



Prognostic Value of Blood-Based Inflammatory Biomarkers in Secondary Hemophagocytic Lymphohistiocytosis

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Received: 21 September 2019 / Accepted: 28 May 2020 / Published online: 3 June 2020
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Abstract

Purpose Hemophagocytic lymphohistiocytosis (HLH) is a rare systematic immune disease manifested with excessive activation of lymphocytes and macrophages. This study was designed to explore the feasible prognostic factors of secondary HLH (sHLH).

Method We retrospectively analyzed 179 patients with newly diagnosed sHLH from January 2016 to May 2019 according to the HLH-2004 protocol. Baseline characteristics and laboratory results were reviewed.

Results The median age of all patients was 53 years, with a male/female ratio of 1.45. The commonest cause of HLH was malignancy. Of the 179 patients, 48.6% presented with Epstein-Barr virus (EBV) infection, 92.8% with hemocytopenia (at least 2 lineages), 60.3% with hypofibrinogenemia, 43.0% with hypertriglyceridemia (≥ 3 mmol/L), 99.4% with high ferritin, 97.8% with fever, 72.1% with splenomegaly, and 72.6% with hemophagocytosis. As to their prognosis, 122 patients died; the median survival was 88 days, with a 2-year survival rate of 26.72%. Univariate analysis confirmed neutrophil-to-lymphocyte ratio > 2.53 , lymphocyte-to-monocyte ratio (LMR) ≤ 4.43 , platelet-to-lymphocyte ratio > 227.27 , red blood cell distribution width > 14.6 , red blood cell distribution width-to-platelet ratio (RPR) > 0.33 , EBV infection, platelet $\leq 34 \times 10^9$ /L, fibrinogen ≤ 1.34 g/L, alkaline phosphatase > 182.4 U/L, adenosine deaminase > 69.2 U/L, and ferritin > 2318 ng/mL were associated with an inferior survival. In a multivariate model, LMR, RPR, and ferritin were considered as three independent factors.

Conclusion Some blood-based inflammatory markers, which can be easily and cheaply detected, are significantly associated with the OS of HLH patients. LMR and RPR, superior to NLR, PLR, RDW, can be taken to predict the OS of patients with HLH.

Keywords Prognostic indicator · clinical manifestation · hemophagocytic lymphohistiocytosis · survival

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) arises from uncontrolled proliferation and activation of lymphocytes and macrophages and leads to excessive secretion of cytokines, infiltration of inflammatory cells, and even organ failure. HLH symptoms include sustained fever, spleen enlargement, cytopenia, coagulation dysfunction, high triglycerides and ferritin level, as well as hemophagocytosis in the bone marrow, liver, or lymph nodes [1]. Though once considered as a pediatric disease, HLH attacks people of all ages. In Japan, 40% of HLH patients are adults [2]. In addition, its global incidence is low, about 1/800000 per year [3].

In regard to its etiology, HLH is classified into primary HLH and secondary HLH. Primary HLH is accompanied with mutations in genes regulating cytotoxic granule activation, polarization, priming, fusion, or function, like PRF1, STX11, UNC13D, STXBP2, RAB27A, LYST, and AP3B1. Secondary HLH (sHLH) is triggered by various pathologies, mainly malignancies, infections, autoimmune disorders [4]. sHLH carries a rapid course and a high mortality [5]. Parikh et al. [6] demonstrated that malignancy-induced HLH (MHLH) acquired a survival significantly inferior to that of non-malignancy-associated HLH (median, 1.4 months vs. 22.8 months). Researchers have strived to explore prognostic markers of HLH. Tabata et al. [7] proposed that a high sIL-2R/ferritin ratio indicated the presence of underlying malignant lymphoma. Elevated serum lactic dehydrogenase, hypofibrinogenemia, platelet $< 40 \times 10^9/L$, and ferritin have been proved to predict worse outcomes [8, 9]. However, the prognostic efficiency of these markers is restricted by high cost, technical obstacles, etc. Feasible biomarkers, like those can be tested in routine blood test, are needed.

Immune dysregulation is involved in inflammatory diseases, autoimmune diseases, and even malignancies. Systemic inflammation is associated with the clinical presentations of neutrophilia and lymphopenia, which raises a possibility of using neutrophil-to-lymphocyte ratio (NLR) to assessing the prognosis of diseases associated with inflammation [10]. Also, increased proinflammatory cytokines (tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-4) and growth factors, secreted by macrophages, are responsible for mediating thrombocytosis and anemia [11]. Platelets participate in inflammation through releasing highly active microparticles. Thus, platelet-to-lymphocyte ratio (PLR) has been used to assess the association between inflammation burden and disease severity in autoimmune diseases [10]. Tumor-associated macrophages (TAMs), derived from circulating monocyte, have demonstrated obvious prognostic ability for various tumors through contributing to promote both tumor progression and reduced immunosurveillance via the inhibition of apoptosis, promotion of angiogenesis. Lymphocyte-to-monocyte ratio (LMR) has been investigated as a prognostic index in

various malignancies [12]. Red blood cell distribution width (RDW), another parameter in routine blood test, elevates under inflammatory circumstances, associated with high mortality [13]. Similarly, red blood cell distribution width-to-platelet ratio (RPR) serves as a noninvasive and economical predictor of colorectal cancer patients' survival [14]. As is well known, dysregulated immune responses and increased cytokines are observed in the progression of sHLH. Contrary to those in acute inflammatory diseases, neutrophils and thrombocytes decrease in sHLH, as a result of high TNF- α , interferon (IFN)- γ , and hemophagocytosis.

In this retrospective study, we evaluated the prognostic efficiency of various indexes for sHLH based on HLH-04 criteria [4]. Our findings may help develop an ideal tool to clinically predict the outcomes of sHLH.

Patients and Methods

Patients

A total of 179 newly diagnosed sHLH cases were recruited in this study. The diagnosis of HLH was established on the 2004 HLH Diagnostic Criteria [4]. At least five of the following criterion had to be satisfied: (1) persistent fever (temperature $> 38.5^\circ\text{C}$ for more than 1 week); (2) splenomegaly; (3) cytopenia referring to at least two of the three lineages—hemoglobin $< 90\text{ g/L}$, platelets $< 100 \times 10^9/L$, and neutrophils $< 1.0 \times 10^9/L$; (4) hypofibrinogenemia (fibrinogen $< 1.5\text{ g/L}$) and/or hypertriglyceridemia ($\geq 3.0\text{ mmol/L}$); (5) hyperferritinemia (serum ferritin $\geq 500\text{ }\mu\text{g/L}$); (6) high levels of soluble IL-2R ($\geq 2400\text{ U/mL}$); (7) low or absent natural killer (NK) cell activity; and (8) hemophagocytosis in bone marrow, spleen, or lymph nodes. On the basis of technical restriction, NK cell activity is not inapplicable in our hospital. PET-CT, in conjunction with B symptoms, was carried out to reveal lymphoma-associated hemophagocytic lymphohistiocytosis (LHLH). The clinical significance of PET-CT in LHLH and non-LHLH has been reported in our previous study [15, 16].

At admission and pretreatment(except small doses of corticosteroids), the following variables were considered: age, sex, presumed etiologies, white blood cell (WBC), absolute neutrophil count (ANC), absolute lymphocyte count, monocyte count, hemoglobin (Hb), RDW, platelet (PLT), fibrinogen(Fib), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), adenosine deaminase (ADA), triglycerides(TG), ferritin, sIL-2R, and $\beta 2$ -microglobulin ($\beta 2$ -MG). Epstein-Barr virus (EBV) infection, fever, splenomegaly, and hemophagocytosis in the bone marrow were also evaluated.

Follow-up was conducted through reviewing inpatient medical records as well as making phone calls. Overall

survival (OS) was calculated as the interval between the time of diagnosis and death from any cause or the last follow-up.

Treatment

Any of the following regimens was adopted as the initial therapy: corticosteroid, corticosteroid+intravenous immunoglobulins (IVIG), CHOP-like chemotherapy, HLH-04, or HLH-94 regimen. Antiviral drugs were used as supportive therapy for patients with viral infection. Red cells, PLT, Fib, or fresh frozen plasma, as supportive care therapy, were transfused when necessary.

In MHLH, 32 patients were assigned to HLH-94 or HLH-04 group; 62 patients to chemotherapy like EPOCH, CHOP, and DEP group; 9 patients to corticosteroid±IVIG group, and 10 patients to early death (< 10 days) group. In non-MHLH (IHLH and AHLH), 18 patients were assigned to HLH-94 or HLH-04 group, 5 patients to chemotherapy like DEP group, 28 to corticosteroid± IVIG group, and 3 patients to early death group. No statistically significant difference was observed in the effect of treatment regimen on LMR and RPR.

Statistical Analysis

All statistical analyses were performed using SPSS (version 20.0, Chicago, IL, USA), GraphPad Prism 5 (La Jolla, CA), STATA/MP statistical software (version 14.0, TX, USA), and MedCalc software (version 15, Belgium). Patients' characteristics were summarized as median ± quartiles through standard descriptive statistics. Continuous variables were compared using the non-parametric Kruskal Wallis *H* test among three groups or Mann-Whitney *U* between two groups. To calculate the optimal cut-off levels for survival, receiver operating characteristic (ROC) analysis was conducted to evaluate the area under the curve (AUC) value and confidence interval (CI). OS was estimated by using Kaplan-Meier method, and the difference between survival curves was assessed by log-rank test in univariate analysis. Multivariate COX analysis (hazard ratios (HR) and 95% CI) was intended to recognize the independent prognostic variable on OS. All subgroup analyses were presented with a COX test for interaction. A *P* value < 0.05 was considered statistically significant.

Results

Clinical Characteristics of the Cohort

From January 2016 to May 2019, a total of 179 subjects with sHLH were enrolled in this retrospective study. The median age at diagnosis was 53 years, ranging from 14 to 86 years. Males accounted for a higher proportion, with a sex ratio of 1.45. Nearly all patients demonstrated hyperferritinemia.

High-grade fever and hemocytopenia (involving at least 2 lineages) were present in more than 90% of the patients (fever, 97.8%; cytopenia, 92.8%). Other clinical and laboratory findings included splenomegaly (129/178), hypofibrinogenemia (108/179), hypertriglyceridemia (77/179), and hemophagocytosis (130/172). Baseline characteristics were described in Table 1. EBV infection was observed in 48.6% of the patients.

Table 1 Clinical characteristics of 179 patients with sHLH

Characteristics	No.	%
Age	53(38–66)	
Sex		
Male	106	59.2
Female	73	40.8
Pathogenesis		
Tumor-associated	113	63.2
Infection-associated	43	24.0
Autoimmune-associated	11	6.1
Unknown reason	12	6.7
EBV virus infection		
Positive	87	48.6
Negative	87	48.6
Unknown	5	2.8
WBC ($\times 10^9/L$)	1.8(1.1–3.2)	
ANC ($\times 10^9/L$)	0.98(0.65–1.8)	
< $1 \times 10^9/L$	95	53.1
$\geq 1 \times 10^9/L$	84	46.9
Hb (g/L) (median, range)	82(71–88)	
<90 g/L	151	84.4
≥ 90 g/L	28	15.6
PLT ($\times 10^9/L$) (median, range)	43(24–69)	
< $100 \times 10^9/L$	170	95.0
$\geq 100 \times 10^9/L$	9	5.0
Fib(g/l) (median, range)	1.34(1–2.01)	
<1.5 g/l	108	60.3
≥ 1.5 g/l	71	39.7
ALP (U/L)	148(84–276.2)	
LDH (U/L)	702(412–1245)	
ADA (U/L)	57.5(34.1–108.4)	
Triglycerides (mmol/L)	2.68(1.75–3.74)	
$\beta 2$ -MG (mg/L)	5.47(3.9–7.86)	
Ferritin (ng/mL)	3141.75(1500–12,565)	
Scd25 (ng/L)	33,819.78(17878–50,935)	
Fever	175	97.8
Splenomegaly	129	72.1
Hemophagocytosis	130	72.6

WBC, white blood cell; ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelet; Fib, fibrinogen; ALP, alkaline phosphatase; LDH, lactic dehydrogenase; ADA, adenosine deaminase; $\beta 2$ -MG, $\beta 2$ -microglobulin

At time of diagnosis, the patients’ median NLR count was 2.32 (0~49.21), the median LMR level was 2.415 (0~44), and the median PLR was 92.325 (2.66~1833.33). Subjects had a median RDW of 16.35 on and RPR of 0.39.

Comparison Between Groups of Different Etiologies

Table 1 displayed the distribution of the underlying diseases in 179 patients with HLH. The major cause was malignancy ($n = 113$, 63.2%), followed by infection ($n = 43$, 24%). Patients with autoimmunity-associated HLH (AHLH) accounted for 6.1%. The remainders had unexplained etiology. No remarkable difference was found in NLR, LMR, and PLR among three groups ($P = 0.727$; $P = 0.232$; $P = 0.095$). RDW showed statistical difference between MHLH and infection-associated HLH (IHLH) group (median value, 16.65 vs. 15.3, $P = 0.047$). The median RPR of patients with MHLH and IHLH was markedly higher than that of patients with AHLH (median value, MHLH 0.43; IHLH 0.33; AHLH 0.22 Fig. 1).

Cut-off Values of Clinical Parameters

According to ROC curve, the survival was predicted with a cut-off of 2.53 using NLR (AUC value 0.623, 95% CI 0.548–0.695), 4.43 using LMR (AUC value 0.587 95% CI 0.511–0.661), 227.27 using PLR (AUC value 0.515 95% CI 0.439–0.590), 14.6 using RDW (AUC value 0.601 95% CI 0.525–0.674), and 0.33 using RPR (AUC value 0.637 95% CI 0.562–0.708) (Fig. 2), respectively. The cut-off values of other indexes were displayed in Table 2.

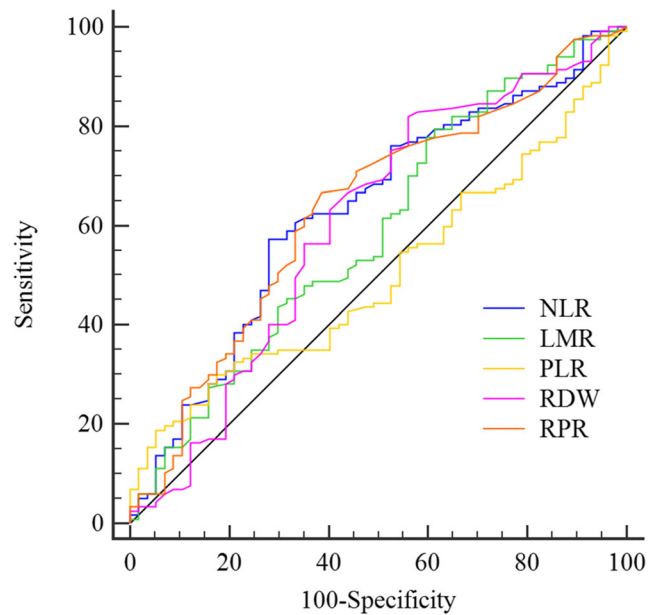


Fig. 2 Receiver operating curve (ROC) for determining the optimal cut-off value of NLR, LMR, PLR, RDW, and RPR

Predictive Factors for HLH Survival

At the end of the study, a total of 122 deaths (68.2%) occurred. The median OS of the cohort group was 88 days (range, 2 to 1210 days), and 2-year survival rate was 26.72%. Patients with MHLH had relatively shorter OS than those with IHLH and AHLH, though no statistical difference was detected (median, 80 vs. 119 days; 80 days vs. undefined). In MHLH

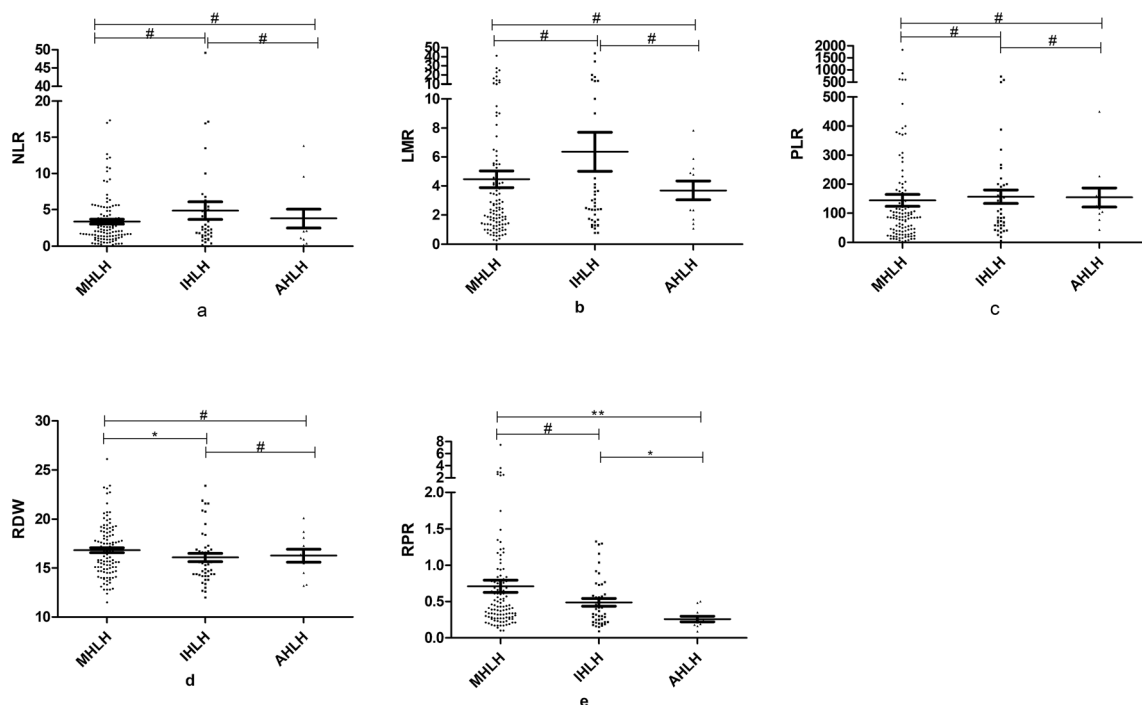


Fig. 1 Comparison of laboratory findings—NLR, LMR, PLR, RDW, and RPR among different etiology groups. # $P > 0.05$, * $P < 0.05$, ** $P < 0.01$

Table 2 Prognostic factors of overall survival of 179 patients with HLH. Prognostic factors of overall survival

Characteristics	OS				
	Univariate analysis		Multivariate analysis		
	Median(days)	<i>P</i>	HR	<i>P</i>	95%CI
Age		0.089			
< 60	107				
≥ 60	60				
Sex		0.042	0.769	0.298	0.469–1.261
Male	60				
Female	106				
Pathogenesis		0.111			
Tumor-associated	80				
Infection-associated	119				
Autoimmune-associated	Not reached				
EBV		< 0.001	1.312	0.264	0.815–2.113
(-)	180				
(+)	50				
ANC		0.640			
< 1 × 10 ⁹ /L	90				
≥ 1 × 10 ⁹ /L	88				
NLR		< 0.001	1.413	0.184	0.848–2.355
≤ 2.53	152				
> 2.53	45				
LMR		0.006	0.459	0.007	0.260–0.812
≤ 4.43	70				
> 4.43	423				
Hb		0.202			
≤ 88 g/L	82				
> 88 g/L	106				
PLT		< 0.001	1.035	0.909	0.570–1.882
≤ 34 × 10 ⁹ /L	40				
> 34 × 10 ⁹ /L	120				
PLR		0.004	1.758	0.076	0.943–3.277
≤ 227.27	106				
> 227.27	40				
RDW		0.008	1.313	0.401	0.695–2.477
≤ 14.6	Not reached				
> 14.6	80				
RPR		< 0.001	2.083	0.024	1.102–3.936
≤ 0.33	276				
> 0.33	46				
Fib		0.003	0.805	0.379	0.496–1.306
≤ 1.34 g/l	60				
> 1.34 g/l	145				
ALP		0.019	1.061	0.819	0.639–1.762
≤ 182.4 U/L	106				
> 182.4 U/L	46				
LDH		0.119			
≤ 1000 U/L	82				
> 1000 U/L	107				
ADA		0.004	1.399	0.244	0.795–2.462
≤ 69.2 U/L	144				
> 69.2 U/L	60				
TG		0.222			
< 3 mmol/L	104				
≥ 3 mmol/L	53				
Ferritin		0.004	1.852	0.020	1.101–3.114
≤ 2318 ng/ml	150				
> 2318 ng/ml	46				

group, the 2-year survival rate was 18.77%, lower than 38.65% in IHLH and 57.72% in AHLH group.

HLH patients with NLR ≤ 2.53 had significantly longer OS compared with those with NLR > 2.53 (152 days vs. 45 days,

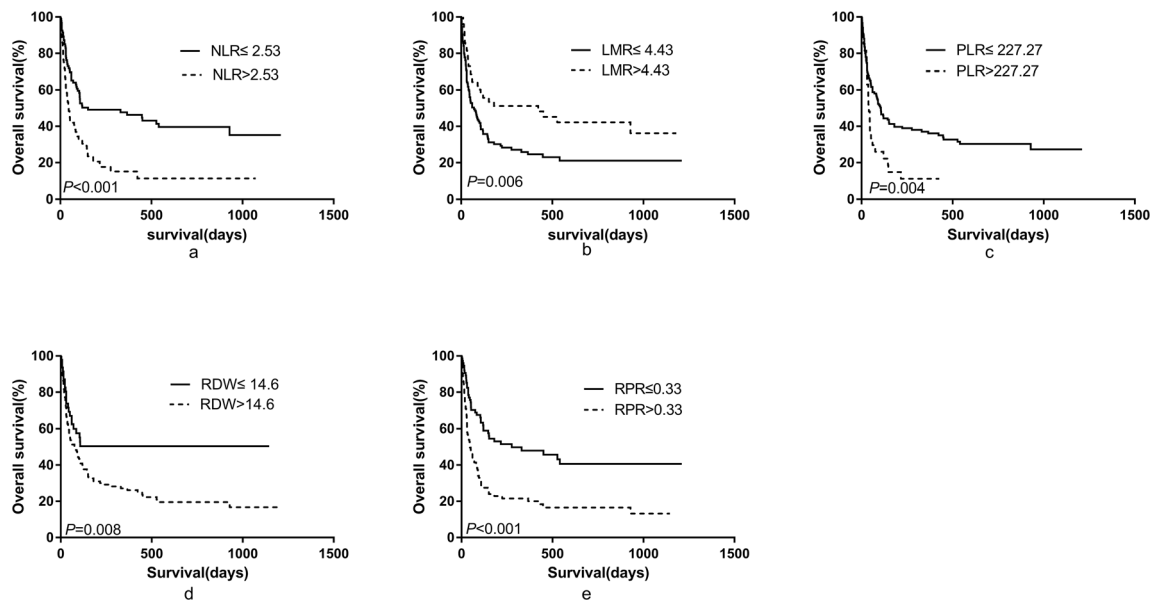


Fig. 3 Univariate analysis of overall survival based on NLR (a), LMR (b), PLR (c), RDW (d), and RPR (e)

$P < 0.001$, Fig. 3a). Median OS was 70 days in patients with $LMR \leq 4.43$ and 423 days in those with $LMR > 4.43$ ($P = 0.006$, Fig. 3b). Remarkably shorter survival was ascertained in high PLR and RDW groups (PLR, 40 days vs. 106 days, $P = 0.004$; RDW, 80 days vs. not reached, $P = 0.008$, Fig. 3c, d). We also observed the significant difference in OS between two RPR groups (276 days vs. 46 days, $P < 0.001$, Fig. 3e). By univariate analysis, male, EBV

infection, $ALP > 182.4$ U/L, $ADA > 69.2$ U/L, and ferritin > 2318 ng/mL were also associated with a worse outcome, whereas $PLT > 34 \times 10^9/L$ and $Fib > 1.34$ g/L were predictive of a better outcome of HLH. In the multivariate Cox model, LMR (HR 0.459, 95% CI 0.260–0.812), RPR (HR 2.083, 95% CI 1.102–3.936), and ferritin (HR 1.852, 95% CI 1.101–3.114) were the independent prognostic factors (Table 2). LMR showed a positive predictive value (PPV) of

Fig. 4 Hazard ratios of LMR index for overall survival according to subgroups. Hazard ratios were derived from a Cox proportional hazards model

Subgroup	HR(95% CI)	P-value	
		Effect	Interaction
Age			
<60	0.68 (0.38, 1.20)	.178	.343
≥ 60	0.43 (0.22, 0.84)	.013	
Sex			
Male	0.53 (0.31, 0.90)	.018	.896
Female	0.55 (0.25, 1.20)	.135	
Pathogenesis			
MHLH	0.57 (0.35, 0.95)	.031	.544
Non-MHLH	0.51 (0.22, 1.19)	.119	
EBV infection			
(+)	0.53 (0.30, 0.94)	.03	.949
(-)	0.53 (0.26, 1.08)	.082	
ANC			
$< 1 \times 10^9/L$	0.49 (0.28, 0.86)	.012	.361
$\geq 1 \times 10^9/L$	0.65 (0.32, 1.34)	.247	
Hb			
≤ 88 g/L	0.60 (0.37, 0.97)	.037	.511
> 88 g/L	0.43 (0.16, 1.17)	.099	
PLT			
$\leq 34 \times 10^9/L$	0.34 (0.16, 0.69)	.003	.075
$> 34 \times 10^9/L$	0.75 (0.43, 1.31)	.316	
Fib			
≤ 1.34 g/l	0.81 (0.46, 1.41)	.45	.133
> 1.34 g/l	0.41 (0.20, 0.81)	.01	
TG			
< 3 mmol/L	0.65 (0.35, 1.22)	.18	.409
≥ 3 mmol/L	0.40 (0.22, 0.75)	.004	
Ferritin			
≤ 2318 ng/ml	0.42 (0.18, 0.93)	.034	.557
> 2318 ng/ml	0.59 (0.35, 0.99)	.047	

73.01% and a negative predictive value (NPV) of 46%; RPR showed a PPV of 78.64% and a NPV of 46.66%.

Subgroup Analysis on the Predictive Power of LMR and RPR in sHLH

We performed subgroup analyses to eliminate the effect of confounding factors, including age, gender, pathogenesis, EBV infection, neutrophils, hemoglobin, platelet, fibrinogen, triglycerides, and ferritin. As showed in Fig. 4, $LMR \leq 4.43$ was also a risk predictor for MHLH ($P = 0.035$), but not for non-MHLH. With regard to RPR, the results remained consistent between the two etiologies, indicating that irrespective of HLH pathogenesis, sHLH with high RPR values was evidently associated with poorer OS compared with those with low values ($P = 0.013$, $P = 0.006$; Fig. 5). Also, LMR and RPR showed obvious predictive significance between one or two subgroups of each variable. Nevertheless, in the overall subgroup analysis, the predictive efficiency of LMR or RPR combined baseline characteristics showed no statistical change.

In MHLH, the pathogenesis included B-NHL (35 cases), T-NHL and NK/T (59 cases), lymphoma diagnosed by PET-CT (12 cases), HL (1 cases), solid tumors (2 cases), and leukemia (4 cases). There was no significant difference in the pathogenesis between LMR and RPR group ($P = 0.710$, $P = 0.673$). Patients with B-NHL acquired a longer survival than T-NHL (107 days vs. 50 days, $P = 0.007$). In the subgroup

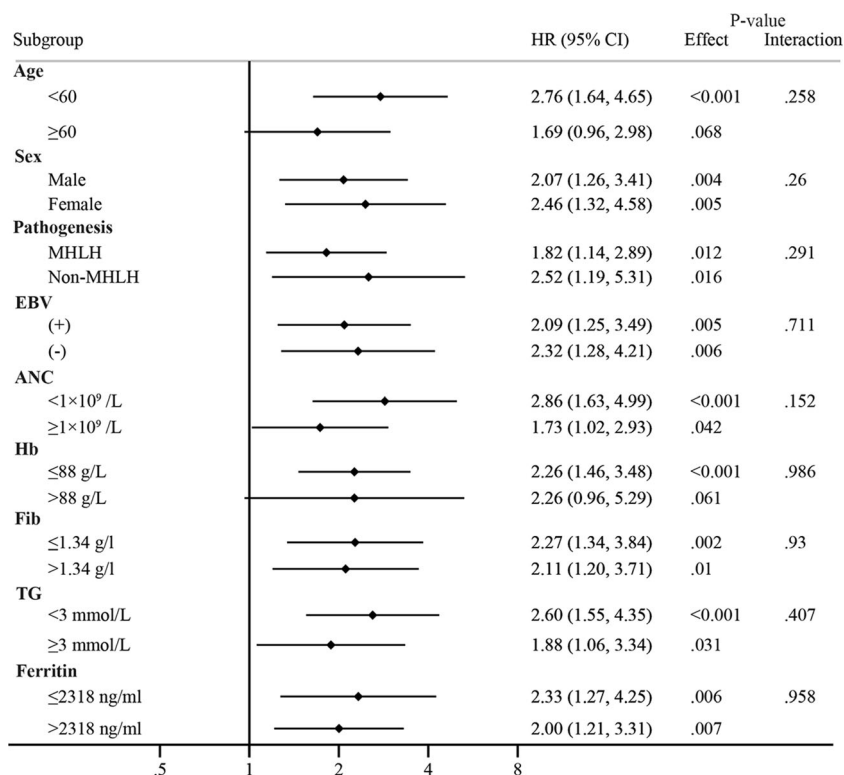
analysis, $LMR \leq 4.43$ or $RPR > 0.33$ were also significant risk predictors for patients with T-NHL ($P = 0.042$; $P = 0.007$), but not those with B-HLH.

Discussion

In this study, routine CBC parameters were used to predict the outcome of sHLH. Our finding showed that patients with MHLH have higher RPR value than those with non-MHLH. Five inflammatory markers (NLR, LMR, PLR, RDW, and RPR) are associated with survival of sHLH. Furthermore, after adjusting other prognostic factors, a low LMR, high RPR, and high ferritin were regarded as independent risk factors for the prognosis of sHLH.

Whether primary or secondary, HLH is accompanied by imbalance of host immune response, during which macrophage activity may become uncontrollable. Hypersecretion of cytokines, known as a “cytokine storm,” appears as the main laboratory feature of HLH. Clinical manifestations of HLH have been increasingly recognized [6, 8, 9, 17, 18]. Almost unexceptionally, 174 adults with HLH demonstrated fever and hyperferritinemia (nearly 100%). In our study, persistent fever, cytopenia, and high ferritin showed up in more than 90% of subjects. Splenomegaly was detected in about 72.1% of patients. The positive rates of hypofibrinogenemia (60.3%) and hypertriglyceridemia (43%) were much lower than those detected with other diagnostic criteria.

Fig. 5 Hazard ratios of RPR index for overall survival according to subgroups



Hemophagocytosis was discovered in 130 patients by bone marrow aspiration, but was not confirmed in another seven patients who had not undergone bone marrow aspiration. Secondary HLH develops from various etiologies, such as malignancy, infection, or autoimmune disorders [19]. In our study, MHLH accounted for the majority, about 63.2%. It has reported that malignancy triggers 52.9% of all HLH cases [17]. The high incidence of MHLH in our study may be explained by the fact that only MHLH patients were admitted, and those HLH patients evolving from infectious and rheumatic diseases were always excluded by our hematological department. Also, contrary to other researches, we enrolled patients with PET-CT confirmed LHLH and B symptoms. We have reported the application of PET-CT in diagnosing LHLH when pathological evidence is unavailable [15, 16]. An absolute SUV max value > 5.5 was highly indicative of the presence of LHLH ($P < 0.001$; sensitivity, 92.9% and specificity, 85.7%; AUC, 0.923). MHLH, a typical lymphoma-associated HLH, has the worst survival among all acquired HLH types [6, 17, 18]. In 114 patients, MHLH brought with a higher early death rate (49.2%) and a shorter median OS (only 2 months) than HLH caused by infections and autoimmune diseases [20]. Our results also revealed that the patients with MHLH acquired a median OS of 80 days and a 2-year survival rate of 18.77%, inferior to those with IHLH or AHLH, though no statistical difference was present. However, this difference was remarkable between MHLH of various cellular origins, being 50 days in T and 107 days in B cell lymphoma ($P = 0.007$). Yu et al. [21] also reported a better prognosis of B cell LHLH than that of T cell NHL. The reason may be that cytokine storm is more serious in T cell lymphoma. In T cell LHLH, the cytokine storm is waged by neoplastic T cells; and the activated T cells may subsequently evoke neoplastic B cells, leading to B cell LHLH [22]. Proper T cell and NK-cell functions are required for clearance of antigenic stimuli and termination of the inflammatory response. Aberrant T cell and NK-cell activations result in excessive cytokine production and sustained macrophage activation.

EBV infection can be found in infection-associated HLH, and lymphoma-associated HLH patients and familial HLH. As the most common etiology, EBV causes approximately 70% of IHLH cases [23]. In our current study, EBV infection was detected in 48.6% of total patients and 62.7% of IHLH patients. It was demonstrated that high levels of proinflammatory factors were inseparable of EBV-related HLH. The clinical outcomes of patients with EBV vary strikingly, ranging from self-limiting to aggressive and fatal. Li et al. [8] showed the association between active infection and shorter survival in IHLH patients (65 days vs. not reached, $P = 0.021$), but not in the MHLH subgroup. However, a multivariate analysis of NK/T cell lymphoma-associated HLH showed peripheral blood EBV positivity was of significant prognostic importance [24]. We analyzed the correlation between EBV

infection and the prognosis of patients in the total group. The results showed that patients infected by EBV obtained a median OS of only 50 days, much shorter than that of patients without infection ($P < 0.001$).

HLH saw a mortality of 68.2% and median OS of less than 3 months in our study. Serum inflammatory cytokines increase in active HLH, including IFN- γ , TNF- α , IL-10, GM-CSF, and IL-18. As TNF- α and IL-10 increase, the lymphocytes decrease, resulting in lymphocyte dysfunction [25, 26]. In turn, inflammatory cytokines are released by lymphocytes for monocyte recruitment and infiltration of tissue. Bakul et al. [27] have described the lymphocyte subpopulations that bring with different outcomes of pediatric HLH. They found that patients with an elevated CD8⁺ lymphocyte count had a better OS. In multivariate analysis of 174 patients [17], lymphocytopenia (lymphocytes $< 0.5 \times 10^9/L$) was confirmed as a risk factor of poor prognosis (HR 1.691, 95% CI 1.062–2.692). Regularly, neutrophils increase in inflammation diseases. However, we found that these molecules decrease in HLH, which may be caused by concentration of TNF- α , INF- γ , and hemophagocytosis. Then, NLR, as a HLH diagnostic criterion, was established based on the above theory. NLR, which can be easily and cheaply detected, has shown its link with the severity or outcome in inflammatory diseases and neoplastic diseases, such as adult-onset Still's disease and follicular lymphoma (FL) [28, 29]. We set the NLR cut-off as 2.53, with AUC value of 0.623 and 95% CI of 0.548–0.695. The univariate analysis result showed that the patients with higher NLR (> 2.53) acquired a survival of less than 2 months, which is consistent with the result in the study on FL [29]. Different cut-off value may be set due to the diseases' varying species and pathogenesis. However, a previous study reported the 30-day OS was significantly different between patients with neutrophils \geq or $< 0.5 \times 10^9/L$ (92.6% vs. 75.4% $P = 0.007$), indicating that severe neutropenia (neutrophils $< 0.5 \times 10^9/L$) is an independent risk factor [30]. Patients with leukopenia or agranulocytopenia are prone to severe opportunistic infection, a common cause of death in HLH. Therefore, we interfered that the prognostic capacity of NLR mainly relies on lymphocytes, not neutrophils.

Similar to neutropenia, thrombocytopenia is also frequently observed in patients with HLH and regarded as another diagnostic principle. A study has provided evidence for the effect of IL-10 on platelet count [31]. Patients with IL-10 ≥ 800 pg/mL showed lower PLT counts, with a median count of $31 \times 10^9/L$. Platelets play an active role in inflammation [32]. Zhou et al. [33] reported sHLH patients with PLT $< 40 \times 10^9/L$ were confronted with a high risk of death and inferior survival, which might result from hemophagocytosis, hypersplenism, and DIC. PLR can be used to evaluate the diagnosis and prognosis of tumors. High PLR has been identified as an independent prognostic factor for the overall survival of patients with stomach cancer [34]. In our study, the

cut-off PLR was calculated to be 227.27, with the AUC of 0.51 (sensitivity 19.8%; specificity 94.7%). After excluding the influence of other inflammatory markers, PLR showed clear predictive capacity.

Previous study showed several chemotactic molecules increased in HLH, like monocyte chemoattractant protein-1, macrophage inflammatory protein-1 β , and IL-8, contributing to the recruitment of monocytes [35]. Also, cytokine IL-10 induced the development of immunosuppressive CD14⁺HLA-DR^{low/-} monocytes [36]. Therefore, circulating serum level of monocytes, such as LMR, can be taken to reflect the status of host immune system. Our results showed that LMR of 4.43 (AUC 0.587) achieved a sensitivity of 77.3% and a specificity of 40.4%. When the patients were divided into two groups based on LMR, the OS of the high LMR group was significantly longer than that of low LMR group. LMR remains prognostic when combined with NLR and PLR. In subgroup analysis of etiologies, high LMR was also correlated with worse OS in MHLH patients, especially T-NHL. This phenomenon may be interpreted by a mechanism involving tumor-associated macrophages (TAMs). Tumor microenvironment plays a key role in the progression of lymphomas [37]. TAMs, deriving from circulating monocytes, exert activity in tumor growth through promoting metastasis, immunosuppression, and tumor angiogenesis. The infiltration of TAMs into tumors may be reflected by monocyte count. Besides, myeloid-derived suppressor cells, another subset of circulating leucocytes, exhibit immunosuppressive activity. A higher level of monocytic myeloid-derived suppressor was associated with a worse prognosis of tumor [12].

RDW is a measure of the size heterogeneity of erythrocyte volume and traditionally used for the differential diagnosis of anemia. In the recent year, RDW has been adopted to mark the systemic inflammatory response in hematological malignancies [13, 38]. A meta-analysis demonstrated that RDW is a risk predictor of cancer-related deaths. Inflammation impairs erythropoiesis by altering the red cell membrane, resulting in the increase of RDW [39]. It has proved the prognostic efficiency of RDW combined with a variety of inflammatory markers, including C-reactive protein, IL-6, soluble TNF receptors I and II, and soluble transferrin receptor [40]. In our study, the results indicated that RDW could efficiently predict the poor OS of patients with sHLH (80 days vs. not reached). Elevated IFN- γ in sHLH acts directly on macrophages to provoke endocytosis, leading to severe anemia [31]. High RDW leads to inadequate production of erythropoietin, nutritional deficiencies (i.e., vitamin B12 and folate), oxidative damage, all corresponding to the poor prognosis in tumor patients [13]. Another interesting finding was that partial patients with MHLH acquired high RDW. This may be explained by the disturbed iron metabolism in the terminal stage of malignancy [41]. HLH usually begins with thrombocytopenia and progresses to pancytopenia. Several studies put

forward that PLT $< 40 \times 10^9/L$ could predict the poor survival [8, 33], which is consistent with the result in our study (PLT cut-off was set to be $34 \times 10^9/L$). There was a strong correlation with RPR and HLH survival. Area under the curve (AUC) for RPR (0.33) was 0.637 (95% CI 0.562–0.708, sensitivity 66.9%, and specificity 61.4%). Similar to LMR, RPR could also predict the survival in subgroups of MHLH and non-MHLH, especially in T-NHL. Besides, RPR values in MHLH patients were significantly higher. Consistent results were also reported in a study on chronic inflammatory autoimmune disease-systemic lupus erythematosus [42].

Hypofibrinogenemia, ADA, and ALP demonstrated to be prognostic for death in univariate analysis. Li et al. [8] showed that the level of fibrinogen in the deaths was significantly lower than that in the survivors. OS was remarkably shorter in patients with fibrinogen < 1.5 g/L. In our study, we reduced this level to 1.34. Activated histiocytes ingest excessive fibrin and/or fibrinogen, leading to hypofibrinogenemia [43]. Cytokines generated by activated T cells/macrophages may destroy liver metabolism, leading to hepatic mitochondrial injury that is a characteristic of HLH [44]. This is why ALP is of prognostic significance in HLH. We have reported that serum ADA could serve as an indicator of underlying lymphoma in sHLH patients [45]. Patients with ADA ≤ 69.2 U/L had longer OS than those with ADA > 69.2 U/L. Ferritin is involved in regulation of iron storage and homeostasis, whose level generally reflects the activation of macrophages in HLH. The present study further showed that patients with hyperferritinemia (ferritin < 2318 ng/mL) acquired a shorter survival.

There are some limitations in our study. First, it is a single-center, retrospective cohort study. The prognostic efficiency of $\beta 2$ -microglobulin and sCD25 was not analyzed due to the number of missing values ($> 40\%$). Second, the study does not include proinflammatory cytokines and/or inflammation markers, such as C-reactive protein, which possibly have a key role in HLH prognosis. Also, partial patients were administered with small dose steroids due to fever or blood products transfusion before collecting the laboratory variables. Finally, the overall AUC value of the LMR and RPR is quite modest, and the findings have not been verified by a clinical cohort study.

Conclusions

Some blood-based inflammatory markers, which can be easily and cheaply detected, are significantly associated with the OS of HLH patients. LMR and RPR, superior to NLR, PLR, RDW, can be taken to predict the OS of patients with HLH.

Authorship Contributions This study was conceived and designed by JH and HQ. JH, GY, WC, and LL contributed to collect retrospective

research data, as well as JH, LD, TT, and JX support to analyze or interpret these data. JH draft the manuscript and GY, JW, XG, and HQ helped revise the manuscript strictly for important content. All authors have read and approved the final manuscript.

Funding Information This work was supported by the National Natural Science Foundation of the People's Republic of China [No. 81570175].

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Ethical Approval All procedures in studies were performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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