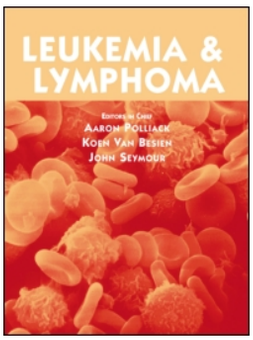

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ORIGINAL ARTICLE

Non-EBV infection-associated hemophagocytic lymphohistiocytosis: a distinct subgroup where pathogen-directed therapy is essential and favorable outcomes are expected

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ABSTRACT

EBV is the most prevalent cause of infection-associated hemophagocytic lymphohistiocytosis (IAHLH), non-EBV IAHLH is observed clinically but less documented. We conducted a retrospective research enrolled 36 cases from 1/1/2015 to 31/12/2019. Intriguingly, 92% cases were immunocompetent individuals prior to the onset of HLH. Clinically, the cardinal features were prolonged high fever, splenomegaly and hemophagocytosis. Bicytopenia occurred in most patients, besides, liver dysfunction was characterized by increased transaminase, bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GGT) and lactate dehydrogenase (LDH). Immunomodulatory agents should be added to control the overwhelming inflammatory storm without delay. Once a certain pathogen was identified as the causative factor of HLH, cytotoxic agents were withdrawn, specific pathogen-directed treatment was initiated. Further, glucocorticoids were tapered off when a stable state of HLH was achieved. After treatment, about 70% patients were fully recovered without relapse. All in all, non-EBV IAHLH is a special group of HLH with admirable outcome.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a fatal hyperinflammatory syndrome resulting from aberrantly activated cytotoxic T cells and macrophages. Clinically, this rare and under-diagnosed disorder is characterized by development of prolonged fever, splenomegaly, cytopenia, hyperferritinemia, and hemophagocytosis in hematopoietic organs [1]. Since it was first described in 1939 by Scott and Robb-Smith, our understanding of HLH, especially in adults, has improved substantially [2]. Dysfunctional NK cells and cytotoxic T cells play a dominant role in the pathophysiology of HLH [3–5]. Additionally, the hypersecretion of proinflammatory cytokines promotes the pathogenic cycle and is responsible for the constellation of features observed in HLH.

HLH occurs in all age groups, with a mean age at diagnosis of nearly 50 years in adults [6]. Traditionally, HLH is classified into primary (genetic) and secondary (reactive) forms. Underlying genetic defects play a pivotal role in the onset of primary HLH. In secondary HLH, an inability to restrict the stimulatory effect of various triggers is generally established [6,7]. In adults, causative factors are mainly external; herpes viral infection is attributed as the most frequent trigger, and malignancies and autoimmune disorders are also common predisposing factors for HLH [5]. Apart from viruses, other pathogens have been implicated in the etiology of HLH, including protozoa, bacteria, fungi, and parasites. Notably, the detection of a pathogen-associated trigger is inadequate to rule out primary HLH, which can also be triggered by an infection [5–7]. This multifaceted disorder is now a challenge for physicians in a wide range of fields, including hematology/oncology, infectious diseases, pneumology, rheumatism and immunology, neurology, and intensive care. The symptoms may not be pronounced on initial presentation, and many features overlap with those of sepsis, thereby delaying diagnosis. Thus, the early detection of HLH is vital for a favorable outcome.

Genetic defects, other predisposing factors, and various triggers contribute to the pathogenesis of HLH; therefore, the management of this rare syndrome is an art and science. The theoretical treatment strategy for HLH requires implementation of the following three simultaneous approaches: supportive intensive

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care, the elimination of triggers, and the suppression of the excessive inflammatory state [6,8]. Standard HLH-94, HLH-04, and salvage DEP regimens have improved outcomes substantially [9,10]. As the most prevalent cause of infection-associated HLH (IAHLH), Epstein–Barr virus (EBV)-HLH demands a graded treatment intensity and duration [1,11]. Apart from cases caused by EBV, trigger-directed therapy is recommended for IAHLH, with recovery rates of approximately 60%–70% with proper management [1]. Studies on non-EBV IAHLH are mostly available as case reports. A considerable sample of retrospective studies may provide a basis for development of diagnostic and therapeutic strategies. In this study, we characterized non-EBV IAHLH, with a focus on clinical aspects and treatment regimens.

Methods

Subjects and objectives

Thirty-six inpatients with non-EBV IAHLH from one single institution (Beijing Friendship Hospital, Capital Medical University) were recruited in this study. Informed consent was obtained from all patients or their guardians, appropriate ethical approval was obtained from Beijing Friendship Hospital in accordance of the Declaration of Helsinki.

Diagnosis and definition

Diagnosis of HLH was established according to the internationally accepted guideline of HLH-2004 [12], clinical course was analyzed by at least three hematologists, and the diagnosis was stringently enforced, meeting five out of eight criteria was a necessity. However, patients with non-EBV IAHLH enrolled in this study fulfilled another four following mandatory criteria: (1) Absence of family history and have negative molecular findings targeted HLH-related genetic defects. (2) No evidence of underlying conditions such as malignancies, autoimmune disorders and other external factors. (3) Definitive evidence of a triggering infectious agent, and the pathogen were considered as the only factor of causing HLH. (4) Negative EBV DNA copies and EBV-encoded small RNA (EBER) in peripheral blood and tissues. A thorough work-up was conducted in search of focus of infection, pathogen was verified by evidence of cultivation, observation by microscope, positive PCR or presence of IgM antibodies. Specialist from infectious department confirmed both the clinical syndrome and the existence of a pathogen.

Table 1. Clinical characteristics of patients with non-EBV infection-associated hemophagocytic lymphohistiocytosis at presentation.

Category	Total patients (percentage)
Sex, Male/Female (number)	21 (57%)/15 (43%)
Median age, range	34.5 years (2–72 years)
Comorbidities	7 (19%)
Immunocompetent host	33 (92%)
Time interval from onset to diagnosis	60 days (13–125 days)
Prolonged fever above 38.5 °C	36 (100%)
Splenomegaly	21 (58%)
Hemophagocytosis in bone marrow	31 (86%)
Central nervous system involvement	4 (11%)
Cutaneous involvement	9 (25%)
Intensive care unit supportive	5 (14%)
Blood transfusion	21 (58%)

Results

Clinical features

As depicted in Table 1, 36 cases fulfilled the criteria for non-EBV IAHLH over a 5-year period (from 1/1/2015 to 31/12/2019) in our database, accounting for 3.55% (36/1013) of all HLH cases over the corresponding period. In this cohort, thirty-four patients were adults and two were children, with a median age of 34.5 years. Only three patients presented with immunocompromised cases prior to the onset of HLH, including one case attributed to pregnancy and two cases attributed to immunosuppressive treatments (for interstitial lung disease and primary biliary cirrhosis). The cardinal features included presence of prolonged high fever (100%), splenomegaly (58%), and hemophagocytosis in the bone marrow (86%). Further, a small group of patients (11%) presented with progressive multiple organ failure with a necessity of being subjected to intensive care.

Non-EBV IAHLH development was associated with exposure to a variety of pathogens, such as viral, bacterial, fungal, and parasitic agents (Table 2). Even after excluding EBV, viral infection remained the most frequent trigger. Cytomegalovirus (CMV) accounted for 28% of all cases caused by non-herpes viruses, and HIV, hantavirus, and acute hepatitis E were also observed as the etiology. Bacterial infections accounted for 36% of all reported cases, among which tuberculosis was the most frequent cause of non-EBV IAHLH development. Parasites and fungi were less frequently reported and might be related to exposure associated with a travel history. Leishmanial infection was not rare accounted for 11% of all non-EBV IAHLH.

Laboratory features

Serial complete blood counts at the onset of disease are summarized in Table 3. Approximately three-

Table 2. Etiological screening of patients with non-EBV infection-associated hemophagocytic lymphohistiocytosis.

Etiology of HLH	Number (%)	Identifies the infectious agent
Viruses	15 (42%)	
Cytomegalovirus	10 (28%)	PCR (blood/alveolar lavage fluid)
HIV	2 (6%)	Serology (HIV-1/HIV-2 antibody)
Human herpesvirus 6	1 (3%)	PCR (blood)
Hantaviruses	1 (3%)	Serology (IgM, IgG)
Hepatic E	1 (3%)	Serology (IgM, IgG) , HEV-RNA
Bacteria	13 (36%)	
Mycobacterium tuberculosis	7 (19%)	Sputum smear or PCR (tissue)
Brucellosis	2 (6%)	Blood cultivation/serum agglutination test
Listeria bacteria	1 (3%)	Blood cultivation
Mycoplasma pneumoniae	2 (6%)	Serology (IgM)
Leprosy bacillus	1 (3%)	PCR (tissue)
Parasites	6 (17%)	
Leishmania	4 (11%)	BM smear + PCR (BM)
Falciparum malaria	1 (3%)	Microscope (smear of blood/BM)
Paragonimus westermani	1 (3%)	Serology (IgM)
Fungi	2 (6%)	
Aspergillus	1 (3%)	Blood cultivation
Histoplasma capsulatum	1 (3%)	BM smear + Mass spectrography (BM)

BM: bone marrow.

Table 3. Characteristics of laboratory feature of the patients at diagnosis of HLH.

Haematological feature	Patients ($N = 36$)
Bicytopenia	31 (86%)
Severe neutropenia ($<0.5 \times 10^9$/L)	8 (22%)
Anemia	
Haemoglobin < 100 g/L	30 (83%)
Haemoglobin < 80 g/L	16 (44%)
Severe thrombocytopenia ($<20 \times 10^9$/L)	8 (22%)
Biochemical features	
Raised transaminases (>200 U/L)	10 (27%)
Increased bilirubin (>2 ULN)	7 (19%)
Decreased albumin (<30 g/L)	18 (50%)
Raised ALP	17 (47%)
Raised γ-GGT	21 (58%)
Increased LDH (>500 U/L)	13 (36%)
Triglycerides (>3.0 mmol/L)	7 (19%)
Ferritin	
>500 ng/mL	35 (97%)
>2000 ng/mL	18 (50%)
Coagulopathy feature	
Fibrinogen < 1.5 g/L	19 (53%)
D-dimer > 1.5 g/L	23 (64%)
FDP > 5.0 mg/L	21 (58%)
Immunological Feature	
Increased CD3+ cell rate	15/28 (54%)
Abnormal CD4/CD8 ratio	15/28 (54%)
Decreased CD19+ cell rate	13/28 (46%)
Decreased CD16 + CD56+ cell rate	18/28 (64%)
Decreased NK cell activity	19/33 (58%)
Increased sCD25	26/33 (79%)
Increased β2 microglobulin (>5.00 mg/L)	6/27 (22%)
Cytokine ($n = 15$, >3ULN)	
IL-1RA	14/15 (93%)
IFN-γ	9/15 (60%)
TFN-α	14/15 (93%)
IL-18	8/15 (53%)

ULN: upper limit of normal; ALP: alkaline phosphatase; γ-GGT: gamma-glutamyl transpeptidase; LDH: lactate dehydrogenase; FDP: fibrinogen degradation products; IL: interleukin; IL-1RA: interleukin-1 receptor antagonist; IFN Interferon; TNF: tumor necrosis factor.

quarters of cases showed presence of leukopenia, and presence of severe or prolonged neutropenia was not uncommon. Anemia was observed in 30 patients (83%) and nearly half of these patients presented with severe anemia (<80 g/L, 44%). Notably, thrombocytopenia developed first, preceding neutropenia or anemia; however, severe thrombocytopenia was less commonly observed.

Biochemical parameter analyses are of utmost importance for obtaining plausible evidence for non-EBV IAHLH development. Altered liver enzyme levels were apparent (34/36, 94%), including aberrantly elevated levels of transaminases in nearly one-third of the cases. Increased levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ-GGT) were relatively common. Fasting triglyceride levels were elevated in more than half of the patients (21/36, 58%) but only exceeded 3.0 mmol/L in a small proportion of patients. As a hallmark of HLH, half of the cases showed development of hyperferritinemia (exceeding 2000 ng/mL). Further, the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were elevated in nearly 75% of the patients, which mirrored systemic inflammation markedly.

Almost half of the patients presented with coagulation abnormalities. Hypofibrinogenemia (<1.5 g/L) was reported in 53% of the patients with non-EBV IAHLH. Moreover, elevated D-dimer levels and fibrinogen degradation products were also noted.

Immunologic dysfunction was detected by serology and cell function assays. Lymphocyte subgroups in peripheral blood were detected in 28 patients, with an increased rate of CD3+ cells in 15 cases (54%) and a decreased CD4/CD8 ratio in 39% of the cases; these two indices might not be closely associated with outcomes. Further, we have previously established a rapid and reliable cytometry-based NK cell activity assay

Table 4. Treatment and outcome of the patients with non-EBV IAHLH.

	Treatment		Outcome	
Etiology of HLH (number)	Pathogen-directed	Immunomodulatory	Recovery	Died/loss to follow-up
Virus				
Cytomegalovirus (10)	Ganciclovir/Foscarnet	IVIG + GC/VP-16/ruxolitinib	7	2/1
HIV (2)	Antiretroviral	Ruxolitinib	1	1
Human herpesvirus 6 (1)	Foscarnet	GC	0	1
Hantaviruses (1)	Ribavirin	IVIG + GC	1	0
Hepatic E (1)	Ribavirin	IVIG + GC	0	1
Bacteria				
Mycobacterium tuberculosis (7)	Antituberculous	IVIG + GC/VP-16/DEP regimen[a]	4	2/1
Brucellosis (2)	Rifampin + doxycycline	GC/VP-16/HLH 94[b]	1	1
Listeria bacteria (1)	Glycopeptide Antibiotic	IVIG + GC/VP-16/DEP regimen[a]	0	1
Mycoplasma pneumoniae (2)	Azithromycin	IVIG + GC	2	0
Leprosy bacillus (1)	Antileprotic	GC	1	0
Parasites				
Leishmania (4)	Stibogluconate	GC/VP-16/DEP regimen[a]	4	0
Falciparum malaria (1)	Artemisinin	IVIG/GC	1	0
Paragonimus westermani (1)	Praziquantel	–	1	0
Fungi				
Aspergillus (1)	Amphotericin B	IVIG/VP-16/DEP regimen[a]	1	0
Histoplasma capsulatum (1)	Amphotericin B	VP-16/DEP regimen[a]	1	0

IVIG: intravenous immunoglobulin; GC: glucocorticoid; VP-16: etoposide; DEP regimen [a]based on Wang et al. [10]; HLH 94 [b]based on Ehl et al. [14].

[13]. Low NK cell activity was identified in 58% of the reported cases. Moreover, an elevated level of alpha chain of soluble IL-2 receptor (sCD25) was observed in 79% of the patients.

Surrogate markers of macrophage activation, such as β2 microglobulin and cytokines, were examined. Nearly three-quarters of patients exhibited increased β2 microglobulin levels; however, only a small proportion (6/27, 22%) of patients exhibited aberrantly high levels (>5.00 mg/L). In terms of cytokines, interleukin-1 receptor antagonist (IL-1RA) and tumor necrosis factor (TNF-α) levels were remarkably elevated in almost all patients (14/15, 93%). Moreover, increased levels of interferon-γ (IFN-γ) and interleukin (IL)-18 were frequently observed.

Treatments and outcomes

As a primary treatment, supportive care was initiated immediately. Considering the time-consuming process of identifying the underlying cause, immunomodulatory agents were administered to control the overwhelming inflammatory storm without delay, including intravenous immunoglobulin administration, one dose of etoposide (100–150 mg), and low-dose glucocorticoids. For persistent or relapsed HLH, the HLH-94 or DEP regimen was applied [10,14,15]. After a pathogen was identified as a causative factor, specific antimicrobial agents were administered and cytotoxic agents were withdrawn. Further, glucocorticoids were tapered off when a stable state of HLH was achieved; alternatively, ruxolitinib was added to control the development of excessive inflammation.

As illustrated in Table 4, ten patients were diagnosed with CMV-IAHLH, 70% achieved recovery, and two patients died (one succumbed to severe sepsis after the DEP regimen and the other succumbed to liver failure occurring at the onset of HLH). For HIV-IAHLH, antiretroviral treatment was a priority, and one patient with central nervous system involvement died after a short duration. Brucellosis-IAHLH was not uncommon, and one patient died from progressive disease due to pathogen-targeted treatment interruption. The diagnosis of *Leishmania*-IAHLH is a challenge. One case was admitted owing to the development of HLH relapse even after treatment with the DEP regimen; after the presence of *Leishmania* was subsequently confirmed, treatments using cytotoxic agents and glucocorticoids were stopped, stibogluconate was administered, and the patients were cured of HLH. Tuberculosis-IAHLH was prevalent; two patients died, one presented with untreated tuberculosis, and the other experienced multiple organ failure. *Listeria*-induced HLH development was rare and may led to the death of the affected individual if without timely pathogen-targeted treatment.

Patients underwent regular clinical follow-up. Among all 36 patients, 69% showed recovery with a median follow-up of 20.5 months and no recurrence or reactivation of HLH was observed. Overall mortality was 25.0%, and involvement of the central nervous system and development of severe sepsis and multiple organ failure were attributed as the main causes of

death. Furthermore, among the patients who died, most were immunocompetent prior to the onset of HLH.

Discussion

Based on the accumulated data, EBV-associated HLH is undoubtedly the most common subgroup of IAHLH [16,17]. Intensive and prolonged treatment regimens are imperative for patients with EBV-HLH [1,11]. With respect to prognosis, patients with EBV-HLH present with a critical clinical course and a relatively high mortality rate [5,11]. Most importantly, pathogen-directed therapy is not applicable to EBV-HLH treatment owing to the lack of availability of effective drugs. Therefore, non-EBV IAHLH and EBV HLH differed significantly. However, relatively little is known about non-EBV IAHLH. Therefore, we aimed to establish the characteristics of non-EBV IAHLH and proposed appropriate treatments.

Thirty-six patients with non-EBV IAHLH were eligible for our study, accounting for 3.55% of all patients with HLH present at our center; this was lower than the frequency in a multicenter nationwide survey conducted in China (197 of the 601 cases, 7.8%) and markedly lower than the estimates reported in Japan (138 of the 567 cases, 24.3%) and in a worldwide survey (850 of the 2197 cases, 38.7%) [6,16,17]. The lower prevalence may be explained by the consideration of a more rigorous definition of IAHLH; however, the discrimination of HLH from sepsis may be difficult. In this study, the clinical course was analyzed by at least three hematologists, diagnosis was stringent (requiring fulfillment of at least five out of the eight criteria), and convincing evidence for the presence of a specific pathogenic microorganism was necessary. Intriguingly, 81% of the cases comprised originally healthy individuals without underlying comorbid conditions, which were rarely documented.

In our study, only 29% of the patients with tuberculosis-associated HLH presented with comorbidities, which was markedly lower than a previously reported estimate of 54% [18]. The time to diagnosis was long, with an average of 60 days, which was markedly longer than that previously reported [19]; thus, early diagnosis should be a priority. Consistent with HLH, development of prolonged high fever was the most common presenting feature [19,20]. Viral infection was the most frequent trigger, particularly that caused by CMV. Compared with previous estimates (206 of the 1138 cases, 18%) [6], bacterial infections accounted for a greater proportion (36%) of cases in our study, among which 54% were related to tuberculosis.

Most patients present with bicytopenia at diagnosis, and blood transfusion is indispensable. These manifestations may mimic many clinical courses, such as diseases involving bone marrow failure. Compared with previous data [21], more patients in our study showed the presence of liver dysfunction, characterized by increased transaminase, ALP, γ-GGT, and LDH levels. A decreased level of lipoprotein lipase contributed to high triglyceride levels; however, the threshold of $>$3.0 mmol/L was fulfilled in only 19% of the cases, in marked contrast to the 70% coincidence rate reported previously [21]. Ferritin is mainly secreted by macrophages or hepatocytes and acts as an effective screen for inflammation and HLH [12]. Almost all patients included in this study showed the presence of hyperferritinemia (97%).

As an acute phase protein, fibrinogen is theoretically increased in infectious diseases; thus, decreased fibrinogen levels should be regarded as a prominent indicator for the development of imminent immune disorders; further, the decreased levels result in the exhibition of pronounced constellation of features in HLH. Further, ESR and CRP levels were also elevated in three-quarters of patients, which were consistent with systemic inflammation.

In a lymphocyte subgroup analysis, consistent with previous findings, an increased CD3+ cell rate and a decreased CD4/CD8 ratio were observed at the onset of HLH [22–24]. However, we found that these parameters might not be associated with outcomes in HLH. Further, it has been reported that the CD4/CD8 ratio increases as HLH progression is controlled [23]. Low NK activity was detected in the more than half of the cases. NK activity was reevaluated in five patients after treatment; all patients recovered and NK activity returned to normalcy. Therefore, NK activity is related to outcomes and should be tested regularly.

sCD25 is a surrogate index which reflects the activation of T cells, and elevated sCD25 levels are correlated with the disease status with high specificity [25,26]. Consistent with previous data [6], elevated sCD25 expression occurred in most cases. As a marker of macrophage activation, β2 microglobulin expression is substantially higher in HLH and is an independent poor prognostic factor for overall survival [27]. In the present study, β2 microglobulin expression was elevated, and higher levels were associated with poor outcomes. Elevated serum IL-1RA levels are reported to be positively correlated with disease activity, and IFN-γ, TNF-α, and IL-18 levels are increased in active

HLH [28,29]. In this study, pronounced hypercytokinemia was also observed.

To date, no generally accepted treatment protocol has been established for non-EBV IAHLH. Successful management has been described in case reports, with supportive and pathogen-directed therapy yielding good results [20]. However, administration of immunomodulatory agents has also been recommended [1,18,19]. Notably, the process of identifying the underlying cause is time-consuming, and all forms of HLH should be initially treated without delay considering its high mortality rate. However, cytotoxic agents and glucocorticoids impair the ability to resist infection. Therefore, once a certain pathogen is identified as the causative factor, antimicrobial agents should be administered to clear the triggering infection. Cytotoxic agents should be withdrawn, and glucocorticoids should be tapered off in stable HLH. Fortunately, most patients achieve positive outcomes without recurrence.

An equally encouraging observation is that ruxolitinib can be used as a coadjutant in improving clinical symptoms, especially persistent fever. The Janus kinase 1/2 (JAK1/2) inhibitor ruxolitinib can dampen HLH by targeting deleterious immune cells [30]. Despite the paucity of data related to the use of ruxolitinib in secondary HLH, our findings support the potential role of ruxolitinib as a coadjutant in the treatment of non-EBV IAHLH.

All in all, our study shows that the non-EBV IAHLH is a special group of HLH. Considering the potential effect of impairing immune system, cytotoxic agents and glucocorticoid should be cautiously applied, pathogen-directed therapy is the most critical treatment.

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Disclosure statement

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References

[1] La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. Blood. 2019;133(23):2465–2477.
[2] Scott RB, Robb-Smith AH. Histiocytic medullary reticulocytosis. Lancet. 1939;234(6047):194–198.
[3] de Saint Basile G, Fischer A. Defective cytotoxic granule-mediated cell death pathway impairs T lymphocyte homeostasis. Curr Opin Rheumatol. 2003;15(4):436–445.
[4] Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science. 1999;286(5446):1957–1959.
[5] Schram AM, Berliner N. How I treat hemophagocytic lymphohistiocytosis in the adult patient. Blood. 2015;125(19):2908–2914.
[6] Ramos-Casals M, Brito-Zeró N P, Ló Pez-Guillermo A, et al. Adult haemophagocytic syndrome. Lancet. 2014;383(9927):1503–1516.
[7] Janka GE, Lehmberg K. Hemophagocytic syndromes-an update. Blood Rev. 2014;28(4):135–142.
[8] Janka GE, Lehmberg K. Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. Hematology Am Soc Hematol Educ Program. 2013;2013:605–611.
[9] Trottestam H, Horne A, Aricò M, for the Histiocyte Society, et al. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. Blood. 2011;118(17):4577–4584.
[10] Wang Y, Huang W, Hu L, et al. Multicenter study of combination DEP regimen as a salvage therapy for adult refractory hemophagocytic lymphohistiocytosis. Blood. 2015;126(19):2186–2192.
[11] Wang J, Wang Y, Wu L, et al. PEG-aspargase and DEP regimen combination therapy for refractory Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. J Hematol Oncol. 2016;9(1):84.
[12] Henter JI, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124–131.
[13] Zhang J, Wang Y, Wu L, et al. Application of an improved flow cytometry-based NK cell activity assay in adult hemophagocytic lymphohistiocytosis. Int J Hematol. 2017;105(6):828–834.
[14] Ehl S, Astigarraga I, von Bahr Greenwood T, et al. Recommendations for the use of etoposide-based therapy and bone marrow transplantation for the treatment of HLH: consensus statements by the HLH

Steering Committee of the Histiocyte Society. J Allergy Clin Immunol Pract. 2018;6(5):1508–1517.
[15] Brisse E, Wouters CH, Matthys P. Hemophagocytic lymphohistiocytosis (HLH): a heterogeneous spectrum of cytokine-driven immune disorders. Cytokine Growth Factor Rev. 2015;26(3):263–280.
[16] Ishii E, Ohga S, Imashuku S, et al. Nationwide Survey of Hemophagocytic Lymphohistiocytosis in Japan. Int J Hematol. 2007;86(1):58–65.
[17] Pei R, Wang Z, Wang Y, et al. A multicenter retrospective etiological analysis of 601 patients with hemophagocytic lymphohistiocytosis in China. Zhonghua Nei Ke Za Zhi. 2015;54:1018–1022.
[18] Brastianos PK, Swanson JW, Torbenson M, et al. Tuberculosis-associated haemophagocytic syndrome. Lancet Infect Dis. 2006;6(7):447–454.
[19] Nair V, Das S, Sharma A, et al. A clinicopathological analysis of 26 patients with infection-associated haemophagocytic lymphohistiocytosis and the importance of bone marrow phagocytosis for the early initiation of immunomodulatory treatment. Postgrad Med J. 2013;89(1050):185–192.
[20] Giri PP, Pal P, Ghosh A, Sinha R. Infection-associated haemophagocytic lymphohistiocytosis: a case series using steroids only protocol for management. Rheumatol Int. 2013;33(5):1363–1366.
[21] Madkaikar M, Shabrish S, Desai M. Current Updates on Classification, Diagnosis and Treatment of Hemophagocytic Lymphohistiocytosis (HLH). Indian J Pediatr. 2016;83(5):434–443.
[22] An Q, Fang DH, Xuan CM, et al. Lymphocyte subsets in children with hemophagocytic lymphohistiocytosis (HLH) and its clinical significance. Eur Rev Med Pharmacol Sci. 2018;22(7):2000–2004.
[23] An Q, Wang Y, Hu S, et al. Clinical significance of lymphocyte subset changes in hemophagocytic lymphohistiocytosis of children. Exp Ther Med. 2016; 12(6):3549–3552.
[24] Dalal BI, Vakil AP, Khare NS, et al. Abnormalities of the lymphocyte subsets and their immunophenotype, and their prognostic significance in adult patients with hemophagocytic lymphohistiocytosis. Ann Hematol. 2015;94(7):1111–1117.
[25] Shiota Y, Sato T, Ono T. Serum levels of soluble CD25 (soluble interleukin 2 receptor) in asthmatic patients. Arerugi. 1993;42:914–919.
[26] Filipovich AH. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. Hematol Am Soc Hematol Educ Program. 2009;2009(1):127–131.
[27] Jiang T, Ding X, Lu W. The prognostic significance of Beta2 microglobulin in patients with hemophagocytic lymphohistiocytosis. Dis Markers. 2016;2016:1–6.
[28] Mazodier K, Marin V, Novick D, et al. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood. 2005;106(10): 3483–3489.
[29] Henter JI, Andersson B, Elinder G, et al. Elevated circulating levels of interleukin-1 receptor antagonist but not IL-1 agonists in hemophagocytic lymphohistiocytosis. Med Pediatr Oncol. 1996;27(1):21–25.
[30] Albeituni S, Verbist KC, Tedrick PE, et al. Mechanisms of action of ruxolitinib in murine odels of hemophagocytic lymphohis. Blood. 2019;134:147–159.