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Original Article

Peripheral blood lymphocyte counts in patients with infectious mononucleosis or chronic active Epstein-Barr virus infection and prognostic risk factors of chronic active Epstein-Barr virus infection

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Abstract: Objective: To explore the peripheral blood lymphocyte counts and analyze the prognostic risk factors for the death in patients with chronic active Epstein-Barr virus (CAEBV) infection. Methods: Clinical data of 64 patients infected with CAEBV (CAEBV group) and 64 patients with infectious mononucleosis (IM group) in our hospital were retrospectively analyzed. Meanwhile, 64 healthy individuals came for physical examination were enrolled in the control group. The three groups were compared for white blood cell count, lymphocyte count, and levels of peripheral blood NK cells, B cells, $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+CD28^+$, $CD8^+CD28^+$, $CD4^+CD25^+$, DR^+CD8^+, $CD38^+CD8^+$, $CD4^+$ and $CD8^+$ naive T cells and subsets of memory T cells. Patients infected with CAEBV were further divided into a survival subgroup and a death subgroup according to the survival outcome. The data were processed using univariate analysis and multivariate logistic regression analysis. Results: Compared with the control group, the IM group had higher levels of white blood cell count, lymphocyte count, $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+CD25^+$, DR^+CD8^+, $CD38^+CD8^+$, effector-memory $CD4^+CD62L^-CD45RO^+$ and effector-memory $CD8^+CD62L^-CD45RO^+$, but lower levels of NK cells, B cells, $CD4^+CD28^+$, $CD8^+CD28^+$, naive $CD4^+CD62L^+CD45RA^+$ and naive $CD8^+CD62L^+CD45RA^+$ (all $P<0.05$). Compared with the control group, the CAEBV group had lower levels of white blood cell count, lymphocyte count, $CD3^+$, $CD4^+$, $CD8^+$, NK cells, B cells, $CD4^+CD28^+$, $CD8^+CD28^+$, naive $CD4^+CD62L^+CD45RA^+$ and naive $CD8^+CD62L^+CD45RA^+$, but higher levels of $CD4^+CD25^+$, DR^+CD8^+, $CD38^+CD8^+$, effector-memory $CD4^+CD62L^-CD45RO^+$ and effector-memory $CD8^+CD62L^-CD45RO^+$ (all $P<0.05$). Univariate analysis and multivariate logistic regression analysis showed that EBV DNA>105 copies/mL, platelet count $<50\times10^{12}$/L, albumin <30 g/L and serum ferritin >5000 μg/L were independent risk factors for the death of patients with CAEBV. Conclusion: Patients infected with CAEBV showed imbalance of lymphocyte subsets and immune dysfunction. EBV DNA>105 copies/mL, platelet count $<50\times10^{12}$/L, albumin <30 g/L and serum ferritin >5000 μg/L are risk factors of death in patients with CAEBV.

Keywords: Chronic active Epstein-Barr virus, peripheral blood, lymphocyte subsets, prognosis

Introduction

Epstein-Barr virus (EBV) is one of the herpes viruses known to infect humans. EBV usually infects B lymphocytes and is closely associated with a variety of B cell derived diseases. According to the epidemiological data, the infection rate of EBV worldwide is over 90% [1]. Primary infection of EBV is mostly acute. The patients may have no typical clinical symptoms or develop infectious mononucleosis (IM) [2, 3]. For some patients without obvious immunodeficiency, they can have continuous active replication of the virus without getting into the latent infection state. The patients may also have viral replication in large numbers again after getting into the latent infection state when infected with EBV. Therefore, IM symptoms appear repeatedly in patients and lead to complications like interstitial pneumonia, hemophagocytic syndrome and diffuse intravascular coagulation, accompanied by abnormal changes in EBV antibodies and a significant increase in viral load. This phase is clinically referred as chronic active EBV (CAEBV) infection [4, 5]. The pathogenesis of CAEBV remains to be clarified.

Studies have believed that it may be closely related to the abnormal immune function of patients [6]. Currently, there is no unified gold standard for the diagnosis and treatment of CAEBV. Clinically, serological specific antibody detection, quantitative detection of serum EBV-DNA are mainly used, but they all have certain limitations. Therefore, it is of great clinical significance to explore the biological indicators and the risk factors that affect the prognosis of patients with CAEBV infection [7-10]. Studies have found that EBV can infect T lymphocytes, natural killer cells and B lymphocytes, causing disorder in the body immune mechanism. We therefore believe that the expression of peripheral blood lymphocytes can reflect the condition of patients with CAEBV to a certain extent [4, 11]. This study aimed to explore the expression of peripheral blood lymphocytes and analyze the risk factors affecting the prognosis of patients infected with CAEBV, so as to provide evidence for the clinical diagnosis and treatment of CAEBV.

Materials and methods

General data

In this retrospectively study, a total of 64 patients infected with CAEBV admitted to our hospital from January 2017 to January 2021 were enrolled in the CAEBV group, and 64 patients with IM were enrolled in the IM group. Meanwhile, 64 healthy people came for physical examination, were enrolled in the control group. All research subjects and their families knew about the study and signed an informed consent. This study was approved by the Ethics Committee of our hospital.

Diagnostic criteria

The diagnostic criteria for IM were as follows [12]. (1) Patients developed acute fever, angina, hepatosplenomegaly and lymphadenectasis. (2) The hemogram results of patients showed increased proportion of lymphocytes (>50%) and atypical lymphocytes (>10%). (3) Patients were detected with positive EBV capsid antigen antibody or positive peripheral blood EBV DNA. (4) HIV and CMV were tested negative.

Patients infected with CAEBV met the standards established by Okano et al. in 2005 [12]. (1) Patients showed persistent or recurrent symptom of IM. (2) Patients showed increased level of early antigen and EBV capsid antigen antibody or peripheral blood EBV DNA. (3) Chronic disease course could not be explained by other known diseases.

Inclusion and exclusion criteria

Inclusion criteria were as follows. (1) Patients were over or equal to 18 years old. (2) Patients enrolled in IM group met the IM diagnostic criteria and those in CAEBV group met the CAEBV diagnostic criteria. Subjects in the control group were healthy people who had a physical examination within the past 1 month. (3) Patients and their families knew and agreed to participate in this study. (4) Patients did not have other serious diseases. (5) Patients did not have infectious diseases in the past 3 months.

Exclusion criteria were as follows. (1) Patients combined with myocarditis, thrombocytopenia or hepatitis. (2) Patients took glucocorticoids or immunosuppressive agents within the past 3 months. (3) Patients accompanied by infection, disease of immune system or other serious diseases. (4) Patients infected with other herpes viruses. (5) Patients had a family genetic disease. (6) Patients with a history of drug allergy. (7) Those with poor compliance.

Methods

Venous blood (5 mL * 3 tubes) was collected from all subjects at enrollment (IM group and CAEBV group before treatment).

The first tube of blood sample was measured for the white blood cell count, lymphocyte count, NK cell count and B cell count using an automatic biochemical analyzer (BS400, Mindray, China). The experiment in each group was repeated for 3 times.

The second tube of blood sample was measured for levels of $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, $CD4^+CD28^+$, $CD8^+CD28^+$, $CD4^+CD25^+$, DR^+CD8^+, $CD38^+CD8^+$, naive $CD4^+CD62L^+CD45RA^+$, naive $CD8^+CD62L^+CD45RA^+$, effector-memory $CD4^+CD62L^-CD45RO^+$, and effector-memory $CD8^+CD62L^-CD45RO^+$ using Flow cytometry (EPICS-XL, Beckman, USA). Monoclonal antibody (10 μL) and 100 μL of whole blood were added to each test tube. After mixing, the cells were incubated at room tempera-

Table 1. General data of the three groups

Group	Number of cases (n)	Sex (n)		Age (year)	BMI (kg/m^2)
		Male	Female		
Control group	64	33	31	42.1±4.4	23.1±2.2
IM group	64	35	29	41.5±5.8	23.4±1.9
CAEBV group	64	35	29	42.8±5.2	23.2±2.4
Statistic	0.168	0.168		1.032	0.315
P value	0.921	0.919		0.359	0.731

Note: BMI: body mass index; IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

ture, washed, and adjusted and corrected for the light and liquid paths of Flow cytometry. The coefficient of variation was determined to be less than 2%, and the boundary between negative and positive of fluorescence staining was established. In each tube, 5000 cells were read and tested. The experiment in each group was repeated for 3 times. All lymphocyte monoclonal antibodies were purchased from Immunotech, USA.

The third tube of blood sample was used to measure EB DNA level using quantitative fluorescence PCR. DNA extraction kit was used to obtain EBV DNA from peripheral blood lymphocytes. EBV detection kit was used for quantitative fluorescence PCR (TC100 PCR machine, Bio-RAD, USA), with a lower limit of 500 copies/mL. The reaction system was pre-denaturation at 95°C for 30 s, 95°C for 5 s and 60°C for 30 s for 35 cycles. The experiment in each group was repeated for 3 times. The reagents were purchased from Shanghai Beyotime, China.

Patients in the CAEBV group were followed up for 1 year to record their survival status. Then, they were further divided into a survival subgroup (49 cases) and a death subgroup (15 cases).

Outcome measures

There were 3 main outcome measures. First, levels of $CD3^+$, $CD4^+$, and $CD8^+$ were compared among the three groups. Second, levels of the functional subgroups of T cells, regulatory T cells and activated T cells ($CD4^+CD28^+$, $CD8^+CD28^+$, $CD4^+CD25^+$, DR^+CD8^+, $CD38^+CD8^+$) were compared among the 3 groups. Third, the levels of $CD4^+$ and $CD8^+$ naive T cells, and subsets of memory T cells were compared among the 3 groups.

There were 3 sets of secondary outcome measures. First, the white blood cell count, lymphocyte count, peripheral blood NK cells and B cells were compared among the three groups. Second, patients in the CAEBV group were followed up and further divided into a survival subgroup and a death subgroup. General data of patients in the two subgroups were collected, including age, gender, white blood cell count, lymphocyte count, platelet count, total bilirubin, AST, ALT, albumin, serum ferritin and T lymphocyte subgroup levels. Last, indicators with statistical differences in univariate analysis were further analyzed by multiple logistic regression analysis (forward LR).

Statistical analyses

SPSS 22.0 software was used for statistical analysis. The measurement data conforming to the normal distribution were represented by the mean ± standard deviation ($\bar{x}$±sd), and comparison among multiple groups was conducted using one-way analysis of variance followed by LSD-t test for pairwise comparison. The measurement data that did not conform to the normal distribution were represented by M (P_{25}, P_{75}), and comparison among multiple groups was conducted using Kruskal-Wallis H test followed by Mann-Whitney U test for pairwise comparison. Count data were processed with the use of χ^2 test. The indicators with statistical differences in univariate analysis were further analyzed by multivariate logistic regression analysis. A difference of $P<0.05$ was considered statistically significant.

Results

Comparison of general data among three groups

There were no significant differences in age, gender, and body mass index (BMI) among the 3 groups (all $P>0.05$). See **Table 1**.

Comparison of white blood cell count and lymphocyte count among the three groups

Compared with the control group, the IM group had higher white blood cell count and lymphocyte count, while the CAEBV group showed lower results in both indicators (all $P<0.05$).

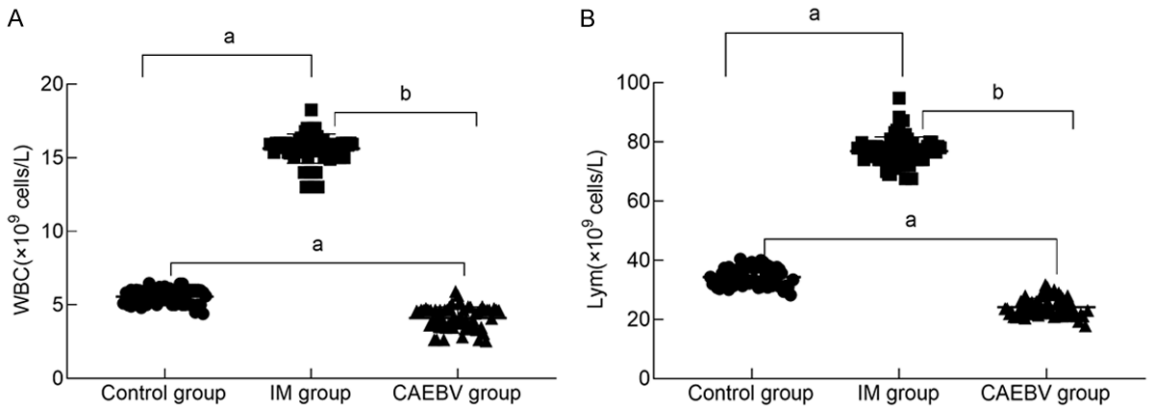

Figure 1. Comparison of white blood cell count and lymphocyte count among the three groups. A: White blood cell counts in the three groups; B: Lymphocyte counts in the three groups. Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus; WBC: white blood cell.

Table 2. Comparison of the levels of B cells, NK cells and $CD3^+$, $CD4^+$, and $CD8^+$ among the three groups

Group	$CD3^+$	$CD4^+$	$CD8^+$	B cells	NK cells
Control group (n=64)	2.11±0.34	0.81±0.29	0.59±0.23	0.33±0.10	0.38±0.09
IM group (n=64)	8.24±0.27[a]	0.98±0.22[a]	7.22±1.35[a]	0.09±0.03[a]	0.25±0.07[a]
CAEBV group (n=64)	0.29±0.11[a,b]	0.22±0.09[a,b]	0.23±0.11[a,b]	0.05±0.02[a,b]	0.07±0.02[a,b]
F	15762.81	217.263	1575.738	389.664	347.224
P	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

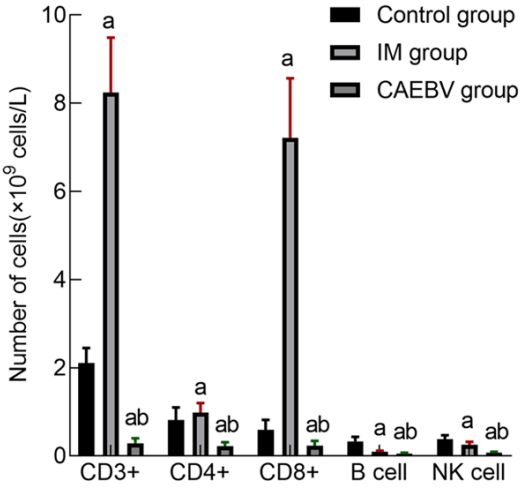

Figure 2. Comparison of the levels of B cells, NK cells and $CD3^+$, $CD4^+$, and $CD8^+$ among the three groups. Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

The levels of white blood cell count and lymphocyte count between the IM group and CAEBV group were statistically different (both P<0.05). See **Figure 1**.

Comparison of levels of B cells, NK cells, $CD3^+$, $CD4^+$ and $CD8^+$ cells among the three groups

Compared with the control group, the IM group showed higher levels of $CD3^+$, $CD4^+$ and $CD8^+$ cells, while lower levels of NK cells and B cells (all P<0.05), and the CAEBV group had lower levels in all the 5 indictors above (all P<0.05). The levels of $CD3^+$, $CD4^+$, $CD8^+$, NK cells and B cells in IM group and CAEBV group were statistically different (all P<0.05). See **Table 2** and **Figure 2**.

Comparison of T cell functional subgroups, regulatory T cells and activated T cells among the three groups

Compared with the control group, the IM group had higher levels of $CD4^+CD25^+$, DR^+CD8^+ and $CD38^+CD8^+$ cells, while lower levels of $CD4^+CD28^+$ and $CD8^+CD28^+$ cells, and the CAEBV group showed similar trend when comparing

Table 3. Comparison of the levels of T cell functional subgroups, regulatory T cells and activated T cells

Group	$CD4^+CD28^+$	$CD8^+CD28^+$	$CD4^+CD25^+$	DR^+CD8^+	$CD38^+CD8^+$
Control group (n=64)	98.52±8.84	48.33±5.98	4.41±0.94	44.32±5.56	16.71±3.36
IM group (n=64)	71.18±7.93[a]	21.45±5.31[a]	5.98±1.01[a]	94.82±9.05[a]	95.32±8.04[a]
CAEBV group (n=64)	90.20±6.51[a,b]	39.05±4.56[a,b]	5.03±0.64[a,b]	83.17±6.25[a,b]	35.75±6.72[a,b]
F	205.608	422.291	51.899	833.926	2666.624
P	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

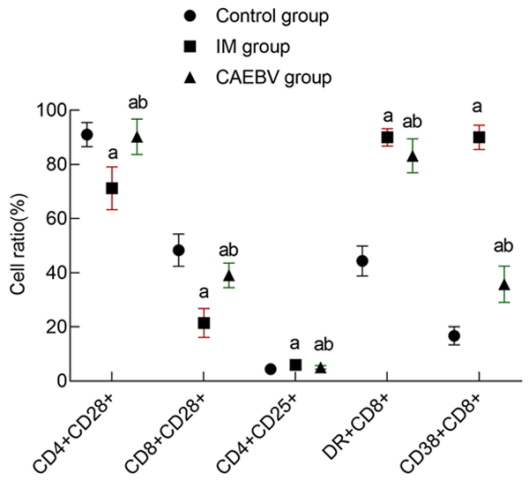

Figure 3. Comparison of the levels of T cell functional subgroups, regulatory T cells and activated T cells. Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

with the control group (all P<0.05). While the levels of $CD4^+CD28^+$, $CD8^+CD28^+$, $CD4^+CD25^+$, DR^+CD8^+ and $CD38^+CD8^+$ cells in IM group and CAEBV group were still statistically different (all P<0.05). See **Table 3** and **Figure 3**.

Comparison of $CD4^+$ and $CD8^+$ naive T cells and memory T cell subsets among the three groups

Compared with the control group, the IM group had higher levels of effector-memory $CD4^+CD62L^-CD45RO^+$ cells and effector-memory $CD8^+CD62L^-CD45RO^+$ cells, while lower levels of naive $CD4^+CD62L^+CD45RA^+$ cells and naive $CD8^+CD62L^+CD45RA^+$ cells (all P<0.05). The CAEBV group showed opposite results when comparing to the control group (all P<0.05). The levels of $CD4^+$ and $CD8^+$ naive T cells and memory T cell subsets in the IM group and CAEBV group were statistically different (all P<0.05). See **Table 4** and **Figure 4**.

Comparison between the survival subgroup and the death subgroup

As of the follow-up, a total of 15 patients died. There were significant differences in the number of EBV DNA copies, platelet count, total bilirubin, AST, albumin, serum ferritin, $CD4^+CD28^+$, $CD38^+CD8^+$ cells, naive $CD4^+CD62L^+CD45RA^+$ cells and naive $CD8^+CD62L^+CD45RA^+$ cells levels between the survival subgroup and the death subgroup (all P<0.05). See **Table 5**.

Results of logistic regression analysis

Continuous data that were statistically significant from the results of univariate analysis were assigned for categorical values. EBV DNA>10^5 copies/mL was assigned as 1, EBV DNA≤10^5 copies/mL as 0, platelet count <50×10^{12}/L as 1, platelet count ≥50×10^{12}/L as 0, AST>75.92 U/L as 1, AST≤75.92 U/L as 0, albumin <30 g/L as 1, albumin ≥30 g/L as 0, serum ferritin >5000 μg/L as 1, serum ferritin ≤5000 μg/L as 0, $CD4^+CD28^+$ <91.94% as 1, $CD4^+CD28^+$ ≥91.94% as 0, $CD38^+CD8^+$ >33.94% as 1, $CD38^+CD8^+$ ≤33.94% as 0, naive $CD4^+CD62L^+CD45RA^+$ <29.87% as 1, naive $CD4^+CD62L^+CD45RA^+$ ≥29.87% as 0, naive $CD8^+CD62L^+CD45RA$ <18.94% as 1, and naive $CD8^+CD62L^+CD45RA$ ≥18.94% as 0. Logistic regression analysis (forward LR) was then performed. The results showed that EBV DNA>10^5 copies/mL, platelet count <50×10^{12}/L, albumin <30 g/L and serum ferritin >5000 μg/L were independent risk factors for death in patients with CAEBV (all P<0.05). See **Table 6**.

Table 4. Comparison of the levels of $CD4^+$ and $CD8^+$ naive T cells and memory T cell subsets among the three groups

Group	Naïve $CD4^+CD62L^+CD45RA^+$	Effector-memory $CD4^+CD62L^-CD45RO^+$	Naïve $CD8^+CD62L^+CD45RA^+$	Effector-memory $CD8^+CD62L^-CD45RO^+$
Control group (n=64)	58.31±4.45	14.71±2.58	56.67±3.41	13.58±4.97
IM group (n=64)	19.35±4.42[a]	36.94±5.22[a]	5.98±1.25[a]	65.48±3.05[a]
CAEBV group (n=64)	28.64±3.95[a,b]	23.05±4.71[a,b]	17.33±4.59[a,b]	25.22±4.28[a,b]
F	1447.066	431.629	3610.413	2721.599
P	<0.001	<0.001	<0.001	<0.001

Note: Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

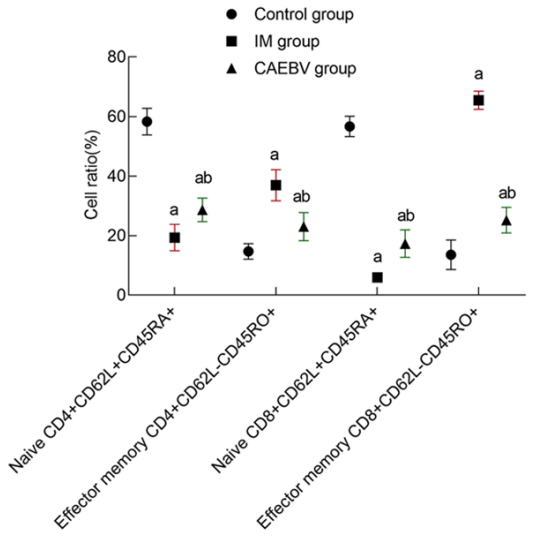

Figure 4. Comparison of the levels of $CD4^+$ and $CD8^+$ naive T cells and memory T cell subsets among the three groups. Compared with the control group, [a]P<0.05; compared with the IM group, [b]P<0.05. IM: infectious mononucleosis. IM: infectious mononucleosis; CAEVB: chronic active Epstein-Barr virus.

Discussion

Studies have shown that when EBV infects the human body, the viral envelope glycoprotein can bind to the B lymphocyte membrane receptor CD21 for latent infection and then promote the replication of EBV. However, T cell-mediated immune response can eliminate infected B lymphocytes [13-15]. Therefore, auxiliary examination of the expression level of T lymphocytes is of clinical significance for the diagnosis and treatment of the disease.

White blood cells and lymphocytes play an important role in resisting stimuli from external factors, while NK cells are the main undertakers of the body's natural immunity. T lymphocyte subsets play an important role in the body's immune mechanism, among which $CD3^+$ and $CD4^+$ cells can promote the body's cellular immune response, while $CD8^+$ cells can inhibit the body's immune response. Under normal physiological conditions, $CD4^+$ and $CD8^+$ are in a dynamic balance. The results of this study showed that compared with the normal population, there were differences in the white blood cell count, lymphocyte count, levels of B cells, NK cells and T lymphocyte subsets in patients with IM or CAEBV, suggesting dysfunction of the immune system of patients with both diseases. In patients with IM, the white blood cell count and lymphocyte count increased, while the levels of B cells and NK cells decreased. Studies have shown that the EBV can make NK cells lose the ability to clear the virus, promote the proliferation of T cells, and form a strong cellular immune response, thereby recognizing the infected B lymphocytes and promoting their apoptosis [16, 17]. Additionally, this process can also inhibit the proliferation and differentiation of B lymphocytes [18, 19]. Therefore, in the acute phase of EBV infection, patients may show a significant increase in the level of T lymphocytes, and a significant decrease in the levels of B cells and NK cells. Without timely and effective treatment, the interaction between T lymphocytes and B lymphocytes can be enhanced, and the activity of B cells is restricted, leading to a further decline in the level of B cells [20]. The results of this study also showed significantly decreased white blood cell count, lymphocyte count, levels of B cells and NK cells in patients with CAEBV, which indicated that the immune disorder was more serious in patients CAEBV. Among the T lymphocyte subsets, the levels of $CD3^+$, $CD4^+$ and $CD8^+$ significantly increased in the IM group but decreased in the CAEBV group as

Table 5. Comparison of indicators between the survival subgroup and the death subgroup

Indicator	Survival subgroup (n=49)	Death subgroup (n=15)	Statistic	*P* value
Male (n)	26	9	0.223	0.637
Age (year)	42.1±4.9	43.2±4.7	0.768	0.446
EBV DNA>10^5 copies/mL	17	14	15.811	<0.001
White blood cell count (×10^9/L)	4.01±1.34	3.87±1.01	0.373	0.711
Lymphocyte count (×10^9/L)	24.38±2.33	23.89±1.97	0.737	0.464
Platelet count <50×10^{12}/L	11	12	16.522	<0.001
Total bilirubin >50 μmol/L	4	8	15.381	<0.001
ALT (U/L)	149.51±12.34	152.42±14.82	0.762	0.449
AST (U/L)	75.92±11.05	124.57±13.44	14.173	<0.001
Albumin <30 g/L	10	12	18.079	<0.001
Serum ferritin >5000 μg/L	10	12	18.079	<0.001
$CD3^+$ (×10^9/L)	0.32±0.21	0.26±0.15	1.027	0.309
$CD4^+$ (×10^9/L)	0.25±0.19	0.20±0.13	0.951	0.345
$CD8^+$ (×10^9/L)	0.26±0.11	0.21±0.15	1.41	0.164
B cells (×10^9/L)	0.05±0.03	0.04±0.02	1.208	0.232
NK cells (×10^9/L)	0.08±0.04	0.07±0.02	1.298	0.201
$CD4^+CD28^+$ (%)	91.94±6.71	87.42±4.31	2.451	0.017
$CD8^+CD28^+$ (%)	40.26±9.42	38.09±6.45	0.832	0.409
$CD4^+CD25^+$ (%)	4.97±1.45	5.10±1.87	0.283	0.778
DR^+CD8^+ (%)	82.09±8.46	84.09±8.14	0.808	0.422
$CD38^+CD8^+$ (%)	33.94±5.14	38.48±4.08	3.126	0.003
Naive $CD4^+CD62L^+CD45RA^+$ (%)	29.87±.3.31	27.02±3.52	2.876	0.006
Effector-memory $CD4^+CD62L^-CD45RO^+$ (%)	22.62±4.45	23.98±3.17	1.099	0.276
Naive $CD8^+CD62L^+CD45RA^+$ (%)	18.94±3.45	15.22±2.73	3.819	<0.001
Effector-memory $CD8^+CD62L^-CD45RO^+$ (%)	24.48±3.87	26.18±4.97	1.391	0.169

Note: ALT: alanine transaminase; AST: aspartate transaminase; EBV: Epstein-Barr virus.

Table 6. Results of logistic regression analysis

Risk factor	B value	SE	Wald χ^2	*P* value	OR	95% CI
EBV DNA>10^5 copies/mL	0.314	1.152	4.445	0.016	8.671	4.457, 11.359
Platelet count <50×10^{12}/L	0.345	1.223	11.347	<0.001	13.822	10.045, 16.358
Albumin <30 g/L	2.661	4.795	10.229	<0.001	14.502	11.389, 18.357
Serum ferritin >5000 μg/L	0.287	1.822	5.735	0.004	14.511	11.342, 18.409

Note: EBV: Epstein-Barr virus.

compared with those in the control group. This result suggests that $CD3^+$, $CD4^+$ and $CD8^+$ are highly activated in patients with IM, which is conducive to the elimination of EBV. In patients with CAEBV, T lymphocytes are declined, which makes it difficult to clear the EBV, leading to persistent EBV infection.

CD28 is the second signal for the normal activation of T cells. Previous studies have found that patients with acute and early EBV infection have a temporary incompetent state of down-regulating CD28 cells, possibly because of the temporary non-responsive state of activated T cells in the process of participating in the immune response to the virus [21]. The results of this study showed that compared with the normal population, the levels of T cell functional subgroups ($CD4^+CD28^+$ and $CD8^+CD28^+$) decreased in IM group. Though the levels of T cell functional subgroups in CAEBV group also decreased but were higher than those in the IM

group. Possible mechanism is that the non-decline state of CD28 cells was absent in the early stage of acute CAEBV infection, and this may also be a characteristic of the chronic activity of EBV infection. The CD25 molecule is involved in suppressing the over-activated autoimmune response. $CD4^+CD25^+$ cells, as a regulatory T cells, can suppress immune response and maintain body tolerance. The results of this study showed that the level of $CD4^+CD25^+$ increased in patients with IM, while the levels in patients with CAEBV patients was between those in patients with IM and in the normal population. This may be related to the chronicity of excessive inflammatory response in patients with CAEBV. DR^+CD8^+ and $CD38^+CD8^+$, as activated T cells, are specific markers of virus-induced cellular immune activation [22]. The results of this study showed that compared with the controls, the levels of DR^+CD8^+ and $CD38^+CD8^+$ significantly increased in both IM and CAEBV groups, but the levels in CAEBV groups were lower than those in the IM group. This is possibly related to the mechanism of viral clearance at acute early phase and the entry into chronic active phase, but the specific mechanism still needs to be further explored. Naive T cells can be transformed into effector-memory T cells after being stimulated by antigens. The results of this study showed that compared with the normal population, the IM group and the CAEBV group showed decreased level of naive T cells (naive $CD4^+CD62L^+CD45RA^+$ and naive $CD8^+CD62L^+CD45RA^+$) and increased level of effector-memory T cells (effector-memory $CD4^+CD62L^-CD45RO^+$ and effector-memory $CD8^+CD62L^-CD45RO^+$). This may be related to the immune escape mechanism that causes disorder in naive T cell formation. Compared with the IM group, the CAEBV group had a lower level of naive T cells while a higher level of effect memory T cells. We believe that it is related to the chronic active phase of CAEBV.

We further explored the possible risk factors that affected the prognosis of CAEBV. The results showed that EBV DNA>10^5 copies/mL, platelet count <50×10^{12}/L, albumin <30 g/L and serum ferritin >5000 μg/L were independent risk factors for death in patients with CAEBV. A high viral load often indicates that the patient may have a persistent infection, which aggravates the disease condition. Platelets play important roles in coagulation function as well as regeneration and repair of vascular endothelial cells. Significant drop of platelet level may cause spontaneous bleeding of internal organs, which can be life-threatening in some severe cases [23]. Decrease in albumin, on the one hand, may lead to endocrine disorders and increase the risk of infection, on the other hand, indicates severe systemic consuming and poor prognosis in patients [24]. Serum ferritin is closely related to the body's inflammatory response. Higher level of serum ferritin usually indicates more severe inflammatory response of the patient. Therefore, it is necessary to pay close attention to the above indictors and take control measures in time during clinical diagnosis and treatment.

The innovations of this study included the followings: (1) We provided new ideas for the clinical diagnosis of CAEBV by analyzing the differences in lymphocyte levels between patients with CAEBV and patients with IM. (2) The risk factors affecting death in patients with CAEBV were obtained through follow-up, which provided relevant basis for targeted clinical interventions. However, this study also has the following limitations: the sample size included in the study was small, and the indicators were not dynamically monitored, so further exploration of the possible mechanisms using larger sample studies are still needed.

In summary, patients infected with CAEBV have imbalanced lymphocyte subsets and immune dysfunction. EBV DNA>10^5 copies/mL, platelet count <50×10^{12}/L, albumin <30 g/L and serum ferritin >5000 μg/L are the risk factors that affect the death of patients with infectious mononucleosis or chronic active Epstein-Barr virus infection.

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Disclosure of conflict of interest

None.

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