immune response, but also an immunological organ, playing a key role in the hyperinflammatory pathogenesis of HLH [29, 31]. Liver can directly respond to cytokines and produce inflammatory cytokines such as IL-33 and IL-8 as part of the immune response as well as injury and repair [32]. Elevated aminotransferase, bilirubin, and higher proportion of liver enlargement all indicate severer liver injury and inflammation, leading to adverse outcomes.

Coagulation disorders are frequent in HLH, which are reported in more than half of patients, including hypofibrinogenemia, prolonged PT, elevated d-dimer levels, and even

disseminated intravascular coagulation (DIC) [33–35]. An isolated decrease in plasma fibrinogen sometimes is the most frequently described [33]. Previous studies have reported that 50 to 80% of HLH patients have developed hypofibrinogenemia, and decreased fibrinogen levels appear to be associated with case fatality [34, 35]. In this study, hypofibrinogenemia was found in up to 56.8% HLH patients but was not correlated with in hospital death. Compared with the non-early death group, we found more significant prolonged PT and APTT in the early death group, which were both influence factors for prognosis. Meanwhile, FDP, d-dimer, and triglyceride were not statistically different between the two groups. The precise mechanism of coagulation disorders is not fully understood. Perhaps the most critical hypothesis is the inflammatory persistent state of HLH patients, including stimulated macrophages secrete proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 [34]. Other potential mechanisms may include DIC and liver dysfunction, both of which can be resulted from coagulation disorders [34].

Our study had its limitations. Firstly, it is a single center, bidirectional observational cohort study including retrospective data. In addition, this study is performed in the National-Level Rheumatology Center of China, resulting in the significantly increased proportion of MAS patients compared with previous studies. All of these factors could lead to bias in the selection of study population. Secondly, cytokine storms play a critical role in the pathogenesis of HLH, but our study does not include cytokines such as IL-1, IL-6, TNF-α, and IFN-γ, which may also be potential prognostic indicators in HLH. Thirdly, HLH is diagnosed according to HLH-2004 criteria, which may be too strict for adult secondary HLH as a commonplace. Otherwise, what needs to be emphasized is that the majority of the patients do not have leukopenia, elevated TG, or decreased FIB when at admission, for the above abnormal laboratory tests are developed during hospitalization. Meanwhile, hemophagocytosis and levels of ferritin, triglyceride, fibrinogen, and sCD25 which are included in the diagnosis of HLH have no correlation with the prognosis in this study. All above suggest that it is necessary to search for more sensitive and specific biomarkers for the diagnosis and prognosis of HLH. And further prospective multicenter researches with larger samples are urgently needed to explore more meaningful clinical indicators for the diagnosis and treatment of adult HLH.

## Conclusion

The study has presented the detailed clinical characteristics of adult sHLH, explored the risk factors related with in-hospital prognosis, and found that factors such as increasing age (≥ 38 years), cytopenia ≥ 2 lines, decreased platelets ($\leq 50 \times 10^9$/L) at admission, elevated AST (≥ 135 U/L), prolonged PT (≥ 14.9 s) and APTT (≥ 38.5 s), EBV, and fungal infection could predict the risk of early deaths in adult sHLH patients. The findings will assist clinicians to timely identify HLH patients who are at a substantial risk of poor prognosis and make better treatment decisions accordingly.

**Author contributions** Contribution to the concept or design of the work was carried out by Yuanyuan Pei, Jihong Zhu, Yuan Jia, and Yin Su. Access to informed consent was carried out by Yuanyuan Pei, Ranran Yao, Lingjie Cao, Renge Liang, and Ziye Wang. Yuanyuan Pei, Ranran Yao, and Renge Liang participated in clinical data entry and statistics. Yuanyuan Pei was responsible for data statistical analysis and article writing. Jia Yuan and Yin Su were responsible for the revision of this article. All authors read and approved the final manuscript.

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**Data availability** The datasets used or analyzed about the study could be available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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