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ORIGINAL ARTICLE

Characteristics of Virology and Immune Inflammation of Epstein-Barr Virus Infection Related Non-Neoplastic Diseases in Children

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SUMMARY

Background: **The goal was to study the difference of virological, immunologic, and inflammatory indicators between Epstein-Barr associated infectious mononucleosis (EBV-IM) and EBV associated hemophagocytic lymphohistiocytosis (EBV-HLH) and to explore the evaluation indicators for monitoring the therapeutic efficacy of EBV-HLH.**

Methods: **Twenty children with EBV-IM (IM group) and 10 children with EBV-HLH (HLH group) were selected. Virology indicators were detected; the absolute count of lymphocyte, and lymphocyte subsets were detected; the levels of immunoglobulin and ferritin were assayed.**

Results: **Compared to the IM group, the HLH group showed a decrease in EBV-specific VCA-IgM antibody levels ($U = 29.0$, $p = 0.006$) and an increase in EBV-specific NA-IgG antibody levels ($U = 17.0$, $p = 0.001$), while there was no significant difference in EB-DNA loads ($t = 0.417$, $p = 0.680$). The counts of lymphocytes, and various lymphocyte subsets in the HLH group were lower than those in the IM group. Inflammatory markers in the HLH group were significantly higher than those in IM group. Dynamic monitoring of virological, immunological, and inflammatory indicators in HLH patients during treatment showed that EBV DNA gradually decreased in patients with good prognosis. Inflammatory indicators significantly decreased and returned to normal, lymphocyte count significantly increased and returned to normal during treatment. However, patients with poor prognosis showed rebound increase in EBV DNA and inflammatory indicators in the later stage of treatment, while lymphocyte count further decreased with the recurrence of the disease. Conclusions: Exhausted and damaged immune function in host by persistent stimulation of EB viral antigen is one of the main pathogeneses of EB-HLH. Lymphocyte count and serum ferritin level are effective indicators to monitor the therapeutic efficacy during the treatment to HLH.**
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KEYWORDS

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INTRODUCTION

Epstein-Barr virus (EBV) is a kind of DNA virus which belongs to the gamma herpesviridae family [1]. Humans are susceptible to EBV, with 90% of adults having positive antibodies to EBV [2]. EBV infection in children can induce immune dysfunction, including EBV-associated infectious mononucleosis (EBV-IM), EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH), and chronic active EBV infection, etc., while a few pa-

tients may possibly develop into malignant tumors [2-4].

The main immunological feature of IM is the robust proliferation and activation of $CD8^+$ T cells, accompanied by the reduction of B cells and $CD4^+$ T cells [5]. Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome, is a group of clinical syndromes caused by a variety of pathogenic factors caused by excessive proliferation of lymphocytes and tissue macrophages, producing a large number of inflammatory factors, causing inflammation in multiple organs [6]. The main characteristics of HLH patients are accompanied by high fever, rapid disease progression, and high mortality [7,8]. It has been widely accepted that host immune response plays a key role in clearing EB virus, especially the cellular immune response which may determine the outcome of the disease [9,10]. It has been reported that the virological character of EBV infection is related to the severity of disease and its clinical characteristics [11,12].

In this study, we analyzed the difference of virological and immunological indicators between pediatric patients with EB-IM and pediatric patients with EB-HLH, including EB viral load, EBV-specific antibodies levels, lymphocyte subsets, immunoglobulin levels, and inflammatory indicators. In addition, kinetic analysis of inflammatory indicators was performed in patients with EBV-HLH to find a valuable parameter for diagnosis of EBV infection related non-neoplastic diseases and monitoring the efficacy of treatment.

MATERIALS AND METHODS

Study subjects

A total of 30 patients who visited the Department of Pediatrics, the Second Affiliated Hospital of Anhui Medical University, from January 2020 to November 2023 with EBV infection were selected. All subjects were divided into two groups: 20 patients with EBV-IM and 10 patients with EBV-HLH. All patients with IM met the diagnostic criteria provided by the Chinese Medical Association (CMA) [13], which include any three of the following symptoms: fever, pharyngitis, tonsillitis, enlarged lymph nodes in the neck, liver enlargement, and spleen enlargement; in addition, laboratory indicators indicate that a lymphocyte count $\geq 5.0 \times 10^9$/L, the atypical lymphocyte count $\geq 1.0 \times 10^9$/L, and the EBV antibody test met one of the following conditions: 1) The VCA-IgM antibody was initially positive and later turned negative. 2) The titer of VCA-IgG antibody was increased by more than 4 times. 3)The antibody titer of EA was transiently increased. 4) VCA-IgG antibodies are initially positive and EBNA antibodies later become positive. Diagnosis of HLH According to the HLH 2004 diagnostic criteria of the International Histological Cell Society [14]: 1) Fever, duration $\geq$ 7 days, maximum body temperature $\geq$ 38.5°C. 2) Enlarged spleen (subcostal $\geq$ 3 cm). 3) Cytopenia (not due to hypoplasia or hyperplasia of bone marrow, Hb < 90 g/L, PLT < 100×10^9/L, N < 1.0×10^9/L). 4) Hypertriglyceridemia and/or hypofibrinogenemia (Triglyceride $\geq$ 2.0 mmol/L, or an increase of $\geq \pm 3$ standard deviations from normal. Fibrinogen $\leq$ 1.5g/L, or a decrease $\leq$ normal ± 3 standard deviations). 5) The presence of hemophagocytes found in bone marrow or spleen or lymph nodes, no evidence of malignant disease. 6) Reduced NK cell activity. 7) Serum ferritin level $\geq$ 500 ng/L. 8) soluble CD25 $\geq$ 2,400 U/mL. Pediatric patients who meet any five of the above eight criteria can be diagnosed.

Detection of serological markers of EBV infection

Serum levels of IgG, IgM, and IgA antibodies against different EBV antigens were measured using the corresponding chemiluminescence kit (Shenzhen Snibe Diagnostic). EBV DNA load was quantitatively measured by real-time PCR using EBV DNA diagnostic kit (Sansure Biotech), according to the manufacturer's instructions.

Analysis of immune function

EDTA anticoagulated blood (2 mL) was collected. Counts of white blood cells, monocytes, lymphocytes, neutrophils, and platelets were detected. Lymphocyte subsets were analyzed by flow cytometry, including $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, $CD19^+$ B lymphocytes, natural killer (NK) cells. Humoral immunity was analyzed by serum IgA, IgG, and IgM levels which were detected by immune scattering turbidimetry.

Detection of inflammatory indicators and anticoagulant function

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels were detected by Beckman AU5800 Automatic Clinical Chemistry Analyzer. Serum ferritin level was detected by electrochemiluminescence (ECL) (Roche ECL analysis System). Procalcitonin (PCT) levels were detected by dry-type immunofluorescence quantitative analyzer (Base Egg Biotechnology). Plasma prothrombin time (PT), international standardized ratio (PT-INR), prothrombin activity (PT-%), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-dimer (D-D), fibrin degradation product (FDP), and antithrombin III (AT-III) activity were detected by Sysmex Automatic Blood Coagulation Analyzer CS-5100.

Statistical analysis

SPSS 25.0 software was used for statistical analysis. The Shapiro-Wilk method was used for normality test. Normal distribution data were expressed by $\bar{x} \pm s$. The independent sample *t* test was used to compare normal distribution data. Non-normal distribution data were recorded by median (quartile) [M (P25, P75)]. Mann-Whitney U test was performed for comparison of non-normal distribution data. The chi-squared (χ^2) test was performed to compare categorical data. Spearman's cor-

Table 1. Virology indicators of patients in the IM group and the HLH group.

	IM group	HLH group	Statistical value	p-value
Number of cases	20	10	-	-
Gender (male/female)	10/10	4/6	$\chi^2 = 0.268$	0.605
Age	4.45 ± 1.47	6.78 ± 4.35	t = 1.565	0.153
Lg EBV-DNA	5.46 ± 1.02	5.66 ± 1.47	t = 0.417	0.680
VCA-IgM (AU/mL)	29.16 (11.29, 30.00)	2.25 (1.17, 15.02)	U = 29.000	0.006
VCA-IgA (AU/mL)	4.17 (2.26, 5.90)	0.76 (0.03, 2.75)	U = 22.000	0.002
VCA-IgG (AU/mL)	18.89 (5.84, 50.00)	31.42 (13.71, 50.00)	U = 67.000	0.519
EA-IgA (AU/mL)	3.90 (1.83, 6.14)	0.54 (0.01, 1.56)	U = 13.000	0.000
EA-IgG (AU/mL)	0.88 (0.25, 2.22)	0.38 (0.15, 0.97)	U = 50.500	0.138
NA-IgG (AU/mL)	0.14 (0.01, 0.24)	37.54 (4.26, 50.00)	U = 17.000	0.001

Table 2. Comparison of peripheral blood cell count and immune function between IM and HLH groups.

	IM group	HLH group	Statistical value	p-value
WBC (x 10^9/L)	13.19 (10.60, 17.39)	2.29 (1.41, 3.77)	t = 9.404	< 0.0001
N (x 10^9/L)	2.73 (1.81, 3.56)	0.67 (0.34, 2.39)	t = 2.107	0.006
M (x 10^9/L)	0.88 (0.61, 1.69)	0.11 (0.09, 0.73)	U = 62.000	0.002
PLT (x 10^9/L)	170.50 (147.25, 233.75)	41.00 (36.00, 134.00)	U = 33.000	0.006
LC-%	72.20 (64.70, 74.45)	29.80 (24.45, 61.20)	t = 3.700	0.005
LC (x 10^9/L)	9.12 (7.28, 13.01)	0.80 (0.46, 1.73)	t = 11.030	< 0.0001
$CD3^+$ T cells frequency	84.45 (78.60, 90.00)	80.10 (75.55, 82.55)	t = 1.697	0.101
$CD3^+$ T cells count	5,878.50 (3,938.75, 9,712.00)	944.00 (448.00, 1,637.50)	U = 3.000	< 0.0001
$CD4^+$ T cells frequency	15.85 (10.65, 18.60)	32.80 (22.60, 37.80)	t = 4.358	0.002
$CD4^+$ T cells count	1,153.50 (925.50, 1,511.75)	314.00 (143.00, 781.00)	U = 18.000	0.000
$CD8^+$ T cells frequency	63.35 (53.48, 71.18)	45.40 (34.60, 56.90)	t = 3.623	0.001
$CD8^+$ T cells count	4,311.50 (2,897.50, 8,175.25)	632.00 (286.50, 647.50)	U = 2.000	< 0.0001
$CD19^+$ B cells frequency	4.25 (3.00, 5.63)	10.80 (7.10, 15.15)	t = 4.337	0.002
$CD19^+$ B cells count	353.50 (261.00, 478.00)	127.00 (58.00, 220.50)	t = 4.312	0.000
NK cells frequency	8.20 (5.43, 14.33)	10.00 (3.30, 11.80)	t = 0.764	0.451
NK cells count	667.50 (386.00, 1,233.50)	83.00 (22.00, 274.50)	U = 9.000	< 0.0001
IgM	1.63 (1.12, 1.89)	7.89 (6.68, 9.62)	U = 1.000	0.000
IgG	12.87 (10.78, 13.98)	1.64 (0.28, 2.33)	t = 14.020	< 0.0001
IgA	2.04 (1.38, 2.66)	0.65 (0.41, 0.77)	t = 5.805	< 0.0001

relation analysis was used for correlation analysis. For all statistical analyses, a p-value < 0.05 was considered significant

RESULTS

Virological characteristics of pediatric patients with IM or HLH

This study included 30 pediatric patients with EBV infection. Twenty patients (10 males and 10 females) were diagnosed as infectious mononucleosis and 10 patients (4 males and 6 females) were diagnosed as hemophagocytic lymphohistiocytosis. There was no signifi-

Table 3. Comparison of inflammatory indicators between IM and HLH groups.

	IM group	HLH group	Statistical value	p-value
Ferritin	**152.00 (79.40, 184.00)**	**7,894.00 (1,292.00, 24,573.50)**	**t = 3.011**	**0.017**
PCT	**0.19 (0.16, 0.24)**	**1.95 (0.19, 3.80)**	**U = 35.000**	**0.055**
ALT	**47.00 (32.50, 122.50)**	**167.00 (119.50, 402.50)**	**U = 29.500**	**0.003**
AST	**59.50 (45.50, 71.75)**	**194.00 (101.00, 433.00)**	**U = 21.000**	**0.001**
LDH	**543.00 (438.00, 622.25)**	**811.00 (441.00, 1,962.50)**	**t = 2.324**	**0.048**

Table 4. Comparison of anticoagulant activity between IM and HLH groups.

	IMG group	HLH group	Statistical value	p-value
PT-S	**11.05 (10.48, 12.03)**	**11.90 (11.65, 13.50)**	**t = 2.161**	**0.042**
PT-INR	**0.95 (0.89, 1.03)**	**1.02 (1.00, 1.16)**	**t = 2.239**	**0.036**
PT-%	**102.95 (87.55, 118.35)**	**87.20 (66.45, 89.45)**	**t = 2.098**	**0.048**
APTT	**29.05 (26.18, 30.18)**	**31.30 (24.10, 34.80)**	**t = 0.572**	**0.580**
TT	**19.95 (18.63, 20.53)**	**21.70 (18.10, 23.40)**	**U = 44.500**	**0.256**
FIB	**2.30 (2.01, 2.71)**	**2.05 (1.54, 3.04)**	**U = 50.500**	**0.448**
D-D	**0.72 (0.49, 1.15)**	**13.16 (2.69, 51.67)**	**U = 11.000**	**0.001**
FDP	**4.70 (2.33, 6.50)**	**46.65 (9.04, 124.48)**	**U = 13.000**	**0.001**
AT-III	**114.05 (98.25, 128.80)**	**98.10 (74.30, 129.40)**	**t = 1.223**	**0.235**

cant difference of gender and age between the IM group and the HLH group ($p > 0.05$) (Table 1). Among all the subjects, there was no difference of EB viral load, EBV antigen-specific IgG antibodies against early antigen (EA), and viral capsid antigen (VCA) between IM and HLH patients ($t = 0.417$, $U = 50.5$, $U = 67.0$, all $p > 0.05$). However, serum levels of VCA-IgM, EA-IgA, and VCA-IgA antibodies in the HLH group were significantly lower than those in the IM group, while serum levels of nuclear antigen NA-IgG were significantly higher ($U = 29.0$, 13.0, 22.0, all $p < 0.05$) (Table 1).

Comparison of immune-inflammatory biomarker between IM and HLH group

It was shown that the count of white blood cell (WBC), neutrophil (N), lymphocyte, monocyte (M), and platelet (PLT) in the HLH group were significantly lower than those in the IM group ($p < 0.01$). Lymphocyte subsets analysis showed that the count of $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, $CD19^+$ B lymphocytes and NK cells in HLH group were significantly lower than those in IM group ($p < 0.001$). Compared to the IM group, the frequency of $CD8^+$ T lymphocytes was significantly decreased ($p = 0.001$), while the frequency of $CD4^+$ T lymphocytes and $CD19^+$ B lymphocytes increased ($p < 0.01$) in the HLH group, which was due to the robust proliferation of $CD8^+$ T cells in pediatric patients with EB-IM. But there was no difference in the frequency of $CD3^+$ T lymphocytes and NK cells between IM and HLH group ($p = 0.101$; $p = 0.451$, see Table 2).

To reflect humoral immunity, the immunoglobulins were analyzed including IgG, IgA, and IgM. The result showed that serum IgM level in HLH group was significantly increased ($p < 0.001$), while serum IgG and IgA levels were significantly decreased ($p < 0.0001$, see Table 2).

It was shown that inflammatory biomarkers including ferritin, LDH, ALT, and AST in the HLH group were significantly higher than those in the IM group ($p < 0.05$, see Table 3). Procalcitonin (PCT) seemed to be increased, but there was no statistical difference ($p = 0.055$, see Table 3).

Comparison of anticoagulant activity between the IM group and HLH group

Anticoagulant activity of hemostatic biomarkers was also analyzed. Compared to the IM group, plasma D-dimer (D-D) and fibrin degradation product (FDP) levels in the HLH group were significantly increased ($p = 0.001$). Also, plasma prothrombin time (PT) and international normalized ratio (PT-INR) were slightly increased ($p < 0.05$); while prothrombin activity (PT-%) was decreased ($p = 0.048$) (Table 4). However, there were no significant differences of activated partial thromboplastin time (APTT), thrombin time (TT), fi-

Table 5. Correlation analysis between EBV DNA load and other laboratory detection indicators in IM and HLH groups.

	IM group lg EBV-DNA		HLH group lg EBV-DNA	
Parameters	***r* value**	**p-value**	***r* value**	**p-value**
EA-IgG (AU/mL)	**-0.184**	**0.437**	**-0.111**	**0.650**
VCA-IgM (AU/mL)	**-0.053**	**0.826**	**0.502**	**0.028**
EA-IgA (AU/mL)	**-0.009**	**0.970**	**-0.392**	**0.097**
VCA-IgG (AU/mL)	**0.042**	**0.862**	**-0.052**	**0.836**
VCA-IgA (AU/mL)	**0.078**	**0.743**	**-0.582**	**0.009**
NA-IgG (AU/mL)	**-0.339**	**0.144**	**-0.663**	**0.002**
WBC (x 10^9/L)	**0.230**	**0.329**	**-0.250**	**0.110**
N (x 10^9/L)	**0.236**	**0.316**	**-0.163**	**0.303**
M (x 10^9/L)	**0.280**	**0.231**	**0.108**	**0.498**
PLT (x 10^9/L)	**-0.504**	**0.024**	**0.071**	**0.655**
LC-%	**-0.255**	**0.278**	**-0.084**	**0.596**
LC (x 10^9/L)	**0.148**	**0.533**	**-0.255**	**0.103**
$CD3^+$T proportion	**0.566**	**0.009**	**-0.007**	**0.985**
$CD3^+$T count	**0.266**	**0.258**	**0.090**	**0.792**
$CD4^+$T proportion	**-0.304**	**0.193**	**-0.510**	**0.109**
$CD4^+$T count	**0.030**	**0.899**	**-0.266**	**0.429**
$CD8^+$T proportion	**0.572**	**0.008**	**0.347**	**0.296**
$CD8^+$T count	**0.268**	**0.254**	**-0.219**	**0.515**
$CD19^+$B proportion	**-0.076**	**0.750**	**-0.013**	**0.971**
$CD19^+$B count	**0.150**	**0.529**	**-0.033**	**0.924**
NK cells proportion	**-0.542**	**0.014**	**0.152**	**0.655**
NK cells count	**-0.226**	**0.339**	**0.267**	**0.428**
Ferritin	**-0.131**	**0.592**	**0.056**	**0.739**
PCT	**0.376**	**0.137**	**0.238**	**0.582**
ALT	**0.229**	**0.332**	**0.163**	**0.315**
AST	**0.330**	**0.156**	**0.054**	**0.743**
LDH	**0.065**	**0.786**	**0.373**	**0.030**
PT-S	**-0.084**	**0.776**	**0.448**	**0.005**
PT-INR	**-0.026**	**0.930**	**0.378**	**0.021**
PT-%	**-0.109**	**0.710**	**-0.423**	**0.009**
APTT	**-0.390**	**0.169**	**0.437**	**0.007**
TT	**0.302**	**0.292**	**-0.173**	**0.305**
FIB	**-0.459**	**0.099**	**-0.013**	**0.941**
D-D	**0.066**	**0.823**	**-0.046**	**0.840**
FDP	**0.075**	**0.800**	**0.033**	**0.885**
AT-III	**-0.256**	**0.376**	**-0.455**	**0.029**

brinogen (FIB), and antithrombin III activity (AT-III) between the IM group and HLH group (APTT, p = 0.580; TT, p = 0.256; FIB, p = 0.448; AT-III, p = 0.235) (Table 4).

Correlation analysis between EBV viral load and other different laboratory parameters in pediatric patients with IM or HLH

Correlation analysis between EBV DNA viral load and other different laboratory parameters showed that EBV DNA viral load was positively correlated with the frequency of $CD3^+$ T cells (r = 0.566, p = 0.009), the fre-

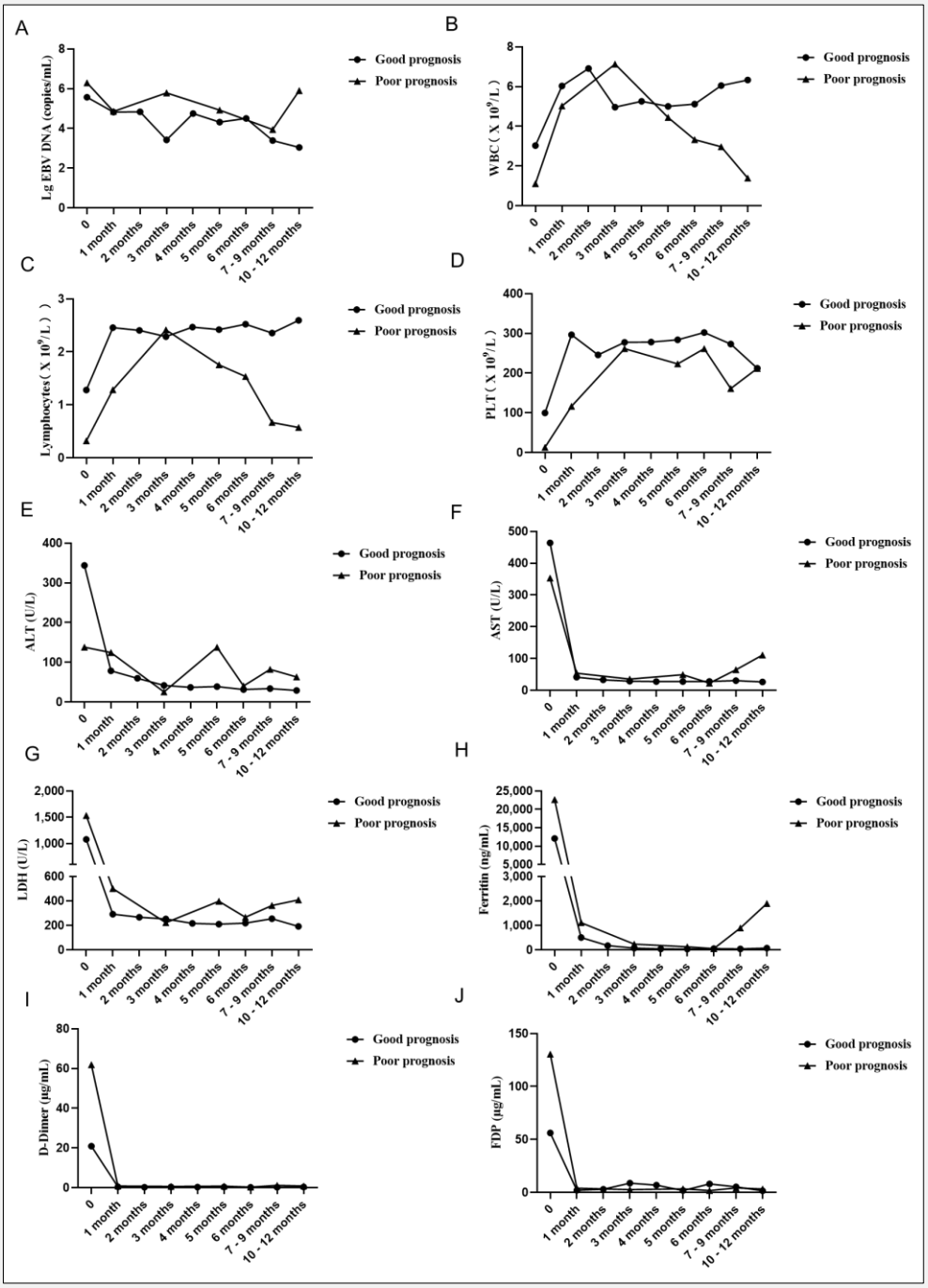

Figure 1. Dynamic changes of relevant indicators during treatment of HLH patients with good and poor prognosis.

EB treatment - HLH each of the laboratory indexes in the process of dynamic change, A - peripheral blood EB - DNA loads, B - WBC count, C - Lymphocyte count, D - Platelet count, E - ALT level, F - AST level, G - LDH level, H - Ferritin level, I - D-dimer concentration, J - Fibrin degradation products.

quency of $CD8^+$ T cells (r = 0.572, p = 0.008), but negatively correlated with the count PLT (r = -0.504, p = 0.024) and the frequency of NK cells (r = -0.542, p = 0.014) in patients with IM. It was also found that EBV DNA viral load was positively correlated with VCA IgM (r = 0.502, p = 0.028), serum LDH level (r = 0.373,

p = 0.03), PT (r = 0.448, p = 0.005), PT-INR (r = 0.378, p = 0.021), and APTT (r = 0.437, p = 0.007), but negatively correlated with VCA-IgA (r = -0.582, p = 0.009), NA-IgG (r = -0.663, p = 0.002), PT-% (r = -0.423, p = 0.009) , AT-III (r = -0.455, p = 0.029) in patients with HLH (Table 5).

Kinetic analysis of immune-inflammatory biomarkers in patients with HLH during treatment

The kinetic changes of serum immune-inflammatory biomarkers in patients with HLH who received treatment were analyzed retrospectively. According to different outcomes of these patients, 9 patients did not relapse with good prognosis, 1 patient had recurrence with multiple organ damage and poor prognosis. As shown in Figure 1, X axis represents the time of blood sample collection; Y axis represents the value of the measured index. Day 0 was the time of treatment. After the treatment, the EBV DNA viral load continued to decline. The inflammatory biomarkers including serum ferritin, ALT, AST, LDH, and the anticoagulant activity biomarkers including plasma D-dimer and FDP were significantly decreased and continued to be in the normal reference range. WBC, lymphocyte and PLT count significantly increased and continued to remain in the normal reference range after about one month of treatment in patients with good prognosis (Figure 1). However, in patients with poor prognosis, EBV DNA viral load fluctuated greatly. The counts of WBC and lymphocyte increased gradually and then decreased sharply when recurrence occurred. Inflammatory biomarkers including serum ferritin, ALT, AST, and LDH decreased in patients with treatment and then increased significantly when recurrence occurred. There was no significant change of anticoagulant activity in biomarkers (Figure 1).

DISCUSSION

After the primary infection of EB virus, most patients have no clinical symptoms. After infection, EB virus may establish latent infection in a few B lymphocytes for life. Most primary infections of EB virus are acute infections. Children with primary EBV infection in children is mostly infectious mononucleosis with good prognosis; children with persistent EBV infection, in which the virus cannot be cleared effectively, may progress to hemophagocytic lymphohistiocytosis.

In this study, pediatric patients with EB virus infection were divided into infectious mononucleosis (IM) group and hemophagocytic lymphohistiocytosis (HLH) group. The serum virological marker, immunological and inflammatory indexes were compared between IM and HLH group. It was shown that there was no significant difference in EB virus DNA load between the two groups. IgM antibody responses to virus capsid antigen (VCA) in IM group was significantly higher than that in HLH group. and it was positive, suggesting that IM was caused by EBV infection which was similar with the study by G. Papaevangelou et al. [15]. Therefore, the anti-VCA IgM antibody has been considered as the diagnostic marker of EBV-related IM. However, IgG antibody responses to nuclear antigen in the HLH group was significantly higher than that in the IM group, indicating the persistent EBV infection, and abnormal immune function of patients against EBV infection.

The main target cells for EBV infection and persistence are B lymphocytes. This will stimulate the proliferation and activation of $CD8^+$ T cells and then clear the infected B lymphocytes. Meanwhile, a large quantity of inflammatory cytokines produced cause fever, lymphadenopathy, angina, and other clinical symptoms. The count of WBC, monocyte, lymphocyte, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and NK lymphocytes of IM group were significantly higher than those of the HLH group, suggesting that a large number of lymphocytes proliferated during the acute phase of IM, especially CD8+ T cells which are activated and kill the infected target cells. This result is similar to the study of Wang Yun et al. [16]. In addition, the levels of serum total IgG and IgA in the HLH group were lower than those in the IM group. Although the level of serum total IgM was significantly higher in the HLH group than that in the IM group, the serum level of EB virus specific-IgM in the HLH group was significantly lower than that in the IM group, indicating that the virus antigen continued to persist and stimulate, the level of non-specific IgM increased, and the specific humoral immune function was deficient. It indicated the impairment of immune response against EBV and could not eliminate the virus effectively. The deficiency or exhaustion of cellular and humoral immune function after EBV infection is the main cause of secondary hemophagocytic lymphohistiocytosis. Whether EB-HLH is related to abnormal humoral immune disorder still needs further study.

Abnormal expression of inflammatory biomarkers has been observed with EBV infection. The levels of ferritin, ALT, AST, and LDH in the HLH group were significantly higher than those in the IM group. There was a positive correlation between LDH and EBV DNA load. It indicated that HLH is an aggressive condition with systemic inflammatory response syndrome [17]. Coagulation dysfunction is common in HLH patients. FIB level less than 1.5 g/L is one of the diagnostic criteria for HLH. Hypofibrinogenemia occurs in 50 - 80% of HLH patients [18]. In this study, FIB level was under normal reference range in most HLH patients; APTT, TT, D-D, and FDP were significantly higher than the upper limit of the normal reference range. Plasma levels of D-D and FDP in the HLH group were significantly higher than in the IM group. High levels of D-D and FDP indicate hyperfibrinolysis. It has been shown that both D-D and FDP can serve as diagnostic indicators for HLH patients and may be associated with poor prognosis [19,20].

During EBV infection, the interaction between the virus and host determines the outcome of infection. The cor-

relation between immune-inflammatory markers and EBV DNA load was analyzed. It was found that the proportion of $CD3^+$ T cells and $CD8^+$ T cells in IM patients is positively correlated with the EBV DNA load. This suggests that in the acute phase of IM, the virus stimulates the proliferation of T cells, especially $CD8^+$ T cells, which play a crucial role in virus clearance. The value of APTT, TT, PT-S, and PT-INR is positively correlated with EB virus load in the HLH group, while the value of PT% and AT-III was negatively correlated with EBV DNA load. It indicates that coagulation dysfunction demonstrates the severity of HLH. In addition, kinetic analysis of the immune-inflammatory markers was performed in HLH patients during the first year of treatment. It showed that in patients with good prognosis, EBV DNA gradually decreased in patients; the inflammatory indicators decreased and returned to normal during the first month of treatment; the count of lymphocytes increased and returned to normal during the first month of treatment. It indicates the resolution of inflammation, recovery of immune function and coagulation function after treatment in patients with good prognosis. However, in patients with poor prognosis, EBV DNA and inflammatory markers showed a rebound increase; the count of WBC and lymphocyte declined again along with the recurrence of the disease.

In summary, patients with IM have normal immune function, while patients with HLH have persistent virus infection, impaired immune function, systemic inflammatory response, and coagulation dysfunction. Comprehensive analysis of virological and immune-inflammatory characteristics contributes to the differential diagnosis of EB-HLH. Kinetic analysis of immune-inflammatory markers such as ferritin and LDH is beneficial for evaluating therapy efficacy of HLH.

Declaration of Interest:

The authors declare no conflict of interest.

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