Early predictors of Epstein-Barr virus infection in patients with severe fever with thrombocytopenia syndrome

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Abstract

Background Epstein-Barr virus (EBV) can be reactivated and proliferated with fatal outcome in immuno-compromised people, but the clinical consequences of EBV infection in patients with severe fever with thrombocytopenia syndrome (SFTS) remain uncertain. In this study, we investigated the infection rate, the influence and the early predictors of EBV infection in SFTS patients.

Methods In this retrospective study, SFTS patients who were treated in the First Affiliated Hospital of Nanjing Medical University from May 2011 to August 2021 were enrolled and divided into infected and non-infected groups. We compared the demographic characteristics, clinical manifestations and signs, laboratory tests and prognosis, and explored the risk factors of EBV infection by receiver operating characteristic (ROC) curve and logistic regression.

Results A total of 120 hospitalized SFTS patients with EBV-DNA testing were enrolled in this study. Patients with EBV infection had statistically significant higher mortality rate (32.0% vs. 11.43%, $P = 0.005$). Compared with the non-infected group, the EBV-infected group had higher levels of C-reactive protein (CRP), creatine-kinase (CK), fasting blood glucose (FBG), blood urea nitrogen (BUN), D-dimer, and $CD56^+$ cell counts, lower levels of immunoglobulin G (IgG), IgM, complement 3 (C3), and C4. The proportion of patients with age ≥ 60 years and ferritin > 1500.0 ng/ml in the EBV-infected group was significantly higher than that in the non-infected group. The results of ROC analysis showed that the cut-off values of CRP, IgG, C3, C4, and $CD56^+$ cell counts to predict EBV infection were 13.2 mg/l, 12.5 g/l, 1.1 g/l, 0.6 g/l, 0.3 g/l, and 94.0 cells/μl. Multivariable logistic analysis showed that age ≥ 60 years old, CRP > 13.2 mg/l, BUN > 5.4 mmol/l, ferritin > 1500.0 ng/ml, IgG < 12.5 g/l, IgM < 1.1 g/l, C4 < 0.3 g/l, and $CD56^+$ cell counts > 94.0 cells/μl were the independent risk factors of EBV infection in SFTS patients.

Conclusions SFTS combined with EBV infection is associated with high morbidity and mortality. It is necessary to strengthen screening for EBV infection and its early predictive markers after admission in SFTS patients.

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Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a severe tick-borne disease caused by Dabie banda-virus (DBV), which is a negative-strand RNA virus and belongs to the genus *Bandavirus* in family *Phenuiviridae* [1]. This viral hemorrhagic fever disease was first identified in 2009 in the rural areas of China [2]. After DBV infection, the patients were characterized by fever, headache, thrombocytopenia, leukopenia, gastrointestinal symptoms, diffuse intravascular coagulation, multiple organ dysfunction syndrome (MODS) and shock [3]. The emergence of SFTS has had a huge impact on population health mainly in East and Southeast Asia. Despite the fact that the case fatality rate (CFR) of SFTS ranges between 10.5% and 35%, there are currently no effective treatments available for SFTS patients [4–6]. Previous studies have proved that ribavirin can suppress the replication of DBV in vivo and in vitro [7, 8]. However, there is no specific clinical evidence to prove if the antivirus effect of ribavirin can be useful to increase the survival rates of SFTS patients [3]. Controlling the progression and reducing the mortality of SFTS is still challenging.

The Epstein-Barr virus (EBV), which is a ubiquitous gamma herpesvirus infects more than 90% of the adulthood worldwide [9]. Most immunocompetent people have no obvious manifestations after infection, but in immunocompromised individuals, EBV may be reactivated and proliferated with fatal outcome. Previous studies have presented that EBV infection can affect the progression and outcome of several diseases, including coronavirus disease 2019 (COVID-19) and multiple sclerosis (MS) [10, 11]. Cytokine storm, which is characterized by imbalanced immune responses with an exaggerated systemic inflammation, played a crucial role in the deterioration of SFTS [12, 13]. However, there is no specific evidence to support the relationship between EBV infection and the prognosis of SFTS up to date. Moreover, the risk factors associated with EBV infection in SFTS patients have never been investigated. This study systematically assessed the impact that different potential factors have on the risk of EBV infection in patients with SFTS, and explore the early predictors.

Materials and methods
Study design and participants

From May 2011 to August 2021, the clinical records of SFTS patients who were hospitalized in the First Affiliated Hospital of Nanjing Medical University were collected in this retrospective case-control study. All these patients should undergo a complete clinical examination at the time of admission, including the plasma EBV DNA levels. Patients were divided into two groups according to EBV-DNA levels. The peripheral whole blood samples of SFTS patients were collected in tubes containing Ethylenediaminetetraacetic acid (EDTA). EBV PCR Fluorescence Quantitative Diagnostic Kit (Daan Gene Co., Guangzhou, China) was used to extract DNA from whole blood and a real-time quantitative PCR with ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA) was used to quantify EBV-specific sequences. It was performed at the department of Laboratory Medicine of the hospital. EBV DNA load > 500 copies/ml was defined as positive and ≤ 500 copies/ml was defined as a negative level. This research was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

Data collection

The retrospective analysis evaluated information from the electronic medical record system, including epidemiological, demographic characteristics (age, gender, basic diseases, smoking and drinking history), clinical data (clinical manifestations and signs), laboratory tests (routine blood tests, renal and liver function tests, and inflammatory biomarkers), treatment, and prognosis. All patients were followed up for 30 days post-discharge.

Diagnostic criteria

Based on the confirmed definition published by the Chinese Ministry of Health in 2010 [14], SFTS was diagnosed according to the following criteria: (1) patients having a history of bite from a tick in the two weeks prior to onset of illness, or having been to hilly terrain, forest region or mountainous area in epidemic seasons. (2) patients presented with fever, fatigue, anorexia and so on with acute-onset. (3) laboratory tests indicated low white blood cell and platelet counts. (4) patients who were tested to meet one of the following four criteria: positive for DBV RNA in peripheral blood using polymerase chain reaction; seroconversion in immunoglobulin G (IgG) antibody specific for DBV; compared with the samples during acute stage, the specific antibody was detected more than four times during convalescent stage; the isolation of DBV from the samples. Central nervous system (CNS) manifestation was identified as at least one of the following symptoms: muscle and limb tremor, cognitive dysfunction, lethargy, confusion, stupor, delirium, coma, dysphoria, convulsion and seizures [15, 16].

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Statistical analysis

We used IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 (Graph-Pad Software, San Diego, USA) for all statistical analysis. Normal and non-normal continuous variables were presented as means with standard deviations (SDs) and medians with inter-quartile range (IQR) and analyzed by Mann-Whitney U test. Categorical variables were presented as number with percentages and analyzed by chi-square test or Fisher's test. Variables were considered statistically significant with a *P*-value of <0.05. Receiver operating characteristic (ROC) curve analyses were used to evaluate the best cut-off values and the prognostic value of the clinical indicators. The risk factors for EBV infection were performed by multivariate logistic regression analysis and the results were described as the odds rations (ORs) and 95% confidence intervals (CIs). Survival analysis comparisons between the two groups were analyzed by the log rank (Mantel-Cox) test with the 30-day follow-up period.

Results

Overview of clinical information

Figure 1 showed the flow chart for inclusion and exclusion of SFTS patients. Among 142 SFTS patients with EBV-DNA testing from May 2011 to August 2021, 120 patients were included in the final analysis according to the inclusion and exclusion criteria. There were 50 cases (41.7%) in EBV-infected group and 70 cases (58.3%) in non-infected group. Comparisons of demographics, clinical symptoms and signs, laboratory findings, and other clinical information were presented in Table 1 (at the end of the article). The proportion of SFTS patients who were at least 60 years old was 70.0% in EBV-infected group, and that in the non-EBV infection group was 50.0%, the difference was statistically significant (*P*<0.05). Twenty-two (44.0%) were males in EBV-infected group, while 36 (51.4%) were males in non-infected group. In terms of underlying diseases there was no significant difference between the two groups in our study. The patients had

symptoms and signs as fever, cough, myalgia, gastrointestinal symptom, and CNS manifestation with no significant difference.

As shown in Table 1, the median levels of inflammatory and immunological markers including C-reactive protein (CRP), IgG, IgM, complement 3 (C3), C4, and CD56+ cell counts in EBV-infected patients were 12.7 (IQR 5.0-18.9) mg/l, 10.9 (IQR 7.3–13.3) g/l, 0.8 (IQR 0.6–1.1) g/l, 0.6 (IQR 0.4–0.7) g/l, 0.3 (IQR 0.2–0.3) g/l, and 146.6 (IQR 97.5–246.0) cells/μl. The levels of CRP and CD56+ cell counts in EBV-infected group were significantly higher than those in non-infected group and the levels of IgG, IgM, C3, and C4 were lower (*P*<0.05). The proportion of patients whose ferritin level was more than 1500.0 ng/ml was 92.0% in EBV-infected group and 74.3% in non-infected group (*P*<0.05). With respect to coagulative indicators, the level of D-dimer was higher in EBV-infected group than in non-infected group (*P*<0.05). In addition, there was significant difference of creatine-kinase (CK), fasting blood glucose (FBG), and blood urea nitrogen (BUN) between the two groups, while other biochemical indexes were no significant difference.

Outcomes of the EBV-infected group and the non-infected group

The proportion of patients admitted to intensive care unit (ICU) was higher in EBV-infected group, but the difference was not statistically significant (*P*>0.05). In addition, the median duration of hospital stay was 8.0 (IQR 6.0–13.0) days in EBV-infected group and 7.0 (IQR 4.0–11.0) days in non-infected group with significant difference (*P*<0.05). For these SFTS patients, the mortality rate was 32.0% for the EBV-infected group and 11.4% for the non-infected group with significant difference (*P*<0.05). As shown in Fig. 2, the infected group always had a higher mortality than the non-infected group during the one-month follow-up period. In addition, the difference of mortality rate between the two groups increased significantly after the first two weeks.
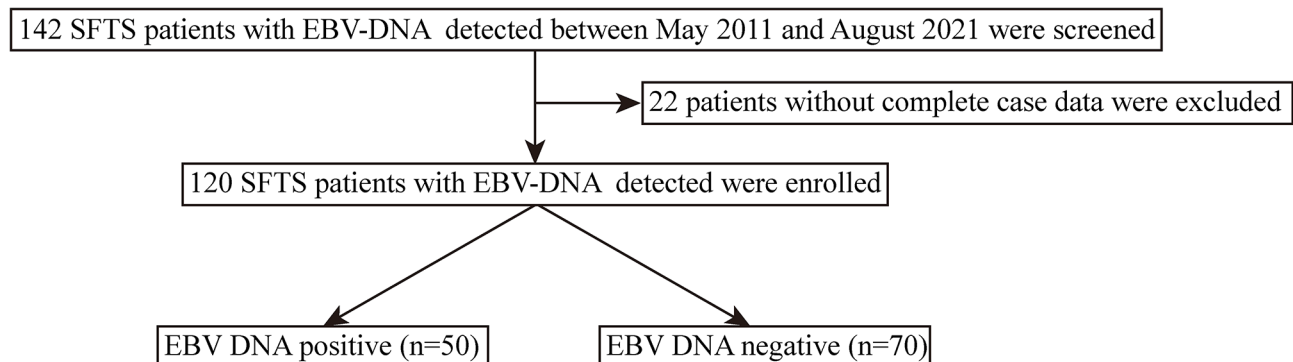

Fig. 1 Flow chart of patient included in this study. SFTS, severe fever with thrombocytopenia syndrome

Table 1 Comparisons of clinical characteristics between EBV infected and non-infected groups in SFTS patients

Index	EBV infected (*n* = 50)	EBV non-infected (*n* = 70)	*P* value
Demographic			
Age ≥ 60years	35 (70.0%)	35 (50.0%)	0.028
Sex			0.422
Male	22 (44.0%)	36 (51.4%)	
Female	28 (56.0%)	34 (48.6%)	
Farmer	32 (64.0%)	50 (71.4%)	0.388
History of bite from a tick	8 (16.0%)	22 (31.4%)	0.054
Time from onset to treatment, days	7.0 (3.0,9.0)	7.0 (5.0,10.0)	0.153
Smoking	17 (34.0%)	15 (21.4%)	0.125
Alcohol drinking	10 (20.0%)	11 (15.7%)	0.542
Underlying conditions			
Hypertension	17 (34.0%)	17 (24.3%)	0.244
Diabetes	9 (18.0%)	7 (10.0%)	0.204
Cardiovascular and cerebrovascular disease	5 (10.0%)	8 (11.4%)	0.804
Clinical symptoms and signs			
Maximum body temperature (℃)	39.0 (38.7,39.5)	39.0 (39.0,39.6)	0.258
Cough	23 (46.0%)	22 (31.4%)	0.104
Myalgia	17 (34.0%)	19 (27.1%)	0.419
Fatigue	38 (76.0%)	56 (80.0%)	0.600
Headache	20 (40.0%)	30 (42.9%)	0.754
Chest distress	15 (30.0%)	15 (21.4%)	0.285
Nausea	17 (34.0%)	34 (48.6%)	0.111
Abdominal pain	11 (22.0%)	16 (22.9%)	0.912
Diarrhoea	16 (32.0%)	29 (41.4%)	0.293
Gastrointestinal bleeding	2 (4.0%)	10 (14.3%)	0.123
Muscle twitching	8 (16.0%)	8 (11.4%)	0.468
CNS manifestation	41 (82.0%)	49 (70.0%)	0.134
Rash	9 (18.0%)	15 (21.4%)	0.643
Chemosis	2 (4.0%)	8 (11.4%)	0.264
Pharyngeal swelling	3 (6.0%)	7 (10.0%)	0.655
Petechiae and ecchymoses	16 (32.0%)	19 (27.1%)	0.564
Respiratory sound abnormalities	22 (44.0%)	24 (34.3%)	0.281
Lymphadenopathy	15 (30.0%)	25 (35.7%)	0.513
Pulmonary infection	26 (52.0%)	37 (52.9%)	0.926
Pleural effusion	11 (22.0%)	9 (12.9%)	0.185
MODS	17 (34.0%)	18 (25.7%)	0.325
HPS	2 (4.0%)	4 (5.7%)	1.000
Laboratory findings at admission			
Leukocytes (×10^9/l)	2.1 (1.5–4.3)	2.5 (1.4–5.2)	0.692
Neutrophils (×10^9/l)	1.4 (0.8-2.0)	1.3 (0.8–3.7)	0.809
Hemoglobin (g/l)	134.5 (123.0-151.0)	128.0 (119.0-145.0)	0.097
Platelets (×10^9/l)	42.5 (26.8–63.3)	42.0 (29.8–64.3)	0.762
PCT (ng/ml)	0.3 (0.1–0.5)	0.2 (0.1–0.3)	0.073
CRP (mg/l)	12.7 (5.0-18.9)	7.2 (3.5–12.7)	0.047
Ferritin>1500.0 ng/ml	46 (92.0%)	52 (74.3%)	0.026
PT (s)	12.2 (11.5–12.8)	12.0 (11.3–12.8)	0.767
TT (s)	28.5 (23.0-41.1)	24.8 (20.3–35.1)	0.078
APTT (s)	47.0 (39.1–54.8)	42.8 (36.2–48.7)	0.059
Fibrinogen (g/l)	1.9 (1.7–2.4)	2.1 (1.7–2.5)	0.296
D-dimer (mg/l)	3.5 (1.7-8.0)	1.8 (1.0-4.1)	0.007
ALT (U/l)	118.1 (60.2-172.2)	91.2 (50.5–140.0)	0.125
AST (U/l)	284.7 (140.9-522.3)	205.3 (114.7-422.5)	0.207
Albumin (g/l)	32.1 (28.9–36.0)	31.9 (28.5–35.5)	0.731

Table 1 (continued)

| Index	EBV infected (*n* = 50)	EBV non-infected (*n* = 70)	*P* value
CK (U/l)	780.5 (379.5-1488.5)	340.5 (154.0-1012.0)	0.004
FBG (mmol/l)	8.4 (6.3–12.7)	6.6 (5.5–8.7)	0.006
BUN (mmol/l)	6.3 (4.9-9.0)	5.2 (4.1–7.1)	0.009
Cr (µmol/l)	67.1 (59.0-89.6)	67.6 (56.0-87.8)	0.505
ADA (mg/l)	42.1 (28.5–53.3)	44.6 (27.8–65.3)	0.502
IgG (g/l)	10.9 (7.3–13.3)	12.8 (10.0-15.4)	0.009
IgA (g/l)	2.3 (1.5-4.0)	1.8 (1.3–2.6)	0.053
IgM (g/l)	0.8 (0.6–1.1)	1.1 (0.70–1.9)	0.024
C3 (g/l)	0.6 (0.4–0.7)	0.7 (0.5–0.8)	0.019
C4 (g/l)	0.3 (0.2–0.3)	0.3 (0.2–0.3)	0.038
CD3$^+$ T-cell counts (cells/µl)	635.2 (486.7-933.4)	644.0 (450.3-852.9)	0.593
CD4$^+$ T-cell counts (cells/µl)	298.5 (157.9-469.3)	243.0 (147.8-365.3)	0.323
CD8$^+$ T-cell counts (cells/µl)	340.5 (211.8-470.8)	382.6 (202.7-557.2)	0.499
CD4$^+$/CD8$^+$ ratio	1.0 (0.6–1.7)	1.2 (0.6–1.9)	0.346
CD19$^+$ T-cell counts (cells/µl)	292.3 (128.45–629.8)	198.6 (115.0-372.0)	0.066
CD56$^+$ cell counts (cells/µl)	146.6 (97.5–246.0)	106.9 (45.8-181.8)	0.026
DBV RNA load (lg copies/ml)	6.3 (5.8–6.9)	5.8 (5.0–7.0)	0.172
Outcome			
ICU transfer	16 (32.0%)	12 (17.1%)	0.058
Length of hospital stay (days)	8.0 (6.0–13.0)	7.0 (4.0–11.0)	0.033
Mortality	16 (32.0%)	8 (11.4%)	0.005
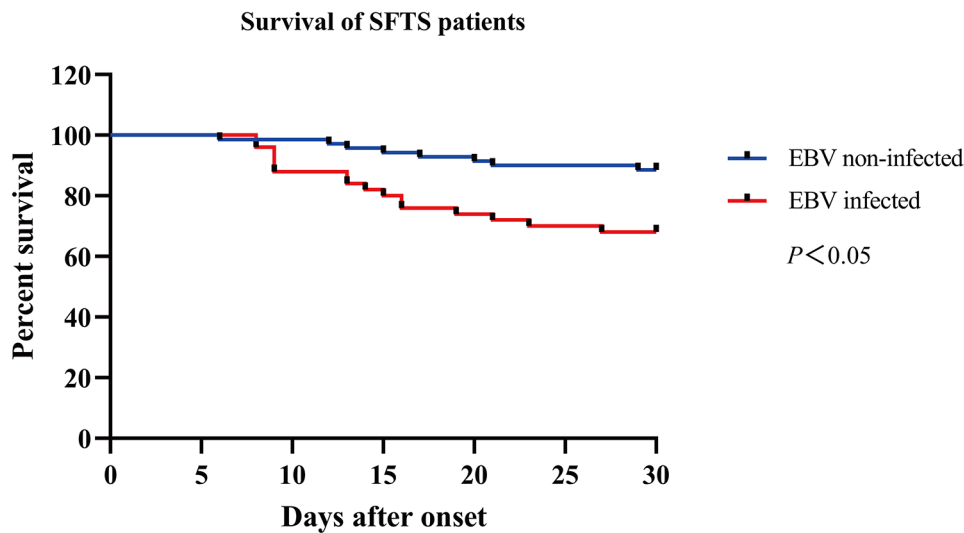
Fig. 2 Survival curves were compared between SFTS patients infected and non-infected by EBV.

Risk factors for EBV infection in SFTS patients

Table 2 showed the results of ROC analysis of immune and inflammatory biomarkers based on the comparable findings in Table 1. The cut-off values of CRP, IgG, IgM, C3, C4, and CD56$^+$ cell counts to predict EBV infection were 13.2 mg/l, 12.5 g/l, 1.1 g/l, 0.6 g/l, 0.3 g/l and 94.0 cells/µl, with sensitivities of 50.0%, 72.0%, 74.0%, 60.0%, 58.0%, and 78.0% and specificities of 77.1%, 51.4%, 51.4%, 64.3%, 64.3%, and 47.1%, respectively. As shown in Table 3, cut-off values of CK, FBG, BUN, and D-dimer to predict the incidence of EBV infection in SFTS patients

were 299.5 U/l, 7.7 mmol/l, 5.4 mmol/l, and 2.2 mg/l, with sensitivities of 82.6%, 61.7%, 70.0%, and 69.4%, and specificities of 50.0%, 68.1%, 55.7%, and 59.4%, respectively.

Multivariate logistic regression analysis was adopted to identify if the significant indexes in Table 1 correlated with EBV infection in SFTS patients. The results were summarized in Table 4, which showed that the following several significant risk factors remained: age ≥ 60 years old (OR 3.196, 95% CI 1.046–9.763, *P* = 0.041), CRP > 13.2 mg/l (OR 3.097, 95% CI 1.077–8.908, *P* = 0.036), BUN > 5.4 mmol/l (OR 4.670, 95% CI

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Table 2 ROC analysis of immune and inflammatory biomarkers predicting EBV infection in SFTS patients

Index	Cut off value	AUC (95%CI)	*P* value	Sensitivity	Specificity
CRP (mg/l)	13.2	0.606 (0.503–0.710)	0.047	50.0	77.1
IgG (g/l)	12.5	0.640 (0.539–0.741)	0.009	72.0	51.4
IgM (g/l)	1.1	0.621 (0.520–0.723)	0.024	74.0	51.4
C3 (g/l)	0.6	0.626 (0.523–0.729)	0.019	60.0	64.3
C4 (g/l)	0.3	0.612 (0.509–0.714)	0.038	58.0	64.3
CD56$^+$ cell counts (cells/μl)	94.0	0.620 (0.518–0.721)	0.026	78.0	47.1

Table 3 ROC analysis of other factors predicting EBV infection in SFTS patients

Index	Cut off value	AUC (95%CI)	*P* value	Sensitivity	Specificity
CK (U/l)	299.5	0.659 (0.559–0.759)	0.004	82.6	50.0
FBG (mmol/l)	7.7	0.651 (0.548–0.754)	0.006	61.7	68.1
BUN (mmol/l)	5.4	0.640 (0.541–0.739)	0.009	70.0	55.7
D-dimer (mg/l)	2.2	0.648 (0.546–0.749)	0.007	69.4	59.4

Table 4 Multivariate logistic regression analysis of factors predicting EBV infection in SFTS patients

Index	β	OR	95%CI	*P* value
Age ≥ 60 years	1.162	3.196	1.046–9.763	0.041
CRP > 13.2 mg/l	1.130	3.097	1.077–8.908	0.036
BUN > 5.4 mmol/l	1.541	4.670	1.424–15.311	0.011
Ferritin > 1500.0 ng/ml	2.364	10.638	1.990-56.868	0.006
IgG < 12.5 g/l	1.278	3.589	1.218–10.580	0.020
IgM < 1.1 g/l	2.097	8.141	2.469–26.844	0.001
C4 < 0.3 g/l	1.193	3.296	1.132–9.598	0.029
CD56$^+$ cell counts > 94.0 cells/μl	1.742	5.710	1.839–17.732	0.003

1.424–15.311, *P* = 0.011), ferritin > 1500.0 ng/ml (OR 10.638, 95% CI 1.990-56.868, *P* = 0.006), IgG < 12.5 g/l (OR 3.589, 95% CI 1.218–10.580, *P* = 0.020), IgM < 1.1 g/l (OR 8.141, 95% CI 2.469–26.844, *P* = 0.001), C4 < 0.3 g/l (OR 3.296, 95% CI 1.132–9.598, *P* = 0.029), and CD56$^+$ cell counts > 94.0 cells/μl (OR 5.710, 95% CI 1.839–17.732, *P* = 0.003).

Discussion

DBV has been identified that it can affect the cellular and humoral immune responses of patients, which may contribute to the high possibility of EBV infection in SFTS patients [17]. And given the increasing popularity and mortality of SFTS, we performed the first study to date to reveal the strong evidence that EBV infection is strongly associated with the worse outcome in SFTS patients. Meanwhile, our multivariate analysis clearly showed the risk factors for EBV infection in SFTS patients.

Referred to as one of the most common viruses, EBV can persist for the remainder of the life in individuals following the acute phase of infection and can be reactivated by primary or secondary immunodeficiency [18]. Immunosuppressive agents, high-dose corticosteroid treatment, acute virus coinfection have been identified as the most common risk factors for EBV infection in several studies, which were based on immunodeficient or immunosuppressed patients [19–21]. In the present study, we focused on patients with SFTS. DBV has been confirmed to have the ability to attenuate cellular and humoral immune responses. Aberrant production of pro- and anti-inflammatory cytokines are produced in severe cases, resulting in systemic inflammatory response syndrome (SIRS) and mixed/compensatory anti-inflammatory response syndrome (MARS/CARS) [22]. SIRS coupled with MARS/CARS can increase the occurrence of secondary infection, multi-organ failure and fatal outcomes [23]. The immune damage including cellular immunosuppression and cytokine storms provided the probability that EBV infection has a strong association with SFTS. Our study evaluated a cohort of 120 hospitalized patients with SFTS, of which 41.7% patients were EBV-DNA positive.

In this study, we found that EBV infection contributed to prolonged length of hospital stay, higher rates of ICU admission, and increased mortality of SFTS patients. Prior studies have also shown that SFTS patients with microbial infections exhibit a higher mortality rate compared to those without co-infections [24]. Several studies have highlighted that EBV infection substantially elevates mortality rates when juxtaposed with uninfected patients across various conditions such as COVID-19, multiple myeloma, and acute-on-chronic liver failure (ACLF) [25–27]. On the one hand, increasing evidence proved that in critically ill patients with SFTS, the mortality might be associated with an excessive upregulation of proinflammatory mediators, which may further be triggered by EBV infection [28]. On the other hand, studies have indicated that an older age and elevated levels of BUN serve as valuable prognostic indicators of mortality in SFTS patients [29]. Our study has identified BUN levels > 5.4 mmol/l and an age of ≥ 60 years as predictors of EBV infection in SFTS patients. EBV infection likely serves as a marker of illness severity in SFTS patients, aligning well with our understanding of herpes virus co-infections.

In this research, several immune and inflammatory biomarkers were confirmed to be useful to the prediction of EBV infection in SFTS patients. CRP and ferritin, biochemical markers of inflammation, have been reported to be associated with poor prognosis in SFTS patients and can be significantly increased when tissues are extensively damaged by DBV [30–33]. Our study confirmed the link between CRP and ferritin levels and EBV infection in SFTS patients. Hyperinflammatory response impaired immunocompetence were leaded by DBV and then EBV infection, which results in a worse outcome. This may have a strong association with the outbreak of inflammatory cytokines after EBV infection, which still needs further mechanism studies to confirm.

From the perspective of other laboratory indicators, we observed that the levels of complement and immunoglobulins can serve as biomarkers for predicting DBV-associated immune dysfunction and EBV infection in SFTS patients. The production of immunoglobulins plays a strong part in the adaptive immune system by binding to pathogens and facilitating their elimination [34]. Previous studies have suggested deficiency in immunoglobulins may lead to immunodeficiency and severe infections [35]. The abnormality of immunoglobulins in relation to EBV infection risk in SFTS patients has not been investigated. In our cohort of SFTS, we discovered that low IgM and IgG levels were risk factors for EBV infection. The complement system can also label pathogens through the alternative, lectin and classical pathways for promoting an inflammatory immune response [36]. C4 is a key component of the complement system and can be decreased through consumption after DBV infection [37]. Our result showed that lower serum concentrations of C4, which indicates excessive complement activation and product consumption were associated with the risk of EBV infection in SFTS.

In addition, there was no significant difference in lymphocyte subsets between the two groups except for $CD56^+$ cells (NK cells), which might due to the small sample size and individual difference. NK cells have been identified as an important immunomodulatory part in both innate immune and adaptive immune response. Individuals with NK cell deficiency can be more susceptible to herpes virus [38]. In contrast, in our multivariable model, higher level of NK cells had an increased risk of EBV infection. Various studies have proved that the proportion and absolute count of NK cells in patients with acute phase and severe SFTS was higher than in convalescent cases and mild SFTS [39]. It was supposed that increased numbers of NK cells may contribute to the extensive tissue damage in SFTS patients, which has been proved to be correlated with EBV infection [40].

To our knowledge, this study is the first large population-based study to prove that SFTS patients with EBV infection are at increased risk for worse prognosis. There was a significant dysregulation of several essential components of the immune and inflammatory system in SFTS patients with EBV infection. Acute-phase proteins, complement factors, immunoglobulins and lymphocyte subsets are dysregulated in SFTS patients, which indicate disruptions of innate and adaptive immunity and contribute to the high risk of EBV infection. Results of our study also provide the insight that further research is needed to determine if anti-herpesvirus drugs can improve the prognosis in SFTS patients with EBV infection. In addition, due to the limited data of cases in this study, it was not possible to further explore the prognosis in SFTS patients with different levels of EBV infection. Finally, efforts need to be maintained to elucidate the impact of SFTS in the reactivation of latent EBV.

Conclusions

In conclusion, EBV infection may be frequent in SFTS patients and increase the mortality. The levels of acute-phase proteins, complement factors, immunoglobulins and lymphocyte subsets have crucial predictive values for the early identification of EBV infection in SFTS patients. It is necessary to strengthen screening for EBV infection and its early predictive markers after admission in these patients. Attention should be paid to SFTS patients with dysregulation of several essential components of the immune and inflammatory system.

Author contributions
QP and YD participated in the research design, writing of the paper, performance of the research, and data analysis; NH and ZT participated in the research design, revision of the paper, and data analysis; PS, NJ, LS, ZF, RW, XH and KJ participated in the research design and data analysis. JL was responsible for the conceptualization and reviewed and edited the manuscript. All authors approved the final version for publication and agree to be held accountable for all aspects of the work.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This research was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (protocol no. 2023-SR-336). However, no informed consent was signed. Due to the retrospective nature of the study, except for personal information, only the clinical data of anonymous patients were collected without infringing on the rights and interests of the patients.

Consent for publication
Written informed consent for publication was obtained from all participants and their parents.

Competing interests
The authors declare no competing interests.

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