Lymphoma associated hemophagocytic syndrome: A single-center retrospective study

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Abstract. To improve the understanding of lymphoma associated hemophagocytic syndrome (LAHS) and find an effective treatment for this fatal disease, 57 patients with LAHS were retrospectively reviewed. The most common histopathological type was extranodal natural killer (NK)/T-cell lymphoma, nasal type (ENKL) (45.61%). Patients with B-cell LAHS were significantly older (P<0.001), and exhibited a higher triglyceride level (P=0.012), lower serum ferritin level (P=0.014) and lower plasma Epstein-Barr virus DNA (P<0.001) compared with patients with T/NK-cell LAHS. The median survival time of all patients was 43 days, and patients with B-cell (n=14) and T/NK-cell (n=43) LAHS had a median survival time of 55 and 40 days, respectively (P=0.797). Compared with patients who were treated based on HLH-2004 protocols combined with multidrug chemotherapy, those without chemotherapy had a reduced prognosis (P=0.002). The patients that underwent hematopoietic stem cell transplantation (HSCT) following chemotherapy had a significantly improved overall survival (OS) compared with patients that did not undergo HSCT (P=0.001). Patients with B-cell LAHS treated with rituximab (P=0.015) and patients with ENKL treated with L-asparaginase/pegaspargase (L-asp/peg) (P=0.009) had an improved prognosis compared with patients not treated with these drugs. In the T/NK-cell LAHS group, patients treated with chemotherapy containing gemcitabine did not exhibit an improved OS compared with those not treated with gemcitabine (P=0.326). Furthermore, multivariate analysis demonstrated that long diagnosis time and poor performance status were independent prognosis factors for all patients with LAHS. The present study indicated that survival time does not differ between patients with B-cell LAHS and patients with T/NK-cell LAHS. Early diagnosis and appropriate immunochemotherapy plus HSCT are essential to achieve improved outcomes. The outcome of patients with B-cell LAHS may be significantly improved following treatment with rituximab. L-asp/peg-containing regimens are promising treatments for patients with NK/T-cell LAHS.

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Introduction

Hemophagocytic syndrome (HPS) or hemophagocytic lymphohistiocytosis (HLH), which was first reported as virus-associated HPS by Risdall *et al* (1), represents an uncontrolled immune response triggered by various stimuli. The excessive activation of lymphocytes and macrophages produces a high level of inflammatory cytokines, including interferon (IFN)-γ, interleukin (IL)-12, IL-18 and tumor necrosis factor (TNF)-α (2). The cytokine storm and infiltration of activated macrophages are responsible for features, including persistent fever, hepatosplenomegaly, pancytopenia and hemophagocytosis in bone marrow, liver and other organs (2-4). HPS is classified into primary and secondary forms. Primary HPS generally occurs in infants or young children with a clear genetic or familial inheritance (2). Due to a variety of underlying conditions, secondary HPS development may be triggered by infections, autoimmune disorders, malignancies and immunosuppression (3,5).

Lymphoma is the most common underlying condition of malignancy-associated HPS. HPS may occur as an initial presentation of lymphoma, as well as a complication at the relapsed or advanced stage of lymphoma (6). It was reported that the most common type of lymphoma-associated HPS (LAHS) was T/natural killer (NK)-cell lymphoma and there were fewer cases derived from B-cell lymphomas (7,8). Currently, LAHS is considered to be life-threatening. Few systematic reports are available on LAHS (9). The median survival time for patients

with NK/T-cell and other types of T-cell lymphoma is 28 and 33 days, respectively (6). Han *et al* (8) reviewed 29 patients with LAHS and determined that the median survival time was only 36 days. Furthermore, there are ongoing discussions in various aspects of LAHS, including specific indicators for early diagnosis, therapeutic regimens and hematopoietic stem cell transplantation (HSCT) (9-11). Therefore, in the present study, the clinical features, treatment and prognosis factors of 57 patients with LAHS were analyzed. To the best of our knowledge, the present study used the largest cohort of patients with LAHS. Furthermore, the differences between B-cell and T/NK-cell LAHS were discussed in order to improve the understanding of LAHS and attempt to find an appropriate treatment for this disease.

Patients and methods

Patient selection. A total of 57 patients diagnosed with LAHS (34 males and 23 females) were selected from the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China), who enrolled from December 2008 to March 2016. The median age was 36 years old with an age range of 4-76 years old. All patients underwent laboratory tests, including blood routine test, liver and kidney function, lactate dehydrogenase, β2-microglobulin, serum ferritin, coagulation function and polymerase chain reaction tests for Epstein-Barr virus (EBV)-DNA. Bone marrow smears and biopsies were assessed.

Diagnosis of lymphoma was confirmed according to the World Health Organization classification of hematopoietic and lymphoid tumors in 2008 (12). Diagnosis of HPS was based on the Histiocyte Society HLH-2004 pediatric diagnostic criteria (13). NK cell activity and soluble CD25 levels were not evaluated. The stage of lymphoma was evaluated by Ann Arbor staging system (14) through computed tomography (CT) scans or positron emission tomography (PET)/CT. Performance status was assessed based on the Eastern Cooperative Oncology Group (ECOG) scale (15). Immuno-chemotherapy was based on HLH-2004 protocols (13). Etoposide-based regimens were followed, including dexamethasone, cyclosporine A (CSA), intravenous immunoglobulins and intrathecal therapy. The treatment response was assessed according to Cheson *et al* (16).

Statistical analysis. The data was presented as the mean ± standard deviation. The sample normality detection was assessed using a Shapiro-Wilk test. Clinical and laboratory data of patients were assessed using Pearson's χ^2 test, Fisher's exact test, Kruskal-Wallis test, independent-samples Student's t-test or Mann Whitney U test. Overall survival (OS) time, measured as the period from diagnosis to mortality or the last follow-up, was estimated by the Kaplan-Meier method. Survival rates were compared by the log-rank test. Univariate analysis using Cox proportional hazards model was used to calculate hazard ratios of prognostic factors for patients with LAHS. Multivariate analysis using Cox proportional hazards model was used to identify the potential independent effects of those factors. $P<0.05$ was considered to indicate a statistically significant difference. The software package SPSS 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Results

Patient characteristics. Patient characteristics are summarized in Table I. There were 43 patients diagnosed with T/NK-cell lymphoma, accounting for 75.44% of all patients with LAHS. The most frequent histopathological type was extranodal natural killer/T-cell lymphoma, nasal type (ENKL) (45.61%). The other types included diffuse large B-cell lymphoma (24.56%), anaplastic large cell lymphoma (10.53%), peripheral T cell lymphoma, not otherwise specified (8.77%), progressive NK/T-cell leukemia (PNKTL) (5.26%), subcutaneous panniculitis-like T-cell lymphoma (3.51%) and hepatosplenic T-cell lymphoma (1.75%). The majority of patients (92.98%) were classified into Ann Arbor III-IV stage. Among all patients, 21 had a history of lymphoma (had been previously diagnosed with lymphoma) and were diagnosed with HPS at the advanced Ann Arbor stage of lymphoma. The most frequent symptom was fever (100%), followed by splenomegaly (92.89%), multicavity effusion (56.14%), hepatomegaly (43.86%), jaundice (31.58%) and edema (31.58%). A total of 56 patients exhibited thrombocytopenia (98.25%), 48 patients (84.21%) had a high level of aspartate aminotransferase, 47 patients (82.46%) had an elevated level of serum ferritin and 39 patients had hemophagocytosis in the bone marrow (68.42%).

The comparison of clinical features and laboratory data between patients with B-cell and T/NK-cell LAHS are listed in Tables II and III. Compared with patients with T/NK-cell LAHS, patients with B-cell LAHS were older ($P<0.001$), had a higher level of triglycerides ($P=0.012$), and a lower level of serum ferritin ($P=0.014$) and the number of copies of EBV DNA ($P<0.001$). The differences in the remaining features were not statistically significant.

Treatment and survival. Following a median follow-up of 33 days (range, 5-1,133 days), 52/57 patients (91.23%) had succumbed. The median survival time of all patients was 43 days (range, 5-1,133 days). Survival curves are depicted in Fig. 1. The median survival time of patients with B-cell LAHS and T/NK-cell LAHS was 55 (range, 11-1,133 days) days and 40 days (range, 5-809 days), respectively ($P=0.797$). The 0.5, 1 and 2-year OS rates for patients with B-cell LAHS were 16.0, 16.0 and 8.0%, respectively. The rates for OS for patients with T/NK-cell LAHS were 26.0, 17.0 and 13.0%, respectively. Compared with 41 patients who were treated with the HLH-2004 regimen combined with multidrug chemotherapy (median survival time, 55 days), those who only received the HLH-2004 regimen (and did not receive chemotherapy for lymphoma) had a significantly reduced prognosis (median survival time, 25 days) ($P=0.002$; Fig. 2A). Of the 57 patients, five underwent autologous or allogeneic HSCT following chemotherapy and had a significantly improved OS (median survival time, 1,110 days) compared with the 52 remaining patients without HSCT (median survival time, 36 days) ($P=0.001$; Fig. 2B).

Of 14 patients with B-cell LAHS, five were treated with CSA or dexamethasone and four patients faced rapid disease progression in a short time. A total of 3 patients with B-cell LAHS received chemotherapy plus rituximab, and two patients exhibited complete remission (CR). The survival time of the 3 patients who received chemotherapy plus rituximab

Table I. Characteristics of all patients with lymphoma associated hemophagocytic syndrome.

Characteristics	Patients, n (%)
Sex (male)	
Male	34 (59.65)
Female	23 (40.35)
B-cell lymphoma	14 (24.56)
DLBCL	14 (24.56)
T/NK/-cell lymphoma	43 (75.44)
ENKL	26 (45.61)
PNKL	3 (5.26)
PTCL, NOS	5 (8.77)
ALCL	6 (10.53)
SPTL	2 (3.51)
HSTL	1 (1.75)
Ann Arbor Stage I-II	4 (7.02)
Ann Arbor Stage III-IV	53 (92.98)
Previous lymphoma history	21 (36.84)
Symptoms and signs	
Fever	57 (100)
Splenomegaly	53 (92.89)
Hepatomegaly	25 (43.86)
Multicavity effusion	32 (56.14)
Jaundice	18 (31.58)
Edema	18 (31.58)
Laboratory data	
ANC <1.5x10^9/l	41 (71.93)
Hb <90 g/l	36 (63.16)
PLT <100x10^9/l	56 (98.25)
ALT >40 U/l	46 (80.70)
AST >40 U/l	48 (84.21)
ALB <30 g/l	38 (66.67)
TBIL >25 μmol/l	23 (40.35)
LDH >500 U/l	40 (70.18)
FIB <1.5 g/l	40 (70.18)
TG >3.0 mmol/l	22 (38.60)
Ferritin >1,000 μg/l	47 (82.46)
EBV DNA copies >10^2	39 (68.42)
BM hemophagocytosis	39 (68.42)

DLBCL, diffuse large B cell lymphoma; NK, natural killer; ENKL, extranodal NK/T cell lymphoma, nasal type; PNKTL, progressive NK/T-cell leukemia; PTCL, NOS peripheral T-cell lymphoma, not otherwise specified; ALCL, systemic anaplastic large cell lymphoma; SPTL, subcutaneous panniculitis like T-cell lymphoma; HSTL, hepatosplenic T-cell lymphoma; ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; LDH, lactate dehydrogenase; FIB, fibrinogen; TG, triglyceride; EBV, Epstein-Barr virus; BM, bone marrow.

(median, 645 days) was significantly longer compared with 6 patients who did not receive rituximab (median, 53 days) (P=0.015; Fig. 3A). For patients with T/NK-cell LAHS, 17 patients underwent chemotherapy with gemcitabine, and they did not exhibit a significantly improved OS (median, 56 days), compared with 15 patients not treated with gemcitabine (median, 27 days) (P=0.326; Fig. 3B). For patients with ENKL, 13 patients received chemotherapy regimens with L-asparaginase (L-asp) or pegaspargase (peg), and they had an improved prognosis (median survival time, 56 days) compared with 7 patients not treated with these drugs (median survival time, 20 days) (P=0.009; Fig. 3C).

Univariate and multivariate analysis for prognostic factors. The results of the univariate analysis of patients with LAHS are presented in Table IV. Among the patients with B-cell LAHS, it was determined that OS was significantly associated with serum ferritin level (P=0.036). However, the following factors predicted poor OS for patients with T/NK-cell LAHS and all patients with LAHS: Long diagnosis time (P<0.001 for both); high ECOG scores (P=0.024 and P=0.005, respectively); low hemoglobin (P=0.023 and P=0.005, respectively); and high EBV DNA copies (P=0.011 and P=0.004, respectively). Furthermore, low level of fibrinogen was also a negative prognostic factor (P=0.036) for all patients with LAHS. Other baseline characteristics were not significantly associated with prognosis.

As presented in Table V, multivariate analysis was performed using the Cox proportional hazards model to assess the potential independent prognostic factors. Results demonstrated that diagnosis time (P=0.021) and ECOG scores (P=0.022) were independent predictors of all patients with LAHS. Furthermore, diagnosis time (P=0.003) was an independent predictor of patients with T/NK-cell LAHS. The median survival time of patients with long diagnosis time (>20 days) and high ECOG score (3-5 scores) was 25.3 and 30.9 days, respectively. However, the median survival time of patients with a short diagnostic time (≤20 days) and low ECOG score (0-2 scores) was 85.0 and 383.8 days, respectively. For patients with T/NK-cell, the median survival time of patients with long and short diagnosis time were 23.7 and 213.1 days, respectively.

Discussion

To the best of our knowledge, this is one of the largest cohort of patients with LAHS in a study. The present study demonstrated that LAHS, a subtype of secondary HPS, has specific clinical features, prognostic factors and outcomes. A total of 57 patients with LAHS were retrospectively reviewed in the present study. A total of 43 patients were diagnosed with T/NK-cell LAHS. Patients with ENKL and PNKTL accounted for half of all patients with LAHS. Although a number of reports stated that there were an increasing number of B-LAHS cases (17,18), the majority of reported cases of LAHS remained as T/NK-cell lymphoma (8,9,19), which was consistent with the present study.

In the present study, patients with B-cell LAHS and T/NK-cell LAHS shared similar clinical features and laboratory data; however, patients with B-cell LAHS were older, which was similar to the previous studies by Han *et al* (8) and Sano *et al* (9). Patients with T/NK-cell LAHS also presented a higher level of serum ferritin compared with patients with

Table II. Clinical features of patients with B-cell LAHS and T/NK-cell LAHS.

Patients' Characteristics	B-cell lymphoma (n=14)	T/NK-cell lymphoma (n=43)	P-value
Sex			0.397[a]
Male	7	27	
Female	7	16	
Age (years); mean ± SE	51.1±4.0	33.1±2.2	<0.001[c]
IPI score			0.720[d]
0-1	0	2	
2-3	8	19	
4-5	6	22	
ECOG			0.822[a]
0-2	4	9	
3-5	10	34	
Splenomegaly			1.000[b]
Yes	13	41	
No	1	2	
Hepatomegaly			0.479[a]
Yes	5	20	
No	9	23	
Multicavity effusion			0.931[a]
Yes	8	24	
No	6	19	
Jaundice			0.958[a]
Yes	5	13	
No	9	30	
Edema			0.958[a]
Yes	5	13	
No	9	30	
Previous lymphoma history			0.169[a]
Yes	3	18	
No	11	25	
Diagnosis time (days); median (range)	22.5 (6.0-42.0)	20.0 (5.0-90.0)	0.993[e]

LAHS, lymphoma associated hemophagocytic syndrome; SE, standard error; IPI, International Prognostic Index; ECOG, Eastern Cooperative Oncology Group scale. [a]P-value for Pearson's χ^2 test, [b]P-value for Fisher's exact test, [c]P-value for independent-samples Student's t-test (Age observes the normal distribution by means of Shapiro-Wilk test), [d]P-value for Kruskal-Wallis test, [e]P-value for Mann Whitney U test.

B-cell LAHS. In a study conducted by Yu *et al* (18), serum ferritin level was significantly higher in patients with T-cell LAHS, which was consistent with the present result. They also considered that a higher ferritin level may be associated with reduced survival outcome in patients with T-cell LAHS, as hyperferritinemia may indicate elevated cytokine activation and result in activating hepatic proinflammatory mediators through the nuclear factor-κB signaling pathway (18). Allen *et al* (20) reported that a ferritin level >10,000 μg/l was 90% sensitive and 96% specific for HPS; however, a high level of ferritin alone is just indicative, and the diagnosis of HPS may not be confirmed.

In the present study, plasma EBV DNA was determined to be higher in patients with T/NK-cell LAHS compared with patients with B-cell LAHS, whereas Yu *et al* (18) indicated that there was no significant difference between patients with T-cell and B-cell lymphoma in the presence of an EBV infection. Opposing results may be due to numerous reasons. Firstly, the constituent ratio of the underlying disease was different. In the present study a total of 2/3 of the T/NK-cell lymphoma accounted for ENKL, whereas ENKL accounted for <6% according to Yu *et al* (18). Furthermore, in the study by Yu *et al*, patients were only checked if they were EBV-positive, whereas in the present study overall plasma EBV DNA copies were detected. EBV serves an important role in T/NK-cell lymphoma as well as EBV-associated HPS, and it may cause oncogenesis or occur in tumor-associated lymphocytes as a function of immune dysregulation (21). A number of previous studies have confirmed that plasma EBV-DNA was a prognostic marker for ENKL (22,23). EBV-infected T cells selectively upregulate TNF-α expression, which may activate macrophages in combination with IFN-γ and other

Table III. Laboratory data of patients with B-cell LAHS and T/NK-cell LAHS.

Features median (range)	B-cell lymphoma (n=14)	T/NK-cell lymphoma (n=43)	P-value
ANC ($x10^9$/l)	1.3 (0.0-3.0)	0.8 (0.0-5.3)	0.082[a]
Hb (g/l); mean ± SE	84.5±6.1	83.4±3.0	0.858[b]
PLT ($x10^9$/l)	26.5 (7.0-96.0)	22.0 (2.0-129.0)	0.475[a]
Liver function			
ALT (U/l)	81.5 (12.0-755.0)	108.0 (5.0-578.0)	0.404[a]
AST (U/l)	140.0 (15.0-1227.0)	160.0 (6.0-1294.0)	0.904[a]
ALB (g/l); mean ± SE	26.5±1.1	28.9±0.8	0.128[b]
TBIL (μmol/l)	21.1 (5.7-356.3)	18.0 (6.7-500.5)	0.753[a]
IBIL (μmol/l)	8.5 (2.5-106.5)	7.6 (0.7-108.0)	0.948[a]
DBIL (μmol/l)	12.3 (2.3-294.2)	11.4 (3.1-451.5)	0.867[a]
Coagulation function			
FIB (g/l)	1.9 (0.3-4.8)	1.1 (0.5-3.9)	0.093[a]
LDH (U/l)	942.5 (227.0-2597.0)	854.0 (131.0-14851.0)	0.838[a]
β2-MG (mg/l); mean ± SE	7.5±1.0	6.6±0.6	0.467[b]
TG (mmol/l)	3.7 (0.9-11.7)	2.6 (0.8-8.2)	0.012[a]
Ferritin (μg/l)	1335.7 (316.0-2129.0)	2000.0 (760.9-11816.0)	0.014[a]
BM hemophagocytosis			
Yes	10	29	1.000[c]
No	4	14	
EBV DNA copies			
$>10^2$	3	33	<0.001[c]
$<10^2$	11	10	

LAHS, lymphoma associated hemophagocytic syndrome; SE, standard error; ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; IBIL, indirect bilirubin; DBIL, direct bilirubin; FIB, fibrinogen; LDH, lactate dehydrogenase; β2-MG, β2-microglobulin; TG, triglyceride; BM, bone marrow; EBV, Epstein-Barr virus. [a]P-value for Mann Whitney U test (median), [b]P-value for independent-samples Student's t-test, [c]P-value for Pearson's χ^2 test.

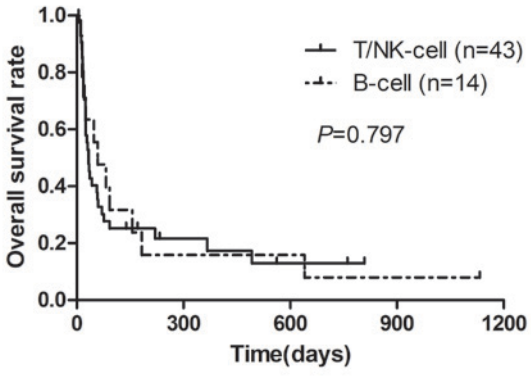

Figure 1. Kaplan-Meier survival analysis of patients with B-cell and T/NK-cell LAHS. The median survival time of B-cell LAHS was 55 days, 0.5, 1 and 2-year OS rates were 16.0, 16.0 and 8.0%, respectively. The median survival time of T/NK-cell LAHS was 40 days, and 0.5, 1 and 2-year OS rates were 26.0, 17.0 and 13.0%, respectively. NK, natural killer; LAHS, lymphoma associated hemophagocytic syndrome; OS, overall survival.

cytokines (24,25). The elevated levels of cytokines secreted by EBV-infected cells cause a series of clinical manifestations (25); however, Ohno *et al* (24) demonstrated that EBV involvement was not detected in patients with B-cell LAHS, which indicated that EBV infection was not involved in the onset of B-cell LAHS. Instead, numerous reactive CD3+ T cells were detected in the bone marrow of all patients with B-cell LAHS, and these reactive T cells were functionally activated, thus indicating that they may be responsible for cytokine production in B-cell LAHS (24).

In the present study, the OS of patients with LAHS was poor. The median survival time was 43 days. Previous studies also reported an inferior OS of patients with LAHS. Barba *et al* (26) demonstrated that lymphoma was one of the factors associated with increased mortality in patients with HPS. Tong *et al* (19) conducted a study of 28 patients with aggressive T-cell LAHS and indicated that the median survival time of the patients was 40 days. However, Yu *et al* (18) reviewed 30 patients with LAHS and reported that the median survival time was 231 days. They indicated that the improved survival may be due to rituximab treatment and HSCT in patients with B-cell LAHS and T-cell LAHS, respectively.

In the present study, although patients with B-cell LAHS exhibited a longer survival time compared with patients with T/NK-cell LAHS, this difference was not statistically significant (P=0.797). These results were confirmed by Yu *et al* (18). However, a number of studies indicated that B-cell lymphoma was associated with a better prognosis compared with T/NK-cell lymphoma (9,27). The potential reasons for the

Table IV. Prognostic factors by univariate analysis.

	B-cell lymphoma			T/NK-cell lymphoma			All patients		
Risk factor	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Sex (Male/Female)	3.822	0.972-15.030	0.055	0.524	0.260-1.054	0.070	0.844	0.468-1.524	0.574
Lymphoma history (No/Yes)	0.313	0.069-1.416	0.131	1.578	0.774-3.217	0.210	1.174	0.639-2.154	0.605
Diagnosis time (>20 days/≤20 days)	1.208	0.385-3.793	0.746	5.594	2.392-13.082	0.000	3.198	1.732-5.906	0.000
ECOG (3-5/0-2)	5.929	0.728-48.299	0.096	2.914	1.151-7.375	0.024	3.258	1.437-7.389	0.005
IPI score (4-5/0-3)	1.334	0.403-4.418	0.637	1.049	.0529-2.082	0.890	1.109	0.616-1.997	0.731
Multicavity effusion (Yes/No)	3.311	0.829-13.222	0.090	1.583	0.780-3.212	0.203	1.715	0.939-3.132	0.079
ANC (≤1.0x10^9/l/>1.0x10^9/l)	0.557	0.164-1.894	0.348	0.640	0.300-1.365	0.249	0.964	0.496-1.874	0.914
Hb (≤90 g/l/>90 g/l)	3.668	0.741-18.155	0.111	2.566	1.141-5.767	0.023	2.745	1.359-5.545	0.005
PLT (≤25x10^9/l/>25x10^9/l)	1.85	0.531-6.449	0.199	1.625	0.775-3.406	0.199	1.714	0.919-3.194	0.090
ALT (>80 U/l/≤80 U/l)	0.818	0.258-2.596	0.733	0.906	0.442-1.859	0.788	0.925	0.508-1.685	0.798
AST (>80 U/l/≤80 U/l)	1.069	0.318-3.590	0.914	0.701	0.341-1.441	0.334	0.799	0.433-1.476	0.474
TBIL (>25 μmol/l/≤25 μmol/l)	1.385	0.550-2.196	0.790	1.385	0.444-4.325	0.575	1.152	0.637-2.082	0.640
DBIL (>10 μmol/l/≤10 μmol/l)	1.797	0.479-6.736	0.385	1.377	0.686-2.764	0.368	1.499	0.817-2.752	0.192
IBIL (>14 μmol/l/≤14 μmol/l)	2.601	0.615-11.010	0.194	0.733	0.298-1.803	0.499	0.898	0.429-1.878	0.775
β2-MG (>5 mg/l/≤5 mg/l)	1.578	0.412-6.038	0.505	0.916	0.451-1.862	0.809	1.090	0.589-2.019	0.784
FIB (<1.5 g/l/≥1.5 g/l)	1.951	0.559-6.817	0.295	2.830	0.985-8.134	0.053	2.090	1.051-4.157	0.036
TG (≥3 mmol/l/<3 mmol/l)	2.609	0.566-12.019	0.219	1.559	0.742-3.276	0.241	1.610	0.890-2.911	0.115
Ferritin (≥2,000 μg/l/<2,000 μg/l)	3.911	1.092-14.010	0.036	0.637	0.319-1.269	0.199	0.966	0.536-1.741	0.908
EBV DNA (≥10^5/<10^5)	6.161	0.558-67.968	0.138	2.834	1.274-6.304	0.011	2.508	1.336-4.706	0.004
BM hemophagocytosis (Yes/No)	0.648	0.182-2.312	0.504	0.608	0.291-1.271	0.186	0.625	0.332-1.177	0.145

ECOG, Eastern Cooperative Oncology Group scale; IPI, International Prognostic Index; ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; β2-MG, β2-microglobulin; FIB, fibrinogen; TG, triglyceride; EBV, Epstein-Barr virus; BM, bone marrow; CI, confidence interval; NK, natural killer.

Table V. Prognostic factors by multivariate analysis.

	T/NK-cell lymphoma			All patients		
Risk Factor	Hazard ratio	95%CI	P-value	Hazard ratio	95%CI	P-value
Diagnosis time (>20 days/≤20 days)	3.901	1.586-9.597	0.003	2.182	1.123-4.230	0.021
ECOG (3-5/0-2)	2.318	0.867-6.541	0.092	2.814	1.164-6.803	0.022
Hb (≤90 g/l/>90 g/l)	1.826	0.761-4.382	0.177	1.950	0.915-4.157	0.084
FIB (<1.5 g/l/≥1.5 g/l)	2.747	0.902-8.369	0.075	1.448	0.700-2.994	0.318
EBV DNA ($\geq 10^5/<10^5$)	1.525	0.619-3.756	0.359	1.834	0.947-3.551	0.072

ECOG, Eastern Cooperative Oncology Group scale; Hb, hemoglobin; FIB, fibrinogen; EBV, Epstein-Barr virus; NK, natural killer; CI, confidence interval.

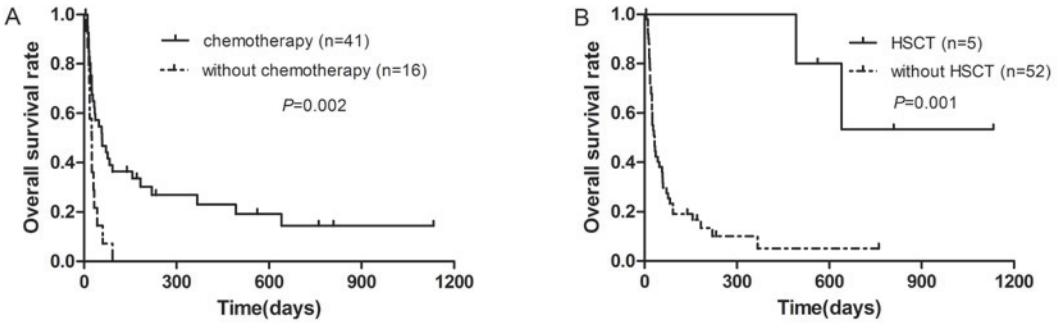

Figure 2. (A) Kaplan-Meier survival analysis of patients treated based on HLH-2004 protocol and chemotherapy vs. patients not treated with chemotherapy (median survival time, 55 vs. 25 days). (B) Patients treated with HSCT vs. patients not treated with HSCT (median survival time, 1,110 vs. 36 days). HSCT, hematopoietic stem cell transplantation.

inconsistencies between the results of these previous studies and the present study was that there were five patients with B-cell LAHS who only received CSA or dexamethasone in the present study.

The median diagnosis time in the present study was 22 days. Univariate and multivariate analysis identified that a long diagnosis time was a poor prognostic factor for patients with LAHS. Numerous factors may influence the diagnosis. For patients with lymphoma suspected of having HPS, misdiagnosis often occurs as fever and pancytopenia may also be caused by severe infection or myelosuppression following chemotherapy. For patients without lymphoma, once the diagnosis of HPS was established, the underlying diseases were difficult to identify, due to a number of patients being too weak to receive biopsies.

Numerous attempts have been made for an early diagnosis of LAHS. It is reported that PET-CT may act as a significant tool to assess patients with LAHS, as it is highly sensitive in detecting neoplasms of the majority of histologic subtypes of lymphoma, and also demonstrates extensive 18-fluorodeoxyglucose (FDG) uptake in tumor tissues (28). It was also reported that the maximum standardized uptake values of patients with malignancy-associated HPS, particularly lymphoma, was statistically higher compared with those with an infection or rheumatosis-associated HPS. Therefore, PET-CT may serve an important role in differential diagnosis of secondary HPS (29). Furthermore, FDG uptake may reflect the level of cytokine storm to a certain extent and be a prognostic factor for patients with LAHS (30). Tabata *et al* (10) reviewed 57 LAHS cases and 53 benign disease-associated HPS cases, and indicated that the serum soluble IL-2 receptor (sIL-2R) level and the sIL-2R/ferritin ratio may act as useful markers for distinguishing underlying lymphoma from other causes in patients with HPS. Maruoka *et al* (31) identified that IFN-inducible protein 10 (IP-10) and monokine induced by IFN-γ (MIG) were useful markers for early diagnosis of LAHS. The sensitivity and specificity for the diagnosis were 100 and 95%, respectively. The serum level of IP-10 and MIG in T or NK/T-cell LAHS were higher compared with those in B-cell LAHS.

HLH-1994 (32) or HLH-2004 protocols are validated treatments for primary HPS. However, the efficacy of these treatment protocols for LAHS is poorly understood. It is generally considered that the most important treatments for LAHS are combined chemotherapy regimens that target malignancy lymphomas (6,18). In the present study, patients who were treated according to the HLH-2004 protocol and with multidrug chemotherapy exhibited improved outcomes compared with those who did not receive chemotherapy, which demonstrates that it is equally important to treat primary diseases as well as treating HPS. Furthermore, the survival time of three patients with B-cell LAHS who received regimens containing rituximab was significantly

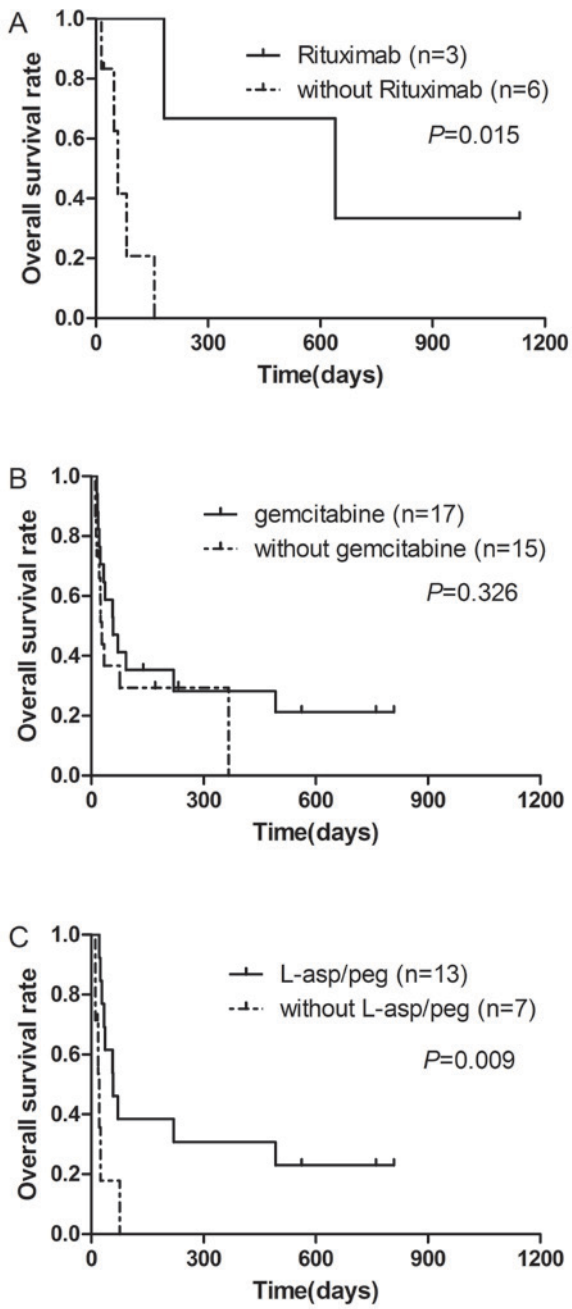

Figure 3. (A) Kaplan-Meier survival analysis of patients with B-cell LAHS treated with chemotherapy plus rituximab vs. patients not treated with rituximab (median survival time, 645 vs. 53 days). (B) Patients with T/NK-cell LAHS treated with gemcitabine-based chemotherapy vs. chemotherapy without gemcitabine (median survival time, 56 vs. 27 days). (C) Patients with NK/T-cell LAHS treated with chemotherapy containing L-asp/peg vs. chemotherapy without L-asp/peg (median survival time: 56 vs. 20 days). LAHS, lymphoma associated hemophagocytic syndrome; L-asp/peg, L-asparaginase/pegaspargase; NK, natural killer.

longer compared with those who did not receive rituximab. This survival benefit may be due to introducing rituximab. To date, gemcitabine-based combination chemotherapy has been demonstrated to be highly successful in improving the treatment outcome of T/NK-cell lymphoma (33-35). However, in the present study patients with T/NK-cell LAHS who were treated with gemcitabine did not exhibit survival advantage. L-asp/peg-based regimens were analyzed in the ENKL group and led to an improved prognosis. L-asp/peg-containing regimens have been indicated to be highly effective for patients with NK/T-cell lymphoma (35). The prior studies also demonstrated that the significant efficacy and safety profile of the peg-based regimen in the treatment of newly diagnosed and relapsed/refractory ENKL (35-37). The anticancer effect of L-asp is not affected by multidrug resistance gene due to its unique mechanism (37). Since ENKL cells cannot synthesize asparagine themselves, tumor cell proliferation is suppressed under the effect of hydrolyzing asparagine by L-asp (38).

It was reported that patients with primary HPS may achieve long-term survival following a treatment regime of immunochemotherapy combined with HSCT (11). A large prospective study of 249 patients with familial, refractory or recurrent HPS indicated that the 5-year survival rate of 124 patients who underwent HSCT was 66±8% (39). A study comparing reduced intensity conditioning (RIC) regimens with myeloablative conditioning (MAC) regimens demonstrated that the overall 3-year survival following HSCT was 43% for patients with MAC and 92% for patients with RIC (40). However, HSCT has rarely been reported as a treatment of LAHS, and the efficacy remains unknown. It was reported that a number of patients with LAHS achieved CR and obtained long-term survival from allogeneic or autologous HSCT (8,18,41,42). The present study indicated that HSCT may improve the outcome of patients with LAHS. However, further research is required to investigate the role of HSCT in LAHS treatment.

The present study has several limitations. The study was retrospectively conducted from a single center, and the number of patients involved was relatively small. Additionally, NK cell activity and soluble CD25 levels were not analyzed.

In conclusion, LAHS is relatively common but has the worst prognosis of secondary HPS types, and it poses a challenge to clinicians (43). The results of the present study demonstrated that the survival time did not differ between patients with B-cell and T/NK-cell LAHS. Early diagnosis and immunochemotherapy plus HSCT may lead to better outcomes. Treatment of the underlying lymphoma in patients with LAHS ought to be treated at the same time as the implementation of countermeasures for the suppression of the extreme inflammation triggered by HPS. The outcome of patients with B-cell LAHS may be significantly improved following treatment with rituximab. L-asp/peg-containing regimens are promising treatments if NK/T-cell lymphomas are recognized as an underlying disease. Prospective multicenter studies with larger sample sizes are required for an optimal treatment for LAHS.

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Availability of data and materials

All data analyzed during this study are included in this published article.

Authors' contributions

YC, GS and MZ designed the study, interpreted the results and wrote the manuscript. MC, XF, LH and LZ performed the data analysis and statistical analysis. LL, XL, ZS, JW, XZ, ZL, FN and JY performed literature research and the clinical data acquisition.

Ethics approval and consent to participate

This study was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent for the collection of medical information was obtained from all patients.

Consent for publication

Informed consent for the collection and publication of medical information was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

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