Development and Initial Validation of a Novel Prognostic Model in Adult Secondary Hemophagocytic Lymphohistiocytosis Patients: Experience From a Single-center Retrospective Study

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Abstract

Objectives: To describe the prognostic factors of adult patients diagnosed with secondary hemophagocytic lymphohistiocytosis (HLH), a rare disease caused by excessive immune activation and uncontrolled cytokine storm, and to establish and initially validate the predictive value of a prognostic model.

Methods: We conducted a retrospective cohort study of 204 adult secondary HLH patients from January 2010 to December 2018 (the development cohort, n=161) and January 2019 to December 2020 (the validation cohort, n=43). All patients met ≥5 HLH-2004 criteria. Prognostic variables to death within three months at diagnosis were selected by logistic regression models, and performance of the predictive model was assessed in development and validation cohort, respectively.

Results: Of all patients, malignancies were the most common trigger, accounting for 61.3%. Patients with poor prognosis showed lower hemoglobin (P<0.001), platelets (P<0.001), albumin (P=0.007) and fibrinogen (P=0.002), and higher levels of total bilirubin (P=0.007), LDH (P=0.033), BUN (P=0.006), and TG (P=0.015). Furthermore, patients with poor prognosis had a higher ISTH score (P<0.001). In the development cohort, a predictive model was established based on four variables (splenomegaly, hemoglobin, LDH and ISTH score) recognized by the multivariate logistic regression, with the cut-off value of 0.277 reaching a sensitivity of 87.7%, specificity of 49.0% and AUC of 0.731 (95%CI 0.651-0.810, P<0.001). Besides, the model could perform well in the validation cohort, with a sensitivity of 53.8%, specificity of 40.0% and accuracy of 44.2%.

Conclusions: Our predictive model provides a possibility of forecasting the prognosis of adult patients within three months at diagnosis of secondary HLH, while more large sample, multicenter, randomized controlled clinical research are needed to confirm it.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH), sometimes referred to as hemophagocytic syndrome (HPS), is a rare, life-threatening systematic hyperinflammatory disorder that can rapidly deteriorate to disseminated intravascular coagulation (DIC), multiple organ failure and death[1]. In general, the in-hospital mortality of HLH was reported ranging from 20–75%, with even greater risk for death in critically ill patients[2]. It is clinically characterized by persistent high fever, hemocytopenia, hepatosplenomegaly, and activated macrophage infiltration in hematopoietic organs. According to the International Histiocyte Society guidelines, a diagnosis of HLH requires a molecular evidence or at least five out of eight HLH-2004 criteria[3]: fever, splenomegaly, hemocytopenias, hypertriglyceridemia and/or hypofibrinogenemia, hyperferritinemia, elevated soluble interleukin-2 receptor (sIL-2R), decreased or deficient natural killer cell activity, and hemophagocytosis in bone marrow/spleen/adenoids. Broadly, it is classified into primary (familial) and secondary (acquired) hemophagocytic lymphohistiocytosis, with the latter accounting for approximately 90% of HLH patients[4]. Compared to primary HLH resulting from genetic defects, more underlying triggers were identified in secondary HLH, including infections, malignancies, autoimmune disease, acquired immunodeficiency, and drug therapies[1, 5]. Recent studies suggest that infections and drugs are the two main causes of secondary HLH, while epidemiological data from China showed that about 60% are caused by malignant neoplasms especially hematological malignancies[6, 7].

Hemophagocytic lymphohistiocytosis may occur at any age, although a nationwide survey of HLH in Japan indicated that over half of the patients were juveniles younger than fifteen years old[8]. For the past several decades, it seems that hemophagocytic lymphohistiocytosis has been well described in pediatric patients, however

few scientific analyses of adult HLH has been done, with most of them being case reports or series. Moreover, existing literatures on adult HLH predominantly focus on the diagnosis and clinical therapies, while only a few small studies provide the prognostic data of adult HLH patients. Despite several risk factors associated with poor prognosis have been found, it is still difficult to transform into clinical practice with the lack of verification of these indicators. Therefore, we performed a retrospective cohort study on basis of 204 adult patients diagnosed with secondary hemophagocytic lymphohistiocytosis, introduing a clinically applicable combination of parameters leading to prognostic assessment in hemophagocytic lymphohistiocytosis.

2. Results

2.1 General characteristics of 204 adult patients diagnosed with secondary HLH

Between January 2010 and December 2020, a total of 448 patients diagnosed with hemophagocytic lymphohistiocytosis at discharge were retrieved from the database of West China Hospital of Sichuan University. As shown in Fig. 1, 204 adult HLH patients were finally included in our study. Of them, 114 (55.9%) were male and 90 (44.1%) were female, with the overall average age of 43.42 ± 16.85 years (Table 1). 134 (65.7%) patients were determined with good prognosis at the end of follow-up and 70 (34.3%) had poor prognosis (16 deteriorated and 54 dead). According to the HLH-2004 diagnostic criteria, 191 (93.6%) patients had prolonged fever of 38.5℃ or above, and splenomegaly were found in 173 (84.8%) patients. Two or more lines of hemocytopenia occurred in 174 (85.3%) patients, while 164 participants had elevated triglyceride and/or decreased fibrinogen. All 204 included patients underwent bone marrow biopsy, among them only 87 (42.6%) were found hemophagocytosis histopathologically. Elevated concentrations of ferritin and sIL-2R in sera were discovered in some patients, with 194 of 196 (99.0%) for ferritin and 128 of 137 (93.4%) for sIL-2R.

Malignancies were the most common underlying triggering factor, accounting for 61.3%, followed by infections (31.9%) and autoimmune disorder (3.9%). However, the triggers to HLH in 6 (2.9%) patients remained unclear at their discharge from hospital. Clinical symptoms at the onset were collected in this study, among them 110 (53.9%) patients had symptoms of respiratory system, 73 (35.8%) in digestive and 80 (39.2%) in neural system, respectively. Organ enlargement was also an important objective sign of hemophagocytic lymphohistiocytosis, with 84.8% of patients having obvious splenomegaly, 60.8% having adenomegaly and 36.3% having hepatomegaly on physical and/or radiological examinations. Interestingly, it seemed that patients with good prognosis were more likely to find splenomegaly ($P = 0.009$). Besides, small numbers of patients in our research had clinical manifestations of skin rashes (13.2%), jaundice (11.8%), and hemorrhage (26.0%), and more cases in poor prognosis group had bleedings such as epistaxis, subcutaneous ecchymosis, and gastrointestinal hemorrhage ($P = 0.003$).

On aspect of laboratory results, high loads of EBV-DNA ($\geq 10^2$ copies) in peripheral blood were defined as positive reaction, therefore 128 (62.7%) patients in this study were tested positive for EBV-DNA. In contrast to patients with good prognosis, cases in poor prognosis group showed lower hemoglobin ($P < 0.001$), platelets ($P < 0.001$), albumin ($P = 0.007$) and fibrinogen ($P = 0.002$), and higher levels of total bilirubin ($P = 0.007$), LDH ($P = 0.033$), BUN ($P = 0.006$), and TG ($P = 0.015$). Furthermore, patients with poor prognosis had a significantly higher ISTH score ($P < 0.001$).

Table 1
Demographical and clinical characteristics among patients with secondary HLH.

	All (n = 204)	Good prognosis (n = 134)	Poor prognosis(n = 70)	*P*
Demographical characteristics				
Gender (male/female)	114(55.9)/90(44.1)	76(56.7)/58(43.3)	38(54.3)/32(45.7)	0.768
Age (years)	43.42 ± 16.85	43.35 ± 17.09	43.56 ± 16.49	0.934
Underlying triggering factor				
Infections	65(31.9)	44(32.8)	21(30.0)	0.660
malignancies	125(61.3)	79(59.0)	46(65.7)	
Autoimmune disorder	8(3.9)	6(4.5)	2(2.9)	
Uncertain	6(2.9)	5(3.7)	1(1.4)	
Clinical manifestations				
Respiratory system	110(53.9)	70(52.2)	40(57.1)	0.555
Digestive system	73(35.8)	48(35.8)	25(35.7)	1.000
Neural system	80(39.2)	51(38.1)	29(41.4)	0.653
Organ enlargement				
Liver	74(36.3)	53(39.6)	21(30.0)	0.220
Spleen	173(84.8)	120(89.6)	53(75.7)	**0.013***
Lymph nodes	124(60.8)	81(60.4)	43(61.4)	1.000
Skin rashes	27(13.2)	17(12.7)	10(14.3)	0.828
Jaundice	24(11.8)	14(10.4)	10(14.3)	0.493
Hemorrhage	53(26.0)	26(19.4)	27(38.6)	**0.004***
Laboratory results				
Positive EBV-DNA	128(62.7)	84(62.7)	44(62.9)	1.000
Hemoglobin (g/L)	80.05 ± 18.92	83.90 ± 19.56	72.69 ± 15.22	**< 0.001***
Platelets ($\times 10^9$/L)	39.00(18.00,65.00)	45.00(25.00,77.50)	29.00(14.00,47.25)	**< 0.001***

EBV-DNA: Epstein-Barr virus deoxyribonucleic acid; TB: total bilirubin; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; BUN: blood urea nitrogen; TG: triglyceride; Na^+: sodium ion; Ca^{2+}: calcium ion; PT: prothrombin time; FIB: fibrinogen; ISTH: the International Society on Thrombosis and Hemostasis.

*$P < 0.05$.

	All (n = 204)	Good prognosis (n = 134)	Poor prognosis(n = 70)	*P*
Leukocytes ($\times 10^9$/L)	3.32 ± 3.79	3.46 ± 3.49	3.04 ± 4.31	0.461
Neutrophils ($\times 10^9$/L)	2.30 ± 2.95	2.49 ± 2.98	1.95 ± 2.88	0.213
Lymphocytes ($\times 10^9$/L)	0.66 ± 0.85	0.68 ± 0.88	0.62 ± 0.80	0.631
Monocytes ($\times 10^9$/L)	0.19 ± 0.25	0.20 ± 0.22	0.19 ± 0.31	0.976
TB (μmol/L)	18.75(11.73,44.10)	16.45(10.45,34.70)	27.25(15.90,82.08)	**0.007***
ALB (g/L)	27.76 ± 5.02	28.45 ± 5.00	26.45 ± 4.82	**0.007***
ALT (IU/L)	64.50(28.50,153.50)	54.00(27.75,141.00)	75.50(32.50,169.00)	0.092
AST (IU/L)	91.00(44.25,230.75)	80.50(38.00,197.00)	133.50(54.00,373.75)	0.089
LDH (IU/L)	742.00(480.50,1263.25)	617.50(390.75,1099.00)	905.00(639.75,1607.50)	**0.033***
BUN (mmol/L)	5.58(3.97,7.90)	4.83(3.78,7.43)	6.28(4.68,8.58)	**0.006***
Creatinine (μmol/L)	57.55(46.25,74.00)	57.05(44.75,72.00)	60.50(47.00,81.25)	0.066
TG (mmol/L)	2.72(1.76,3.88)	2.34(1.65,3.71)	3.09(2.16,4.41)	**0.015***
Na^+ (mmol/L)	134.35(131.63,137.85)	134.35(131.88,137.28)	134.20(130.53,138.45)	0.823
Ca^{2+} (mmol/L)	1.92(1.78,2.03)	1.94(1.81,2.03)	1.88(1.74,2.03)	0.089
PT (s)	13.85(12.20,16.65)	13.40(11.98,15.23)	15.15(12.70,18.83)	0.075
FIB (g/L)	1.23(0.80,1.91)	1.39(0.90,2.36)	0.95(0.64,1.29)	**0.002***
Hemophagocytosis	87(42.6)	59(44.0)	28(40.0)	0.655
ISTH score	4.60 ± 1.88	4.10 ± 1.73	5.56 ± 1.80	**< 0.001***

EBV-DNA: Epstein-Barr virus deoxyribonucleic acid; TB: total bilirubin; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; BUN: blood urea nitrogen; TG: triglyceride; Na^+: sodium ion; Ca^{2+}: calcium ion; PT: prothrombin time; FIB: fibrinogen; ISTH: the International Society on Thrombosis and Hemostasis.

*$P < 0.05$.

2.2 Logistic regression analyses and establishment of the clinical prognostic regression model

To further establish a predictive model for poor prognosis of adult secondary HLH patients and evaluate its accuracy, patients were divided into two groups based on their time to our hospital: 161 (78.9%) patients from January 2010 to December 2018 were allocated to the development cohort and the remaining 43 (21.1%) from January 2018 to December 2020 in the validation cohort.

Firstly, the continuous clinical data in the development cohort were converted to categorical variables according to our previous experience or the elevation of laboratory results beyond the upper limit of normal reference. And then, we compared the difference of these variables between patients with different clinical outcomes (Additional file 1), and ten variables with $P < 0.1$ (splenomegaly, hemorrhage, hemoglobin, platelet count, leukocyte count, LDH, creatinine, PT, fibrinogen and ISTH score) were identified. To avoid potential interactions among variables related to bleeding and coagulation, the ISTH score was chosen for the next step, while variables of hemorrhage, platelet count, PT and fibrinogen were excluded. After that, we performed a multivariate logistic regression to verify whether they were still risk factors or not, and the results indicated four independent prognostic factors: splenomegaly (OR 0.351, 95%CI 0.134–0.922, $P = 0.034$), hemoglobin (OR 2.734, 95%CI 1.134–6.592, $P = 0.025$), LDH (OR 2.720, 95%CI 1.096–6.748, $P = 0.031$), and ITSH score (OR 2.262, 95%CI 1.085–4.716, $P = 0.030$) (Table 2). A predictive prognostic model was then developed based on these four variables, with the regression equation shown as follows:

Logit P =-1.047×splenomegaly + 1.006×hemoglobin + 1.001×LDH + 0.816×ISTH score-1.728 (with splenomegaly: 1, without splenomegaly: 0; hemoglobin < 90g/L: 1, hemoglobin ≥ 90g/L: 0; more than twice the normal upper limit of LDH: 1, less than twice the normal upper limit of LDH: 0; ISTH score ≥ 5: 1, ISTH score < 5: 0)

Table 2
Risk factors associated with poor prognosis of adult secondary HLH in the development cohort.

	Univariate			Multivariate		
	OR	95%CI	*P*	OR	95%CI	*P*
Splenomegaly	0.327	(0.134,0.794)	**0.014****	0.351	(0.134,0.922)	**0.034****
Hemorrhage	2.674	(1.310,5.458)	**0.007****			
Hemoglobin < 90g/L	2.370	(1.039,5.408)	**0.040****	2.734	(1.134,6.592)	**0.025****
Platelets < 50×10^9/L	2.343	(1.144,4.799)	**0.020****			
Leukocytes < 3.5×10^9/L	1.944	(0.894,4.226)	**0.094***			
More than twice the normal upper limit of LDH	3.243	(1.387,7.583)	**0.007****	2.720	(1.096,6.748)	**0.031****
Creatinine > 108μmol/L	3.233	(1.005,10.401)	**0.049****			
Prolonged PT > 3s	2.516	(1.269,4.989)	**0.008****			
FIB < 1.5g/L	3.986	(1.713,9.275)	**0.001****			
ISTH score ≥ 5	3.085	(1.551,6.134)	**0.001****	2.262	(1.085,4.716)	**0.030****

LDH: lactate dehydrogenase; PT: prothrombin time; FIB: fibrinogen; ISTH: the International Society on Thrombosis and Hemostasis.

*P< 0.1; **P< 0.05.

2.3 Performance of the prognostic model in the development cohort and cut-off value of *P*

ROC curve was used to compare the predictive ability of the model for clinical outcome of adult patients within 3 months after diagnosis of secondary HLH, and the results showed that the predictive model was superior to any of single indicators, with an AUC of 0.731 (95%CI 0.651–0.810, P< 0.001) (Fig. 2). Besides, the test for calibration showed Hosmer-Lemeshow statistic of 0.577. The Youden's index was calculated to find the optimal cut-off value of *P*. For this predictive model, the best value to distinguish good and poor prognosis of adult secondary HLH patients was 0.277, with sensitivity of 87.7% and specificity of 49.0%. Details of different cut-off values were demonstrated in Table 3.

Table 3
Sensitivity and specificity of the predictive model at each cut-off value.

Cut-off value	Sensitivity (%)	Specificity (%)	Youden's index
0.091	98.2	3.8	2.1
0.134	96.5	4.8	1.3
0.145	96.5	17.3	13.8
0.148	93.0	37.5	30.5
0.213	93.0	38.5	31.4
0.277	87.7	49.0	36.8
0.297	80.7	54.8	35.5
0.321	59.6	69.2	28.9
0.326	57.9	71.2	29.0
0.419	57.9	73.1	31.0
0.517	22.8	95.2	18.0
0.523	17.5	95.2	12.7
0.546	17.5	96.2	13.7
0.659	15.8	97.1	12.9
Youden's index = sensitivity + specificity-1.			

2.4 Initial validation of the model for prognostic evaluation of secondary HLH patients

We compared the difference of clinical parameters between the development and validation group, and the results showed that differences between almost all indicators except fibrinogen levels in the two groups were statistically non-significant (Additional file 2), which meant the general baseline characteristics of patients in different groups was consistent. Subsequently, based on the former logistic regression model and optimal cut-off value, we validated the predictive reliability of the prognostic model in 43 secondary HLH patients from January 2019 to December 2020, and it turned out that the model had a sensitivity of 53.8%, specificity of 40.0% and accuracy of 44.2%.

3. Discussion

In this pilot study, we explored risk factors associated with poor prognosis of secondary hemophagocytic lymphohistiocytosis in adult patients, established a prognostic model on forecasting the clinical outcome of these patients within three months at the initial diagnosis of HLH, and verified its validity and practicability. This is the first report, to our knowledge, that developed and validated a predictive model in adult secondary HLH based on logistic regression analyses. Hemophagocytic lymphohistiocytosis is a rapidly progressive, life-threatening

systematic inflammatory syndrome, characterized by excessive immune activation and uncontrolled cytokine storm that results in myelosuppression and vascular endothelial injury[10]. Patients with HLH tend to have excess length of stay, with almost half of patients being hospitalized for more than two months in our study, and as a result, we compared the clinical outcomes of adult secondary HLH patients within 3 months. Totally, the crude mortality in this study was 26.5%. A previous study in twenty-one countries showed that over 10% of pediatric patients died within two months of diagnosis due to bleeding in visceral organs, opportunistic infections, or multiple organ failure[11]. In contrast to adolescents, the prognosis is worse for adult patients. Otrock et al. reported in their study that the 30-day mortality of adult HLH from admission was 30%, with the range of duration of hospital stay from 1 to 89 days[12]. In another systematic review of critically ill adult hemophagocytic lymphohistiocytosis patients, the overall mortality was 57.8%, and the median time until death in subgroup analysis was 26 days (IQR 10–73 days) [13]. In this study, researchers also found that infections were the most common trigger. The predominant cause of HLH differs in each country, with infections being the main trigger of adult HLH in USA, France, Spain and South Korea, while malignancies were most common in Italy, China and Japan, suggesting the probably specific geographical distribution of triggering agents[14]. In consistence with previous studies[15–17], we found that malignancies especially lymphoma and leukemia were the most frequent cause of adult secondary HLH. While in another research, infections were reported as the most common cause of HLH[18]. A multicenter retrospective etiological analysis of 601 HLH patients in China found that the most common causes of HLH were infections in children child group, infections and malignancies in youth group, and malignancies in middle-aged and elderly group[19]. In summary, diversity of age and geographical distributions might partially explained the difference of triggering factors among studies.

There are many factors affecting the prognosis of secondary hemophagocytic lymphohistiocytosis in adult patients. Liver injury and dysfunction is common in HLH, with severe disease associated with coagulopathy such as hemorrhage and DIC[20]. Our results showed that patients with poor prognosis had worse liver function indexes (TB, ALB and LDH) and were more likely accompanied by hemorrhagic manifestations of epistaxis, subcutaneous ecchymosis, and gastrointestinal hemorrhage. Apart from this, we found in our research for the first time that the ISTH score, is an independent risk factor for poor outcome in HLH patients. The ISTH criteria is widely used to assist diagnosis of hemorrhagic disorders like DIC, and recently it has been found capable of predicting risk of disease worsening in COVID-19 and hematological malignancies[21, 22]. A higher ISTH score in HLH patients with poor prognosis likely reflects the combined effects of hepatic synthetic function, endothelial activation and DIC. Additionally, several risk factors were reported correlated with poor prognosis of HLH in other studies as well. Renal insufficiency (defined as serum creatinine level above the normal reference range for age) was indicated a predictor for early death in adult HLH, with the OR of 4.39[12]. In another study, several blood-based inflammatory markers like lymphocyte-to-monocyte ratio (LMR) and red blood cell distribution width-to-platelet ratio (RPR) were considered as independent factors to predict the overall survival of patients with HLH[16]. A prospective research containing 20 lymphoma-associated HLH patients revealed that interferon-γ secretion capacity of lymphocytes was significantly decreased and it might be used as a predictor of prognosis[23]. From an etiopathogenically driven analysis of adult HLH, researchers found that infection with more than one microbiological agent was the only independent variable associated with mortality[24]. In addition to these traditional predictors, novel biomarkers such as soluble CD163, plasma pentraxin 3 (PTX3) and presepsin displayed critical value for diagnosis and prognosis in patients with secondary HLH[25–27]. Despite many factors were considered to be related to the poor clinical outcomes of HLH, for clinicians, varieties of predictive markers mean the complexity and severity of the disease, and as a result, dynamic monitoring of laboratory indicators other than finding more predictive markers seems more important.

Up to now, few studies have focused on prognostic models for hemophagocytic lymphohistiocytosis. Zhou and colleagues conducted a prognostic model for adult HLH based on three laboratory markers (ferritin, platelets and alanine aminotransferase), which had an obvious improvement in discriminating risk of death in contrast with any of these markers alone[18]. In their study, the cut-off of 0.412 could reach a sensitivity of 76.9% and specificity of 78.9%. However, as the researchers pointed out, this study failed to further verify the accuracy of the model in new groups. External validation is the optimal strategy to control the reliability of predictive models. Unfortunately, the validation strategy is practically almost impossible to apply as HLH is a rare disease. In our study, we allocated 43 newly diagnosed HLH patients to an independent validation cohort and the assessment showed that the predictive model reached a sensitivity of 53.8% and specificity of 40.0%.

There are some limitations to our study. First, with the intrinsic defectiveness of retrospective studies, we had to accept some extent of missing clinical and follow-up data. To avoid these, we didn't use variables with missing values when building regression models, and patients without follow-up data were excluded at the beginning of research. Besides, treatment and several important inflammatory biomarkers such as ferritin, sIL-2R and CRP were not included in analyses, which might affect the precision of the model. Second, this is a small preliminary study, despite there was no statistical discrepancy between the development and validation cohort in demographical characteristics and clinical variables for the modeling establishment, the performance of our prognostic model was far from satisfaction, suggesting possible instability of outcome forecasts and necessity of a larger sample to verify the reliability. Meanwhile, the maximum Youden's index was used to determine the optimal cut-off of our predictive model in this research, assigning equal weights to sensitivity and specificity, yet it was reported that weighted methods might achieve more outstanding discrimination abilities[28]. The third limitation is, over 90% of patients in present study had a trigger of malignancies or infections, indicating that cautions were needed when applying this predictive model. Whatever, considering small number of researches on forecasting models, this study is still of great value in proposing a novel prognostic model for adult secondary HLH patients. Multicenter, prospective randomized controlled trials with larger sample size are further needed to verify the precision of our predictive model.

4. Conclusions

The current study established a predictive model of clinical outcomes of adult patients within three months at diagnosis of secondary hemophagocytic lymphohistiocytosis and initially validated the accuracy of the model, while more large sample, multicenter, randomized controlled clinical research are needed to confirm it.

5. Materials And Methods

5.1 Study population

A retrospective cohort study involving 204 consecutively diagnosed adult hemophagocytic lymphohistiocytosis patients (114 male and 90 female) was conducted at West China Hospital of Sichuan University, Chengdu, China, from January 2010 to December 2020. Eligibility criteria included: (1) patients aged over 18 years old; (2) fulfillment of five or more of the eight HLH-2004 diagnostic criteria. Patients who met any of the following criteria were excluded: (1) genetic abnormality considering primary HLH; (2) the same patient readmitted for recurrent HLH; (3) patients with underlying diseases such as hepatic and/or renal dysfunction, and acquired immunodeficiency syndrome; (4) patients lost to follow-up within three months after diagnosis of HLH.

An initial diagnosis of secondary hemophagocytic lymphohistiocytosis was based on the previously proposed clinical guideline (HLH-2004)[3]: (1) fever; (2) splenomegaly; (3) two or more cell lineage of hemocytopenia (hemoglobin < 90g/L, platelets < 100×10^9/L, neutrophils < 1.0×10^9/L); (4) hypertriglyceridemia (triglyceride > 3.0mmol/L) and/or hypofibrinogenia (fibrigen < 1.5g/L); (5) hemophagocytosis found in bone marrow/spleen/lymph nodes; (6) hyperferritinemia (ferritin ≥ 500μg/L); (7) elevated concentration of soluble interleukin 2 receptor (sIL-2R/sCD25 ≥ 2400u/mL); (8) low or absent activity of natural killer cells. Since that NK cell activity detection was not available in our hospital, the establishment of diagnosis based on the other seven items of diagnostic criteria.

5.2 Data collection and preprocessing

The medical record on each patient was reviewed, and demographical and clinical data were extracted and addressed using a predesigned template. Generally, the medical information collected for this study included: demographical data (sex and gender), clinical characteristics (highest recorded temperature, clinical manifestations, presence of organ enlargement, and underlying triggering factors), laboratory parameters (EB virus loads, hemoglobin, platelets, leukocytes, serum bilirubin levels, hepatic enzymes, lactate dehydrogenase [LDH], albumin, blood urea nitrogen [BUN], creatinine, triglyceride [TG], sodium and calcium levels, prothrombin time [PT], fibrinogen, ferritin, sIL-2R), pathological findings of hemophagocytosis in bone marrow, ISTH score, and clinical outcome within 3 months after diagnosis of secondary HLH. The laboratory results extracted from the medical records were those obtained on the day of diagnosis with HLH, or those obtained up to three days before or after in the absence of data from the same day.

The International Society on Thrombosis and Hemostasis (ISTH) score provides objective measurement of DIC and has been used to predict the mortality of critically ill patients[9]. It proposes a five-step diagnostic algorithm to calculate a DIC score utilizing simple clinical and laboratory parameters: (1) risk assessment including sepsis, trauma, organ damage, malignancies, obstetric events, vascular malformation and aneurysm, hepatic failure, severe poisoning and immune disorders, etc.; the patient with the presence of underlying disorder mentioned above is suitable for this algorithm and should proceed with the scoring system; (2) order global coagulation tests for PT, platelet count, fibrinogen, and fibrin related markers; (3) score the test results, 1) platelet count (> 100×10^9/L = 0, < 100×10^9/L = 1, < 50×10^9/L = 2), 2) fibrin related markers (e.g. D-dimer, fibrin degradation products) with no crease = 0, moderate increase = 2, and strong increase = 3; 3) prolonged PT (< 3s = 0, >3s but < 6s = 1, >6s = 2); 4) fibrinogen level (> 1.0g/L = 0, < 1.0g/L = 1); (4) calculate score; (5) interpretation of results, cumulative score < 5 suggests for non-overt DIC while ≥ 5 means compatible with overt DIC.

The clinical outcome of secondary HLH patients within 3 months after diagnosis was classified as improved (patients had no fever and laboratory results improved), deteriorated (the patient's body temperature was still higher than 38.5℃, and/or laboratory results didn't improve or even worsen), and dead (the patient was dead). Among them, a good prognosis was defined as improvement, while a poor prognosis was defined as deterioration or death. The clinical status of all patients at the end of follow-up was confirmed by hospital clinical records of patients or a telephone call to patients or their family members. This study is in accordance with the Declaration of Helsinki and authorized by the institutional ethics board. Besides, the observational design of the study did not impose the need for getting informed consent from individual patients.

5.3 Prognostic model establishment and validation

Model establishment and validation were based on a split-sample method. In brief, patients admitted to our hospital form January 2010 to December 2018 were assigned to the development cohort (n = 161), and the other 43 participants were reserved as an independent validation cohort. Both groups were compared with demographical and clinical variables.

The prognostic model was established using the data from the development cohort. At first, continuous clinical data were categorized on the basis of prior experience or the elevation beyond the upper limit of normal reference levels, and then, we compared the difference among categorical variables by Chi-square or Fisher's exact tests, and interested covariates ($P < 0.1$) were inputted into a multivariate binary logistic regression using the stepwise selection process to identify independent risk factors of poor prognosis of adult secondary HLH. Once the prognostic model was developed, the predictive reliability was evaluated by the tests for discrimination and calibration. Area under the receiver operating curve (AUC) was used for overall discrimination assessment, and Hosmer-Lemeshow statistic was used for the modeling calibration, with larger values representing better agreement between predicted risk score probability of the model and the real observed outcome. Subsequently, the cut-off value of the predictive model was determined by the maximum Youden's index. For both cohorts, further discrimination performance was assessed through standard measures of sensitivity, specificity and predictive values.

5.4 Statistical analysis

Quantitative data in accordance with normal distribution was presented as mean ± standard deviation (SD), and difference between groups was compared by Student's t-test, while non-parametric Mann-Whitney U test was used for skewness distribution data, indicated as median [interquartile range (IQR)]. Categorical variables were presented as numbers and proportions, and Chi-square or Fisher's exact tests were used for intergroup difference comparisons. Missing data was not included in analyses. Univariate and multivariate binary logistic regression analyses were carried out to recognize independent risk factors for poor prognosis in adult secondary HLH patients and establish predictive models. A two-tailed P value < 0.05 was considered statistically significant. All analyses were performed using SPSS software, version 22.0 (IBM Corp., Armonk, NY, USA).

Abbreviations

HLH, hemophagocytic lymphohistiocytosis; HPS: hemophagocytic syndrome; DIC: disseminated intravascular coagulation; sIL-2R: soluble interleukin-2 receptor; NK cell: natural killer cell; LDH: lactate dehydrogenase; BUN: blood urea nitrogen; TG: triglyceride; PT: prothrombin time; ISTH: the International Society on Thrombosis and Hemostasis; ROC: receiver operating characteristic; AUC: area under curve; SD: standard deviation; IQR: interquartile range; EBV-DNA: Epstein-Barr virus deoxyribonucleic acid.

Declarations

Ethics approval and consent to participate

This study is in accordance with the Declaration of Helsinki and authorized by the institutional ethics board. Besides, the observational design of the study did not impose the need for getting informed consent from individual patients.

Consent for publication

Not applicable.

Availability of data and materials

Please contact author for data requests.

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Competing interests

The authors declare that they have no competing interests.

Author Contributions

DGW carried out the design of the study, participated in collection and analyses of clinical data, and drafted the manuscript. XT participated in the design of the study and draft of manuscript, and revised the manuscript. LW participated in the design of the study, collection of the data and draft of the manuscript. WTZ performed the statistical analyses. SJZ, TLZ and QW participated in collecting the clinical data. HF revised the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

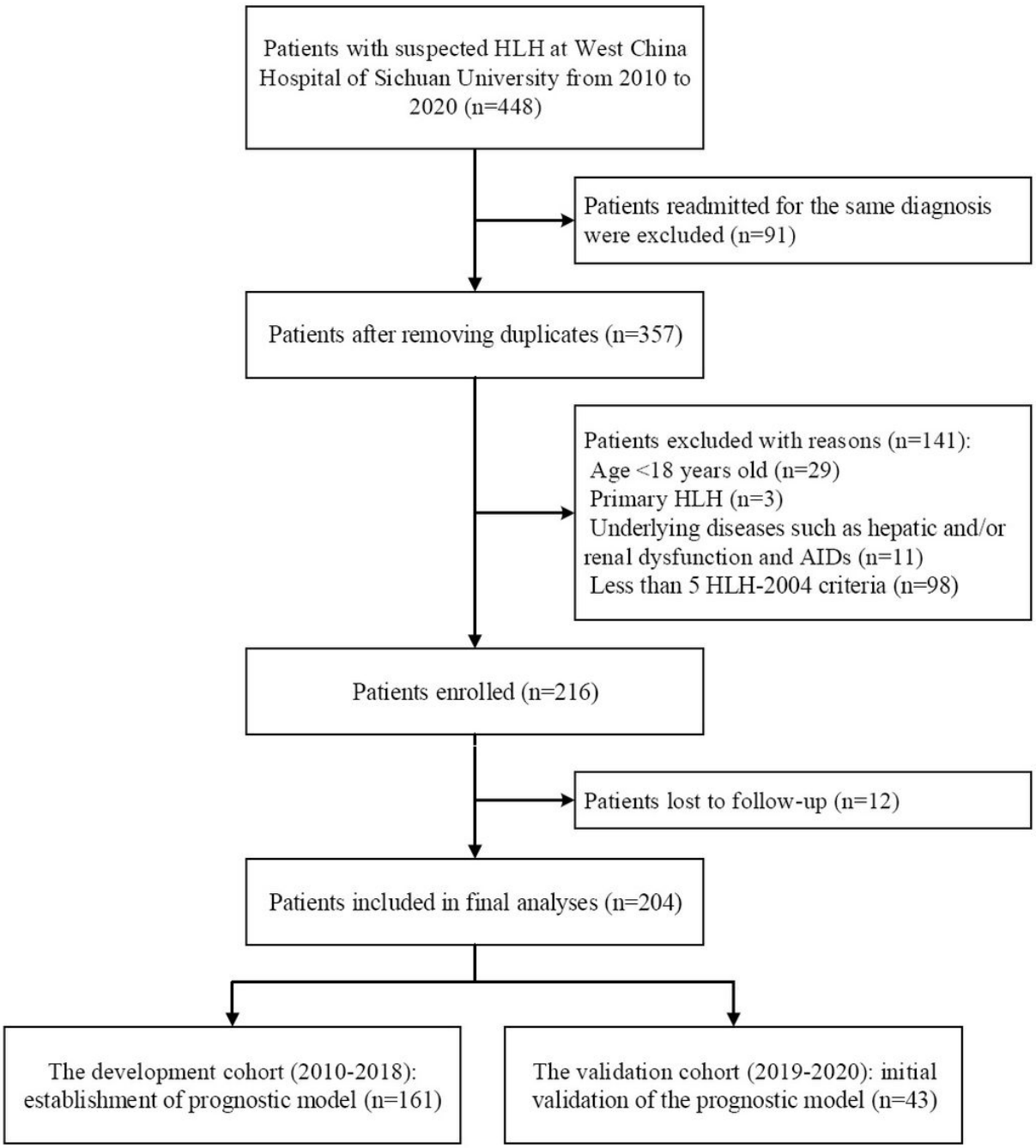

Figure 1

Flow chart of included patients and the simplified model.

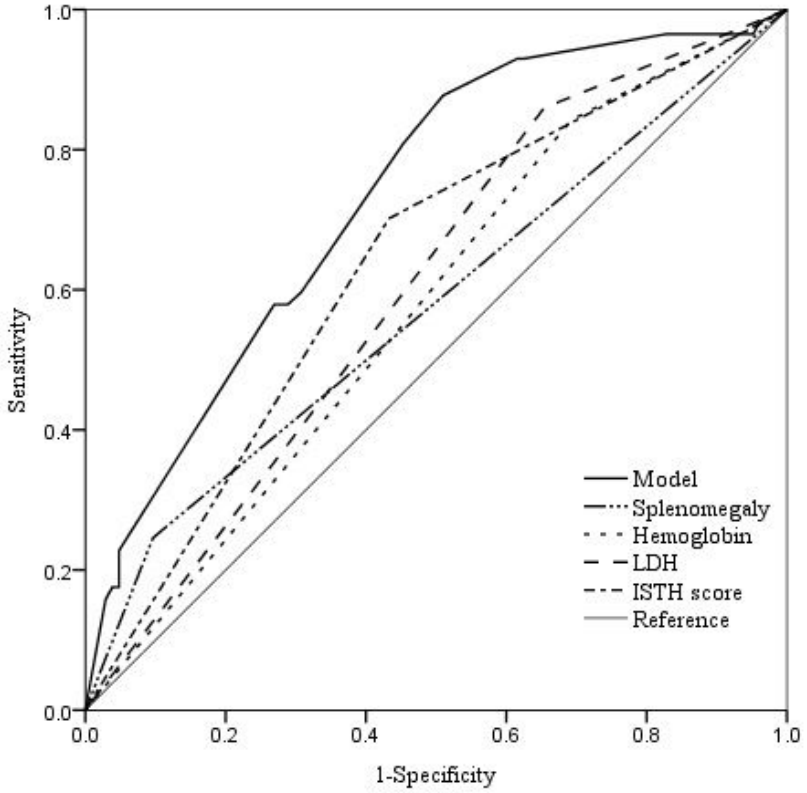

Figure 2

Performance of the prognostic model in the development cohort.

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