ORIGINAL RESEARCH

Clinical laboratory Analysis of EB Virus Associated Hemophagocytic Lymphohistiocytosis in Children

Gailing Hao, MM; Lian Jiang, MM; Li Wen, MM; Weili Guo, MM; Wenjin Geng, MM; Quanheng Li, MM

ABSTRACT

Objective • Epstein-Barr virus (EBV) is a common virus that infects a large portion of the world's population, with most people becoming infected during childhood or adolescence. The objective of this article is to analyze the clinical and laboratory examination results of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) in children, summarize its characteristics, identify critically ill children as soon as possible, and provide a basis for diagnosis and treatment.

Method • The retrospective analysis in this study involved collecting data from 34 cases of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) admitted to Hebei Children's Hospital from January 2019 to December 2022. The inclusion criteria for the cases studied likely included confirmed diagnosis of EBV-HLH based on clinical symptoms, laboratory findings, and possibly viral testing results. Key parameters analyzed in the study may have included clinical manifestations, laboratory test results (e.g., levels of lactate dehydrogenase, sCD25, IL-10, calcium ions, glutathione aminotransferase, ferritin, alanine aminotransferase, D-dimer), survival rates, and other relevant indicators. Additionally, the cases were likely divided into high-risk groups (with multiple organ dysfunction or requiring ventilator-assisted ventilation) and non-risk groups for comparative analysis.

Results • The results showed that 34 cases (100%) of EBV-HLH had elevated levels of lactate dehydrogenase, sCD25, IL-10, and decreased levels of calcium ions. 97.1% of the children had a fever and elevated levels of glutathione aminotransferase and ferritin, with an 8-week survival rate of 91.2%. The levels of alanine aminotransferase, alanine aminotransferase, lactate dehydrogenase, ferritin, D-dimer, and sCD25 in critically ill children were significantly higher than those in the non-critically ill group, with statistical significance ($P < .05$). The decreased levels of calcium ions in EBV-HLH patients suggest potential tissue damage and disruption of calcium homeostasis, contributing to the systemic manifestations of the disease. Compared with non-critical recombinant albumin, the decrease in critical recombinant albumin was statistically significant ($P < .05$).

Conclusion • Significant changes in laboratory results can contribute to the early diagnosis and targeted treatment of EBV-HLH, especially for critically ill children. We should pay timely attention to laboratory examinations, diagnosis and treatment, and avoid or reduce the occurrence of adverse consequences. Based on the results of the study on Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) in children, specific strategies and criteria can be proposed to aid in the early identification of critically ill children with this condition in clinical practice: Clinical Screening, Risk Stratification, Early Intervention, Multidisciplinary Management and Educational Measures. (*Altern Ther Health Med.* 2024;30(5):148-154)

Gailing Hao, MM, Department of Paediatrics; Hebei Medical University. **Gailing Hao**, MM; **Weili Guo**, MM; **Wenjin Geng**, MM; **Quanheng Li**, MM, Department of Emergency Medicine; Hebei Children's Hospital affiliated to Hebei Medical University. **Lian Jiang**, MM, Department of Paediatrics; The Fourth Hospital of Hebei Medical University. **Li Wen**, MM, Department of Haematology Oncology; Hebei Children's Hospital affiliated to Hebei Medical University.

Corresponding author: Lian Jiang, MM
E-mail: jianglianerke@163.com

INTRODUCTION

Epstein-Barr virus (EBV), also known as human herpesvirus type IV, is a ubiquitous and highly prevalent virus that infects the majority of the world's population. In children, EBV infections are commonly transmitted through close contact with infected individuals via saliva. The virus primarily targets B lymphocytes, where it establishes latent infection and can persist asymptomatically or lead to symptomatic illness. Hemophagocytic lymphohistiocytosis (HLH) is a rare but severe syndrome in children that can disrupt the immune system. Epstein-barr virus-associated hemophagocytic lymphohistiocytosis can affect the normal function of hemophagocytic cells (often natural killer cells and CD8+T), destroy infected cells, and cause hemocytopenia and can cause high fever, diarrhea, jaundice, hepatosplenomegalysis and other symptoms. Conventional antiviral treatment alone is ineffective. The massive release of cytokines is thought to be closely related to EBV-HLH.[2] Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome characterized by the dysregulation of the immune response, resulting in uncontrolled activation and proliferation of immune cells. The pathogenesis of HLH involves a complex interplay of genetic predispositions, impaired cytotoxic function, and excessive cytokine production. In the context of HLH triggered by Epstein-Barr virus (EBV) infection, the virus plays a significant role in driving the aberrant immune response that culminates in the development of HLH. EBV-HLH is more common in children than in adults. In order to identify, diagnose, and

provide different treatment plans for different causes and clinical manifestations as soon as possible, we analyzed in detail the laboratory examination results of 34 children with EBV-HLH in the past 4 years, hoping to provide a certain basis for clinical diagnosis and treatment. Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) presents a significant clinical challenge, especially in pediatric patients, due to its potential for rapid disease progression, high morbidity, and mortality rates if left untreated or diagnosed late. Several factors contribute to the severity of EBV-HLH and the challenges it poses in clinical management. EBV-HLH poses a significant clinical challenge in pediatric patients due to its aggressive disease course, diagnostic complexities, treatment resistance, potential complications, and the vulnerability of pediatric populations. Addressing these challenges necessitates a multidisciplinary approach, early recognition, and proactive management to improve outcomes and reduce the morbidity and mortality rates associated with this life-threatening condition.

OBJECTS AND METHODS

Objects

48 children have been collected under the age of 14 who were hospitalized and diagnosed with HLH at Hebei Children's Hospital from January 2019 to December 2022. Among them, 34 children who met the diagnosis of EBV-HLH were enrolled. Among the remaining 14 cases, 2 cases were infected with Leishmania, 2 cases were idiopathic juvenile rheumatoid arthritis, 1 case was considered to be related to lymphoma, 1 case was hemophagy with unclear classification of autoimmune diseases, and the remaining 8 cases were sepsis-related hemophagy. Among the 34 cases, 17 boys and 17 girls had a gender ratio of 1:1 and an average age of 2.97 years (0.2-12 years). 34 enrolled children met the diagnostic criteria for both HLH and EBV.

Diagnosis of HLH: It can be diagnosed if one of the following two criteria is met.[3] (1) The following genetic abnormalities were detected:PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, SH2D1A, XIAP/BIRC4; (2) If the patient meets 5 of the following 8 criteria; (i) Fever ≥38.5°C for more than 7 days; (ii) Splenomegaly; (iii) At least two lineages of peripheral blood cells decreased; (a) Hemoglobin <9 g/dL (in infants: hemoglobin <10 g/dL) (b) Platelets <100 × 10^9/L (c) Neutrophils <1 × 10^9/L (iv) Hypertriglyceridemia (fasting,>265mg/dLor3mmol/L) and/or hypofibrinogenemia (<1.5 g/L) (v) Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver; (vi) NK cell activity was low or absent; (vii) Ferritin >500 μg/L; (viii) sCD25 (soluble IL-2 receptor): >2400 U/mL or elevated±2SD

Diagnosis of EBV: EBV-PCR detection with a result>500 copies/ml indicates a positive result.[4]

When establishing diagnostic criteria for Hemophagocytic Lymphohistiocytosis (HLH) and Epstein-Barr Virus (EBV) infection, it is crucial to consider the rationale behind each parameter to ensure accuracy and clinical relevance.

PCR Result of >500 copies/ml for EBV: This specific threshold for EBV viral load in PCR testing is considered significant because it correlates with active viral replication and proliferation. High EBV viral loads can indicate a more severe and systemic EBV infection, including the potential development of EBV-associated HLH. Monitoring EBV viral load helps in assessing disease activity, response to treatment, and risk stratification in patients with EBV-related disorders.

Diagnostic Criteria for HLH: The diagnostic criteria for HLH include parameters such as fever, splenomegaly, cytopenias, hypertriglyceridemia, and hemophagocytosis. Each criterion reflects different aspects of the hyperinflammatory state and immune dysregulation characteristic of HLH. Fever and splenomegaly are common clinical manifestations, while cytopenias indicate bone marrow involvement. Hypertriglyceridemia and hemophagocytosis reflect the cytokine storm and macrophage activation seen in HLH.

Clinical Significance of Diagnostic Criteria: Understanding the clinical significance of each diagnostic criterion helps in recognizing the pathophysiological basis of HLH and EBV infection. Fever and splenomegaly are cardinal features of systemic inflammation, while cytopenias signify bone marrow suppression and ineffective hematopoiesis. Hypertriglyceridemia reflects abnormal lipid metabolism in HLH, contributing to organ dysfunction. The presence of hemophagocytosis indicates macrophage hyperactivity and tissue damage, supporting the diagnosis of HLH.

By elaborating on the rationale behind selecting specific diagnostic criteria for HLH and EBV infection, researchers and clinicians can gain insight into the disease mechanisms, prognostic implications, and therapeutic implications. This approach enhances the understanding of the diagnostic process and the clinical significance of each criterion, facilitating accurate diagnosis, risk stratification, and management of patients with HLH and EBV-related conditions.

Methods

Analyze the clinical data and laboratory examination results of 34 cases of EBV-HLH, including age, gender, chief complaint, diagnosis time after admission, use of ventilator assisted ventilation, treatment, 8-week survival rate, as well as white blood cells (WBC), neutrophils (NEU), platelets (PLT), hemoglobin (HGB), C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), serum albumin (ALB) Total bilirubin (TBil), Ferritin (FER), calcium ions (Ca2+), partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), D-dimer (DD), fibrinogen (FIB), total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, Apolipoprotein AI(Apo-AI), apolipoprotein B, immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) Total T cells (CD3+), helper T cells (CD4+), suppressive T cells (CD8+), total B cells (CD19+), natural killer cells (CD56+), interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor

necrosis factor- α (TNF- α), γ- Interferon (IFN- γ), Soluble interleukin-2 receptor (s CD25), natural killer cell activity (NK%), CD107a stimulation test, abdominal ultrasound, cerebrospinal fluid, bone biopsy, gene analysis, etc. 34 children were divided into high-risk and non high-risk groups based on whether they had concurrent multiple organ dysfunction or (and) the need for ventilator-assisted ventilation. The laboratory examinations of the two groups of children were compared. Analyze the differences between critical and non-critical recombination to provide a laboratory basis for the early identification of critically ill children.

Here's a suggested breakdown of these parameters into categories with explanations of their significance:

Liver Function Tests: Parameters: Alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, alkaline phosphatase. Explanation: Liver function tests are essential in evaluating liver involvement and monitoring hepatic dysfunction in EBV-HLH. Elevated transaminases and bilirubin levels indicate hepatocellular injury and cholestasis, reflecting the severity of liver inflammation and dysfunction seen in HLH.

Coagulation Profile: Parameters: Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer. Explanation: Coagulation abnormalities are common in EBV-HLH due to the hyperinflammatory state and disseminated intravascular coagulation (DIC). Monitoring coagulation parameters helps in assessing the risk of bleeding and thrombotic complications in patients with HLH.

Inflammatory Markers: Parameters: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, cytokine levels. Explanation: Inflammatory markers provide insights into the systemic inflammation and cytokine dysregulation observed in EBV-HLH. Elevated levels of CRP, ESR, ferritin, and pro-inflammatory cytokines reflect the hyperinflammatory response and disease activity in HLH.

Immune Function Tests: Parameters: Lymphocyte subsets (CD4+, CD8+ T cells, NK cells), immunoglobulin levels. Explanation: Assessing immune function is crucial in understanding the immune dysregulation and impaired immune surveillance in EBV-HLH. Abnormalities in lymphocyte subsets and immunoglobulin levels highlight the immune system's dysfunction and predisposition to viral infections in HLH patients.

Statistical method

Statistical analysis was conducted using SPSS25.0 software. Descriptive statistics are represented by numbers, percentages, arithmetic mean (± SD), and median (minimum and maximum). Intergroup differences adopted χ^2 tests or Fisher's exact tests. The comparison between two sets of normal distribution econometric data was conducted using an independent sample t test, using ($\bar{x} \pm s$) represents the comparison between non-normal distribution econometric data using Mann Whitney U test and M (Q). $P < .05$ is considered a statistically significant difference.

RESULTS

Clinical characteristics and laboratory test results

Among the 34 patients, the male to female ratio was 1:1, with an average age of 2.97 years. 97.1% of them complained of fever. The average number of confirmed days after admission is 4.15 days. Among them, 6 cases required ventilator-assisted ventilation treatment, and 19 cases received etoposide chemotherapy. Due to the fact that the initial induction treatment for hemophagocytic syndrome usually takes 8 weeks, and studies have shown that most deaths occur in the first 8 weeks after diagnosis, we chose 8-week survival as the survival rate. Among the 34 cases, 3 died within 8 weeks, with an 8-week survival rate of 91.2%. The analysis of the three deceased children is shown in Table 3. In the laboratory examination, all 34 cases showed an increase in lactate dehydrogenase, with a median of 850 U/L, and a decrease in calcium ions, with a median of 0.94 mmol/L. 33 cases showed elevated levels of ferritin and glutathione aminotransferase, with a proportion of 97.1% and a median of 14829, respectively μ G/L, 209U/L. 30 cases showed an increase in D-dimer, with a median of 5.03mg/L. In addition, leukopenia, thrombocytopenia, elevated alanine aminotransferase, hepatomegaly, splenomegaly, and gallbladder wall edema are also more significant. Among the 34 cases, 26 cases completed sCD25 testing, and all 26 cases increased (100%), with a median of 20148 pg/ml. 14 cases underwent cytokine testing, and all IL-10 levels increased (100%), with 85.7% of cases having TNF- α, IFN- γ Increase in height. 97% of the 33 cases showed an increase in high-density lipoprotein and apolipoprotein A. Ten cases underwent CD107a stimulation test, and 7 of them showed abnormal degranulation function of NK and/or cytotoxic T lymphocytes (CTL) cells. Gene analysis was completed in 23 cases, of which 2 cases detected blood phagocytic-related genes, all of which were UNC13D. Additionally, other gene variations were found in Table 4, and it is currently uncertain whether these gene variations are related to hemophagocytic syndrome. NK activity was detected in 24 cases, with a 33.3% decrease in activity. Sixteen cases underwent complete cerebrospinal fluid examination, with an abnormality rate of 12.5%. Clinical characteristics and laboratory examination results are shown in Tables 1 and 2.

Comparison results between hazardous and non-hazardous restructuring

Clinical significance of each laboratory marker as follows: Lactate dehydrogenase (LDH): Elevated LDH levels in HLH patients may indicate cellular activation and damage, serving as a marker for assessing disease severity and treatment response. Ferritin: Markedly increased ferritin levels in HLH patients likely reflect the inflammatory response triggered by cellular activation and lysis, making it a diagnostic indicator for HLH. sCD25: Soluble CD25, a form of the IL-2 receptor alpha subunit, is typically elevated in HLH patients. This elevation may suggest abnormal T-cell activation and cytokine release, making sCD25 a useful marker for assessing immune activation and disease activity.

Table 1. Clinical and Laboratory Data of EBV-HLH

Clinical and laboratory data	No. (%) of children with EBV-HLH (min-max)	Variables Median
Sex		
Male	17(50.0)	
Female	17(50.0)	
Age(yr)		1.5 (0.20-12.00)
Fever	33(97.1)	
Treatment by etoposide	19(55.9)	
Underwent mechanical ventilation assisted therapy	6 (17.6)	
Days of diagnosis		3 (1-10)
8-week survival	31(91.2)	
EBV-PCR positive	34(100)	
Haemophilic cells found	24(70.6)	
Splenomegaly	26(76.5)	
Hepatomegaly	29(85.3)	
Edema of gallbladder wall	24(70.6)	
Leukocyte (<4×10^9/L)	26 (76.5)	2.7 (0.6-19.8)
Neutrophil(<1×10^9/L)	18 (52.9)	0.96 (0.13-10.82)
Hemoglobin(<9g/L)	13 (38.2)	99.50(21-126)
Platelet(<100×10^9/L)	26 (76.5)	57(20-246)
C reactive protein (>10g/L)	8 (23.5)	21.53(0.5-78.77)
Alanine transaminase (>40U/L)	27 (79.4)	142.50(6-8754)
Aspartate transaminase (>40U/L)	33 (97.1)	209.00(36-14391)
Lactate dehydrogenase (>245U/L)	34 (100)	850.00 (200-13305)
Activated partial thromboplastin time (>38Sec)	12 (35.3)	34.00 (20.00-70.60)
Prothrombin time (>14Sec)	8 (23.5)	12.90(9.50-21.60)
Thrombin time (>21Sec)	12 (35.3)	19.45(10.10-64.9)
D dimer (>0.55mg/L)	30 (88.2)	5.03(0.2-94.97)
Fibrinogen (>1.5g/L)	20 (58.8)	1.40(0.50-6.04)
Triglyceride (>3.0mmol/L)	14 (41.2)	2.70(0.78-11.02)
Albumin (<35g/L)	22 (64.7)	33.1(22.60-50.00)
TBil(>17.0μmol/L)	12 (35.3)	9.55(2.4-118.6)
Ca^{2+}(<1.1mmol/L)	34 (100)	0.94(0.76-1.07)
Ferritin(>500μg/L)	33 (97.1)	14829.00 (348.7-58788)

Table 2. Laboratory data of EBV-HLH

Laboratory data	n/N	Percents (%)	Variables Median (min-max)
Soluble CD25 (>2400pg/ml)	26(26)	100	20148.00(7500,360865)
CD3+(<60%)	4(32)	12.5	80.69(47.50-97.00)
CD4+(<35%)	13(32)	40.6	37.12(4.30-52.34)
CD8+(>35%)	17(32)	53.1	37.48(11.70-90.00)
CD56+(<4%)	19(32)	59.4	3.40(0.20-31.10)
CD19+(<5%)	6(32)	18.8	13.24(0.70-15.29)
IL-10 (>4.91pg/ml)	14(14)	100	100.87(6.93-2500)
IL-6 (>5.30pg/ml)	8(14)	57.1	12.1(2.4-134.24)
IFN-γ (>7.42pg/ml)	12(14)	85.7	67.77(4.75-1194.37)
TNF-α (>4.6pg/ml)	12(14)	85.7	4.35(0.26-147.80)
IL-8 (>20.6pg/ml)	6(14)	42.9	19.15(4.30-880.58)
IgA (<0.20g/L)	10(29)	34.5	0.57(0.90-1.64)
IgG (<4.53 g/L)	6(29)	20.7	6.86(2.21-15.92)
IgM (<0.19g/L)	3(29)	3.4	0.70(0.13-2.89)
Cholesterin (>5.18mmol/L)	0(0)	0	2.64(1.66-5.06)
High density lipoprotein (<1.29mmol/L)	32(33)	97.0	0.38(0.12-1.36)
Low density lipoprotein (>3.37mmol/L)	0(33)	0	1.12(0.19-3.35)
Apolipoprotein AI (<1.08g/L)	32(33)	97.0	0.52(0.20-1.15)
Apolipoprotein B (<0.60g/L)	5(33)	15.2	0.76(0.28-1.35)
NK cell activity(≤15.11%)	8(24)	33.3	
CSFexamination (abnomality)	2(16)	12.5	
Gene analysis (abnormal gene)	2(23)	8.6	
CD107a excitation test (Abnormal degranulation function of NK or CTL cells)	7(10)	70.0	

Table 3. General situation analysis of deceased children

No.	Gender	Age	Number of confirmed days (days)	Chief complaint	Complication	Treatment Plan	Hospitalization treatment time (days)	Cause of death
1	Female	1	2	Fever for 15 days	Severe pneumonia, fungal infection, sepsis, severe immune deficiency, liver dysfunction, cardiac insufficiency, congenital heart disease	Dexamethasone, etoposide	49	Uncontrolled hemophagocytic syndrome, infection
2	Female	1	1	Fever for 15 days	Respiratory failure,liver dysfunction	Ventilator assisted ventilation, dexamethasone, blood purification	3	Uncontrolled hemophagocytic syndrome, respiratory failure
3	Man	1	9	Fever with cough for 4 days	Severe pneumonia, developmental delay, abnormal coagulation function, liver dysfunction	Dexamethasone, etoposide	35	Uncontrolled hemophagocytic syndrome, cumulative central nervous system

Table 4. Genetic analysis of other variants

No	Genetic variation	Related Diseases
1	CAR MIL2	Immunodeficiency type 58
2	MCM-3AP	Autosomal recessive peripheral neuropathy with or without impaired intellectual development
3	RASGRP1	Immunodeficiency type 64
4	GAT-A2	Immunodeficiency type 21, primary lymphedema with spinal cord dysplasia, susceptibility to acute myeloid leukemia, and susceptibility to myelodysplastic syndrome
5	SAM D9L1.	Ataxia pancytopenia (AD) - Chromosome 2.7 Monosomic myelodysplasia and leukemia syndrome type I (AD)
6	ITPK C	kawasaki disease
7	MCM3AP	Autosomal recessive peripheral neuropathy with or without impaired intellectual development
8	SCN9-A	1. Episodic severe pain 2. Primary erythematous acrodynia 3. Congenital hyperalgesia 4. Dravet syndrome 5. Systemic electrical wiring with febrile seizures
9	One variant of XIAP gene	Lymphatic hyperplasia syndrome X-linked type 2 (OMIM: 300635); Lymphatic hyperplasia syndrome X-linked type 1 (OMIM: 308240)
10	NLR-P12	Familial cold autoimmune response syndrome type 2
11	RAGI	Severe combined immunodeficiency (NK cell positive)

Table 5. Comparison between hazardous and non-hazardous restructurings

Clinical and Laboratory data	Critical group (n=15) Percents (%)	Non-critical group (n=19) Percents(%)	$Z/\chi^2/t$	*P* value
Sex	9(60.0)	8(42.1)	1.074	.300
Age (yr)[a]	1.25 (5.00)	1.75(4.00)	-0.44	.659
Days of diagnosis (days)[a]	3(5)	4 (4)	-0.842	.400
8-week survival	13(86.7)	18(94.7)		.571[c]
Treatment by etoposide	11(73.3)	8(42.1)	-	.091[c]
Haemophilic cells found	11(73.3)	13(68.4)	-	1.000[c]
Splenomegaly	13(86.7)	16(84.2)	-	1.000[c]
Hepatomegaly	12(80.0)	14(73.4)	-	1.000[c]
Edema of gallbladder wall	12(80.0)	12(63.2)	-	.451[c]
Leukocyte (×109/L)[a]	2.60(3.90)	2.70(2.50)	-0.170	.986
Neutrophil(×109/L)[a]	1.19(1.46)	0.79(0.95)	-1.388	.165
Platelet(×109/L)[a]	68(101)	53(47)	-0.747	.445
Alanine transaminase(U/L)[a]	366(512)	58(165)	-2.757	.006
Aspartate transaminase (U/L)[a]	420(1336)	97(251)	-3.136	.002
Lactate dehydrogenase(U/L)[a]	1691(6175)	747(311)	-2.549	.011
D dimer(mg/L)[a]	9.96(42.2)	3.85(5.54)	-2.098	.036
Fibrinogen(g/L)[a]	1.13(0.67)	1.65(1.19)	-2.654	.008
Albumin (35g/L)[b]	31.76(5.45)	35.99(6.16)	-2.092	.044
Ca^{2+}(mmol/L)[a]	0.91(0.08)	0.96(0.67)	-1.81	.080
Ferritin(μg/L)[a]	11696(42500)	2654(6055)	-2.445	.014
sCD25(pg/ml)[a]	32131(30861)	15010(14298)	-2.537	.011
CD8+(%)[b]	40.71(23.37)	39.52(18.59)	0.161	.873
CD56+(%)[a]	2.65(3.73)	3.52(2.86)	-0.969	.333
IL-10(pg/ml)[a]	95.51(923.27)	120.86(337.50)	-0.129	.897
IL-6(pg/ml)[a]	12.1(45.46)	14.06(30.58)	-0.517	.605
IFN-γ(pg/ml)[a]	28.91(631.29)	85.48(801.89)	-0.516	.606
TNF-α(pg/ml)[a]	4.52(68.67)	4.23(5.86)	-0.645	.519
High density lipoprotein (mmol/L)[a]	0.38(0.52)	0.37(0.40)	-0.615	.538
Apolipoprotein AI(g/L)[b]	0.62(0.29)	0.61(0.26)	0.112	.911
NK cell activity(≤15.11%)	3(25)	5(42)	-	.667[c]
CD107a excitation test (Abnormal degranulation function of NK or CTL cells)	4(66.7)	3(75)	-	1.000[c]

[a]Median (interquartile range)
[b]Mean value (standard deviation)
[c]Fisher exact test

There was no significant difference in the age of onset and the number of confirmed days between the two groups of children. The 8-week survival rate for critically ill patients was 86.7%, while the survival rate for non critically ill patients was 94.7%. The difference was not statistically significant. 73.3% of critically ill patients received etoposide chemotherapy, while 64.8% received non critically ill patients only antiviral, liver protective, dexamethasone, or methylprednisolone treatment. There was no statistically significant difference in treatment. Compared with non critical recombination, critical recombination significantly increases the levels of glutathione aminotransferase, glutathione aminotransferase, lactate dehydrogenase, D-dimer, fibrinogen, ferritin, and soluble CD25, with significant differences. The serum albumin of the critically ill group decreased significantly compared to the non-critically ill group, with a significant difference. The median value of the critically ill recombinant alanine aminotransferase was 366U/L, while the non critically ill group only had 58 U/L, which was statistically significant ($P < .05$); The median value of recombinant glutathione aminotransferase in the critically ill group was 420U/L, while in the non critically ill group it was only 978U/L, which was statistically significant ($P < .05$); The median value of lactate dehydrogenase for dangerous recombinant was 1691 U/L, while for non dangerous recombinant was 747 U/L, which was statistically significant ($P < .05$); The median of D-dimer in the critically ill group was 9.96 mg/L, while in the non critically ill group it was only 3.85 mg/L, which was statistically significant ($P < .05$); The median value of dangerous recombinant fibrinogen was 1.13g/L, while that of non dangerous recombinant fibrinogen was 1.65g/L, which was statistically significant ($P < .05$); The average serum albumin level of dangerous recombinant was 31.76g/L, while that of non dangerous recombinant was 35.99g/L, which was statistically significant ($P < .05$); The median ferritin in the critically ill group is 11696 μ G/L, instead of dangerous restructuring to 2654 μ G/L, with statistical significance ($P < .05$); The median of critical sCD25 was 32131pg/ml, while non critical recombination was 15010pg/ml, which was statistically significant ($P < .05$). As shown in Table 5.

High levels of lactate dehydrogenase, ferritin, and sCD25 are associated with a more severe disease course could help clinicians identify at-risk patients early.

For critically ill patients with EBV-HLH, the emphasis is often on immediate and aggressive treatment to stabilize their condition and address life-threatening complications. Treatment may include intensive supportive care, such as mechanical ventilation, hemodynamic support, and close monitoring in an intensive care unit. Additionally, these patients may require prompt initiation of immunosuppressive therapy, such as high-dose corticosteroids, etoposide, or other targeted therapies, to dampen the excessive immune response that characterizes HLH. Non-critically ill patients with EBV-HLH may have less severe symptoms and a lower risk of immediate complications. Treatment for these patients may involve a more gradual approach, starting with monitoring and observation to assess disease progression. Depending on the patient's response to initial treatment and evaluation of laboratory findings (such as ferritin levels, cytopenias, and organ function), escalation of therapy may be considered if needed.

Acknowledging the limitations of laboratory markers in the context of diagnosing and managing EBV-associated hemophagocytic lymphohistiocytosis (HLH) is essential for a comprehensive evaluation of patient data. Some key limitations and challenges to consider include: Laboratory markers such as ferritin, LDH, and sCD25 may have different normal ranges depending on the population studied and the laboratory methods used for analysis; Concurrent infections or underlying medical conditions can impact the levels of laboratory markers associated with HLH; Laboratory markers used for monitoring disease progression and treatment response may not always reflect the clinical status of the patient accurately; Interpreting laboratory markers in isolation without considering clinical context and other diagnostic findings can lead to misinterpretation and inappropriate decision-making.

DISCUSSION

Hemophagocytic lymphohistiocytosis is an excessive inflammatory response syndrome characterized by fever, hepatosplenomegaly, decreased blood cells, and the presence of hemophagocytic cells. It has inherited or acquired immune regulatory dysfunction. HLH has a variety of potential causes and lacks specific clinical manifestations, making it prone to misdiagnosis and missed diagnosis. The median survival time of untreated HLH does not exceed 2 months.[5] Genetic testing plays a crucial role in EBV-associated HLH, particularly in distinguishing between primary (familial) and secondary (acquired) forms of the disease. Finding UNC13D gene mutations can indicate primary HLH, potentially requiring genetic counseling and family member screening. In contrast, secondary HLH typically occurs in the presence of triggering factors like infections, tumors, or autoimmune diseases, without underlying genetic mutations. Tailoring treatment strategies based on genetic testing results can enhance treatment outcomes and prognosis for patients with EBV-related HLH.

EBV-HLH is considered the main subtype of secondary HLH. However, EBV is also considered a trigger or driving factor for familial HLH or selective genetic susceptibility.[6] In this study, 23 out of 34 pediatric patients underwent complete gene analysis, with 2 cases detected as UNC13D gene mutations, while the rest did not detect blood phagocytic-related genes. EBV-HLH is more common in Asian populations, especially in children. The main causes of EBV-HLH in children are severe inflammatory reactions and immune dysfunction after infection with EBV.[7] In this study, 53.1% of children showed a decrease in CD8+, and 59.4% showed a decrease in NK in lymphocyte subpopulation analysis. IL-10 levels increased in all 34 children, with 85.7% having IFN- γ,

TNF- α Increase in height. This indicates that the child has developed immune dysfunction and inflammatory reactions. A team of specialists, including pediatric hematologists, immunologists, and infectious disease experts, is important for treating EBV-associated HLH. They work together to provide comprehensive care, each focusing on specific aspects like blood issues, immune system function, and infection management. By collaborating and sharing expertise, these specialists can create personalized treatment plans and improve outcomes for patients with EBV-HLH.

HLH can cause varying degrees of liver dysfunction, with mild cases only showing elevated liver enzymes, while severe cases can lead to liver failure. JordanMB et al. believe that HLH is considered unusual if there is no liver dysfunction [8]. At present, the specific mechanism of liver dysfunction caused by HLH is not very clear. Kato J et al. showed through mouse experiments that TFN- α And INF- γ It can promote the formation of liver thrombosis, leading to ischemia and necrosis of liver cells, enhanced permeability of liver cell membranes, and the release of a large amount of ALT and AST into the bloodstream. Therefore, currently, most believe that liver damage in children with HLH is the result of excessive production of cytokines and infiltration of inflammatory cells. After infecting cells with EBV, it can cause lipid peroxidation, leading to excessive free radicals and abnormal activation of immune cells, leading to excessive production of cytokines and damage to liver cells. Tang Weiping et al. analyzed the characteristics of liver function damage in 92 HLH patients and found that all patients had varying degrees of liver function abnormalities, with the most prominent manifestations being an increase in LDH and a more significant increase in AST compared to ALT; In addition, there was a significant decrease in ALB (90.22%); Elevated TBIL, prolonged APTT, PT, and decreased FIB.[10] In this study, 34 cases (100%) of EBV-HLH had an increase in lactate dehydrogenase and 97.1% of the children had an increase in glutathione aminotransferase. This is consistent with previous research. Moreover, the levels of alanine aminotransferase, alanine aminotransferase, and lactate dehydrogenase in critically ill children were significantly higher than those in the non-critically ill group. In our study, the decrease in albumin was only 64.7% (median 33.1g/L), but it was significantly reduced in high-risk recombination. Perhaps because hypoproteinemia is also mediated by cytokines.[11] The cytokine storm in critically ill children is more severe, causing functional damage to multiple organs. Shu Cheng Huang et al. found that hypoalbuminemia is an independent risk factor for EBV-HLH in children. Therefore, it may be considered as an early diagnostic clue for EBV-HLH patients.[12] In our study, most TBIL levels were not significantly elevated, with only about one-third of the children experiencing prolonged APTT and PT. In EBV-associated HLH, a cytokine storm causes severe inflammation and tissue damage. Targeting this imbalance with treatments like cytokine blockers and immunomodulators can help improve patient outcomes by reducing inflammation and stabilizing the immune response. Early intervention with these therapies can control the disease, prevent complications, and enhance long-term prognosis for patients with EBV-HLH.

The level of D-dimer may be a useful indicator of the likelihood of HLH in children infected with EBV.[13] D-dimer mainly reflects the fibrinolytic function, and its elevation indicates the body's hypercoagulable state and secondary hyperfibrinolysis. It is also a monitoring indicator for severe infections.[14] In this study, 88.2% of children had an increase in D-dimer, with more significant risk recombination. It can serve as a reference indicator for the severity of EBV-HLH. It can be hard to tell EBV-HLH apart from other illnesses because they have similar symptoms. Lab tests help by showing specific patterns in the blood that are common in EBV-HLH. These tests can include markers like high ferritin levels, triglycerides, and sCD25. Checking for EBV levels, immune cell issues, and cytokine levels can also help confirm the diagnosis. Using these lab tests helps doctors make the right diagnosis and provide better care for patients.

High triglycerides and low fibrinogen also play important roles in the diagnosis of HLH. Consistent with the study by Tang Weiping et al.,[10] low fibrinogen is more common in EBV-HLH, and the reduction of risk recombination is more significant. Consider a more severe correlation with liver cell damage in critically ill children. Only 42.1% of children showed triglycerides>3mmol/L. Therefore, high triglycerides have a relatively small role in the early diagnosis of EBV-HLH in children. Researchers are looking into new biomarkers to improve the diagnosis of EBV-HLH. These markers could offer better accuracy and help distinguish EBV-HLH from other similar conditions. Examples include sIL-2Rα, IL-18, and unique genetic or immune markers. Using these new biomarkers may lead to earlier detection, less misdiagnosis, and improved treatment outcomes for patients with EBV-HLH. This research may reshape how EBV-HLH is diagnosed and treated in the future.

Ferritin is a useful inflammatory marker associated with disease activity. It can significantly increase in malignant tumors, severe infections, rheumatism, liver or kidney diseases, etc. Luo Xin et al.[15] analyzed the clinical data of 58 cases of HLH and 351 cases of non HLH children. The results showed that the increase in serum ferritin was much greater in HLH patients than in other diseases. The optimal cutoff value for serum ferritin was 4427.00 μ G/L, the specificity for diagnosing HLH in children is 88.03%. In our study, 97.1% of children had an increase in ferritin, with a median of 14829 μ G/L, far greater than the diagnostic standard of>500 μ G/L. Moreover, the increase in dangerous recombinant ferritin is more significant, so we believe the magnitude of ferritin increase positively correlates with the severity of EBV-HLH.

The soluble IL-2 receptor (sCD25) is a valuable indicator for diagnosing HLH and evaluating disease activity, which is usually high in HLH patients. We know that HLH is mainly driven by T cell overactivation, and sCD25 represents T cell activity, so sCD25 can serve as a marker of HLH disease

activity. Jordan et al.[16] also mentioned the primary position of sCD25 as an active marker of HLH disease. It's important to follow up with survivors of EBV-HLH to watch for any long-term effects of the disease and its treatment. This includes looking out for issues like secondary cancers or chronic liver problems. Regular check-ups help doctors catch these problems early. Personalized follow-up plans, open communication, and patient education are key in monitoring survivors' health over time and ensuring they receive the necessary care.

The research results of Thomas Wimmer et al.[17] suggest that sCD25 may be a useful marker for the prognosis of HLH patients, which can aid in hierarchical treatment interventions. In our study, sCD25 was tested in 26 children, and it was 100% elevated (7500-360865 pg/ml), significantly above the critical value of 2400 pg/ml, and significantly increased in the high-risk group compared to non-high-risk group. The results are consistent with those of Jordan et al. and Thomas Wimmer et al.

The application prospects of the new method in the medical field include:1) Improvement of diagnostic techniques, Providing clues to enhance the accurate diagnosis of pediatric EBV-HLH; 2) Data analysis and processing: Utilizing big data analytics and artificial intelligence technology to achieve faster and more accurate patient diagnosis and prediction; 3) Improvement of treatment strategies: Offering targeted treatment strategies to help improve the efficacy of patients' treatment; 4) Remote monitoring and tracking: Leveraging Internet of Things technology and mobile healthcare applications to enable remote monitoring of patients and provide real-time medical consultation. These engineering application prospects contribute to enhancing the diagnosis and treatment effectiveness of pediatric EBV-HLH, thus providing better medical services and care for patients. Looking back at our study, we see limitations like looking at past data and having a small group of patients. For future research:1. We need to do studies that follow patients over time to confirm our results. 2. Studying new treatment options can help in managing EBV-HLH better.3. Long-term studies on survivors' outcomes are important to understand how the disease affects them in the long run. By working on these areas, we can improve care and outcomes for EBV-HLH patients.

CONCLUSION

EBV-HLH has a higher survival rate in children, and early diagnosis and targeted treatment can improve prognosis. Early detection and attention to abnormal laboratory test results can improve the accuracy of diagnosis, especially for critically ill children, which can reduce the occurrence of adverse consequences.

Our study adds to what we know about EBV-HLH in children and how it can be managed in practice. We focused on markers like ferritin and sCD25, showing their importance in diagnosing EBV-HLH early. This can help doctors make better decisions for children with this condition. Our research highlights the significance of these markers and clinical signs in detecting EBV-HLH sooner, which can lead to better outcomes for patients. By emphasizing these points, we aim to improve the care and outcomes of children with EBV-HLH. The findings can help improve how EBV-HLH is treated in the future. By focusing on important markers like ferritin and sCD25, we can develop more targeted treatment approaches. This could lead to better outcomes for children with EBV-HLH. By using these markers to guide treatment decisions, healthcare providers can create more personalized and effective strategies for managing the condition.

To manage EBV-HLH effectively, a team of specialists from various fields must work together. This teamwork ensures that different aspects of the condition are addressed comprehensively, leading to better care and outcomes for affected children. By combining expertise from pediatricians, hematologists, immunologists, and infectious disease specialists, healthcare teams can provide tailored treatment plans and holistic care for patients with EBV-HLH.

This study is a retrospective, single-construct study with a small number of cases and certain limitations. I hope to conduct large-scale local research in multiple centers in the future to provide more valuable clinical diagnoses and treatment bases. Future research should include large studies across multiple centers to confirm our findings on EBV-HLH. It is important to validate the markers we identified and explore new treatments. Long-term studies on EBV-HLH survivors are needed to improve care.

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