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ORIGINAL ARTICLE

Development of Laboratory Parameters-Based Formulas in Predicting Short Outcomes for Adult Hemophagocytic Lymphohistiocytosis Patients with Different Underlying Diseases

Jun Zhou[1,2] · Zhi-Qi Wu[1,2] · Tengfei Qiao[3] · Hua-Guo Xu[1,2]

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Abstract

Purpose Hemophagocytic lymphohistiocytosis (HLH) is a severe disease with high mortality. The purpose of this investigation was to build models to predict 30-day death in total and subgroup HLH patients based on available and cheap laboratory parameters.

Method The research contained 431 adults HLH patients from January 2015 to September 2021 in the hospital. Logistic regression and receiver operating characteristic (ROC) were utilized to build models.

Results Results suggested that age, ferritin, lymphocyte (LY), international normalized ratio (INR), thrombin time (TT), globulin, uric acid (UA), chloride, activated partial thromboplastin time (APTT), aspartate aminotransferase (AST), triglycerides (TG), total bilirubin (TB), and indirect bilirubin (IB) were independent factors in HLH and subgroups. Then, models adapted to patients with different underlying diseases were established based on these factors. Area under curve (AUC) of these models was excellent: HLH patients: 0.838 ($p < 0.001$); infection-associated HLH (I-HLH) patients: 0.913 ($p < 0.001$); malignancy-associated HLH (M-HLH): 0.921 ($p < 0.001$) and 0.809 ($p < 0.001$) for two or more different etiologies-associated HLH (Mix-HLH patients). In addition, UA, TT, and chloride were firstly confirmed as independent factors in adult HLH.

Conclusion Four models depending on biomarkers that available and affordable in clinical practice were built. With these models, high-risk patients with different underlying diseases could be easily identified.

Keywords Biomarker · Hemophagocytic lymphohistiocytosis · Model · Mortality · Underlying disease

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a severe disease, accompanied with persistent fever, high levels of ferritin, splenomegaly, cytopenia, cytokine storm, and multi-organ dysfunction [1]. Cytokine storm coming from activated natural killer (NK) and/or cytotoxic T cells was the direct cause of HLH [2]. HLH could be developed at any age [3]. In Japan, from 2001 to 2005, adult patients account for 40% of the total HLH [4]. In adults patients, infection and malignancy are the most commonly reported underlying diseases [5]. In addition, when HLH developed, one or more organ functions would be affected (such as liver, kidney, and coagulation disorders). Thus, the prognosis varies widely [6, 7].

Elevated post-ferritin, interleukin-10, Epstein-Barr virus (EBV) infection, fibrinogen (FIB), platelet (PLT) and lactate dehydrogenase (LDH), blood routine parameters, and their ratios are risk markers and models that included some of these indicators have excellent predictive ability to forecast the outcomes of patients with HLH [3, 8–10]. To malignancy-induced HLH patients, the prognosis is also poor [11, 12]. However, prognostic efficiency and efficacy would be low as these indexes may be expensive and/or have low sensitivity/

Jun Zhou, Zhi-Qi Wu, and Tengfei Qiao contributed equally to this work.

✉ Hua-Guo Xu
huaguoxu@njmu.edu.cn

1 Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

2 Branch of National Clinical Research Center for Laboratory Medicine, Nanjing, Jiangsu, China

3 Department of Laboratory Medicine, Nanjing Lishui District Hospital of Traditional Chinese Medicine, Nanjing, Jiangsu, China

specificity. Thus, feasible, non-invasive, and reasonably predictive models are needed.

In clinical practice, routine blood test, biochemical test, and coagulation test are the most common, readily available, and measured tests. In models, combinations of multi-factors possess markedly better presentation than a single marker [13]. Thus, the purpose of this investigation was to build models to predict 30-day death in total and subgroup HLH patients based on available and cheap factors.

Methods

Patients and Data

The research contained 431 adults with HLH (meeting 5 or more of the 8 HLH-2004 criteria) [14] from January 2015 to September 2021 in the hospital. Inclusion criteria: (1) age ≥ 18 years; (2) newly diagnosed HLH; (3) complete clinical results. Exclusion criteria: patients with unknown outcome. Data such as age, gender, clinical signs, admission laboratory parameters, etiology, medicine, and outcomes.

The primary outcome was defined as death within 30 days when HLH was initially confirmed. The research was consistent with the Declaration of Helsinki and permitted by the hospital (Nanjing, China) (2019-SR-066).

Statistical Methods

Differences in categorical biomarkers (frequencies), continuous parameters (means ± SD), and non-parametric indexes (medians (ranges)) were evaluated by the Chi square test, ANOVA, *T*-test, or Mann-Whitney *U* test as appropriate. Logistic regression was employed to seek out biomarkers of 30-day mortality. Parameters ($p < 0.10$) on univariate analysis were included in the multivariable analysis. Receiver operating characteristic (ROC) was utilized to judge the performance of models in predicting adverse events and selected the ideal points for each of the models and parameters. p-value <0.05 was considered statistically significant. Data analysis was performed by IBM SPSS 21.0 statistical software (IBM SPSS Version 21.0. Armonk, NY).

Results

Characteristics

In total, 431 subjects with HLH (252 males, 179 females) were recruited in the retrospective research. The overall mean age was 52.2 ± 16.5 years. Almost all patients were accompanied with fever (> 90%); EBV infection was remarked in 42.92% of the patients. The overall mortality was 29.7%. Other clinical and laboratory parameters covered splenomegaly (208, 48.26%), lymph node enlargement (200, 46.40%), hemophagocytosis (139, 32.25%), and hyperferritin (2358.9 (15.2, 15,000)). Underlying diseases of HLH were listed as follows: infectious diseases (named infection-associated HLH (I-HLH)) (150, 34.56%); malignancies (named malignancy-associated HLH (M-HLH)) (80, 18.56%); mixed connective tissue disease (named MCTD-HLH) (6, 1.39%); unidentified disease (named Unclear-HLH) (33, 7.66%); and two or more different etiologies-associated HLH which could not be classified into any of the above groups (named Mix-HLH) (162, 37.59%). Other features of the total and subgroups of HLH patients are presented in Table 1. In addition, we also listed and compared the types of infections and malignancies in I-HLH, M-HLH, and Mix-HLH (Supplementary table 1).

Independent Parameters for Total or Subgroup HLH

Based on the follow-up results, patients were assigned to survivors and nonsurvivors. Compared with survivors, there was a distinctly difference in nonsurvivors in the following laboratory data: age, ferritin, EBV infection, lymphocyte (LY), neutrophil, hemoglobin, PLT, prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), FIB, thrombin time (TT), D-dimer, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), triglycerides (TG), albumin, globulin, urea nitrogen (UREA), creatinine (CREA), uric acid (UA), chloride for total HLH (all $p < 0.10$); age, ferritin, EBV infection, LY, PLT, APTT, FIB, TT, D-dimer, ALT, AST, TB, DB, TG, UREA, chloride for I-HLH patients (all $p < 0.10$); ferritin, EBV infection, LY, neutrophil, PLT, PT, INR, APTT, FIB, TT, ALT, AST, TB, DB, IB, TG, globulin, UREA, CREA for M-HLH patients (all $p < 0.10$); age, ferritin, PLT, PT, INR, APTT, TT, D-dimer, AST, TB, DB, IB, TG, UREA, CREA, UA for Mix-HLH patients (all $p < 0.10$) (Table 2).

To select the significant independent markers, ROC was employed to discover the optimal value for each of the continuous factors and models. Then, we reset these parameters in Table 2 with $p < 0.10$ (except for EBV infection, as not all patients were tested for EBV) into categorical parameters.

On univariate and multivariable logistics regression analyses for HLH patients: age (hazard ratio (HR): 2.990, 95% confidence interval (CI): 1.657–5.397, $p < 0.001$), ferritin (HR: 4.264, 95% CI: 2.513–7.238, $p < 0.001$), LY (HR: 0.391, 95% CI: 0.232–0.659, $p < 0.001$), INR (HR: 2.513, 95% CI: 1.382–4.568, $p = 0.003$), TT (HR: 2.077,

Table 1 The demographic, clinical, and laboratorial characteristics of the patients included in the study

Characteristics	Total (n=431)	I-HLH (n=150)	M-HLH (n=80)	Mix-HLH (n=162)	MCTD-HLH (n=6)	Unclear (n=33)	p-value
General							
Gender (male/female), *n*	252/179	73/77	50/30	111/51	16/11	16/17	0.003
Age, y	52.2 ± 16.5	52.8 ± 17.17	51.9 ± 17.6	53.4 ± 15.2	38.0 ± 11.4	47.7 ± 16.2	0.094
Clinical features							
Fever, *n*	408/23	141/9	77/3	155/7	5/1	30/3	0.511
Mean T_{max}, ℃	42	41.3	42.0	42	40	42	0.799
Hepatomegaly	42/389	11/139	13/67	13/149	1/5	4/29	0.206
Splenomegaly	208/223	57/93	47/33	88/74	2/4	14/29	0.010
Lymph node enlargement	200/231	44/106	50/30	92/70	3/3	10/23	<0.001
Rash	68/363	39/111	10/70	27/135	1/5	1/32	0.010
Jaundice	38/393	11/139	10/70	13/149	0/6	2/31	0.595
Edema	68/363	22/128	12/68	29/133	1/5	4/29	0.902
Neurological symptom	100/331	42/108	15/65	35/127	1/5	7/26	0.515
Bone marrow hemophagocytosis	139/292	56/94	24/56	49/113	0/6	10/23	0.272
Laboratory data							
Ferritin (μg/L)	2358.9 (15.2, 15,000)	2038.5 (15.2, 15,000)	2505 (268.9, 15,000)	2671.8 (16.9, 15,000)	9696.25 (188.0, 15,000)	1755.00 (94.8, 15,000)	0.433
EBV infection (+)	186/245	68/82	28/52	77/85	1/5	12/21	0.196
LY ($\times10^9$/L)	0.66 (0.01, 41.07)	0.66 (0.04, 4.48)	0.70 (0.04, 17.82)	0.63 (0.01, 41.07)	0.94 (0.16, 1.62)	0.63 (0.02, 2.53)0.944	0.485
Neutrophil ($\times10^9$/L)	2.28 (0, 62.78)	2.87 (0, 48.29)	1.91 (0, 12.16)	2.01 (0.01, 62.78)	4.09 (0.43, 21.29)	1.86 (0.16, 20.48)	0.018
Hemoglobin (g/L)	95.78 ± 23.89	104.17 ± 22.76	90.09 ± 22.68	90.12 ± 23.00	98.33 ±21.14	98.78 ± 26.04	<0.001
PLT ($\times10^9$/L)	67 (2.00, 512.00)	82.0 (2.00, 512.00)	60.0 (7.00, 409.00)	54.0 (3.00, 343.00)	62.0 (14.00, 221.00)	75.0 (9.00, 309.00)	0.008
PT (s)	14.48 ± 5.27	14.62 ± 8.03	14.34 ± 3.19	14.55 ± 2.82	14.23 ± 3.82	13.89 ±2.27	0.962
INR	1.26 ± 0.47	1.27 ± 0.71	1.25 ± 0.29	1.27 ± 0.26	1.25 ± 0.35	1.21 ± 0.21	0.963
APTT (s)	38.92 ± 17.37	38.59 ± 20.81	38.79 ± 12.83	39.87 ± 17.02	35.97 ± 21.09	36.53 ± 9.58	0.855
FIB (g/L)	2.61 ± 1.56	2.80 ± 1.61	2.63 ± 1.54	2.38 ± 1.52	3.82 ± 2.43	2.61 ± 1.31	0.054
TT (s)	22.02 ± 12.61	22.59 ± 13.80	22.39 ± 15.13	21.67 ± 11.29	21.03 ± 8.39	20.41 ± 5.10	0.900
D-Dimer (mg/L)	3.12 (0.10, 40)	3.25 (0.10, 40)	2.48 (0.10, 40)	3.29 (0.22, 40)	7.45 (0.25, 40)	2.39 (0.28, 40)	0.115
ALT (U/L)	45.8 (6.00, 3249.30)	39.4 (6.00, 2894.10)	46.8 (8.4, 1247.30)	43.1 (7.4, 3249.30)	54.05 (29.7, 300.80)	64.00 (8.20, 593.00)	0.632
AST (U/L)	68.5 (7.60, 4688.10)	66.2 (9.9, 4688.10)	55.9 (12.40, 1208.10)	69.8 (7.60, 3000.00)	50.45 (23.90, 1678.90)	68.50 (15.00, 1502.40)	0.543
TB (μmol/L)	15.4 (4.10, 452.20)	14.2 (4.10, 452.20)	14.7 (5.30, 443.10)	16.1 (4.20, 400.00)	12.95 (8.50, 243.80)	14.10 (5.00, 128.00)	0.148
DB (μmol/L)	7.4 (1.40, 351.00)	6.3 (1.40, 351.00)	7.2 (2.20, 308.40)	8.2 (1.50, 291.10)	8.60 (2.90, 174.70)	6.70 (1.50, 93.20)	0.154
IB (μmol/L)	7.8 (1.80, 138.40)	7.7 (1.80, 112.50)	7.8 (2.40, 138.40)	8.1 (2.20, 108.90)	7.6 (4.00, 19.20)	7.6 (3.10,34.80)	0.121
TG (mmol/L)	2.38 ± 1.61	2.30 ± 1.84	2.26 ± 1.54	2.54 ± 1.53	2.55 ± 1.44	2.18 ± 1.00	0.543
Albumin (g/L)	28.78 ± 5.51	29.32 ± 5.86	28.78 ± 5.32	27.98 ± 5.17	31.93 ± 6.70	29.74 ± 5.33	0.097
Globulin (g/L)	25.73 ± 6.79	27.25 ± 6.66	24.23 ± 5.61	24.83 ± 7.32	29.88 ± 8.98	26.16 ± 5.38	0.002
UREA (mmol/L)	7.03 ± 5.17	6.89 ± 5.68	6.76 ± 5.11	7.65 ± 5.07	5.23 ± 3.08	5.69 ± 3.10	0.231
CREA (μmol/L)	72.46 ± 49.04	74.82 ± 60.95	66.28 ± 31.61	74.72 ± 46.86	66.33 ± 59.36	66.75 ± 27.62	0.646

Table 1 (continued)

Characteristics	Total (n=431)	I-HLH (n=150)	M-HLH (n=80)	Mix-HLH (n=162)	MCTD-HLH (n=6)	Unclear (n=33)	p-value
UA (μmol/L)	263.65 ± 149.36	241.74 ± 143.39	265.21 ± 125.91	288.83 ± 162.68	174.98 ± 92.24	251.95 ± 152.72	0.037
Chloride (mmol/L)	101.49 ± 5.73	101.80 ± 5.98	101.54 ± 5.22	101.31 ± 5.94	99.48 ± 4.90	101.11 ± 4.94	0.829
Mortality (%)	29.7	26.0	30.0	35.2	16.7	21.2	0.349

APTT, activated partial thromboplastin time; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *CREA*: creatinine; *DB*, direct bilirubin; *EBV*, Epstein-Barr virus; *FIB*, fibrinogen; *I-HLH*, infection-associated HLH; *IB*, indirect bilirubin; *INR*, international normalized ratio; *M-HLH*, malignancy-associated HLH; *LY*, lymphocyte; *MCTD*, mixed connective tissue disease; *Mix-HLH*, mixed-cause HLH; *PLT*, platelet; *PT*, prothrombin time; *TB*, total bilirubin; *TG*, triglyceride; *TT*, thrombin time; *UA*, uric acid; *Unclear*, unknown underlying diseases; *UREA*, urea nitrogen

95% CI: 1.193–3.616, $p = 0.010$), globulin (HR: 0.560, 95% CI: 0.301–0.848, $p = 0.010$), UA (HR: 2.637, 95% CI: 1.394–4.988, $p = 0.003$), and chloride (HR: 0.479, 95% CI: 0.285–0.806, $p = 0.006$) remained as independent factors of 30-day death. Model was set up depending on these markers (Table 3) (Table 4).

$$\begin{aligned} Clinical\ model: LogitP =& 1.095 \times age + 1.450 \times ferritin - 0.938 \\ & \times LY + 0.921 \times INR + 0.731 \times TT - 0.682 \\ & \times globulin + 0.969 \times UA - 0.736 \times chloride - 4.355 \end{aligned}$$

After adjusted confounding factors for I-HLH patients: age (HR: 8.813, 95% CI: 2.602–29.848, $p < 0.001$), LY (HR: 0.235, 95% CI: 0.078–0.710, $p = 0.010$), APTT (HR: 5.028, 95% CI: 1.709–14.799, $p = 0.003$), TT (HR: 3.736, 95% CI: 1.233–11.321, $p = 0.020$), AST (HR: 3.656, 95% CI: 1.219–10.965, $p = 0.021$), TG (HR: 4.016, 95% CI: 1.356–11.894, $p = 0.012$), and chloride (HR: 0.214, 95% CI: 0.074–0.615, $p = 0.004$) were still in the multivariate model. A model was developed (Table 3).

$$\begin{aligned} Clinical\ model: LogitP =& 2.176 \times age - 1.499 \times LY + 1.615 \times APTT \\ & + 1.318 \times TT + 1.296 \times AST + 1.390 \times TG - 1.543 \\ & \times chloride - 7.484 \end{aligned}$$

On univariate and multivariable exploration for M-HLH patients: ferritin (HR: 53.839, 95% CI: 7.772–372.961, $p < 0.001$), LY (HR: 0.086, 95% CI: 0.016–0.467, $p = 0.011$), and TB (HR: 12.326, 95% CI: 1.765–86.063, $p = 0.005$) were confirmed as independent indexes. A multivariate model was formed (Table 3).

$$\begin{aligned} Clinical\ model: LogitP =& 3.986 \times ferritin - 2.458 \\ & \times LY + 2.512 \times TB - 6.274 \end{aligned}$$

On univariate and multivariable logistics regression analyses for Mix-HLH patients: ferritin (HR: 2.215, 95% CI: 1.009–4.864, $p = 0.048$), LY (HR: 0.387, 95% CI: 0.175–0.859, $p = 0.020$), APTT (HR: 4.075, 95% CI: 1.470–11.294, $p = 0.007$), IB (HR: 2.516, 95% CI: 1.129–5.609, $p = 0.024$), TG (HR: 3.120, 95% CI: 1.037–9.386, $p = 0.043$), and UA (HR: 4.680, 95% CI: 1.918–11.418, $p = 0.001$) persisted as significant factors of 30 days mortality. A multivariate model was created (Table 3).

$$\begin{aligned} Clinical\ model: LogitP =& 0.759 \times ferritin - 0.948 \times LY + 1.405 \times APTT \\ & + 0.923 \times IB + 1.138 \times TG + 1.543 \times UA - 6.652 \end{aligned}$$

We further explored the distribution of mortality. Figure 1 showed the percentage of mortality by the cumulative number of independent biomarkers. The more risk factors, the higher the mortality.

Performance of Models

ROC was hired to estimate the performance of these clinical models. AUC of these models was listed as follow: HLH patients: 0.842 (95% CI 0.804–0.875, $p < 0.001$); I-HLH patients: 0.913 (95% CI 0.856–0.953, $p < 0.001$); M-HLH: 0.921 (95% CI 0.838–0.969, $p < 0.001$), and 0.809 (95% CI 0.740–0.866, $p < 0.001$) for Mix-HLH patients (Fig. 2).

Discussion

In this study, models for total or subgroup HLH patients were established based on admission laboratory results. LY was an independent marker for total or subgroup HLH patients. For HLH patients, age, ferritin, LY, INR, TT, globulin, UA, and chloride were the most stable factors and the combination of them is the most predictive. For I-HLH patients, age, LY, APTT, TT, AST, TG, and chloride were the most stable factors and the combination of them is the most predictive. For M-HLH patients, ferritin, LY, and TB were the most stable factors and the combination of them is the most predictive. For Mix-HLH patients, ferritin, LY, APTT, UB, TG, and UA were the most stable factors and the combination of them is the most predictive.

Table 2 The laboratorial characteristics of the patients included in the study

Characteristics	HLH			IAHS (*N* = 150)			MAHS (*N*=80)			Mixed (*N* = 162)		
	Survivor (*N* = 302)	Nonsurvivor (*N* = 129)	*p*-value	Survivor (*N* = 111)	Nonsurvivor (*N* = 39)	*p*-value	Survivor (*N* = 56)	Nonsurvivor (*N* = 24)	*p*-value	Survivor (*N* = 105)	Nonsurvivor (*N* = 57)	*p*-value
Gender (male/female), *n*	170/132	82/47	**0.161**	57/54	16/23	**0.270**	36/21	15/9	**1.00**	67/38	44/13	**0.081**
Age, y	50.5 ± 16.9	56.2 ± 14.7	**0.001**	51.00 ± 17.2	58.1 ± 16.1	**0.024**	51.6 ± 18.2	52.4 ± 16.4	**0.862**	51.2 ± 15.9	57.3 ± 13.1	**0.014**
Ferritin (μg/L)	1755.0 (15.2, 15000)	6181.8 (16.9, 15,000)	**<0.001**	1793.7 (15.20, 15,000)	5467.3 (532.30, 15,000)	**0.007**	1551.5 (268.90, 15,000)	6471 (859.10, 15,000)	**<0.001**	2143.4 (19.10, 15,000)	5391 (16.90, 15,000)	**0.004**
EBV infection (+)	114/186	72/57	**<0.001**	45/66	23/16	**0.047**	15/41	13/11	**0.018**	45/60	32/25	**0.107**
LY ($\times10^9$/L)	0.75 (0.02, 4.36)	0.43 (0.01, 41.07)	**<0.001**	0.74 (0.04, 3.09)	0.47 (0.04, 4.48)	**0.026**	0.81 (0.04, 4.13)	0.40 (0.06, 17.82)	**0.055**	0.66 (0.03, 4.36)	0.44 (0.01, 41.07)	**0.167**
Neutrophil ($\times10^9$/L)	2.36 (0.01, 48.29)	2.01 (0.00, 62.78)	**0.058**	3.0 (0.01, 48.29)	2.87 (0.00, 34.86)	**0.861**	2.33 (0.10, 7.50)	1.12 (0.00, 12.16)	**0.050**	2.12 (0.01, 17.60)	1.93 (0.01, 62.78	**0.254**
Hemoglobin	97.02 ± 24.02	92.88 ± 23.45	**0.066**	103.94 ± 23.10	104.82 ± 22.02	**0.836**	92.04 ± 22.39	85.54 ± 23.21	**0.243**	91.22 ± 23.42	88.11 ± 22.28	**0.412**
PLT ($\times10^9$/L)	79.0 (3.00, 447.00)	39.0 (2.00, 512)	**<0.001**	94.0 (9.00, 447.00)	53.0 (2.00, 512.00)	**0.002**	73.0 (7.00, 409.00)	35.0 (7.00, 243.00)	**0.005**	69.0 (3.00, 343.00)	31.0 (3.00, 343.00)	**0.001**
PT (s)	14.09 ± 5.31	15.40 ± 5.08	**0.017**	14.39 ± 8.42	15.26 ± 6.85	**0.564**	13.80 ± 1.81	15.59 ± 4.98	**0.020**	14.11 ± 1.95	15.37 ± 3.85	**0.006**
INR	1.23 ± 0.47	1.34 ± 0.46	**0.014**	1.25 ± 0.74	1.33 ± 0.62	**0.560**	1.20 ± 0.16	1.36 ± 0.45	**0.020**	1.23 ± 0.18	1.35 ± 0.35	**0.005**
APTT (s)	36.11 ± 9.87	45.48 ± 26.88	**<0.001**	35.48 ± 10.34	47.45 ± 35.77	**0.002**	36.83 ± 10.07	43.38 ± 17.07	**0.035**	37.05 ± 9.62	45.07 ± 24.88	**0.004**
FIB (g/L)	2.83 ± 1.56	2.10 ± 1.40	**<0.001**	3.06 ± 1.60	2.07 ± 1.42	**0.001**	3.00 ± 1.54	1.75 ± 1.15	**0.001**	2.47 ± 1.54	2.23 ± 1.47	**0.333**
TT (s)	19.83 ± 6.58	27.14 ± 19.85	**<0.001**	20.13 ± 9.31	29.57 ± 20.69	**<0.001**	19.03 ± 3.69	30.23 ± 25.73	**0.002**	20.05 ± 4.84	24.67 ± 17.58	**0.012**
D-Dimer (mg/L)	2.69 (0.10, 40.00)	5.03 (0.40, 40.00)	**<0.001**	2.89 (0.10, 40.00)	6.81 (0.40, 40.00)	**<0.001**	2.35 (0.10, 40.00)	3.44 (0.58, 40.00)	**0.150**	2.96 (0.22, 40.00)	5.72 (0.41, 40.00)	**0.004**
ALT (U/L)	42.8 (6.00, 2894.10)	61.6 (7.80, 3249.30)	**0.012**	35.1 (6.00, 2894.10)	77.1 (7.80, 2572.00)	**0.009**	40.3 (8.40, 1247.30)	67 (13.90, 512.30)	**0.057**	43.3 (7.40, 394.20)	51.4 (9.00, 3249.30)	**0.431**
AST (U/L)	56.7 (7.60, 2078.00)	105.2 (12.10, 4688.10)	**<0.001**	53.2 (9.90, 2078.00)	127.5 (17.50, 4688.10)	**<0.001**	47.2 (12.40, 1208.10)	120.4 (14.60, 844.00)	**0.001**	63.0 (7.60, 1241.80)	85.8 (12.10, 3000.00)	**0.034**
TB (μmol/L)	14.1 (4.20, 388.00)	18.6 (4.10, 452.20)	**<0.001**	13.4 (4.90, 388.00)	18.2 (4.10, 452.20)	**0.068**	13.6 (5.30, 185.00)	15.7 (7.50, 443.10)	**<0.001**	15.1 (4.20, 297.00)	19.7 (7.60, 400.00)	**0.006**
DB (μmol/L)	6.6 (1.40, 278.50)	9.6 (1.90,351.00)	**<0.001**	5.8 (1.40, 278.50)	8.0 (1.90,351.00)	**0.047**	6.4 (2.20, 132.00)	8.7 (2.90, 308.40)	**<0.001**	7.6 (1.50, 207.30)	10.5 (2.20, 291.10)	**0.017**
IB (μmol/L)	7.4 (2.20, 112.00)	9.4 (1.80, 138.40)	**<0.001**	7.6 (3.40, 112.00)	7.9 (1.80, 112.50)	**0.422**	7.5 (2.40, 59.10)	7.9 (3.50, 138.40)	**<0.001**	7.3 (2.20, 89.70)	9.4 (4.40, 108.90	**0.014**
TG (mmol/L)	2.09 ± 1.14	3.05 ± 2.25	**<0.001**	1.95 ± 1.14	3.28 ± 2.84	**<0.001**	1.88 ± 0.91	3.14 ± 2.24	**0.001**	2.33 ± 1.23	2.93 ± 1.92	**0.017**
Albumin (g/L)	29.30 ± 5.45	27.58 ± 5.46	**0.003**	29.72 ± 5.78	28.18 ± 6.03	**0.161**	29.19 ± 5.17	27.83 ± 5.67	**0.299**	28.42 ± 5.18	27.16 ± 5.10	**0.138**
Globulin (g/L)	26.35 ± 6.40	24.29 ±7.45	**0.004**	27.44 ± 6.05	26.69 ± 8.21	**0.544**	25.48 ± 5.02	21.33 ± 5.95	**0.002**	25.36 ± 7.22	23.86 ± 7.46	**0.215**
UREA (mmol/L)	5.91 ± 3.76	9.68 ± 6.82	**<0.001**	5.91 ± 4.18	9.67 ± 8.06	**<0.001**	5.37 ± 2.96	10.00 ± 7.27	**<0.001**	6.38 ± 3.83	9.99 ± 6.17	**<0.001**
CREA (μmol/L)	67.19 ± 42.23	84.81 ± 60.55	**0.001**	72.61 ± 59.29	81.12 ± 65.86	**0.455**	61.27 ± 23.89	77.98 ± 43.17	**0.029**	65.56 ± 29.11	91.61 ± 65.51	**0.001**
UA (μmol/L)	245.50 ± 121.77	306.14 ± 193.58	**<0.001**	231.67 ± 117.05	270.42 ± 199.56	**0.147**	255.83 ± 120.36	287.08 ± 138.17	**0.312**	255.90 ± 114.44	349.49 ± 214.44	**<0.001**
Chloride (mmol/L)	101.90 ±5.05	100.52 ± 6.99	**<0.001**	102.23 ± 4.95	100.58 ± 8.20	**0.015**	102.14 ± 4.64	100.15 ± 6.26	**0.130**	101.58 ± 5.58	100.82 ± 6.56	**0.442**

APTT, activated partial thromboplastin time; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *CREA*, creatinine; *DB*, direct bilirubin; *EBV*, Epstein-Barr virus; *FIB*, fibrinogen; *I-HLH*, infection-associated HLH; *IB*, indirect bilirubin; *INR*, international normalized ratio; *M-HLH*, malignancy-associated HLH; *LY*, lymphocyte; *MCTD*, mixed connective tissue disease; *Mix-HLH*, mixed-cause HLH; *PLT*, platelet; *PT*, prothrombin time; *TB*, total bilirubin; *TG*, triglyceride; *TT*, thrombin time; *UA*, uric acid; *Unclear*, unknown underlying diseases; *UREA*, urea nitrogen

Table 3 Logistic regression analysis of independent factors and 30-day mortality

Variables	Univariate analysis			Multivariate analysis			
	HR	95% CI	*p*-value	β	HR	95% CI	*p*-value
30-day mortality of total HLH patients (yes vs. no)							
Age > 47y	2.722	1.672–4.433	<0.001	1.095	2.990	1.657–5.397	<0.001
Ferritin> 3678μg/L	4.637	2.984–7.207	<0.001	1.450	4.264	2.513–7.238	<0.001
LY ≤ 0.44×10^9/L	0.293	0.191–0.452	<0.001	–0.938	0.391	0.232–0.659	<0.001
INR > 1.32	3.095	1.896–5.053	<0.001	0.921	2.513	1.382–4.568	0.003
TT > 20.8s	3.589	2.283–5.641	<0.001	0.731	2.077	1.193–3.616	0.010
Globulin ≤ 23.3g/L	0.411	0.270–0.627	<0.001	–0.682	0.506	0.301–0.848	0.010
UA > 375μmol/L	2.772	1.655–4.645	<0.001	0.969	2.637	1.394–4.988	0.003
Chloride ≤ 99.4 mmol/L	0.396	0.258–0.608	<0.001	–0.736	0.479	0.285–0.806	0.006
AUC		0.838					
Clinical model: Logit P = 1.095× age + 1.450 × ferritin – 0.938 × LY + 0.921 × INR + 0.731 × TT – 0.682 × globulin + 0.969 × UA – 0.736 × chloride – 4.355							
30-day mortality of total I-HLH patients (yes vs. no)							
Age > 56y	3.045	1.415–6.556	0.005	2.176	8.813	2.602–29.848	<0.001
LY ≤ 0.36×10^9/L	0.218	0.095–0.497	<0.001	–1.449	0.235	0.078–0.710	0.010
APTT > 37.6s	4.229	1.948–9.179	<0.001	1.615	5.028	1.709–14.799	0.003
TT > 18.7s	5.476	2.369–12.658	<0.001	1.318	3.736	1.233–11.321	0.020
AST > 99.9U/L	4.524	2.091–9.787	<0.001	1.296	3.656	1.219–10.965	0.021
TG > 2.48mmol/L	3.816	1.769–8.271	0.001	1.390	4.016	1.356–11.894	0.012
Chloride ≤ 99.0 mmol/L	0.224	0.103–0.488	<0.001	–1.543	0.214	0.074–0.615	0.004
AUC		0.913					
Clinical model: Logit P = 2.176 × age – 1.499 × LY + 1.615 × APTT + 1.318 × TT + 1.296 × AST + 1.390 × TG – 1.543 × chloride – 7.484							
30-day mortality of total M-HLH patients (yes vs. no)							
Ferritin> 3557.5μg/L	18.333	5.258–63.922	<0.001	3.986	53.839	7.772–372.961	<0.001
LY ≤ 0.53×10^9/L	0.183	0.065–0.515	0.001	–2.458	0.086	0.016–0.467	0.011
TB > 94.8μmol/L	7.800	2.104–28.920	0.002	2.512	12.326	1.765–86.063	0.005
AUC		0.921					
Clinical model: Logit P = 3.986 × ferritin – 2.458 × LY + 2.512 × TB – 6.274							
30-day mortality of total Mix-HLH patients (yes vs. no)							
Ferritin>4009.3μg/L	2.833	1.457–5.510	0.003	0.759	2.215	1.009–4.864	0.048
LY ≤0.44×10^9/L	0.413	0.212–0.801	0.011	–0.948	0.387	0.175–0.859	0.020
APTT > 47.4s	4.533	1.865–11.019	0.001	1.405	4.075	1.470–11.294	0.007
IB > 7.4μmol/L	2.588	1.305–5.132	0.008	0.923	2.516	1.129–5.609	0.024
TG > 3.84mmol/L	4.558	1.718–12.091	0.002	1.138	3.120	1.037–9.386	0.043
UA > 375μmol/L	4.059	1.897–8.686	<0.001	1.543	4.680	1.918–11.418	0.001
AUC		0.809					
Clinical model: Logit P = 0.759 × ferritin – 0.948 × LY + 1.405 × APTT + 0.923 × UB + 1.138 × TG + 1.543 × UA – 6.652							

APTT, activated partial thromboplastin time; *AST*, aspartate aminotransferase; *CI*, confidence interval; *HLH*, hemophagocytic lymphohistiocytosis; *HR*, hazard ratio; *I-HLH*, infection-associated HLH; *IB*, indirect bilirubin; *INR*, international normalized ratio; *M-HLH*, malignancy-associated HLH; *LY*, lymphocyte; *Mix-HLH*, mixed-cause HLH; *TB*, total bilirubin; *TG*, triglyceride; *TT*, thrombin time; *UA*, uric acid; *Unclear*, unknown underlying diseases

Laboratory markers could be useful in monitoring the prognosis of HLH patients. It is reported that old age, low LY, elevated ferritin, high post-treatment ferritin, TB, triglycerides, and AST were positively related with adverse events such as early death, long prognosis in adult HLH patients, or malignancy-associated HLH [3,

Table 4. Diagnostic statistics for clinical predication models

Clinical model	Cutoff value	AUC	*p* - value	Model fixed at maximum Youden's Index			
				Sensitivity	Specificity	PPV	NPV
HLH	0.3486	0.838 (0.800–0.872)	< 0.001	72.1	82.5	63.7	87.4
Logit P = 1.095× age + 1.450 × ferritin – 0.938 × LY + 0.921 × INR + 0.731 × TT – 0.682 × globulin + 0.969 × UA – 0.736 × chloride – 4.355							
I-HLH	0.1804	0.913(0.856–0.953)	<0.001	94.9	76.6	58.7	97.7
Logit P = 2.176 × age – 1.499 ×LY + 1.615 × APTT + 1.318 × TT + 1.296 × AST + 1.390 × TG – 1.543 ×chloride – 7.484							
M-HLH	0.3305	0.921(0.838–0.969)	<0.001	75.0	94.6	85.7	89.8
Logit P = 3.986 × ferritin – 2.458 ×LY + 2.512 × TB – 6.274							
Mix-HLH	0.4034	0.809(0.740–0.866)	<0.001	68.4	80.0	65.0	82.3
Logit P = 0.759 × ferritin – 0.948 ×LY + 1.405 × APTT + 0.923 × IB + 1.138 × TG + 1.543 × UA – 6.652							

APTT, activated partial thromboplastin time; *AST*, aspartate aminotransferase; *CI*, confidence interval; *HLH*, hemophagocytic lymphohistiocytosis; *HR*, hazard ratio; *I-HLH*, infection-associated HLH; *IB*, indirect bilirubin; *INR*, international normalized ratio; *M-HLH*, malignancy-associated HLH; *LY*, lymphocyte; *Mix-HLH*, mixed-cause HLH; *NPV*, negative predictive value; *PPV*, positive predictive value; *TB*, total bilirubin; *TG*, triglyceride; *TT*, thrombin time; *UA*, uric acid; *Unclear*, unknown underlying diseases

Youden's Index=sensitivity+specificity-1

8, 15–19]. Similar results were also concluded in our research. Furthermore, to pediatric HLH patients, INR > 1.5, APTT, TB > 19 μmol/L, and globulin < 20 g/L were risk factors for 7 days and within 30 days prognosis [20]. In this investigation, similar results were gained in adult HLH patients.

Serum bilirubin from the metabolism of heme may be effective in protecting the body from oxidant inflammation and cancers in colorectal cancer [21, 22]. Previous research has described that elevated IB was a risk factor in mortality in the acute phase of ischemic stroke patients [23]. UA, an end product of purine, plays a key role in antioxidants

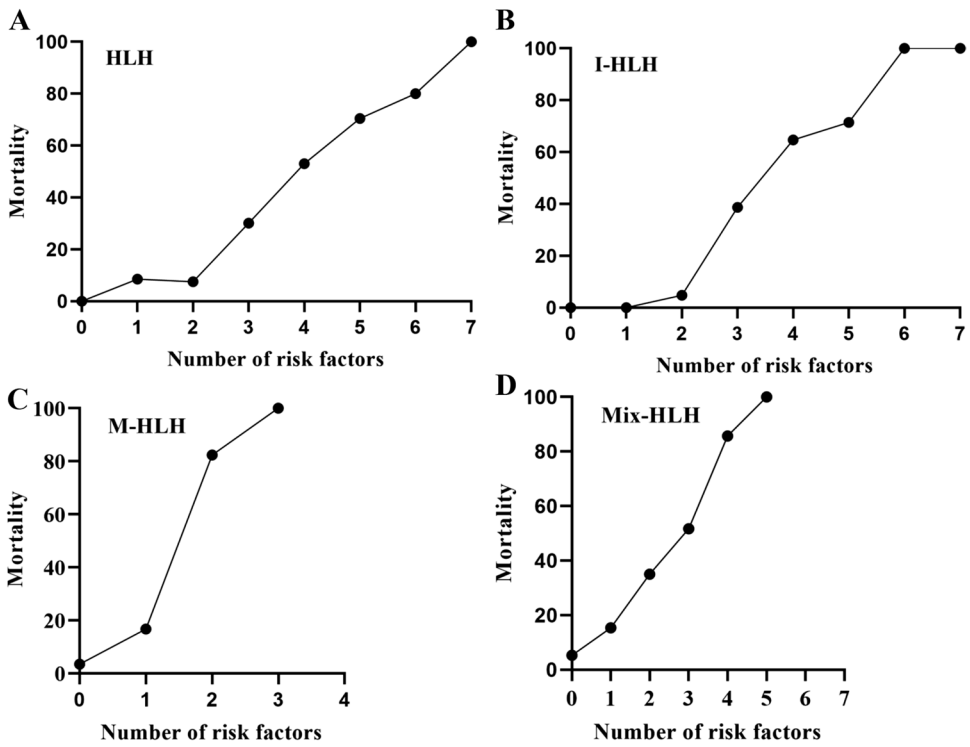

Fig. 1 Distribution of mortality by cumulative number of risk factors. **A** Distribution of mortality for HLH patients. **B** Distribution of mortality for I-HLH patients. **C** Distribution of mortality for M-HLH patients. **D** Distribution of mortality for Mix-HLH patients

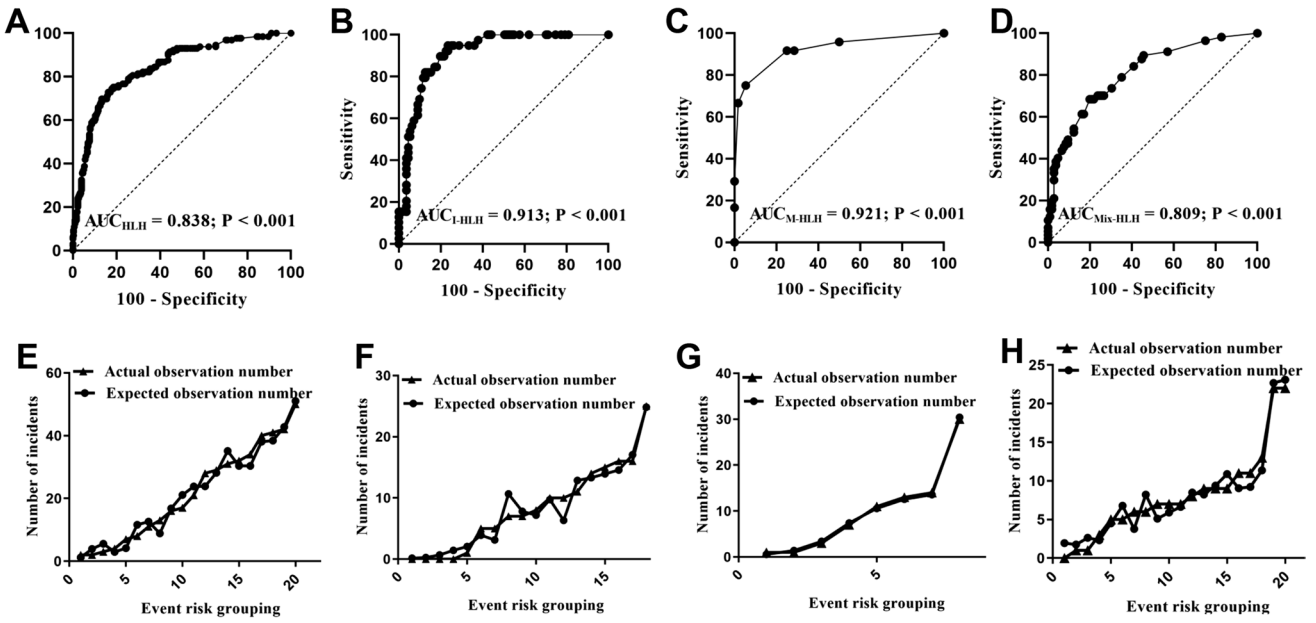

Fig. 2 Performance of the models. **A** AUC$_{HLH}$ for model. **B** AUC $_{I-HLH}$ for model. **C** AUC$_{M-HLH}$ for model. **D** AUC$_{Mix-HLH}$ for model. **E** Calibration curves for HLH model. **F** Calibration curves for I-HLH model. **G** Calibration curves for M-HLH model. **H** Calibration curves for Mix-HLH model.

[24–26]. Chloride ion, a plentiful anion in the extracellular fluid, is an essential part of many functions to work with the body [27]. A high level of serum chloride (≥ 105.4 mmol/L) is an adverse prognosis marker for IgAN patients [28]. Our study also provides clinical data on the prognosis role of the above parameters in adult HLH patients. In addition, the thrombin time (TT), a common coagulation index, has rarely been studied in many diseases. In this research, we firstly found that high TT was an independent and risk factor for HLH patients in 30-day death.

In this research, we built four models based on admission laboratory results. Firstly, we divided the total patients into I-HLH, M-HLH, and Mix-HLH groups according to the underlying diseases. Then, univariate and multivariable logistics regression analyses were applied to select the independent factors to predict 30-day mortality in total and subgroups. We found that independent factors were different in different groups with different values. Therefore, the choice of which laboratory marker should be the primary outcome may depend on the underlying disease. For example, LY is an independent factor to total and subgroup HLH. Ferritin is an independent factor in total, M-HLH, and Mix-HLH. Age, TT, and chloride are independent factors to total and I-HLH.

Strengths of the investigation included that data were from a single center with large samples. Patients were evaluated as total and subgroups with biomarkers that are available and affordable in clinical practice. UA, TT, and chloride were firstly confirmed as independent factors in adult HLH.

As a retrospective research, selection bias is unavoidable. Internal and external validation is lacking. In addition, less information on treatment and genetic testing is included. We encourage these findings to be tested in other studies with multi-centers.

In conclusion, we built four models with biomarkers that are available and affordable in clinical practice. With these models, high-risk patients with different underlying disease could be easily identified.

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Author Contribution Hua-Guo Xu and Jun Zhou designed the study. All the authors contributed to the generation, collection, assembly, analysis, and/or interpretation of data. Jun Zhou wrote the manuscript; Hua-Guo Xu, Zhi-Qi Wu, and Tengfei Qiao revised the manuscript. All the authors have read the manuscript and approved the final manuscript.

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Data availability The data and materials can be found from the corresponding author.

Declarations

Ethics Approval and Consent to Participate The study is in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University (Nanjing, China) (2019-SR-066).

Competing Interests The authors declare no competing interests.

Consent for Publication Patients signed informed consent regarding publishing their data.

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