Yan *et al. Virology Journal* (2025) 22:113
https://doi.org/10.1186/s12985-025-02741-7

Virology Journal

RESEARCH Open Access

Active EBV infection in children: associations between DNA load, infection status, immune status, and disease severity

Haipeng Yan[1†], Xun Li[2†], Xiaohui Zeng[1], Ting Luo[2], Xiao Li[3,4], Longlong Xie[2], Xiangyu Wang[2], Yufan Yang[3], Lan Luo[1], Xiulan Lu[3*] and Zhenghui Xiao[3*]

Abstract

Background This study investigated active Epstein-Barr virus (EBV) infection in children and examined the associations among EBV deoxyribonucleic acid (DNA) load, infection types, disease severity, and immune characteristics.

Methods A total of 35,956 pediatric patients who underwent EBV DNA load testing were included. Patients were categorized based on their EBV DNA levels and infection status.

Results Spearman's rank correlation analysis revealed a positive association between EBV DNA levels and the mortality rate, as well as the incidence rates of acute kidney injury (AKI), respiratory failure, cardiovascular complications, coagulation abnormalities, and liver injury. Mortality risk significantly increased when EBV DNA exceeded 1×10^5 copies/mL (adjusted odds ratio: 10.53, 95% confidence interval: 2.38–46.59, $P < 0.05$). As EBV DNA levels increase, the rise in mortality rate during activation- immunoglobulin G (IgG^+) was more pronounced than that observed during primary infections. Gaussian mixture model clustering identified two immune clusters. Cluster 0 exhibited elevated pro-inflammatory indicators (IFN-γ, IL-6) and anti-inflammatory indicator (IL-10) levels, along with reduced immune cell counts. This cluster showed higher activation-IgG^+ and mortality rates compared with Cluster 1.

Conclusions An elevated EBV DNA load ($> 1 \times 10^5$ copies/mL) in children is associated with increased mortality risk. High pro-inflammatory and anti-inflammatory states, coupled with low immune cell numbers, indicate critical condition. Simultaneous examinations of EBV DNA, antibodies, and immune status are recommended, especially for children with EBV DNA $> 1 \times 10^5$ copies/mL, emphasizing the need for caution in those with activation-IgG^+ and immune dysregulation.

Keywords Epstein-Barr virus, Immune response, Immune dysregulation, Epstein-Barr virus DNA load

[†]Haipeng Yan and Xun Li contributed equally and should be considered as co-first authors.

*Correspondence:
Xiulan Lu
13787252674@163.com
Zhenghui Xiao
xiaozhenghui888@163.com

Full list of author information is available at the end of the article

Background

Epstein-Barr virus (EBV), a member of the gammaherpesvirus family, is a widely prevalent herpesvirus [1] infecting over 90% of adults worldwide. EBV infection commonly occurs during childhood [2–4]. Once infected, EBV establishes persistent infection in B lymphocytes and epithelial cells, typically in a latent form. A significant characteristic of EBV is its ability to reactivate multiple times after the initial infection, leading to secondary or recurrent infections. Both primary infection and reactivation of EBV can result in severe diseases, such as infectious mononucleosis (IM), lymphohistiocytosis (HLH), sepsis, and lymphoma [4–6]. EBV infection can also cause severe damage to distant organs, such as the liver, kidney, respiratory, and nervous system. This organ damage primarily arises from immune-mediated damage caused by lymphocyte infiltration and cytokine release post-EBV infection [4, 6–8], indicating disease progression and increasing the risk of life-threatening complications. Therefore, identifying an effective method to recognize the critical status of EBV infection is crucial for timely intervention to prevent and reduce the risk of severe conditions associated with infection.

EBV is typically diagnosed by detecting EBV-specific antibodies and measuring deoxyribonucleic acid (DNA) loads in body fluids. An elevated EBV DNA load is often associated with severe EBV-related diseases, such as HLH [9], lymphoma [10], and nasopharyngeal carcinoma [11, 12]. Patients with high EBV DNA loads often exhibit more severe disease symptoms and poorer prognosis. A study on lymphoma [13] has shown that patients who are EBV DNA positive have lower progression-free survival and overall survival rates. Reportedly, high EBV DNA levels negatively impact the immune system, contributing to poor outcomes. For instance, a study on the coronavirus disease 2019 (COVID-19) has shown that higher EBV DNA loads correlate with lower immune cells, particularly $CD8^+$ T cells and natural killer (NK) cells, and increased disease severity [14]. However, research on the clinical value of EBV DNA load in pediatric EBV-related diseases remains limited. Although EBV DNA levels have been linked to prognosis in diseases such as HLH, lymphoma, and nasopharyngeal carcinoma, key questions remain unanswered. Specifically, the direct association between EBV DNA load and the risk of organ damage or poor prognosis remains unclear. Additionally, it is unclear whether a specific EBV DNA threshold can reliably guide clinical assessments regarding disease severity. In populations with activated EBV DNA, disease severity differs between patients with primary infection and those with viral reactivation. EBV infection has a synergistic pathogenic effect, leading to immune system damage. Conversely, preexisting immune disorders can trigger EBV reactivation and worsen the clinical condition. This raises the question: Is specific immune status associated with adverse outcomes in individuals with EBV activation?

Therefore, we conducted a study on hospitalized children who underwent EBV testing results to investigate the clinical significance of EBV DNA load in pediatric EBV-related diseases. However, the definition of EBV reactivation remains unclear. In this study, positive EBV immunoglobulin G (IgG) antibodies were used as indicates of EBV reactivation. We analyzed the relationship between EBV DNA levels and organ injury or adverse outcomes during hospitalization in children and explored the differences between primary infection and activation-IgG^+. Additionally, we explored whether a specific immune profile was associated with adverse outcomes in individuals with EBV activation. A comprehensive analysis of these factors provides novel scientific evidence to enhance diagnostic accuracy and treatment strategies.

Methods

Study population

A retrospective analysis was conducted on clinical data from pediatric patients admitted to Hunan Children's Hospital between January 2017 and August 2023 who undergone EBV DNA testing. The inclusion criteria included (1) age < 18 years, and (2) underwent EBV DNA testing with complete clinical data. The study was approved by the Medical Ethics Committee of Hunan Children's Hospital (HCHLL-2022-50) and adhered to the ethical standards outlined in the Helsinki Declaration of 1964 and its subsequent amendments or similar ethical standards. The requirement for written informed consent was waived by the Medical Ethics Committee of the Hunan Children's Hospital.

Measurement of EBV DNA, EBV antibodies, cytokines, lymphocyte subgroups, and liver function markers

Plasma EBV DNA levels were assessed by real-time fluorescence-based quantitative polymerase chain reaction (PCR) using an EBV DNA kit (Sansure Biotech Inc., Ltd of Changsha, Hunan Province, China). Plasma EB viral capsid antigen (VCA) immunoglobulin M (IgM), VCA IgG, early antigen (EA) IgM, and nuclear antigen (NA) IgG levels were measured via chemiluminescence immunoassay using an EBV antibody kit (Shenzhen YHLO Biotech Co, Ltd, China). Serum interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ levels were measured using multiplex bead-based flow cytometry with the cytokine detection kit (Qindao Raisecare Biological Technology Co., Ltd, China). Lymphocyte subsets, including NK cells ($CD3^-$/$CD16^+CD56^+$), total B lymphocytes ($CD3^-CD19^+$), total T lymphocytes ($CD3^+CD19^-$), helper/inducible T lymphocytes ($CD3^+CD4^+$), inhibitory/cytotoxic T lymphocytes (CTLs) ($CD3^+CD8^+$) and T lymphocytes + B

lymphocytes + NK cells were measured by cytometry using a lymphocyte subsets kit (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China). Liver function markers—serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels—were measured using an automated biochemical analyzer with a liver function test kit (Beijing Jiuqiang Biotech, China). The testing procedures were performed by the Laboratory Department of Hunan Children's Hospital following a standardized protocol.

Definition of EBV infection status

EBV infection states were classified based on established criteria [6, 15]: primary infection was defined as (1) the presence of VCA IgM, (2) low-affinity VCA IgG, or (3) a negative result in the EBV antibody test for both VCA IgM and VCA IgG with detection of EBV-DNA level > 400 copies/mL in plasma. Past infection was defined as the presence of high-affinity VCA IgG and/or IgG antibodies against EBV NA (EBNA). Activation-IgG$^+$ was identified as a positive EBV antibody test for VCA IgG, indicating a past infection, along with an EBV-DNA level exceeding 400 copies/mL or the presence of EBV-VCA IgM in plasma. "Unknown status" was defined as patients who had not undergone EBV antibody testing. No-infection was defined as a negative result in the EBV antibody test for both VCA IgM and VCA IgG, with an EBV-DNA level ≤ 400 copies/mL in plasma.

Group definitions

All patients were categorized into six groups based on EBV DNA levels: 1) < 400 copies/mL; 2) > 400 copies/mL and $\leq 1 \times 10^3$ copies/mL; 3) $> 1 \times 10^3$ copies/mL and $\leq 1 \times 10^4$ copies/mL; 4) $> 1 \times 10^4$ copies/mL and $\leq 1 \times 10^5$ copies/mL; 5) $> 1 \times 10^5$ copies/mL < and $\leq 1 \times 10^6$ copies/mL; and 6) $> 1 \times 10^6$ copies/mL. Additionally, patients were further classified into three groups based on their infection status: primary infection group, activated infection (activation-IgG$^+$ group), and the unknown status. Patients were also stratified into survival and death groups based on post-discharge outcomes.

Data collection

Information on underlying and accompanying diseases during hospitalization was extracted from the medical records. Underlying diseases included IM, sepsis, HLH, pneumonia, chronic active EBV (CAEBV), lymphoma, and immunodeficiency. Accompanying diagnoses included toxic encephalopathy, acute kidney injury (AKI), respiratory failure, acute respiratory distress syndrome (ARDS), myocardial injury, heart failure, shock, coagulation dysfunction, disseminated intravascular coagulation (DIC), and liver dysfunction. The diagnosis of CAEBV followed [16]: (1) persistent or recurrent IM-like symptoms for over 3 months; (2) detection of EBV antibodies (EBV-CA and EBV-EA) in tissues or peripheral blood samples, positive cells for EBV-encoded small RNA (EBER) in tissues, or EBV-DNA levels > $10^{2.5}$ copies/mL in plasma and whole blood; and (3) absence of identifiable underlying immunodeficiency disease. The patients were required to meet al.l three criteria for the diagnosis of CAEBV. Lymphoma diagnosis was based on histopathological examination and immunohistochemical analysis according to the 2001 World Health Organization classification of lymphomas [17]. Other diseases were defined as patients who were not diagnosed with any of the following: IM, sepsis, HLH, pneumonia, CAEBV, or lymphoma. The diagnosis of AKI followed the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines, defined as the occurrence of any of the following [18]: (1) an increase in serum creatinine (SCr) by ≥ 0.3 mg/dL (26.5 μmol/L) within 48 h; (2) a known or presumed increase in SCr to 1.5 times the baseline within 7 days; (3) urine output < 0.5 mL/kg/h for 6 h. Respiratory failure was defined as a partial pressure of oxygen (PaO_2) < 60 mmHg in ambient air, diagnosed according to the Berlin definition for ARDS [19]. Myocardial injury was defined as any elevation in cardiac troponin T, I or creatine kinase (CK)-MB. Heart failure met the criteria outlined in the 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure [20]. Shock was defined by low blood pressure requiring either vasopressor, inotropic support, or inadequate tissue perfusion [21, 22]. Coagulopathy was defined as a platelet count $< 80 \times 10^9$/L or a 50% decrease in platelet count from the highest value within 3 days (for patients with chronic hematologic/tumor) or an international normalized ratio (INR) > 2. Liver dysfunction was defined as total bilirubin ≥ 4 mg/dL (not applicable for newborns) or ALT ≥ 2 times the upper limit of normal for age, in accordance with established criteria [23]. Accompanying symptoms were categorized into six groups based on the affected system: (1) renal injury: AKI; (2) respiratory system injury: respiratory failure, ARDS; (3) circulatory system injury: myocardial injury, heart failure, and shock; (4) coagulation dysfunction: DIC; (5) liver injury: liver dysfunction; and (6) immunological dysfunction: cytokine abnormalities and lymphocyte subgroup imbalances at the time of patient admission.

Statistical analysis

Categorical variables were presented as frequencies and percentages. Between-group comparisons of categorical variables were performed using the chi-square test or Fisher's exact test, depending on data distribution. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association among

EBV DNA levels, infection status, and mortality rate. Spearman's rank correlation test was used to analyze the correlation between changes in EBV DNA levels, mortality rates, and organ damage. All hypothesis tests were two-tailed, with a type 1 error rate set at 5%. Multiple comparisons were adjusted using the Bonferroni method. All statistical analyses were performed using IBM SPSS Statistics for Windows version 25 (IBM Corp., Armonk, N.Y., USA) and Python software version 3.10 (Python Software Foundation, Beaverton, OR, USA), with data visualizations created using Python (Python Software Foundation) and GraphPad version 9.0 (San Diego, CA, USA).

To explore potential associations between immune indicators, prognosis, and infection status, we conducted clustering analysis on patients who underwent comprehensive immune parameter testing, including IFN-γ, IL-10, IL-6, IL-4, IL-2, and TNF-α levels; NK counts ($CD3^-/CD16^+CD56^+$); total B lymphocytes ($CD3^-CD19^+$); total T lymphocytes ($CD3^+CD19^-$); helper/inducible T lymphocytes ($CD3^+CD4^+$); inhibitory/CTLs ($CD3^+CD8^+$); and T lymphocytes + B lymphocytes + NK cells. For patients with complete immune indicators, a random split into training and validation sets was conducted at a ratio of 0.5: 0.5. Gaussian Mixture Model (GMM) clustering analysis was applied, with results visualized using principal component analysis. Clinical characteristics, infection status, and prognosis were compared among different clusters.

Results

Patients characteristics

This study included 35,956 pediatric patients (Fig. 1). The distribution of EBV DNA copy numbers was as follows: 90.34% had < 400 copies/mL, 1.84% had 400–1×10^3 copies/mL, 3.87% had 1×10^3 – 1×10^4 copies/mL, 2.12% had 1×10^4 – 1×10^5 copies/mL, 1.21% had 1×10^5 – 1×10^6 copies/mL, and 0.61% had > 1×10^6 copies/mL. The male-to-female ratio was 1.55: 1, and the median age at admission was 4.41 years (ranging: 0.02–17.98 years). Table 1 shows the characteristics of the study population according to EBV DNA copy numbers. The incidence of HLH and IM increased with higher EBV DNA loads ($P<0.001$). Regarding prognosis, the distribution of patients with a high viral load (> 10^5 copies/mL) was higher in the mortality group compared with the survival group ($P<0.001$). Patients with EBV DNA > 1×10^4 copies/mL had a higher pediatric intensive care unit admission rate than those with lower loads; however, the difference was not statistically significant (Table 1). The distributions of infection status in different age and sex groups are presented in Additional Table 1.

EBV DNA levels in different age groups and disease groups

Further analysis was conducted on patients with EBV DNA levels > 400 copies/mL. Patients aged 5–12 years and 12–18 years exhibited lower EBV DNA levels than those aged 1–5 years ($P<0.001$) (Fig. 2A). Among the different disease groups, the CAEBV group had the highest EBV DNA levels, with significant statistical differences compared with the pneumonia and other diagnostic groups ($P<0.001$). The HLH group also exhibited statistically statistical differences compared with the IM, pneumonia, and other diagnostic groups ($P<0.001$) (Fig. 2B).

Distribution of infection status in different diseases

Figure 2C shows the distribution of infection status among the different disease groups. The proportion of activation-IgG^+ decreased sequentially in CAEBV (100%), IM (50.53%), HLH (40.05%), sepsis (15.06%), pneumonia (13.37%), immunodeficiency (7.41%), other diagnosis (4.62%), and lymphoma (4.55%) (Fig. 2C). The pathological types of lymphoma are presented in Additional Table 2.

ALT and AST levels in patients with different EBV DNA loads

Significant differences in AST and ALT levels were observed between patients with different EBV DNA load (AST: H = 1,188.2, $P<0.001$; ALT: H = 1,291.75, $P<0.001$). Both AST and ALT levels increased with increasing EBV DNA levels. Stratified by EBV DNA levels (< 400, 400–1×10^3, 1×10^3 – 1×10^4, 1×10^4 – 1×10^5, 1×10^5 – 1×10^6, > 1×10^6 copies/mL), the median AST levels [M (P_{25}, P_{75}), U/L] were 29.0 (21.6, 42.8), 36.35 (25.7, 69.05), 40.1 (25.9, 75.5), 45.5 (29.4, 93.0), 57.25 (35.7, 129.55), and 88.4 (45.5, 170.3), while the ALT levels [M (P_{25}, P_{75}), U/L] were 18.3 (12.7, 30.7), 27.65 (15.12, 85.52), 29.0 (16.0, 95.41), 36.0 (17.3, 115.25), 61.55 (26.32, 183.0), and 108.8 (34.5, 243.0) (Fig. 2D, E).

Associations between EBV DNA load levels, infection status, and outcomes

Patients with EBV activation-IgG^+ (1.45%) exhibited a higher mortality rate than those with a primary infection (0.62%). Compared with the primary infection group, the adjusted OR (adOR) for the activation-IgG^+ group was 2.48 (95%CI: 1.04–5.89, $P=0.04$). Children with EBV DNA levels of > 1×10^5 copies/mL had a significantly higher risk of mortality. Compared with the 400–1×10^3 copies/mL group, the adOR for the 1×10^5 – 1×10^6 copies/mL group was 11.02 (95%CI: 2.49–48.72, $P=0.002$), while for the > 1×10^6 copies/mL group, the adOR was 12.53 (95%CI: 2.64–59.47, $P=0.002$) (Table 2).

Regarding the correlation between EBV DNA load levels and mortality among patients with EBV DNA activation, a sharp increase in mortality was observed when the

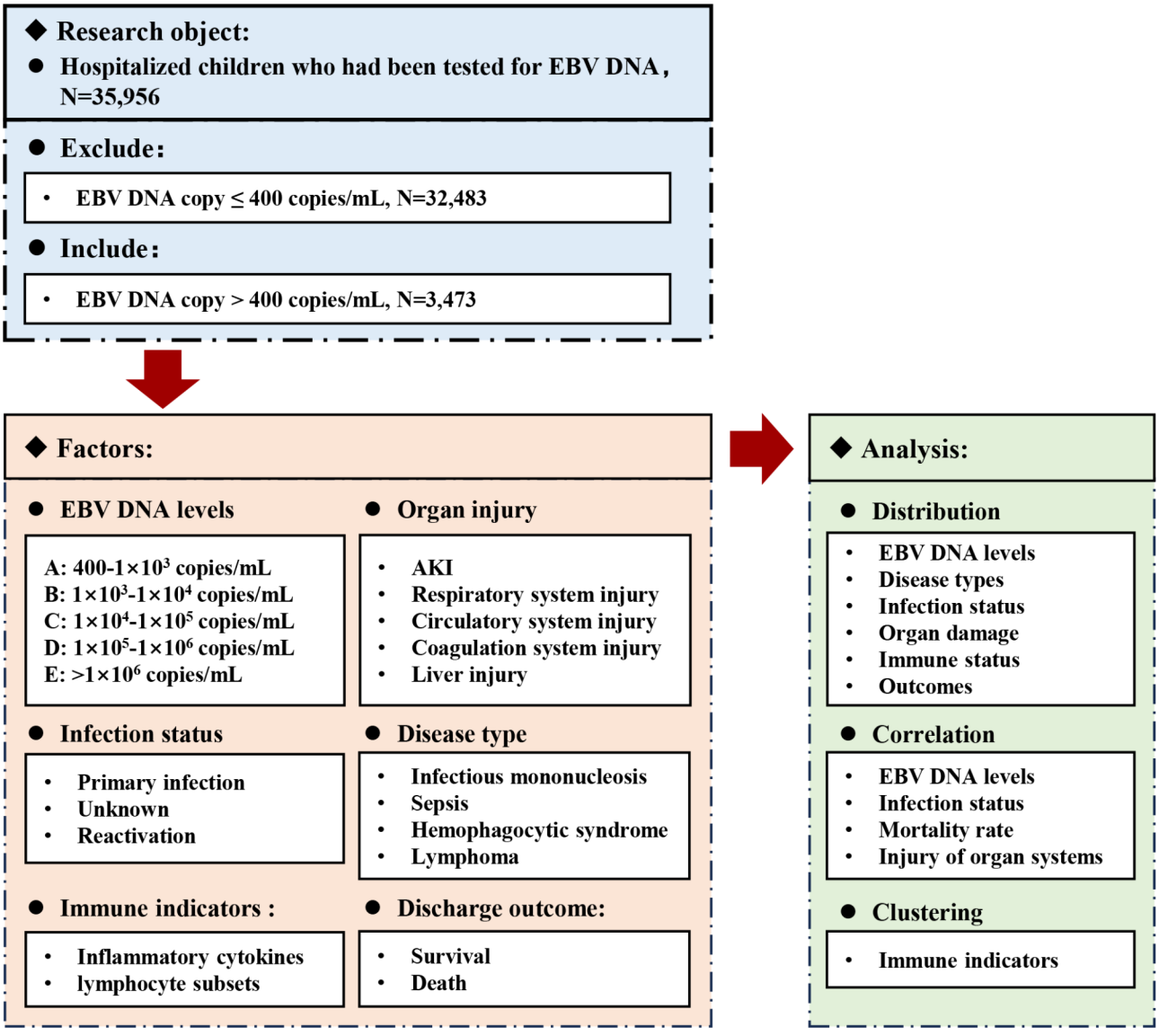

Fig. 1 Study flow chart

EBV DNA was $>1\times10^5$ copies/mL (Fig. 2F). Compared with patients with EBV DNA 400 – 10^3 copies/mL, those with EBV DNA 1×10^5 – 1×10^6 copies/mL had an adOR of 10.53 (95% CI: 2.38–46.59, $P=0.002$) (Table 2). Moreover, the increased rate of mortality in the activation-IgG$^+$ group was higher than that in the primary infection group, with an adOR of 2.48 (95% CI: 1.04–5.89, $P=0.04$) (Table 2).

Mortality rates and incidence of organ injury across different EBV DNA load groups and infection status

Among children with EBV DNA copy > 400 copies/mL, the incidence rates of liver injury, cardiovascular system injury, coagulation system injury, respiratory system injury and AKI were 18.83%, 4.38%, 4.32%, 2.16% and 0.92%, respectively. Spearman's rank correlation trend test analysis indicated that the mortality rate and incidences of respiratory, cardiovascular, coagulation, and liver injuries were positively correlated with EBV DNA load levels ($P<0.05$) (Fig. 3). Among children with primary infection, the mortality rate and coagulation system injury positively correlated with EBV DNA load ($P<0.05$). Respiratory system, cardiovascular system, and liver injuries also showed an increasing trend with higher EBV DNA load levels, although the *P*-values were insignificant ($P>0.05$). Among children with EBV activation-IgG$^+$, the mortality rate and incidences of AKI, respiratory system injury, and coagulation system injury positively correlated with EBV DNA load levels ($P<0.05$). Cardiovascular and liver injuries also showed an increasing trend with higher EBV DNA load levels, although the P-values were insignificant ($P>0.05$) (Fig. 3).

Table 1 General characteristics of the population, N (%)

Variables	*N*	EBV DNA copies/mL, *n* (%)						*P*
	35,956	< 400	400-1×10^3	1×10^3-1×10^4	1×10^4-1×10^5	1×10^5-1×10^6	> 1×10^6	
		n = 32,483	*n* = 663	*n* = 1,393	*n* = 764	*n* = 434	*n* = 219	
Age								<0.001
<1 year	5,025	4,891 (15.06)	25 (3.77)	54 (3.88)	31 (4.06)	19 (4.38)	5 (2.28)	
1–5 years	14,785	12,978 (39.95)	293 (44.19)	672 (48.24)	466 (60.99)	244 (56.22)	132 (60.27)	
5–12 years	13,472	12,066 (37.15)	312 (47.06)	611 (43.86)	247 (32.33)	158 (36.41)	78 (35.62)	
12–18 years	2,674	2,548 (7.84)	33 (4.98)	56 (4.02)	20 (2.62)	13 (3.0)	4 (1.83)	
Sex								0.510
Female	14,111	12,717 (39.15)	248 (37.41)	561 (40.27)	314 (41.1)	182 (41.94)	89 (40.64)	
Male	21,845	19,766 (60.85)	415 (62.59)	832 (59.73)	450 (58.9)	252 (58.06)	130 (59.36)	
Infection Status								<0.001
No-infection	8,269	8,269 (25.46)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Past-infection	6,722	6,722 (20.69)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Primary Infection	969	350 (1.08)	113 (17.04)	200 (14.36)	125 (16.36)	125 (28.8)	56 (25.57)	
Unknown	2,547	16,568 (51.01)	215 (32.43)	382 (27.42)	167 (21.86)	79 (18.2)	38 (17.35)	
Activation-IgG$^+$	17,449	574 (1.77)	335 (50.53)	811 (58.22)	472 (61.78)	230 (53.0)	125 (57.08)	
Diseases								
IM	1,510	347 (1.07)	170 (25.64)	391 (28.07)	224 (29.32)	236 (54.38)	142 (64.84)	<0.001
Sepsis	1,890	1,633 (5.03)	26 (3.92)	78 (5.6)	75 (9.82)	42 (9.68)	36 (16.44)	<0.001
HLH	405	193 (0.59)	15 (2.26)	47 (3.37)	66 (8.64)	53 (12.21)	31 (14.16)	<0.001
Pneumonia	14,264	12,942 (39.84)	249 (37.56)	524 (37.62)	325 (42.54)	149 (34.33)	75 (34.25)	0.012
CAEBV	17	0 (0.0)	2 (0.3)	1 (0.07)	4 (0.52)	5 (1.15)	5 (2.28)	<0.001
Lymphoma	261	240 (0.74)	4 (0.6)	8 (0.57)	6 (0.79)	2 (0.46)	1 (0.46)	0.931
Immunodeficiency	64	61 (0.19)	0 (0.0)	2 (0.14)	0 (0.0)	0 (0.0)	1 (0.46)	0.474
Others diagnosis	19,583	18,393 (56.62)	280 (42.23)	552 (39.63)	245 (32.07)	90 (20.74)	23 (10.5)	<0.001
Department at admission								0.094
General ward	33,988	30,709 (94.54)	635 (95.78)	1,325 (95.12)	710 (92.93)	407 (93.78)	202 (92.24)	
PICU	1,968	1,774 (5.46)	28 (4.22)	68 (4.88)	54 (7.07)	27 (6.22)	17 (7.76)	
Outcome								<0.001
Survival	35,662	32,227 (99.21)	661 (99.7)	1,385 (99.43)	758 (99.21)	420 (96.77)	211 (96.35)	
Death	294	256 (0.79)	2 (0.3)	8 (0.57)	6 (0.79)	14 (3.23)	8 (3.65)	

EBV, Epstein-Barr virus; **IM**, infectious mononucleosis; **HLH**, hemophagocytic lymphohistiocytosis; **CAEBV**, chronic active Epstein-Barr virus infection

GMM clustering analysis of immunological indicators

This study included 323 patients with complete data on immunological indicators. These patients were split into a training set (50%) and a validation set (50%) for GMM clustering analysis of immunological indicators to explore infection status and prognosis in populations with different immune characteristics. Two clusters, Cluster 0 and 1, were identified in the training set. Table 3 summarizes the general characteristics of the two clusters. In Cluster 0 of the training set, the immune status presented elevated pro-inflammatory indicators (IFN-γ, IL-6) and anti-inflammatory indicator (IL-10) levels compared with Cluster 1. The immune cell counts in Cluster 0, including NK cells, total B lymphocytes, helper/inducer T lymphocytes, inhibitory/CTLs, total T lymphocytes, and T + B + NK cells, were lower than those in Cluster 1 (Fig. 4). The immune profiles of both clusters in the validation set resembled those in the training set (Fig. 4).

Compared with Cluster 1, Cluster 0 demonstrated higher mortality rates in both the training and validation sets (Table 3). In the training set, the activation-IgG$^+$ rates for Cluster 0 and 1 were 82.61% and 44.35%, respectively ($P<0.05$), and the mortality rates were 23.91% and 0.87%, respectively ($P<0.05$). In the validation set, the activation-IgG$^+$ rates for Cluster 0 and 1 were 59.26% and 57.04%, respectively ($P=0.775$), and the mortality rates were 37.04% and 2.22%, respectively ($P<0.05$) (Table 3). In the training set, compared with the primary infection group, the activation-IgG$^+$ group had an adOR of 2.48 (95% CI: 1.04–5.89) for developing the immune status of Cluster 1 ($P<0.05$). No statistically significant differences were observed in this correlation in the validation set (Table 4). In both the training and validation sets, Cluster 0 was associated with a higher risk of death than Cluster 1 ($P<0.05$, Table 5).

In all infection status groups, Cluster 0 exhibited higher mortality rates than Cluster 1. In the primary infection group, the mortality rates for Cluster 0 and 1 were 18.18% and 0%, respectively ($P=0.016$). In the group with unknown infection status, the mortality rates for Cluster

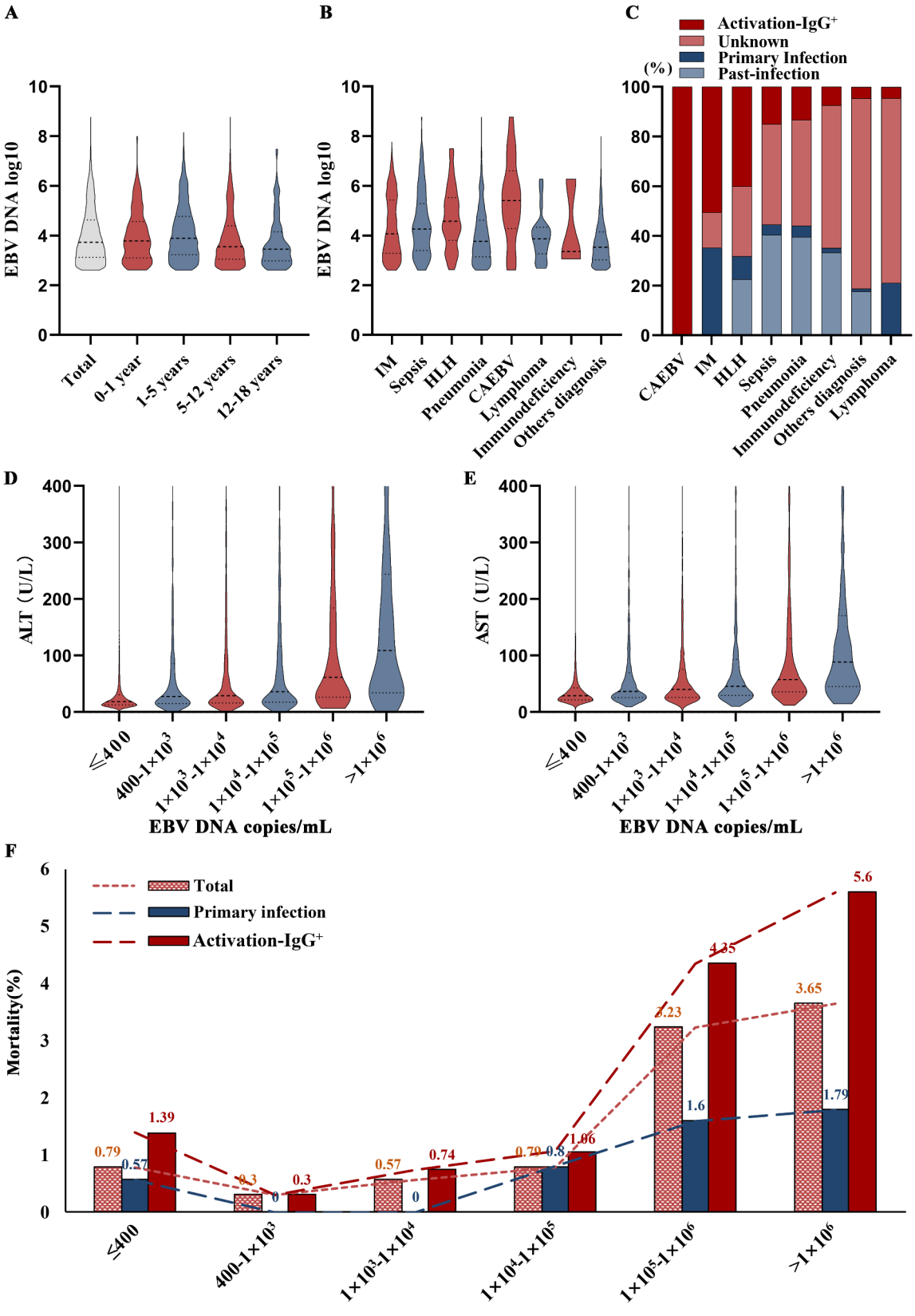

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Distributions of EBV DNA load, infection status, and mortality. (**A**) EBV DNA levels in different age groups. 1–5 years group vs. 5–12 years group, $P < 0.001$; 1–5 years group vs. 12–18 years group, $P < 0.001$. (**B**) EBV DNA levels in different disease types. IM vs. HLH, $P < 0.001$; IM vs. pneumonia, $P < 0.001$; IM vs. others diagnosis, $P < 0.001$; sepsis vs. pneumonia, $P < 0.001$; sepsis vs. others diagnosis, $P < 0.001$; HLH vs. pneumonia, $P < 0.001$; HLH vs. others diagnosis, $P < 0.001$; pneumonia vs. CAEBV, $P < 0.001$; pneumonia vs. others diagnosis, $P < 0.001$; and CAEBV vs. others diagnosis, $P < 0.001$. (**C**) The proportion of primary infections and Activation-IgG$^+$ across different disease types. (**D**) Mortality rates across different EBV DNA levels and infection states. (**E**) Violin plot of ALT levels at different levels of EBV DNA. (**F**) Violin plot of AST levels at different levels of EBV DNA

0 and 1 were 37.5% and 0%, respectively ($P < 0.05$). In the activation-IgG$^+$ group, the mortality rates for Cluster 0 and 1 were 29.63% and 3.12%, respectively ($P < 0.05$) (Table 6).

Discussion

Our study focuses on the correlation between EBV DNA load, infection status, and disease severity. We investigated the differences between infection states to characterize their distinct EBV DNA loads and clinical manifestations. Additionally, we explored the immune characteristics of the EBV DNA-activated pediatric patients, enhancing our understanding of the clinical presentation of pediatric EBV infection and providing insights for improved diagnosis and treatment.

EBV, also known as human herpesvirus 4, is a DNA virus in the herpesvirus family. Its life cycle includes latent and lytic phases. Following infection, EBV enters a latent state within memory B cells. However, under specific pathological conditions, it may reactivate, leading to cell lysis. EBV activation poses significant health risks. Our study, which includes the largest number of cases of activated EBV infection, observed a higher incidence of EBV infection in males compared with females, consistent with the findings of other researches [24]. However, EBV DNA copy numbers did not exhibit significant differences between sexes, suggesting comparable levels of DNA replication across sexes. In our study, the highest incidence of EBV activation occurred in children aged 1–5 years, corresponding to the peak age for IM in China, whereas in Western countries, primary EBV infection tends to occur later [3].

EBV is associated with various pediatric diseases. Primary infection often manifests as IM, while reactivation is more diverse and frequently linked to immunosuppression-related conditions such as sepsis, HLH, or chemotherapy [7]. Our findings indicate that patients with HLH and sepsis exhibit higher EBV DNA loads and increased rates of activation-IgG$^+$. The mechanism of EBV reactivation is a complex process involving cellular immune responses to viral infection, host immune status, and other potential factors, including T-cell and NK-cell functional defects, macrophage activation, and impaired immune surveillance [25–27]. Specifically, pathological stimuli lead to abnormal proliferation of T cells and NK cells, resulting in elevated cytokine levels that promote the EBV reactivation. Inflammatory responses are crucial features of pediatric infectious diseases, such as sepsis and HLH. The release of inflammatory mediators, such as IL-6, creates a conducive microenvironment, allowing EBV to re-enter an active state. In this process, IL-6 activates pathways related to EBV, promoting viral replication [28]. In critically ill patients, prolonged pathological stress can impair cellular immune function [29, 30], and reduce CTL activity, leading to suppressed EBV clearance [31]. Additionally, an increase in regulatory T cells (Treg) may result in an insufficient response to EBV infection [32], further creating favorable conditions for EBV reactivation. Therefore, close monitoring for EBV reactivation is crucial in pediatric patients with conditions that affect immune regulation.

Reactivation of EBV reflects disruptions in host immune function and is associated with an unfavorable prognosis. Moreover, EBV DNA level serves as a key marker of EBV activity. Our study revealed a correlation between high EBV DNA load and in-hospital mortality, with a significant increase in the risk coefficient when the EBV DNA copy number increased to $> 1 \times 10^5$ copies/mL. The in-hospital mortality rate showed a noticeable inflection point as EBV DNA levels increased, particularly among reactivated patients. Additionally, elevated EBV DNA levels were associated with organ injury, including the liver, coagulation system, circulatory system, respiratory system, and kidneys. Prior research suggests that EBV pathogenesis involves B, T, and NK cell activation, along with cytokine production [1]. During the initial infection, EBV first infects B cells, triggering the activation of CD8$^+$ T cells. These activated CD8$^+$ T cells destroy virus-infected cells through cytotoxic effects, leading to necrosis or apoptosis of infected B cells, ultimately leading to reduced B cell count and an increase in CD8$^+$ T cells. Activated T-cells also release cytokines that cause clinical symptoms and injury to various organs [33–35].

To explore the relationship between immune status and post-EBV infection outcomes, we conducted unsupervised clustering analysis on EBV-activated cases using immune indicators. The analysis categorized the patients into two clusters. Based on the analysis of the immune status, EBV infection status, and prognostic characteristics of the two clusters, patients in Cluster 0 exhibited elevated levels of both pro-inflammatory and anti-inflammatory cytokines, such as IFN-γ, IL-10, and IL-6, compared with those in Cluster 1. However, Cluster 0 showed lower expressions of immune cells, including

Table 2 Risk analysis of death by EBV DNA load and infection status

Groups	*N*	Survival, *n* (%)	Death *n* (%)	Crude		Adjusted [a]	
				OR (95% CI)	*P*	OR (95% CI)	*P*
Infection Status							
No-infection	8,269	8,230 (99.53)	39 (0.47)	0.76 (0.32–1.80)	0.534	0.67 (0.28–1.58)	0.357
Past-infection	6,722	6,605 (98.26)	117 (1.74)	2.84 (1.25–6.48)	0.013	2.89 (1.27–6.59)	0.011
Primary Infection	969	963 (99.38)	6 (0.62)	1 (Reference)		1 (Reference)	
Unknown	17,449	17,354 (99.46)	95 (0.54)	0.88 (0.38–2.01)	0.759	0.95 (0.42–2.18)	0.909
Activation-IgG^{+}	2,547	2,510 (98.55)	37 (1.45)	2.37 (0.99–5.62)	0.051	2.48 (1.04–5.89)	0.040
EBV DNA copies/mL							
< 400	32,483	32,227 (99.21)	256 (0.79)	2.63 (0.65–10.58)	0.175	2.47 (0.61–9.94)	0.204
400-1×10^3	663	661 (99.7)	2 (0.3)	1 (Reference)		1 (Reference)	
1×10^3-1×10^4	1,393	1,385 (99.43)	8 (0.57)	1.91 (0.40–9.02)	0.414	1.88 (0.40–8.88)	0.426
1×10^4-1×10^5	764	758 (99.21)	6 (0.79)	2.62 (0.53–13.01)	0.240	2.47 (0.50-12.27)	0.270
1×10^5-1×10^6	434	420 (96.77)	14 (3.23)	11.02 (2.49–48.72)	0.002	10.53 (2.38–46.59)	0.002
> 1×10^6	219	211 (96.35)	8 (3.65)	12.53 (2.64–59.47)	0.001	11.98 (2.52–56.87)	0.002

[a] adjusted for age

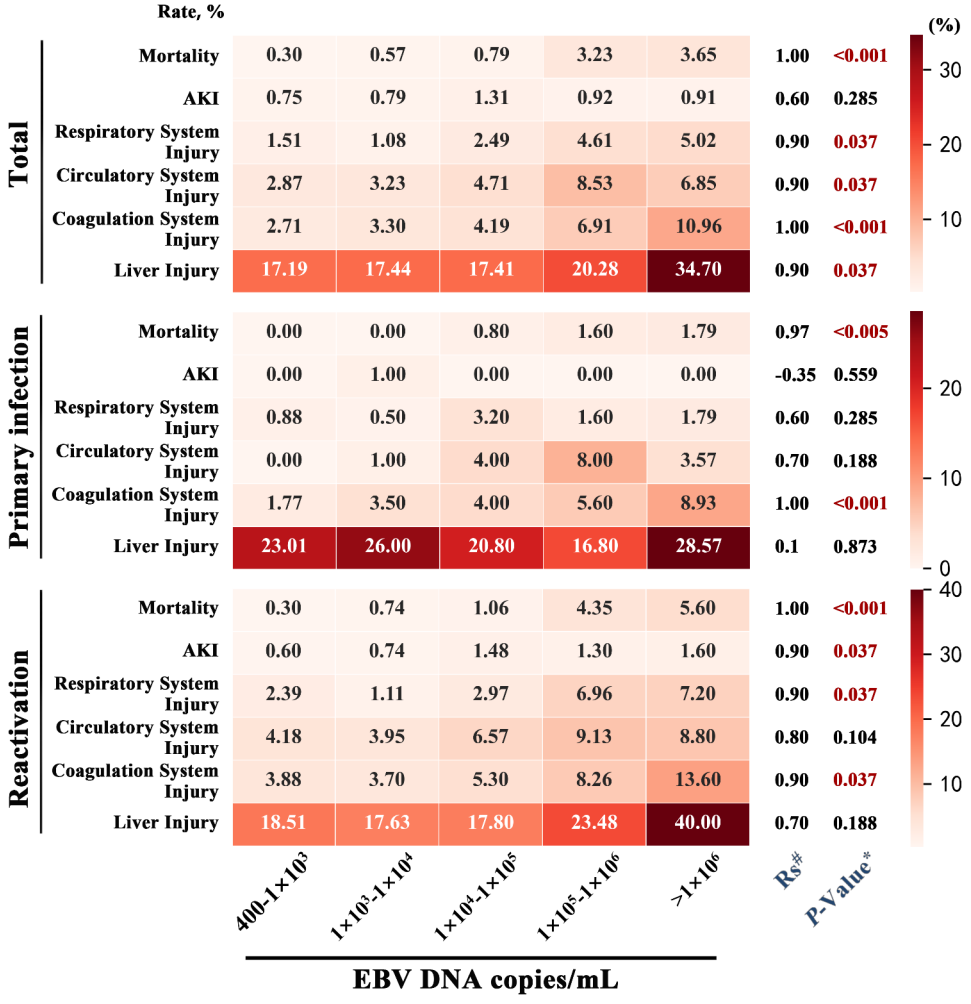

Fig. 3 Heatmap of mortality rates and organ injury across levels of EBV DNA and infection states. **#** The correlation coefficient of Spearman's rank correlation trend test between the incidence rate and EBV DNA levels among patients with EBV DNA copy number > 400 copies/mL. * The P-value of Spearman's rank correlation trend test between the incidence rate and EBV DNA levels among patients with EBV DNA copy number > 400 copies/mL

Table 3 General characteristics of different clusters

Groups	Training Set, *n* (%)			*P*	Validation Set, *n* (%)			*P*
	N	Cluster 0	Cluster 1		*N*	Cluster 0	Cluster 1	
	161	*n* = 46	*n* = 115		162	*n* = 27	*n* = 135	
Age				0.168				0.003
<1 year	3	1 (2.17)	2 (1.74)		2	2 (7.41)	0 (0.0)	
1–5 years	84	26 (56.52)	58 (50.43)		86	18 (66.67)	68 (50.37)	
5–12 years	65	14 (30.43)	51 (44.35)		69	6 (22.22)	63 (46.67)	
12–18 years	9	5 (10.87)	4 (3.48)		5	1 (3.7)	4 (2.96)	
Sex				0.900				0.222
Female	81	24 (52.17)	57 (49.57)		64	14 (51.85)	50 (37.04)	
Male	80	22 (47.83)	58 (50.43)		98	13 (48.15)	85 (62.96)	
Infection Status				< 0.001				0.775
Primary Infection	39	5 (10.87)	34 (29.57)		44	6 (22.22)	38 (28.15)	
Unknown	33	3 (6.52)	30 (26.09)		25	5 (18.52)	20 (14.81)	
Activation-IgG$^+$	89	38 (82.61)	51 (44.35)		93	16 (59.26)	77 (57.04)	
EBV DNA copies/mL				0.803				0.492
400-1 × 10^3	16	3 (6.52)	13 (11.3)		12	1 (3.7)	11 (8.15)	
1 × 10^3-1 × 10^4	33	11 (23.91)	22 (19.13)		44	6 (22.22)	38 (28.15)	
1 × 10^4-1 × 10^5	27	8 (17.39)	19 (16.52)		41	5 (18.52)	36 (26.67)	
1 × 10^5-1 × 10^6	59	18 (39.13)	41 (35.65)		43	10 (37.04)	33 (24.44)	
> 1 × 10^6	26	6 (13.04)	20 (17.39)		22	5 (18.52)	17 (12.59)	
Diseases								
IM	95	7 (15.22)	88 (76.52)	< 0.001	90	3 (11.11)	87 (64.44)	< 0.001
Sepsis	35	22 (47.83)	13 (11.3)	< 0.001	34	18 (66.67)	16 (11.85)	< 0.001
HLH	45	32 (69.57)	13 (11.3)	< 0.001	50	19 (70.37)	31 (22.96)	< 0.001
Pneumonia	75	34 (73.91)	41 (35.65)	< 0.001	83	18 (66.67)	65 (48.15)	0.122
CAEBV	2	1 (2.17)	1 (0.87)	1	1	0	1 (0.74)	1
Lymphoma	5	5 (10.87)	0	0.002	0	0	0	1
Immunodeficiency	0	0	0	1	0	0	0	1
Others diagnosis	10	3 (6.52)	7 (6.09)	1	8	0 (0.0)	8 (5.93)	1
Outcome				< 0.001				< 0.001
Survival	149	35 (76.09)	114 (99.13)		149	17 (62.96)	132 (97.78)	
Death	12	11 (23.91)	1 (0.87)		13	10 (37.04)	3 (2.22)	

EBV, Epstein-Barr virus; **IM**, infectious mononucleosis; **HLH**, hemophagocytic lymphohistiocytosis; **CAEBV**, chronic active Epstein-Barr virus infection;

Other diagnoses include thrombocytopenic purpura, IgA vasculitis, thrombocytopenia, Kawasaki disease, cholestatic hepatitis, hepatic veno-occlusive disease, adult-onset Still's disease, fungal infection, and erythema multiforme

NK cells, total B lymphocytes, helper/inducer T lymphocytes, inhibitory/CTLs, total T lymphocytes and T lymphocytes + B lymphocytes + NK cells compared with Cluster 1. Notably, Cluster 0 had a higher mortality and activation-IgG$^+$ rates, suggesting that the coexistence of high pro-inflammatory and anti-inflammatory states, along with low immune cell numbers, might indicate a critical condition and potentially unfavorable prognosis. Previous studies have suggested that the simultaneous elevation of pro- and anti-inflammatory indicators in response to a sustained immune response is indicative of compensatory anti-inflammatory response syndrome (CARS). Prolonged immune dysregulation may lead to a mixed anti-inflammatory response syndrome (MARS), and sustained immune dysregulation can further result in immune paralysis and apoptosis of immune cells [30, 36]. While excessive inflammatory factors can cause tissue damage, a strong anti-inflammatory response may hinder pathogen clearance, leading to worsening conditions. In this study, the immune profile of Cluster 0 resembled of the MARS state, with a higher proportion of reactivated children in Cluster 0 (pooled proportion, 73.97%) compared to Cluster 1 (pooled proportion, 51.2%). This suggests that reactivated patients may be more prone to this immune imbalance, which is characterized by a high pro-inflammatory and anti-inflammatory coexistence, along with low immune cell numbers. However, regardless of the primary infection or activation-IgG$^+$, Cluster 0 exhibited higher mortality rates compared with Cluster 1. These findings highlight the importance of monitoring immune indicators in patients with activated EBV. Additionally, close monitoring is necessary for an immune state characterized by high pro-inflammatory

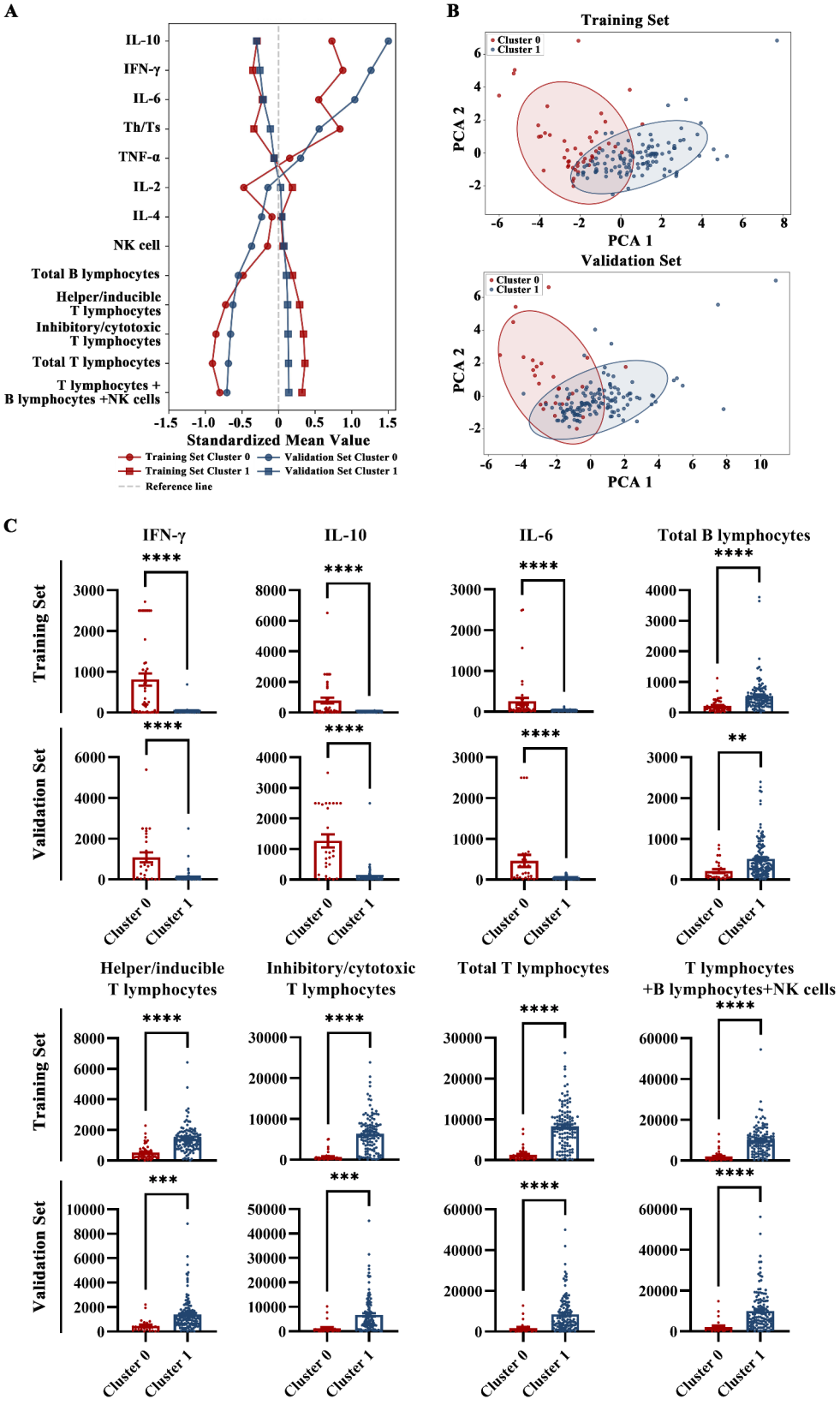

Fig. 4 GMM clustering analysis of immune indicators, PCA visualization, and immune indicators among different clusters. (**A**) Line graph of standardized mean values for different immune indicators across different clusters. (**B**) PCA visualization of the training and validation sets. (**C**) Bar and scatter plots of immune indicators for different clusters in the training set and validation set. * $P<0.05$, ** $P<0.01$, *** $P<0.005$, **** $P<0.001$

Table 4 Associations between clusters and infection status

Groups	Training Set				Validation Set			
	Crude		Adjusted [a]		Crude		Adjusted [a]	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Infection Status								
Primary Infection	1 (Reference)		1 (Reference)		1 (Reference)		1 (Reference)	
Unknown	1.47 (0.32–6.68)	0.617	1.48 (0.33–6.73)	0.611	0.76 (0.28–2.10)	0.596	0.54 (0.14–2.05)	0.361
Activation-IgG$^+$	0.20 (0.07–0.55)	0.002	0.20 (0.07–0.55)	0.002	0.63 (0.17–2.33)	0.49	0.74 (0.26–2.07)	0.561

[a] adjusted for age

Table 5 Associations between clusters and outcomes

Groups	Crude		Adjusted [a]	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Training Set				
Cluster 0	35.83 (4.67-287.32)	0.001	35.93 (4.48-288.35)	0.001
Cluster 1	1 (Reference)		1 (Reference)	
Validation Set				
Cluster 0	25.88 (6.47-103.45)	< 0.01	26.36 (6.34-109.63)	< 0.01
Cluster 1	1 (Reference)		1 (Reference)	

[a] adjusted for age. In logistic regression analysis, dependent variable cluster 1 is assigned a value of 1 and cluster 0 is assigned a value of 0

Table 6 Associations between immune clusters and outcomes according to infection status

Groups	*N*	Cluster 0	Cluster 1	OR (95% CI) [a]	*P*
		n = 8	*n* = 50		
Primary Infection					
Survival	81	9 (81.82)	72 (100)	1	
Death	2	2 (18.18)	0 (0)	0.03 (0.01–0.59) *	0.016
Unknown					
Survival	55	5 (62.5)	50 (100)	1	
Death	3	3 (37.5)	0 (0)	0.03 (0.01–0.31) *	0.002
Activation-IgG$^+$					
Survival	162	38 (70.37)	124 (96.88)	1	
Death	20	16 (29.63)	4 (3.12)	0.08 (0.02–0.24)	< 0.001

[a] As the contingency table had an initial count of zero, a value of 1 was added to each cell for calculation of OR (95% CI)

and anti-inflammatory states, along with low immune cell numbers.

This study has some limitations. The sample size for comprehensive immune indicators was limited. Consequently, we could not dynamically assess the relationship between changes in EBV DNA copy number and the immune system, a facet that will be addressed in future research. Additionally, this study was conducted at a single-center design, necessitating further validation to confirm the generalizability of our conclusions.

Conclusion

In children with EBV activation-IgG$^+$, an EBV DNA copy number exceeding 10^5 copies/mL was associated with a significantly increased mortality risk. Activation-IgG$^+$ posed a higher mortality risk than primary infection, and an elevated EBV DNA copy number correlated with complications involving the liver, coagulation system, circulatory system, respiratory system, and AKI. In children with EBV activation-IgG$^+$, a combination of high pro-inflammatory and anti-inflammatory activity, along with low immune cell numbers, may be indicative of a critical condition and poor prognosis.

Abbreviations

EBV	Epstein-Barr virus
AKI	Acute kidney injury
IM	Infectious mononucleosis
HLH	Lymphohistiocytosis
COVID-19	Coronavirus disease 2019
NK	Natural killer
PCR	Polymerase chain reaction
VCA	Viral capsid antigen, TNF, tumor necrosis factor
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CAEBV	Chronic active EBV
ARDS	Acute respiratory distress syndrome
KDIGO	Kidney Disease: Improving Global Outcomes
ORs	Odds ratios
Cis	Confidence intervals
GMM	Gaussian mixture model
adOR	Adjusted OR
CARS	Compensatory anti-inflammatory response syndrome

MARS Mixed anti-inflammatory response syndrome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02741-7.

Supplementary Material 1: Additional table 1: Distribution of infection status at different age and sex groups.

Supplementary Material 2: Additional table 2: Distribution of pathological types of lymphoma.

Author contributions

HY and XLi contributed to the study's conception, analysed and interpreted the data, and wrote the manuscript. ZX and XLu contributed to the study design, interpreted the data, and revised the manuscript. XHZ, TL, LX, LXie，XW, YY and LL performed chart reviews, interpreted the data, and revised the manuscript. ZX and XLu designed the study, interpreted the data, and revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the Hunan Provincial Natural Science Foundation of China (No. 2022JJ40203, grant to HY; 2023JJ40351, grant to LXie), the National Natural Science Foundation of China (Young Scientists Fund, No. 82102285, grant to XLi), the Hunan Provincial Science and Technology Department Project (2020SK1014-3 and 2020SK2114, grant to XLu), the Hunan Provincial Key Laboratory of Emergency Medicine for Children (No. 2018TP1028, grant to ZX), and the Scientific Research Project of Hunan Provincial Health Commission (No. 202217012704, grant to HY; B202217018341, grant to LXie). The study sponsors have no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Medical Ethics Committee of the Hunan Children's Hospital (HCHLL-2022-50). Informed consent was waved because of the retrospective design. The authors had no access to information that could identify individual participants during and after data collection.

Competing interests

The authors declare no competing interests.

Author details

[1]Internation Inpatient Ward & Hunan Provincial Key Laboratory of Emergency Medicine for Children, The Affiliated Children's Hospital of Xiangya School of Medicine, Central South University (Hunan Children's Hospital), Changsha, China
[2]Pediatrics Research Institute of Hunan Province & Pediatric Intensive Care Unit & Hunan Provincial Key Laboratory of Emergency Medicine for Children, School of Medicine, The Affiliated Children's Hospital of Xiangya, Central South University (Hunan Children's Hospital), Changsha, China
[3]Pediatric Intensive Care Unit & Hunan Provincial Key Laboratory of Emergency Medicine for Children, The Affiliated Children's Hospital of Xiangya School of Medicine, Central South University (Hunan Children's Hospital), Changsha, China
[4]Hengyang Medical School, University of South China, Hengyang, China

Received: 5 November 2024 / Accepted: 14 April 2025
Published online: 23 April 2025

References

1. Damania B, Kenney SC, Raab-Traub N. Epstein-Barr virus: biology and clinical disease. Cell. 2022;185(20):3652–70.
2. Lunn RM, Jahnke GD, Rabkin CS. Tumour virus epidemiology. Philosophical Trans Royal Soc B: Biol Sci 2017;372(1732).
3. Chan KH, Dowd JB, Palermo T, Brite J, McDade TW, Aiello A. Seroprevalence of Epstein-Barr virus infection in U.S. Children ages 6–19, 2003–2010. PLoS ONE 2013;8(5).
4. Ye Z, Chen L, Zhong H, Cao L, Fu P, Xu J. Epidemiology and clinical characteristics of Epstein-Barr virus infection among children in Shanghai, China, 2017–2022. Front Cell Infect Microbiol 2023;13.
5. Lin W, Xiong G, Zhang B et al. Epstein-Barr virus (EBV) infection in Chinese children: A retrospective study of Age-Specific prevalence. PLoS ONE 2014;9(6).
6. Shi T, Huang L, Chen Z, Tian J. Characteristics of primary Epstein-Barr virus infection disease spectrum and its reactivation in children, in Suzhou, China. J Med Virol. 2021;93(8):5048–57.
7. Yang Y, Gao F. Clinical characteristics of primary and reactivated Epstein-Barr virus infection in children. J Med Virol 2020.
8. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr Virus - Induced disease. Annu Rev Immunol. 2015;33(1):787–821.
9. Zhang J, Qin S, Jin Z, et al. The clinical significance and prognostic role of Whole-Blood Epstein-Barr virus DNA in Lymphoma-Associated hemophagocytic lymphohistiocytosis. J Clin Immunol. 2023;43(6):1302–10.
10. Okamoto A, Yanada M, Inaguma Y, et al. The prognostic significance of EBV DNA load and EBER status in diagnostic specimens from diffuse large B-cell lymphoma patients. Hematol Oncol. 2015;35(1):87–93.
11. Li H, Cao D, Li S et al. Synergistic association of hepatitis B surface antigen and plasma Epstein-Barr virus DNA load on distant metastasis in patients with nasopharyngeal carcinoma. JAMA Netw Open 2023;6(2).
12. Chan KCA, Woo JKS, King A, et al. Analysis of plasma Epstein - Barr virus DNA to screen for nasopharyngeal Cancer. N Engl J Med. 2017;377(6):513–22.
13. Qiu L, Si J, Kang J, et al. A retrospective analysis of EBV-DNA status with the prognosis of lymphoma. J Cell Mol Med. 2022;26(20):5195–201.
14. Paolucci S, Cassaniti I, Novazzi F, et al. EBV DNA increase in COVID-19 patients with impaired lymphocyte subpopulation count. Int J Infect Dis. 2021;104:315–9.
15. Naughton P, Healy M, Enright F, Lucey B. Infectious mononucleosis: diagnosis and clinical interpretation. Br J Biomed Sci. 2021;78(3):107–16.
16. Okano M, Kawa K, Kimura H, et al. Proposed guidelines for diagnosing chronic active Epstein-Barr virus infection. Am J Hematol. 2005;80(1):64–9.
17. Chan JKC. The new world health organization classification of lymphomas: the past, the present and the future. Hematol Oncol. 2001;19(4):129–50.
18. Palevsky PM, Liu KD, Brophy PD, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. Am J Kidney Dis. 2013;61(5):649–72.
19. Force ADT, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012;307(23):2526–33.
20. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2016;37(27):2129–200.
21. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis campaign: international guidelines for management of Sepsis and septic shock: 2016. Intensive Care Med. 2017;43(3):304–77.
22. Aneja RK, Carcillo JA. Differences between adult and pediatric septic shock. Minerva Anestesiol. 2011;77(10):986–92.
23. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics*. Pediatr Crit Care Med. 2005;6(1):2–8.
24. Shi J, Ma W, Li W. Epidemiologic features of children with Epstein-Barr virus associated diseases in Hangzhou, China. J Med Virol. 2019;92(8):1277–82.
25. Ford M, Orlando E, Amengual JE. EBV reactivation and lymphomagenesis: more questions than answers. Curr Hematol Malig Rep. 2023;18(6):226–33.
26. Mertowska P, Mertowski S, Smolak K, et al. Could immune checkpoint disorders and EBV reactivation be connected in the development of hematological malignancies in immunodeficient patients?? Cancers. 2023;15:19.
27. Murata T, Sugimoto A, Inagaki T et al. Molecular basis of Epstein - Barr virus latency establishment and lytic reactivation. Viruses 2021;13(12).
28. Tao Q, Zhang G, Tsang CM et al. Enhanced IL-6/IL-6R signaling promotes growth and malignant properties in EBV-Infected premalignant and cancerous nasopharyngeal epithelial cells. PLoS ONE 2013;8(5).

29. Duggal NA, Snelson C, Shaheen U, Pearce V, Lord JM. Innate and adaptive immune dysregulation in critically ill ICU patients. Sci Rep 2018;8(1).
30. Liu D, Huang S-Y, Sun J-H et al. Sepsis-induced immunosuppression: mechanisms, diagnosis and current treatment options. Military Med Res 2022;9(1).
31. Antia R, Hawkins JB, Delgado-Eckert E, Thorley-Lawson DA, Shapiro M. The cycle of EBV infection explains persistence, the sizes of the infected cell populations and which come under CTL regulation. PLoS Pathog 2013;9(10).
32. Li J, Qian C-N, Zeng Y-X. Regulatory T cells and EBV associated malignancies. Int Immunopharmacol. 2009;9(5):590–2.
33. Freeman ML, Burkum CE, Jensen MK, Woodland DL, Blackman MA. γ-Herpesvirus reactivation differentially stimulates Epitope-Specific CD8 T cell responses. J Immunol. 2012;188(8):3812–9.
34. Farina A, Rosato E, York M, Gewurz BE, Trojanowska M, Farina GA. Innate immune modulation induced by EBV lytic infection promotes endothelial cell inflammation and vascular injury in scleroderma. Front Immunol 2021;12.
35. Deng Y, Chatterjee B, Zens K, et al. CD27 is required for protective lytic EBV antigen - specific CD8 + T-cell expansion. Blood. 2021;137(23):3225–36.
36. Bone RC. Immunologic dissonance: a continuing evolution in our Understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). Ann Intern Med. 1996;125(8):680–7.

Publisher's note