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Rituximab-containing immuno-chemotherapy regimens are effective for the elimination of EBV for EBV-HLH with only and mainly B lymphocytes of EBV infection

Guang-Qiang Meng, Jing-Shi Wang, Yi-Ni Wang, Na Wei, Zhao Wang *

Department of Hematology, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

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ABSTRACT

Patients with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) have a poor prognosis. This study investigated the efficacy of rituximab-containing immuno-chemotherapy regimens for EBV-HLH. In this study, 15 patients were treated with rituximab-containing regimens. The treatment efficacy and adverse events were evaluated. In 10 patients, EBV DNA became negative after the first course of treatment. The lymphocyte types infected by EBV in the 10 patients were only infected with B cells and mainly infected with B cells. In the other 5 patients, the EBV DNA of peripheral blood mononuclear cells (PBMC) before and after treatment with the regimens had no statistical difference ($P = 0.111$). In addition, in these 5 patients, EBV mainly infected T and NK cells. Among the 5 patients without a significant decline in EBV DNA of PBMC, 2 patients received allogeneic hematopoietic stem cell transplantation and turned negative for EBV DNA. This study suggests that rituximab-containing regimens are effective therapy for EBV-HLH with only and mainly B lymphocytes infected by EBV, especially for eliminating EBV.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder characterized by uncontrolled activation of the immune system and excessive release of inflammatory cytokines. The clinical features are fever, hepatosplenomegaly, pancytopenia, coagulopathy, hypertriglyceridemia, and hemophagocytic phenomena. HLH is divided into primary and secondary HLH. The primary HLH is a dominant inherited disorder and has genetic defects. Secondary HLH is most commonly caused by infection, malignancy, and autoimmune disorders [1,2]. Epstein-Barr virus (EBV) infection-related HLH (EBV-HLH) is the most common type of infection-related HLH [3].

Presently, the first-line treatment of EBV-related HLH is mainly based on the HLH-94 program and then followed by allogeneic hematopoietic stem cell transplantation (allo-HSCT) [4,5]. However, patients with EBV-HLH still have very poor survival. For example, a previous study found that approximately 30% of patients with EBV-HLH did not respond to this treatment regimen [4]. Therefore, new treatment options have been explored, including the B cell-targeting monoclonal antibody rituximab. Based on its effective treatment of EBV-related B-lymphocyte post-transplant lymphoproliferative disease (PTLD) [6,7], rituximab has been applied for the treatment of EBV-HLH. Some studies had found that rituximab-containing regimens could improve the clinical status in most patients with EBV-induced HLH and reduce EBV load and diminish inflammation [8,9].

For patients with EBV-HLH in Asia, EBV mainly infected T and NK cells. Previous studies of rituximab-containing EBV-HLH did not analyze the different lymphocyte subgroups infected by EBV. Rituximab is a targeted drug for B cells. Does the rituximab-containing regimen have therapeutic effects on EBV-infected T and NK lymphocytes? In this study, EBV-infected lymphocyte types were analyzed. It was found that rituximab was mainly effective for patients with EBV-HLH who were only infected and those who were mainly infected with B cells by EBV.

2. Materials and methods

2.1. Patients

A total of 265 EBV-HLH patients were admitted to our center between June 2017 and December 2019, of which 229 were tested for

* Corresponding author.
E-mail addresses: menggq2001@163.com (G.-Q. Meng), wangjingshi987@126.com (J.-S. Wang), catenny@hotmail.com (Y.-N. Wang), applewei80@live.cn (N. Wei), wangzhao@ccmu.edu.cn (Z. Wang).

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EBV-infected lymphocyte subgroups, and 36 were not tested for EBV-infected lymphocyte subgroups. Among them, 15 patients with EBV-HLH received rituximab-containing chemo-immunotherapeutic regimens. We retrospectively evaluated the 15 patients with EBV-HLH who received rituximab-containing chemo-immunotherapeutic regimens. The eligibility criteria for including patients in this study were as follows: (1) patients met the HLH-04 diagnostic criteria [10]; (2) high values for EBV-DNA copies or high numbers of cells containing EBV-encoded small RNA (EBER) were detected in the peripheral blood or tissues [11]. Patients with primary HLH and lymphoma-associated HLH with EBV infection were excluded. Patients with primary HLH-related genes tested positive by whole-exome sequencing were excluded, as were patients with lymphoma confirmed by biopsy pathology.

2.2. Data collection and outcomes

Data were collected on the following: patients' general conditions, including age and sex, and body temperature at diagnosis. Laboratory examinations included blood routine examination, liver function, triglyceride levels, fibrinogen levels, serum ferritin levels, LDH levels, β2-MG levels, NK cell activity, soluble CD25 level, liver and spleen size, hemophagocytosis, peripheral blood and serum EBV DNA, EBER of tissues, EBV-infected lymphocyte subgroups, and survival time and prognosis.

Positive EBV in serum and peripheral blood mononuclear cell (PBMC) was defined as EBV-DNA copy number ≥500 copies/ml. The detection method of EBV-infected lymphocyte subgroups was as follows: After taking peripheral blood from patients to isolate mononuclear cells (1 million cells), CD4+ T, CD8+ T, CD19+ B, and CD56+ NK cells were sorted out with magnetic beads to verify the purity and extract each subpopulation DNA, using real-time quantitative PCR instrument to detect the EBV-DNA content of each lymphocyte subgroups. Among the 229 patients who underwent EBV-infected lymphocyte subgroup analysis, 13 patients (5.7%) were categorized into only EBV-infected B lymphocyte subgroups.

All 15 patients received the treatment with rituximab-containing regimens. The dosages of rituximab were as follows: 375 mg/m^2, 300 mg/each time, and 100 mg/each time, and the applied treatment course was 1–4 courses. Seven patients received the first course of rituximab as induction therapy, and 8 patients received salvage therapy for recurrence. Three patients (20%) received rituximab weekly, while 9 patients (60%) received it every two weeks. Three patients (20%) received a single dose therapy, 4 patients (26.7%) received two doses, 4 patients (26.7%) received three doses, and 4 patients (26.7%) received four doses.

All 15 patients received combination therapy with etoposide and other drugs. Two patients (13.3%) received R-EP (VP16 and methylprednisolone/dexamethasone) regimen, one of whom also received ruxolitinib. Eleven patients (73.3%) received R-DEP (liposomal doxorubicin, VP16, and methylprednisolone) regimen; one of them also received ruxolitinib and another also received PEG-aspargase. One patient (6.7%) received the regimen of R combination with HLH-94. One patient (6.7%) received the regimen of R-ECOP (VP16, cyclophosphamide, vincristine, and dexamethasone). During the treatment with rituximab, 2 patients (13.3%) received anti-viral medications and 2 (13.3%) received intravenous immunoglobulin, while 2 patients (13.3%) underwent allogeneic HSCT.

The efficacy of rituximab-containing regimens for the treatment of EBV-HLH was assessed according to the revised efficacy criteria proposed by United States HLH collaborative group [12]. According to the standards, treatment results of HLH, including complete response (CR), partial response (PR), and no response (NR), were evaluated. EBV negative was defined when DNA copies in PBMC and plasma were both <500 copies/ml. Adverse effects and complications were monitored during the treatment, including infusion-related toxicities of rituximab, infection, abnormal liver function, cytopenia, and cardiac dysfunction.

2.3. Data analysis

SPSS 20.0 software (SPSS, Chicago, IL) was used for statistical analysis. Age, clinical manifestations, relevant laboratory tests, and survival of the patients were statistically analyzed. In this study, the patients' age and relevant laboratory tests were normally distributed data, which were analyzed using parametric tests and are presented as mean ± SD. The data without a normal distribution are presented as median and range. The *t*-test was used to compare the data of two groups with a normal distribution. $P < 0.05$ was considered to be of statistical importance.

3. Results

3.1. General patient characteristics and laboratory findings

Fifteen patients with EBV-HLH had received the treatment with rituximab-containing regimens. Eight cases (53%) were male, while 7 cases (47%) were female (male-to-female ratio of 1.14:1). The median age of the patients was 37 years, and the age ranged from 1 to 68 years (Table 1), 11 of them (73.3%) being ≥18 years.

The results showed that the incidence rates at the time of diagnosis were 100% in fever, 93.3% in hepatosplenomegaly, 75% in hemophagocytosis, 73.3% in cytopenia for at least two cell lines, and 75% in NK cell function depressed. Other laboratory findings are presented in Table 1.

The EBV DNA in the PBMC of the 13 patients was >5.0E+02 copies/ml and ranged from 5.8E+02 to 1.00E+07 copies/ml (median of 1.70E+04 copies/ml). A total of 12 patients were tested for plasma EBV DNA, and 7 patients had EBVDNA load > 5.0E+02 copies/ml, with a mean of 6.53E+04 ± 1.56E+05 copies/ml.

The 15 patients tested positive for EBV-infected lymphocyte subgroups. Three patients had EBV infection of both T (CD4+ and/or CD8+) and B (CD19+) lymphocyte subgroups, and 1 case was mainly infected with B lymphocytes (EBV infected CD19+ cells were more than CD4+ and/or CD8+ cells). Two patients had EBV infection of both B (CD19+) and NK (CD56+) lymphocyte subgroups, and 1 case was

Table 1
Baseline characteristics of 15 patients with EBV-HLH.

Characteristics	N(%) / mean ± SD
Gender	
Male	8 (53%)
Female	7 (47%)
Age (years), mean ± SD	32.60 ± 23.42
Fever	15 (100%)
Hepatosplenomegaly	14 (93.3%)
Hemophagocytosis in bone marrow	12 (75%)
Cytopenia for at least two cell lines	11 (73.3%)
Laboratory data, mean ± SD	
ANC (×10^9/L) (1.8–6.3 × 10^9/L)	1.51 ± 1.98
Hb (g/L) (130–175 g/L)	88.20 ± 20.19
PLT (×10^9/L) (125–350 × 10^9/L)	92.10 ± 97.83
ALT (U/L) (9–50 U/L)	145.53 ± 100.12
AST (U/L) (15–40 U/L)	231.15 ± 197.83
ALB (g/L) (40–55 g/L)	33.83 ± 4.30
TBIL (μmol/L) (3.42–17.10 μmol/L)	29.59 ± 31.10
LDH (U/L) (120–250 U/L)	789.27 ± 591.03
β2-MG (mg/L) (1.09–2.53 mg/L)	3.62 ± 1.48
FIB (g/L) (2–4 g/L)	2.03 ± 1.08
TG (mmol/L) (0.57–1.70 mmol/L)	2.60 ± 1.20
Ferritin (μg/L) (24.00–336.00 μg/L)	64437.90 ± 227220.35
sCD25 (pg/ml) (<6400 pg/ml)	25637.24 ± 45678.99
NK cell function (depressed)	12 (75%)

Abbreviations: SD, Standard Deviation; ANC, absolute neutrophil count; Hb, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; LDH, lactate dehydrogenase; β2-MG, β2-microglobulin; FIB, fibrinogen; TG, triglycerides; NK, natural killer.

mainly infected with B lymphocytes (EBV infected CD19+ cells were more than CD56+ cells). Among them, 3 patients had EBV infection of T, B, and NK lymphocyte subgroups simultaneously, and 1 case was mainly infected with B lymphocytes (EBV infection with more CD19+ cells than CD4 and/or CD8 cells and CD56+ cells). EBV only infected B lymphocyte subgroups in 7 patients. At the time of diagnosis, the mean of PBMC EBV DNA in patients infected with EBV only and those mainly infected with B lymphocytes was 1.19E+04 ± 1.37E+04 copies/ml, and that in patients with T and NK lymphocytes mainly infected with EBV was 3.00E+06 ± 4.27E+06 copies/ml, with no statistical significance ($P = 0.193$) (see Table 2).

3.2. Responses to treatment

Four cases (26.7%) achieved CR, and 10 cases (66.7%) achieved PR, with 1 case (6.7%) NR after the 2 weeks of the first rituximab-containing regimen. The total effective rate was 93.3%. Of the 7 cases of induction therapy, 1 case achieved CR, and 5 cases achieved PR, while 1 case was NR. Of the 8 cases of salvage therapy, 3 achieved CR and 5 achieved PR Table 3.

After the first treatment with rituximab-containing regimen, EBV DNA of PBMC and plasma were detected, and EBV DNA of 10 patients (50%) were negative. Of the 10 patients with negative EBV after the first treatment, 7 patients were only infected with B lymphocyte subgroup cells, 1 patient infected with T, B, and NK lymphocyte subgroup cells, 1 patient infected with T and B lymphocyte subfroups cells, and 1 patient infected with B and NK lymphocyte subgroups cells. And the latter three cases were mainly infected with B lymphocyte subgroup. Of the 5 patients who were still positive for EBV after the first treatment, 2 patients were infected with T and B lymphocyte cells, 2 patients were infected with T, B, and NK lymphocyte cells, and 1 patient was infected with NK lymphocyte cells. Among the latter 5 patients tested for EBV-infected lymphocyte subgroups, EBV mainly infected T and NK lymphocyte cells Table 4.

After all rituximab-containing regimens were completed, EBV DNA could still be positive in 5 patients whose EBV did not turn negative after the first course, and EBV DNA was positive in 2 of 10 cases who turned negative after the first course. The duration of EBV negative in the 2 patients was 3 and 4 months, respectively. In the two patients whose EBV were positive again, EBV-infected lymphocyte subgroups were tested, and it was found that EBV mainly infected T and NK lymphocytes.

Of the 5 patients with persistent EBV positive, 2 patients received allo-HSCT and EBV turned negative, whereas 3 patients remained positive. In 5 patients with EBV persistent positive before and after the first course or after the end of all treatment courses, no significant decrease in EBV DNA load was found ($P > 0.05$) (see Table 5).

3.3. Side effects of rituximab

Two patients developed infusion-related toxicities, including chills (n = 2) and fever (n = 2). The side-effect symptoms of the two patients were alleviated by suspending the drug infusion and slowing the infusion rate of rituximab. One month later, one patient was tested positive for herpes simplex virus and then turned negative after anti-viral treatment. No patients experienced reactivation of hepatitis B, hepatitis C, varicella-zoster virus, cytomegalovirus, or parvovirus.

3.4. Survival time

By the time of follow-up on May 14, 2020, 11 (73.3%) patients were alive with a median duration of survival of 318 days since EBV-HLH diagnosis (range, 202–671 days). Two patients died due to HLH (n = 1), with transplant-related complications (n = 1). Two patients were lost to follow-up. Of the 11 surviving patients, 4 patients were still positive for EBV, 2 of whom were children (4 years and 2 years old) who continued to be positive for EBV, and 2 patients received EBV negative after the first treatment and then became positive. All the 11 surviving patients exhibited no evidence of activity and recurrence of EBV-HLH.

4. Discussion

HLH is a rare, life-threatening disease characterized by uncontrolled immune responses. HLH is divided into primary and secondary HLH. The common causes of secondary HLH are infection, malignant tumors, and autoimmune diseases. In secondary HLH, infection plays an important role, especially EBV infection [3]. EBV-HLH accounts for approximately 70% of infection-associated HLH, with a high incidence in Asians [13]. The treatment of EBV-HLH remains challenging, and previous studies have found that ≥30% of patients die of the disease or its complications [14,15]. The survival of patients with EBV-HLH is still poor, with 1-year overall survival (OS) of only 25.0% according to a previous study [16]. The main treatment principles for EBV-HLH are suppression of hyperinflammation, elimination of EBV, and replacement of the defective immune system. Therefore, for patients with EBV-HLH, if the EBV load can be eliminated or significantly reduced, the patients' symptoms and survival rate can be improved.

EBV initially infects naive B cells, and it can reside in the memory B cell pool of normal healthy individuals for a long time [17]. B cells become the main "factory" and reservoir of EBV. EBV can infect B and non-B cell populations in patients with EBV-HLH. Rituximab is an anti-CD20 monoclonal antibody that can destroy CD20 positive B cells. Thus,

Table 2
EBVDNA and EBV-infected lymphocyte subsets of 15 patients with EBV-HLH at diagnosis.

	EBVDNA (copies/ml) at diagnosis		EBV-infected lymphocyte subsets (EBV copies per 1 million cells)			
	PBMC	Plasma	CD4+	CD8+	CD19+	CD56+
1	6.30E+05	8.60E+03	<500	2.10E+06	4.30E+05	<500
2	1.70E+04	8.50E+02	2.40E+04	7.30E+04	3.90E+04	<500
3	1.60E+05	–	BLD	BLD	3.70E+03	9.30E+03
4	4.20E+06	–	BLD	1.00E+05	2.10E+05	2.70E+06
5	1.00E+07	–	3.10E+03	BLD	6.30E+04	1.20E+05
6	1.20E+04	<5.00E+02	<5.00E+02	1.90E+05	1.20E+07	9.10E+04
7	<5.00E+02	4.70E+03	1.80E+03	BLD	5.20E+04	BLD
8	2.90E+03	<5.00E+02	BLD	BLD	2.50E+04	2.30E+03
9	<5.00E+02	5.00E+03	BLD	BLD	6.80E+04	BLD
10	3.70E+04	4.20E+05	BLD	BLD	2.80E+05	BLD
11	2.80E+04	1.50E+04	BLD	BLD	1.60E+06	BLD
12	1.20E+03	<5.00E+02	BLD	BLD	1.40E+04	BLD
13	1.10E+04	<5.00E+02	BLD	BLD	1.00E+05	BLD
14	5.80E+02	<5.00E+02	BLD	BLD	2.30E+03	BLD
15	2.30E+03	2.60E+03	BLD	BLD	3.00E+05	BLD

Abbreviations: PBMC, peripheral blood mononuclear cell; 1.00E+01, 1.00×10^1; –, not tested; BLD, below the limits of detection

Table 3
Clinical characteristics, efficacy and prognosis of 15 patients with EBV-HLH treated with rituximab-containing regimen.

	Gender	Age (years)	Disease states before rituximab treatment	Treatment	Rituximab dose	Course of treatment with rituximab	Evaluation after 2 weeks	HSCT	Survival
1	Female	4	Salvage	R-DEP	300 mg	3	CR	No	Alive
2	Female	2	Salvage	R-LDEP	100 mg	3	PR	No	Alive
3	Male	26	Induction	R-EP-Ru	100 mg	2	NR	Yes	Die
4	Male	55	Induction	R+HLH-94	375 mg/m^2	1	PR	Yes	Alive
5	Male	37	Induction	R-EP	375 mg/m2	2	PR	No	Die
6	Female	3	Salvage	R-DEP	300 mg	1	PR	No	Alive
7	Male	56	Salvage	R-ECOP	375 mg/m2	4	PR	No	LFU
8	Female	19	Salvage	R-DEP	375 mg/m2	4	CR	No	Alive
9	Female	37	Induction	R-DEP	375 mg/m2	2	PR	No	Alive
10	Male	48	Induction	R-DEP	375 mg/m2	3	PR	No	Alive
11	Male	49	Salvage	R-DEP	375 mg/m2	1	PR	No	Alive
12	Male	68	Induction	R-DEP	375 mg/m2	4	PR	No	LFU
13	Male	1	Salvage	R-DEP	375 mg/m2	2	CR	No	Alive
14	Female	62	Salvage	R-DEP + Ru	375 mg/m2	3	PR	No	Alive
15	Female	22	Induction	R-DEP	375 mg/m2	4	CR	No	Alive

Abbreviations: R, rituximab; VP16, etoposide; Ru, ruxolitinib; NR, no response; PR, partial response; CR, complete response; LFU, loss to follow up

Table 4
Changes in EBVDNA of 15 patients with EBV-HLH before and after treatment.

	EBVDNA before rituximab treatment (copies/ml)		EBVDNA after the first course of rituximab treatment (copies/ml)		EBVDNA after the first course of rituximab	EBVDNA at the end of treatment (copies/ml)		EBVDNA at the end of treatment	EBVDNA after HSCT	EBVDNA at follow-up
	PBMC	Plasma	PBMC	Plasma		PBMC	Plasma			
1	6.30E+05	8.60E+03	3.90E+05	<5.00E+02	Positive	3.90E+05	<5.00E+02	Positive	–	Positive
2	1.70E+04	8.50E+02	3.60E+03	BLD	Positive	8.50E+02	<5.00E+02	Positive	–	Positive
3	1.60E+05	–	1.50E+06	4.30E+05	Positive	5.50E+02	2.10E+04	Positive	Negative	Negative
4	4.20E+06	–	1.50E+05	<500	Positive	7.90E+03	BLD	Positive	Negative	Negative
5	1.00E+07	–	2.30E+04	2.20E+04	Positive	1.59E+06	1.59E+04	Positive	–	Positive
6	1.20E+04	<5.00E+02	BLD	BLD	Negative	BLD	BLD	Negative	–	Negative
7	<5.00E+02	4.70E+03	<5.00E+02	<5.00E+02	Negative	<5.00E+02	<5.00E+02	Negative	–	Negative
8	2.90E+03	<5.00E+02	BLD	BLD	Negative	BLD	BLD	Negative	–	Negative
9	<5.00E+02	5.00E+03	<5.00E+02	<5.00E+02	Negative	<5.00E+02	<5.00E+02	Negative	–	Negative
10	3.70E+04	4.20E+05	<5.00E+02	<5.00E+02	Negative	<5.00E+02	<5.00E+02	Negative	–	Negative
11	2.80E+04	1.50E+04	BLD	BLD	Negative	BLD	BLD	Negative	–	Negative
12	1.20E+03	<5.00E+02	BLD	BLD	Negative	BLD	BLD	Negative	–	Negative
13	1.10E+04	<5.00E+02	<5.00E+02	<5.00E+02	Negative	9.60E+03	<5.00E+02	Positive	–	Positive
14	5.80E+02	<5.00E+02	BLD	<5.00E+02	Negative	BLD	<5.00E+02	Negative	–	Negative
15	2.30E+03	2.60E+03	<5.00E+02	<5.00E+02	Negative	4.40E+03	<5.00E+02	Positive	–	Positive

Abbreviations: PBMC, peripheral blood mononuclear cell; 1.00E+01, 1.00 × 10^1; BLD, below the limits of detection.

Table 5
Changes of EBVDNA before and after treatment with rituximab in patients whose EBV mainly infected T and NK cells.

	EBVDNA (PBMC) (copies/ml)	*P* value
Before rituximab treatment	3.00E+06 ± 4.27E+06	
After the first course of rituximab treatment	4.13E+05 ± 6.27E+05	$P = 0.276$ (t = 1.261)
After all rituximab treatments	3.98E+05 ± 6.87E+05	$P = 0.190$ (t = 1.577)

Abbreviations: PBMC, peripheral blood mononuclear cell; *P*, compared with before treatment; 1.00E+01, 1.00 × 10^1.

rituximab can reduce EBV load, which in turn reduces EBV-induced overactive immune responses [8,18,19]. There have been studies to treat EBV-HLH with a chemotherapeutic regimen containing rituximab and achieved good results. For example, Chellapandian et al. retrospectively reported the clinical study of 42 patients with EBV-induced HLH who received rituximab-containing regimens [8]. Rituximab-containing regimens improved clinical status in 43% of patients and appeared to be well tolerated. The authors of this previous study believed that rituximab-containing regimens could reduce viral load and diminish inflammation. Al Asad et al. reported an adult patient with EBV-related HLH [20] who achieved complete remission after treatment with rituximab alone, without the conventional chemotherapy. However, there are no studies of EBV-HLH patients with different lymphocyte subgroups (B, T, and NK) for EBV infection with rituximab treatment.

In our study, in 5 of our 15 patients, EBV mainly infected T and NK cells. After rituximab-containing regimens, the EBV load of 5 patients did not decrease significantly. In the other 10 patients with EBV-HLH, EBV only and mainly infected the B cells. The EBV load decreased significantly after the first course of rituximab-containing regimens. The reason may be that rituximab only acts on B cells by destroying B cells in order to eliminate EBV in B cells. However, rituximab has no effect on T and NK cells; therefore, EBV in T and NK cells may not be eliminated. Moreover, in the previously reported rituximab-containing EBV-induced HLH, some patients had EBV-DNA positive B cell lymphoma, and rituximab has been confirmed to have specific treatments for the effect of the above disease [8,21,22]. However, in our research, all of them had EBV-HLH and no other diseases. Although the rituximab-containing regimens had a difference in reducing EBV load in patients with EBV-HLH with EBV infected different lymphocyte groups, there is no significant difference in the efficacy of HLH. Therefore, we believe that in the rituximab-containing regimens for EBV-HLH, rituximab may be mainly used to reduce the EBV load of infected B cells, and chemotherapy drugs are mainly used to control HLH. In the 5 patients with

mainly T and NK cells infected with EBV, the EBV load of all patients did not decrease after 1–3 courses. Two cases of EBV turned negative after allo-HSCT. Therefore, for patients with mainly T and NK cells infected with EBV, allo-HSCT remains the only possible curative treatment. In the 10 cases with only and mainly B cell infection, after the first course of treatment, the EBV load fell to the normal range or was undetectable. However, in 2 patients, EBV DNA became positive again after 3 and 4 months, and then the infected cell subgroups were T and NK cells. The reason may be that EBV was initially infected with T and NK cells, but only few cells were infected and were not detected. It was suggested that EBV DNA should be closely monitored even after EBV DNA turns negative.

For the side effects of rituximab, two patients developed infusion-related toxicities, including chills and fever, which were alleviated by slowing the infusion rate of rituximab. One patient was tested positive for herpes simplex virus and then turned negative after anti-viral treatment. No patients experienced reactivation of hepatitis B, hepatitis C, varicella-zoster virus, cytomegalovirus, or parvovirus. Overall, the data suggested that rituximab was safe for EBV-HLH.

In summary, in this study, we found that the rituximab-containing regimen was effective for patients with only and mainly B lymphocytes infected with EBV. However, this study is a retrospective analysis, with a small number of cases and mostly rituximab combination therapy. It needs to be further validated by prospective large-scale rituximab-containing therapy in patients with EBV-HLH with different lymphocyte subgroups for EBV infection. However, the study suggests the importance of detecting specific cell subgroups of EBV infection in patients with EBV-HLH and provides important evidence for future prospective clinical trials.

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CRediT authorship contribution statement

Guang-Qiang Meng: Conceptualization, Methodology, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Jing-Shi Wang:** Conceptualization, Methodology, Investigation, Visualization. **Yi-Ni Wang:** Formal analysis, Methodology, Visualization. **Na Wei:** Formal analysis, Methodology, Visualization. **Zhao Wang:** Conceptualization, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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