

# Clinical features and outcomes of patients with hemophagocytic lymphohistiocytosis at onset of systemic autoinflammatory disorder and compare with Epstein–Barr virus (EBV)-related hemophagocytic lymphohistiocytosis

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## Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disease. In clinical practice, we have observed that some HLH patients who have features of systemic autoinflammatory diseases (SAIDs) exhibit unique clinical manifestations and outcomes different from other HLH patients.

We analyzed data from 25 HLH patients who were considered to have SAIDs; data were collected from patients of our center between January 1, 2015 and September 1, 2018.

The median age of the patients was 1.75 years. In the early phase, all patients had a fever and 92% of patients had a rash; 96% of patients had high white blood cell count (WBC), C-reaction protein, and erythrocyte sedimentation rate. With progression, the above laboratory results decreased gradually. During the HLH period, we compared SAIDs-related HLH and Epstein-Barr virus (EBV)-related HLH and found that rash was more common (92%,  $P < .001$ ) and splenomegaly was less common (64%,  $P = .023$ ) in SAIDs-related HLH. Further, WBC, ferritin, and Interleukin-6 levels in SAIDs-related HLH patients were higher than those in EBV-related HLH patients. In contrast, hemoglobin, triglyceride, sCD25, Interleukin-10, and interferon- $\gamma$  levels in SAIDs-related HLH patients were lower compared with those in EBV-related HLH patients. SAIDs-related HLH patients received a modified HLH-2004 protocol at our center. Most patients had a good prognosis.

We provide a summary of the unique clinical and laboratory features, treatment protocols, and outcomes of SAIDs patients with HLH at onset. The findings indicate that these patients had a better response to corticosteroids and cyclosporin compared with EBV-related HLH patients.

**Abbreviations:** ALT = glutamic-pyruvic transaminase, CRP = C-reaction protein, ESR = erythrocyte sedimentation rate, FMF = familial Mediterranean fever, G-CSF = granulocyte-colony stimulating factor, HIDS = hyper-IgD syndrome, HLH = hemophagocytic lymphohistiocytosis, HSCT = hematopoietic stem cell transplantation, Ig = immunoglobulin, KD = Kawasaki disease, MKD = mmevalonate kinase deficiency, SAID = systemic autoinflammatory disease, SF = serum ferritin, sJIA = systemic juvenile idiopathic arthritis, SLE = systemic lupus erythematosus, TRAPS = tumor necrosis factor (TNF) receptor associated periodic syndrome, WBC = white blood cell count, WES = whole exome sequencing.

**Keywords:** hemophagocytic lymphohistiocytosis, macrophage activation syndrome, systemic autoinflammatory disease

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## 1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening systemic inflammatory disease caused by a cytokine storm, that is, an overwhelming activation of cytotoxic T cells and well-differentiated macrophages. HLH can be classified as either primary or secondary HLH. When HLH is secondary to rheumatic diseases, it is also called macrophage activation syndrome (MAS). Many studies have shown that MAS is associated with many kinds of rheumatic diseases, including lupus erythematosus (SLE), juvenile idiopathic arthritis (sJIA), Kawasaki disease (KD), scleroderma, and so on.<sup>[1,2]</sup>

Systemic autoinflammatory disorders (SAIDs) were first described in 1999,<sup>[3]</sup> however, there remains no clear unanimous diagnostic criteria for the syndrome. As previously reported, SAIDs are characterized by excessive systemic inflammation leading to recurrent fever, rashes, and interleukin-1 (IL-1) production.<sup>[4]</sup> However, there is no evidence of either infection or specific antibody involvement.<sup>[5]</sup> A high level of inflammatory indicators can be detected in these patients; however, levels often return to normal intermittently.<sup>[6–8]</sup> Although genetic mutations may help to confirm a definite diagnosis, more than 60% of SAIDs patients do not have mutations of SAIDs-related genes.<sup>[3,5,9]</sup> Thus, symptoms and experimental examinations play a vital role in the diagnostic process.

SAIDs can be categorized genetically as either monogenetic or multigenetic SAIDs based on relevant gene mutations. Monogenetic SAIDs include familial Mediterranean fever (FMF), tumor necrosis factor (TNF) receptor associated periodic syndrome (TRAPS), periodic fever syndrome mevalonate kinase deficiency (MKD), hyper-IgD syndrome (HIDS), and so on, while multigenetic SAIDs include sJIA, Crohn disease, and several other rare diseases.<sup>[3]</sup> Clinicians are familiar with sJIA and can detect MAS after sJIA has been diagnosed; however, when HLH only is observed at the onset of disease, it is not easy to determine the underlying cause. In a previous study, MAS cases with monogenic SAIDs were evaluated.<sup>[10]</sup> Among the 9 observed cases, 8 patients (88.9%) were alive after receiving effective treatments; thus, it appeared that these patients had better outcomes compared with ordinary HLH patients. Most of these patients experienced HLH after diagnosis of SAIDs. However, there are many patients with SAIDs-related HLH who have onset of HLH before receiving an SAIDs diagnosis; these patients are often overlooked by clinicians.

In clinical practice, we noted that it is easy to recognize and diagnose HLH after the patient has been diagnosed with a rheumatic disease. However, other HLH-onset patients do not receive timely and appropriate diagnosis and treatment. To improve outcomes for these patients, it is necessary to identify common features observed among these patients. We found that, at the onset of disease, these patients always had a fever and rash, while arthritis was seldom present. These patients did not respond to anti-infection therapy, had elevated white blood cells (WBC) count (especially neutrophils), erythrocyte sedimentation rate (ESR), and C-reaction protein (CRP), but did not meet the criteria for sJIA. Onset of HLH was observed to occur quickly after onset of the disease (usually within 1 to 2 weeks). As the disease progressed, HLH was usually diagnosed first and patients were usually transferred to the hematology department. After extensive examination, it was still difficult to identify protopathy, and SAIDs were highly suspected. After treatment, these patients appeared to have better outcomes compared with other HLH patients, such as Epstein-Barr virus (EBV)-related HLH patients,

even with low-intensity immunosuppression treatment. Therefore, it appears that HLH occurring at onset of SAIDs is associated with unique clinical and experimental features, as well as treatment and prognosis, compared with other HLH patients. Identifying and distinguishing these patients may help to avoid misdiagnosis and unnecessary overtreatment, and may assist in prognostic speculation by hematologists.

In this article, we summarize the clinical features, laboratory results, treatment protocol, and prognosis of patients with SAIDs-related HLH. The purpose of this study was to assist in early identification of these patients so that they can receive appropriate treatment, avoid overtreatment, and obtain a good prognosis.

## 2. Methods

### 2.1. Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Children's Hospital.

### 2.2. Patients

We analyzed data from 25 HLH patients who were considered to have SAIDs; data were collected from patients of our center between January 1, 2015 and September 1, 2018. HLH diagnosis was based on the HLH-2004 protocol.<sup>[11]</sup> SAIDs was the most likely diagnosis of each patient based on the presentation described in previous studies, including the presence of fever, transient arthralgia, serum cavity effusion, high WBC count with neutrophilia and CRP, and elevated ESR, usually without autoantibodies.<sup>[7,12,13]</sup> Meanwhile, many related examinations were taken to exclude infection, autoimmune diseases, lymphoma, and other malignant diseases, including copies of EBV-DNA, Cytomegalovirus-DNA and Human Parvovirus B19-DNA, antibodies of EBV, Human Parvovirus B19, Leishmania, etc, various of autoantibodies and tumor markers, cranial magnetic resonance imaging, pulmonary high-resolution computed tomography, positron emission tomography, and so on. However, because their symptoms and laboratory results were consistent with the diagnostic criteria for HLH, their short disease course was not consistent with a diagnosis of sJIA or other SAIDs, they could not receive rheumatic therapy.<sup>[14]</sup>

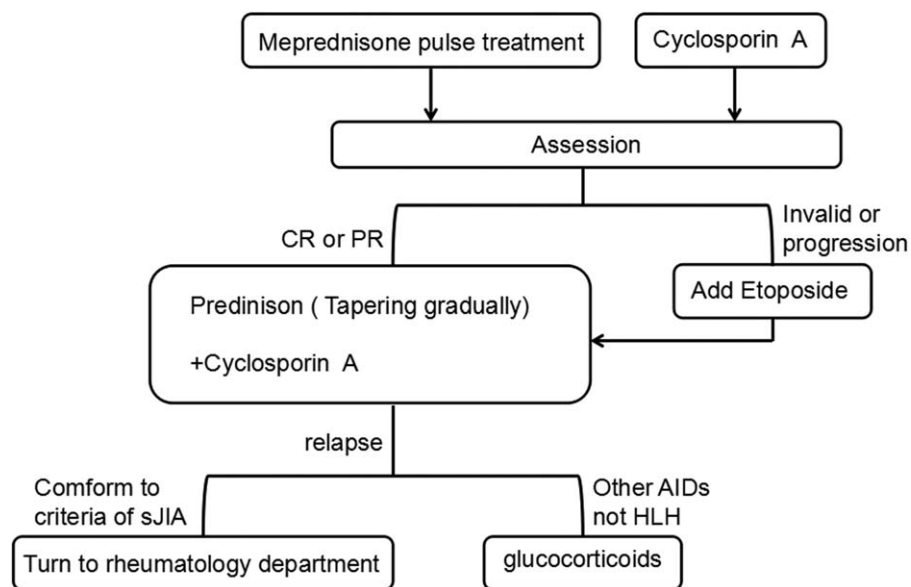
To compare with patients with SAIDs-associated HLH, we also collected data of 49 patients with EBV-associated HLH from January 1, 2015 and January 1, 2018. These patients were selected according to matched age and gender. Related examinations were taken to exclude other cause of HLH.

Seven patients underwent whole exome sequencing (WES) and 13 patients underwent the HLH and immunodeficiency gene package. Related data were collected.

It should be noted that we only examined patients who suffered from HLH at the onset of SAIDs and excluded patients who presented with HLH after onset of SAIDs.

### 2.3. Therapeutic regimen

Once patients were diagnosed with HLH, they immediately received a modified HLH-94/04 protocol (with or without etoposide according to the severity of disease) (Fig. 1). Maintenance treatment with a corticosteroid and ciclosporin was usually given until the 40th week.



**Figure 1.** The therapeutic regimen of hemophagocytic lymphohistiocytosis (HLH) at onset of systemic autoinflammatory disease (SAID).

Three patients with sJIA as primary disease received prednisone and/or other immunosuppressors while under the care of the rheumatism department; these patients had passed the acute phase of HLH after receiving the modified HLH-2004 protocol or after disease relapse (sJIA not HLH). The diagnostic criteria for sJIA were consistent with previous studies.<sup>[15,16]</sup> Six patients relapsed (fever and increased inflammatory markers, not HLH) after drug withdrawal or during the course of treatment; these patients still did not meet the diagnostic criteria for sJIA and continued to receive glucocorticoid therapy.

#### 2.4. Statistical analysis

Median and range of the data which did not conform to a normal distribution were displayed. GraphPad Prism 5 statistical analysis software (GraphPad Software, Inc, CA) was used to compare with different groups. Mann–Whitney *U* tests were used to compare with data of skewed distribution.  $P < .05$  was considered to be significant difference, and  $P < .01$  was considered to be very significant difference.

### 3. Results

#### 3.1. Patients

A total of 25 HLH patients from our center, who were considered to have SAIDs, were enrolled in this study; patients attended our center between January 1, 2015 and September 1, 2018. Among them, 14 were male and 11 were female; the median age was 1.75 years (range from 2 mo–13 y).

#### 3.2. Clinical manifestations

All 25 patients presented with fever and most patients (23/25, 92%) had a rash. Only 16 patients (64%) had splenomegaly. In contrast, patients with EBV-HLH seldom had a rash (20%,  $P < .001$ ) and almost always had splenomegaly (90.24%,  $P = .023$ ). Seventeen patients (68%) showed mild lymphadenopathy in the neck, submaxillary, or groin. Joint swelling or

arthralgia is a common symptom in connective tissue diseases, but it was only present in 7 patients at diagnosis (28%). Among them, 5 patients had joint fluid; 3 patients were later diagnosed with sJIA in the rheumatic department, while the remaining patients who were not diagnosed with sJIA exhibited transient arthralgia for less than 6 weeks, without arthrocele.

Serous membrane fluid is also an important symptom in rheumatic immune diseases; 15 of our patients (60%) had serositis. Among them, 13 had polyserositis (effusion in 2 or more sites). Specifically, 11 patients had pleural effusion, 10 had ascites, 6 had pericardial effusion, 4 had joint fluid, 4 had pelvic effusion, and 1 had left hydrocele.

Some patients also exhibited several rare symptoms. Three patients had seizures (12%), 3 (12%) had acroesthesia, and 1 (4%) had severe headache. Among them, 1 patient (4%) had both acroesthesia and headache.

All of the above symptoms refer to the clinical manifestations observed at the onset of disease.

#### 3.3. Laboratory examinations

At the onset of fever, patients usually had a high WBC count (median  $15 \times 10^9/L$ ). Neutrophils were predominant with a median rate of 72.3%. However, the median levels of hemoglobin and platelets were normal (108 g/L and  $283 \times 10^9/L$ , respectively). Six patients (26.1%) had an increased number of platelets, more than  $400 \times 10^9/L$ . It is important to note that other inflammatory indicators were also abnormal, such as CRP and ESR, with medians of 67 g/L and 43 mm/h, respectively. Despite these high inflammatory indicators, there was no evidence of infection in these patients; thus, anti-infection therapy was invalid and not suitable. The above data can be found in Table 1.

After a period of time (from 7 to 39 d; median 22 d), 1 or more hemocyte lineages were found to gradually decrease to below normal. CRP and ESR were also further decreased compared with the early stage of the disease. At the diagnosis of HLH, the median WBC count and percentage of neutrophils were

**Table 1**  
Laboratory results of autoinflammatory disorders-related hemophagocytic lymphohistiocytosis (HLH).

Items	Minimum	Maximum	Median
WBC <sup>1</sup> ( $\times 10^9/L$ )	8.1	39.6	15.0
HB <sup>1</sup> (g/L)	80	144	108
PLT <sup>1</sup> ( $\times 10^9/L$ )	7	791	283
CRP <sup>1</sup> (mg/L)	12.7	243.0	67.0
ESR <sup>1</sup> (mm/h)	5	321	43
WBC <sup>2</sup> ( $\times 10^9/L$ )	0.5	17.81	5.23
HB <sup>2</sup> (g/L)	54	100	74
PLT <sup>2</sup> ( $\times 10^9/L$ )	8	317	80
CRP <sup>2</sup> (mg/L)	2	160	63.5
ESR <sup>2</sup> (mm/h)	2	68	5
SF (ng/mL)	653	69,360	9956
sCD25 (pg/mL)	203.4	44000.0	16967.0
ALT (U/L)	12	1942	214
ALB (g/L)	18.0	35.0	28.8
FIB (g/L)	0.50	4.37	1.48
TG (mmol/L)	0.56	5.8	2.37
NK activity (%)	8.77	17.76	15.22
IL-6 (pg/mL)	2.62	1327.29	74.3
IL-10 (pg/mL)	0	90.93	12.49
IFN-r (pg/mL)	0	1797	2.40
TNF (pg/mL)	0	134.5	4.64
IL-2 (pg/mL)	0	7.69	0
IL-4 (pg/mL)	0	3.31	0.05
NK $\Delta$ CD107a (%)	0	14.45	9.09
CTL $\Delta$ MFI (%)	0.6	3.8	2.1

ALB=albumin, ALT=transaminase, CRP=C-reaction protein, CTL=cytotoxic T cell, ESR=sedimentation rate, FIB=fibrinogen, HB=hemoglobin, IFN=interferon, IL=interleukin, PLT=platelet, sCD25=soluble-CD25, SF=ferritin, Superscript 1=at the onset of disease, Superscript 2=at the time of HLH diagnosis, TG=triglyceride, TNF=tumor necrosis factor, WBC=white blood cell, Without superscript=at the time of HLH diagnosis.

$5.23 \times 10^9/L$  and 55.7%, respectively. Seven patients (28%) had a normal WBC count ( $4.0 \times 10^9/L$  to  $10.0 \times 10^9/L$ ), while a WBC below  $4 \times 10^9/L$  was observed in 44% of patients. To be noted, 7 patients (28%) still had a high WBC count although it had reduced gradually. Meanwhile, hemoglobin, platelets, CRP, and ESR were all obviously decreased compared with at HLH diagnosis, with medians of 74 g/L,  $63.5 \times 10^9/L$ , 32 mg/L, and 5 mm/h, respectively. It should be noted that CRP had not decreased to normal. Relevant data can be found in Table 1.

Hemophagocytosis was found in the bone marrow of 19 patients (76%) during the HLH period. All of the 25 patients had increased ferritin (SF), ranging from 653 to 69,360 ng/mL, with a median of 9956 ng/mL; this was much higher than among patients with EBV-related HLH (Table 2). Soluble-CD25 (sCD25) is also a vital indicator in the diagnostic criteria for HLH, and we observed increased levels of sCD25 in our patients. sCD25 levels greater than 6400 pg/mL are considered abnormal. In total, 88% (22/28) of our patients showed high levels of sCD25 with a median of 16,978 pg/mL; this appears to be much higher than among EBV-HLH patients (Table 2). Further, glutamic-pyruvic transaminase (ALT) was increased (more than 40 U/L) in 23 patients (92%), with a median level of 214 U/L (range from 12 to 1942 U/L). Since we found that most patients had serous cavity fluid, we also examined albumin levels, which were slightly decreased (median level of 28.8 g/L, range from 18.0 to 35.0 g/L).

On the other hand, there was little change in fibrinogen and triglyceride levels during HLH period, with medians of 1.48 g/L

**Table 2**  
Comparison of laboratory results of EBV-HLH and AID-HLH.

Items	Median (EBV-HLH)	Median (AID-HLH)	P value	Mann-Whitney U
WBC	1.875	5.23	<.001 <sup>‡</sup>	188.5
HB	85.5	74.0	.001 <sup>†</sup>	239.0
PLT	50	63.5	.096	345.0
CRP	25	32	.299	239.5
TG	3.2	2.37	.003 <sup>†</sup>	271.5
FIB	1.17	1.48	.051	348.0
SF	2454	9956	.032 <sup>*</sup>	333.0
NK activity	14.7	15.22	.184	325.0
sCD25	33081	16978	.009 <sup>†</sup>	281.0
IL-6	19.57	74.3	.005 <sup>†</sup>	267.0
IL-10	50.685	12.49	.004 <sup>†</sup>	266.5
IFN-r	18.27	2.87	.010 <sup>*</sup>	273.0
TNF	2.6	4.64	.143	366.0
IL-2	0.585	0	.057	313.0
IL-4	1.52	0.05	.060	327.0
NK $\Delta$ CD107a	10.37	9.09	.082	170.5
CTL $\Delta$ MFI	2	2.1	.356	230.5
ALT	120.8	214	.054	360.0
ALB	28.3	28.8	.074	278.5

AID-HLH=autoinflammatory disorders related hemophagocytic lymphohistiocytosis, ALB=albumin, ALT=transaminase, CRP=C-reaction protein, CTL=cytotoxic T cell, EBV-HLH=EBV related hemophagocytic lymphohistiocytosis, FIB=fibrinogen, HB=hemoglobin, IFN=interferon, IL=interleukin, PLT=platelet, sCD25=soluble-CD25, SF=ferritin, TG=triglyceride, TNF=tumor necrosis factor, WBC=white blood cell.

<sup>\*</sup> P < .05.

<sup>†</sup> P < .01.

<sup>‡</sup> P < .001.

and 2.37 mmol/L, respectively. NK cell activity less than 15.11% is defined as decreased activity. In our study, 11 out of 23 (47.8%) patients showed decreased NK activity, with a median activity level of 15.22%.

Cytokines are important in the pathogenesis of HLH. In this study, protein levels of several kinds of cytokines in plasma were also detected, including interleukin-6, interferon- $\gamma$ , interleukin-10, and tumor necrosis factor. Their median levels were 74.3 pg/mL (range from 2.62 to 1327.29 pg/mL), 2.40 pg/mL (range from 0 to 1797 pg/mL), 12.49 pg/mL (range from 0 to 90.93 pg/mL), and 4.64 pg/mL (range from 0 to 134.5 pg/mL), respectively. Among them, protein levels of IL-6 seemed to be higher than those of EBV-related HLH patients, and the levels of IL-10 and IFN- $\gamma$  were lower than those of EBV-related HLH patients (Table 2).

CD107a is another important indicator of the function of NK and CTL cells. We measured the levels of CD107a in 19 patients. Ten patients (52.63%) showed abnormal degranulation function of NK cells (absence: 5 patients; reduction: 5 patients), while the remaining 9 patients had normal degranulation function. On the other hand, 89.5% of patients showed abnormal degranulation function of CTL cells. Thus, abnormal degranulation function in CTL cells was more common. These reductions in function recovered gradually after appropriate treatment.

In the early phase, echocardiograms showed enlarged left ventricle and decreased diastolic function in 3 patients (12%). These observations were transient and recovered after 1 or 2 weeks of treatment of the primary disease.

Among our 25 patients, 7 underwent WES, but only 1 was found to have the UNC13D heterozygous mutation; all other patients showed no significant disease-related mutation in WES.

Thirteen patients underwent blood and immunodeficiency disease-related gene package; 2 had the UNC13D heterozygous mutation. Patients who did not receive gene test mainly had a financial problem. Unfortunately, no SAIDs-related genes were observed and no valuable conclusions could be drawn from the data.<sup>[17]</sup>

### 3.4. Comparison to EBV-related HLH

We also compared the data of SAIDs-related HLH patients to data from patients from our center with EBV-related HLH. In the hemogram, the WBC count was much higher in patients with SAIDs-related HLH compared with those with EBV-related HLH ( $P < .001$ ), while hemoglobin levels were lower in the former group than the latter one ( $P = .001$ ). Although there was no difference in CRP between the 2 groups ( $P = .299$ ), ferritin, an important inflammatory marker, was significantly higher in SAIDs-related HLH patients than EBV-related HLH patients ( $P = .032$ ). We also detected a difference in the levels of triglyceride between the 2 groups ( $P = .003$ ), but there were no differences in the levels of platelets and fibrinogen (see Table 2).

The cytokine profile of HLH patients has drawn great attention over recent years, and we found significant differences in the levels of IL-6, IL-10, and IFN- $\gamma$  between SAIDs-related HLH patients and EBV-related HLH patients. In the SAIDs patients, levels of IL-6 were much higher while levels of IL-10 and IFN- $\gamma$  were much lower compared with EBV-related HLH patients ( $P = .005$ ,  $P = .004$ , and  $P = .010$ , respectively). Further, protein levels of sCD25 in SAIDs-related HLH patients were lower than those in EBV-related HLH patients ( $P = .009$ ).

There were no significant differences between the groups in terms of NK activity, transaminase levels, and albumin levels (Table 2).

### 3.5. Treatments

After each patient was diagnosed with HLH, they underwent a variety of examinations, such as bone marrow aspiration and biopsy, flow cytometer examination, examination of infection and immune-related markers, imaging examinations, and so on. In the meantime, each patient immediately received the modified HLH-2004 protocol (with or without etoposide according to the severity of disease). Based on disease severity, 2 patients received a glucocorticoid only, 7 patients were given a glucocorticoid and cyclosporine, 16 received the HLH-2004 protocol including etoposide (among them, 4 patients began with a glucocorticoid and cyclosporine but failed to achieve complete or partial remission at the first assessment; thus, etoposide was administered. Etoposide was stopped once the patient achieved complete remission at the fourth week; otherwise, 8 weeks of treatment was given). In total, 88% of patients achieved complete or partial remission at the evaluation during the fourth week. Maintenance treatment included a glucocorticoid and cyclosporine, without etoposide. Treatment was stopped after the 40th week of treatment, if the patient was well. Three patients were diagnosed with sJIA<sup>2</sup>; one had been transferred to the rheumatologic department after MAS was controlled (diagnosed during first hospitalization), and the other 2 patients were not diagnosed until they relapsed (fever, rash, and high levels of inflammatory indicators, but not HLH) during or after maintenance treatment. One patient relapsed (fever, rash, and high levels of inflammatory indicators, but not HLH) on the 37th week and was diagnosed as

highly suspicious SAIDs by the rheumatologic department. These patients were all prescribed a glucocorticoid with or without cyclosporine by the rheumatism outpatients office.

### 3.6. Prognosis

HLH patients with a primary SAIDs disease seem to have a better prognosis than other HLH patients, such as primary HLH patients and EBV-related HLH patients. Among our 25 patients, four patients who were diagnosed as sJIA or suspicious SAIDs were still taking prednisone and/or other immunosuppressors and visited the rheumatologic department regularly to adjust the dosage. Three of these four patients relapsed (sJIA or SAIDs, not HLH). The remaining 21 patients were followed up regularly by the hematologic department. Among them, 18 patients had stopped treatment after evaluation at the 40th week. Although three patients relapsed (SAIDs, not HLH) at 11 months, 2 months after stopping treatment, and on the 22nd treatment week, respectively, they achieved remission after being given prednisone (10 mg/kg, 3 d, 2 mg/kg, 4 wks, then tapering down). Therefore, the recurrence rate was 24%. Fortunately, after relapse, they still had a good response to glucocorticoids. To our surprise, all patients were alive at the latest follow-up on June 1, 2019. It should be noted that recurrence refers to relapse of SAIDs, including fever, rash, high WBC count, high level of neutrophils, high ESR and CRP, no response to anti-infection therapy, and rarely the presence of arthritis. Thus, these patients could not be rediagnosed as HLH because of insufficient indicators.

## 4. Discussion

Due to its relatively short history, there is lack of recognition of specific clinical manifestations and definitive diagnostic criteria for SAIDs; thus, it is difficult to differentiate SAIDs from other diseases such as infections and cancer, especially at the early phase of disease.<sup>[3,7]</sup> In clinical practice, SAIDs-related HLH is often neglected. In a previous study of SAIDs-related HLH, most patients suffered from HLH after diagnosis of SAIDs.<sup>[10]</sup> The current study investigated SAIDs patients who presented with HLH at onset.

To assist in the early identification of these patients, we have summarized the common clinical manifestations and laboratory results. At the onset of disease, these patients often presented with fever and rash, as well as high WBC count, CRP, and ESR, but there was no evidence of infection; antibiotics were not effective. Splenomegaly was not common in patients with SAIDs-related HLH compared with patients with EBV-related HLH.

In this paper, we compared SAIDs-related HLH patients with EBV-related HLH patients and found that SAIDs-related HLH patients had higher WBC count and neutrophil number, as well as higher ferritin and IL-6 levels, but lower hemoglobin, triglyceride, sCD25, IL-10, and IFN- $\gamma$  levels compared with EBV-related HLH patients. Doctors can use these clues to distinguish HLH patients and select the most appropriate treatments. Although not all markers were significantly different between the 2 types of HLH patients, ALT, albumin, NK cell activity, and CD107a in NK cells and CTL cells should also be monitored when considering a patient's diagnosis.

Many reports have shown that WES can assist in the diagnosis of SAIDs, but about 60% of patients with SAIDs could not be found positive gene mutations. Among the 25 patients in this study, 7 underwent WES and 13 underwent investigation for

genes related to blood and immunodeficiency diseases. Only three of these patients had heterozygous mutations of UNC13D. Patients who did not take gene test mainly had a financial problem. More studies should be performed to examine whether there are relationships between UNC13D and HLH secondary to SAIDs. Specifically, WES should be used, including analysis of SAIDs-related genes.

Most of our patients with SAIDs-related HLH received a modified HLH-2004 protocol in the acute phase of the disease. After overcoming the severe phase, they often only took prednisone and cyclosporin, with or without etoposide (unless necessary). Patients without severe disease did not require etoposide. However, for the patients who did not achieve remission, it was necessary to use etoposide to assist them to acquire remission. All patients showed good prognosis and achieved long-term survival, which is in contrast to patients with EBV-associated HLH.<sup>[18]</sup> Based on their good outcomes, hematopoietic stem cell transplantation (HSCT) was not suggested for these patients. More research should be done to identify the most effective diagnostic and treatment scheme in the future.

Among our patients, 6 relapsed during or after treatment (from 18 wks after commencement of treatment to 11 mo after stopping treatment). However, it should be noted that the recurrent manifestation included fever, rash, high WBC count, CRP, and ESR, and no response to anti-infective therapy. Thus, the relapse was a recurrence of SAIDs, not HLH. Only 2 patients exhibited joint symptoms, and these patients were later diagnosed with sJIA at the rheumatologic department. After receiving glucocorticoids, all of these patients achieved remission. Thus, relapse is not catastrophic; periodic monitoring and prompt treatment are key.

In this study, we analyzed several cytokines, including IFN-, TNF, IL-6, IL-10, and so on. Meanwhile, granulocyte-colony stimulating factor (G-CSF) is also an important factor in the formation of cytokine storm of HLH.<sup>[19]</sup> Granulocytopenia and agranulocytosis are common in HLH patients, and administering G-CSF is an effective and widely used strategy. However, it had been reported that G-CSF could promote the production of cytokines and hemophagocytosis could be exacerbated by administration of G-CSF for severe neutropenia.<sup>[20–23]</sup> Their research might help to reveal the mechanism of G-CSF worsen the condition of HLH patients and decide which patient could receive G-CSF therapy. Further study about the G-CSF pathway should be done in the future to answer these questions.

In summary, doctors should pay more attention to the identification of different types of HLH patients. If patients exhibit the above characteristics of SAIDs, HLH-onset-SAIDs should be highly suspected. The selection of appropriate treatment and the avoidance of toxic effects are important. Thus, the important questions are, how do we balance hypotoxicity and effectiveness, and should etoposide be included in the treatment regime. This study indicates that it is crucial to achieve early remission and closely monitor HLH patients. We hope HLH-onset SAIDs will come to the attention of hematologists, resulting in more patients receiving optimal treatment.

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### Author contributions

RZ and TW designed the work; ZL, LZ and HL collected data; YZ, HM, DW, XZ and QZ analyzed data; YZ and RZ wrote the article.

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