BRITISH JOURNAL OF HOSPITAL MEDICINE

ARTICLE

Reactivation of Epstein-Barr Virus Among Neurointensive Care Patients: A Prospective Observational Study

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Abstract

Aims/Background Approximately 90% of the population is seropositive for Epstein-Barr virus (EBV), and its reactivation has been reported to be associated with increased morbidity and mortality in critically ill patients. However, the clinical significance of EBV reactivation among patients in neurointensive care units (NICU) has rarely been investigated. This study aimed to demonstrate the association between EBV reactivation and clinical outcomes in neurocritically ill patients.

Methods Clinical data were collected from 179 patients admitted to the NICU of the Chinese PLA General Hospital between October 2021 and January 2024. These patients were divided into two groups based on EBV infection status: the EBV reactivation group (n = 80) and the non-EBV reactivation group (n = 99). Gender, age, laboratory test results, diagnosis and functional prognosis were compared between the two groups to evaluate the clinical significance of EBV reactivation in neurocritically ill patients.

Results A total of 179 patients were included in this study, of which 80 (44.69%) had EBV reactivation. Patients with EBV reactivation demonstrated higher levels of serum lactic dehydrogenase (32.50% versus 16.16%, p = 0.010), C-reactive protein (36.25% versus 21.21%, p = 0.026), immunoglobulin G (26.25% versus 12.12%, p = 0.015), cerebrospinal fluid leukocyte counts (67.50% versus 47.47%, p = 0.007), interleukin-6 (68.75% versus 42.42%, $p < 0.001$), and interleukin-10 (33.75% versus 17.17%, p = 0.010). There was no significant difference in 6-month mortality between patients with and without EBV reactivation (7.50% versus 3.03%, p = 0.302). However, patients with EBV reactivation exhibited poorer functional prognosis compared to those without (42.50% versus 26.26%, p = 0.022). Central nervous system lymphoproliferative disorders are more common in patients with EBV reactivation.

Conclusion EBV reactivation is frequent among immunocompetent, neurocritically ill patients and is associated with poorer functional prognosis but not with increased 6-month mortality. Furthermore, EBV reactivation is associated with systemic inflammation.

Key words: Epstein-Barr virus; prognosis; reactivation infection; immunocompetent; neurointensive care medicine

Submitted: 21 February 2025 Revised: 28 April 2025 Accepted: 30 April 2025

How to cite this article:
Xu X, Zhao J, Yang X, Liu R, Wang Y, Ma Y, He M, Yang F, Zhang J. Reactivation of Epstein-Barr Virus Among Neurointensive Care Patients: A Prospective Observational Study. Br J Hosp Med. 2025. https://doi.org/10.12968/hmed.2025.0152

Introduction

Epstein-Barr virus (EBV) is a ubiquitous infectious agent in the human population and becomes latent, causing lifelong asymptomatic infection (Wong et al, 2022). The latent virus can be periodically reactivated under certain conditions (Shareena and Kumar, 2023). EBV-infected cells, free viruses, and gene products have been detected in the cerebrospinal fluid (CSF) of patients with neurological

injuries, suggesting that latent viruses can invade the central nervous system (CNS) either directly or indirectly through infected B lymphocytes. Reactivated EBV in the CNS may promote the proliferation of glial cells, and B lymphocytes and T lymphocytes, thereby contributing to the onset and progression of neurological disease (Lee et al, 2021; Soldan and Lieberman, 2020; Zhang et al, 2022). Previous studies have reported that the incidence rate of EBV reactivation in CNS disorders ranges from 5% to 28.9% (Lee et al, 2021; Lupia et al, 2020; Musukuma-Chifulo et al, 2023). However, the mechanism and pathogenicity of EBV reactivation in these patients remain unclear.

Recently, an increasing number of studies have investigated the effects of EBV reactivation on patients. Musukuma-Chifulo et al (2023) suggested that the detection of EBV DNA in CSF may not only indicate CNS infection but could also suggest reactivation in the context of another CNS pathogen infection or immunodeficiency. In immunocompromised patients, EBV reactivation generally results in lymphoproliferative disorder (Sausen et al, 2023). It has been reported that immunocompetent, critically ill patients with EBV reactivation faced increased morbidity (Guiouillier et al, 2024; Libert et al, 2015). These patients experienced fewer ventilator-free days and a higher incidence of acute respiratory distress syndrome (ARDS), infections, and septic shock during hospitalization compared to those without EBV reactivation.

Similarly, a retrospective study by Lee et al (2021) concluded that the detection of EBV DNA in CSF was associated with encephalitis and poor prognosis in immunocompetent patients with CNS disorders. Other studies have identified EBV infection as a significant risk factor for multiple sclerosis (Aloisi et al, 2023; Farrell, 2023; Vietzen et al, 2023). A longitudinal study has demonstrated that EBV-seropositive individuals have a significantly higher risk of developing multiple sclerosis compared to EBV-seronegative individuals (Bjornevik et al, 2022).

However, the clinical significance of EBV reactivation in patients with CNS disorders, particularly immunocompetent patients, remains poorly understood. To address this gap, we conducted a study to evaluate whether EBV reactivation in immunocompetent, neurocritically ill patients is associated with 6-month survival and functional prognosis. Additionally, we aimed to identify the factors associated with EBV reactivation in this category of patients.

Methods

General Information

Clinical data from 179 patients in the neurointensive care units (NICU), who were admitted to the Chinese PLA General Hospital between October 2021 and January 2024, were collected and analyzed. The patients were divided into EBV reactivation group (n = 80) and non-EBV reactivation group (n = 99) based on the EBV infection status. This study was approved by the Ethics Committee of the Chinese PLA General Hospital (Approval ID: S2022-314-01). All procedures followed the ethical principles outlined in the Declaration of Helsinki (World Medical

Association, 2013), and informed consent was obtained from the patients or legal guardians of all the participants.

Inclusion and Exclusion Criteria

Inclusion criteria of this study are as follows:

(a) Patients aged ≥14 years;

(b) Patients with fever ≥37.3 °C for more than 3 days;

(c) Patients with abnormal CSF examination results, defined as leukocytosis ($>10 \times 10^6$/L) and/or elevated protein concentration (>400 mg/L) and/or decreased glucose levels (<2.8 mmol/L) (Ou et al, 2023);

(d) Patients with abnormal neuroimaging findings, which are characterized by abnormal signals in the brain parenchyma, spinal cord, or cerebrospinal meninges on cranial magnetic resonance imaging (MRI) (Ou et al, 2023);

(e) Patients, or whose legal guardians, who had given their consent;

(f) Patients with complete medical records.

Exclusion criteria of the present study are as follows:

(a) Patients with a history of malignancy, or human immunodeficiency virus (HIV) infection, or receiving long-term immunosuppressive therapy or glucocorticoids;

(b) Patients with uncertain EBV infection status;

(c) Patients with contraindications to lumbar puncture or refusal of lumbar puncture;

(d) Patients who refused to participate in the study or were lost to follow-up.

Data Collection and Follow-up

On admission to the NICU and during the second week after admission, EBV testing was performed on blood and CSF samples from all enrolled patients. EBV serology was conducted using chemiluminescence, and EBV DNA was quantified using polymerase chain reaction (PCR) (forward primer: 5′-CGTCTCCCCTTTG GAATGG-3′, reverse primer: 5′-GAAATAACAGACAATGGACTCCCTTAG-3′, and EBV ebna1 probe: 6Fam-5′-CCTGGACCCGGCCCACAA-3′) and metagenomic next-generation sequencing (mNGS). Prior EBV infection was indicated by the presence of Epstein-Barr nuclear antigen (EBNA)-immunoglobulin (Ig) G and virus capsid antigen (VCA)-IgG. EBV reactivation was defined as any of the following: EBV-VCA-IgA ≥40 U/mL, EBV-VCA-IgM ≥40 U/mL, EBV DNA >1000 copies/m, or a specific read number of EBV ≥3 detected via mNGS.

The Modified Rankin Scale (mRS) score (van Swieten et al, 1988) were calculated at admission and after 6 months post-discharge to assess neurological function. An mRS score of 0–2 indicated functional independence, whereas a score of 3–6 indicated poor functional outcomes. All scores were assessed by two neurologists. Patient characteristics, including age, gender, underlying diseases or conditions, serological test, and CSF examination results (all CSF specimens came from a lumbar puncture), were documented. Further information, such as diagnosis, ventilator utilization, mortality, and functional outcomes at six months, was prospectively recorded.

Definition of CNS Disorders

The diagnostic criteria for tuberculous meningitis were based on Thwaites' criteria (Marais et al, 2010), whereas other CNS infectious diseases were diagnosed in accordance with the guidelines of the Practical Neurology (5th edition) (Lv and Zhou, 2021). Fungal infections were confirmed based on pathogenic evidence. Immune-mediated CNS diseases are a group of conditions caused by abnormal immune system attack on the CNS, typically manifesting as an inflammatory or demyelinating response in the nervous system (Co et al, 2017).

Currently, there is no universal consensus regarding the diagnosis of central nervous system lymphoproliferative disorder (CNS-LPD). In this study, the diagnoses were based on the criteria for chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS) (Tobin et al, 2017), and recommendations for chronic active EBV infection (CAEBV) with CNS involvement (Ou et al, 2023). The diagnostic criteria applied in this study were as follows:

(a) Histopathologic and immunohistochemical diagnosis of CNS-LPD;

(b) Meeting the diagnostic criteria for EBV reactivation;

(c) Evidence of CNS involvement: (i) presence of neurological symptoms and signs; (ii) abnormal CSF examination; (iii) abnormal neuroimaging findings;

(d) Responsiveness to glucocorticoid therapy, as indicated by the relief of clinical symptoms, improvement in abnormal CSF indicators, or reduction in lesion number or volume on imaging following glucocorticoid treatment;

(e) Exclusion of other CNS diseases, such as inflammatory demyelinating diseases, infectious diseases, gliomas, and other CNS malignant tumors.

A definite diagnosis of CNS-LPD was made if criterion (a) was satisfied. A diagnosis of probable CNS-LPD was made if criteria (b), (d), (e), and at least one of (c) were met.

Statistical Analysis

Statistical analyses were performed using R, version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria), and Kaplan-Meier curves were generated using GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA). Expressed as absolute values and proportions, qualitative data were analyzed using chi-square or Fisher's exact test. The chi-square test was used when the theoretical T value was ≥ 5 and the total number of cases was ≥ 40; and the Fisher's exact test was used when the theoretical T-value satisfied $1 \leq T < 5$ or the total number of cases was <40 or when the p-value obtained from the test was close to the test level of α. Normality was tested using the Shapiro-Wilk test. Quantitative variables that followed a non-normal distribution are presented as medians with interquartile range (Q1 and Q3) and were analyzed using Mann-Whitney test. Kaplan-Meier curves were plotted to evaluate survival outcomes in patients with and without EBV reactivation. Survival times of patients with EBV reactivation at admission and during the second week of admission were not compared due to the low overall mortality rate in the sample. A p-value < 0.05 was considered statistically significant.

Table 1. Clinical characteristics of the study population.

	All patients (*n* = 179)	EBV reactivation group (*n* = 80)	Non-EBV reactivation group (*n* = 99)	χ^2/z	*p*-value
Age, years	37 (26, 53)	42 (24, 55)	36 (28, 52)	–0.473	0.636
Male, *n* (%)	121 (67.60)	53 (66.25)	68 (68.69)	0.120	0.729
mRS score at admission	2 (1, 4)	2 (1, 4)	2 (1, 3)	–0.178	0.859
Medical history, *n* (%)					
Diabetes or IGT	40 (22.35)	18 (22.50)	22 (22.22)	0.002	0.965
Hypertension	29 (16.20)	16 (20.00)	13 (13.13)	1.538	0.215
CHD	7 (3.91)	4 (5.00)	3 (3.03)	-	0.702
Serological test result, *n* (%)					
Liver impairment	54 (30.17)	27 (33.75)	27 (27.27)	0.881	0.348
Kidney impairment	8 (4.47)	3 (3.75)	5 (5.05)	-	0.733
Elevated LDH	42 (23.46)	26 (32.50)	16 (16.16)	6.577	0.010
Elevated CRP	50 (27.93)	29 (36.25)	21 (21.21)	4.970	0.026
Elevated IL-6	78 (43.58)	39 (48.75)	39 (39.39)	1.575	0.209
Elevated IL-10	6 (3.35)	3 (3.75)	3 (3.03)	-	1.000
Elevated IgG	33 (18.44)	21 (26.25)	12 (12.12)	5.874	0.015
Elevated IgA	11 (6.15)	5 (6.25)	6 (6.06)	-	1.000
Elevated IgM	3 (1.68)	2 (2.50)	1 (1.01)	-	0.587
$CD3^+$ T-lymphocyte	74.14 (66.38, 78.88)	74.34 (67.12, 79.85)	72.01 (66.34, 78.57)	–1.039	0.299
$CD4^+$ T-lymphocyte	40.24 (32.70, 47.33)	40.18 (30.88, 47.53)	40.24 (34.81, 47.34)	–0.332	0.740
$CD8^+$ T-lymphocyte	27.79 (23.01, 33.99)	28.14 (23.76, 36.47)	27.16 (22.11, 31.55)	–1.214	0.225
$CD4^+/CD8^+$ T-lymphocyte	1.41 (1.06, 1.95)	1.32 (0.92, 1.96)	1.44 (1.23, 1.87)	–1.3173	0.188
B-lymphocyte	14.49 (9.25, 20.30)	14.90 (9.41, 20.14)	14.14 (9.06, 21.19)	–0.087	0.930
NK cell	9.87 (5.92, 15.22)	10.12 (5.86, 14.54)	9.76 (6.12, 16.11)	–0.500	0.617
CSF involvement, *n* (%)					
Elevated intracranial pressure	93 (51.96)	39 (48.75)	54 (54.55)	0.595	0.440
Elevated leukocyte count	101 (56.42)	54 (67.50)	47 (47.47)	7.216	0.007
Elevated protein concentration	123 (68.72)	59 (73.75)	64 (64.65)	1.706	0.192
Decreased glucose levels	44 (24.58)	25 (31.25)	19 (19.19)	3.470	0.062
Reduced chloride levels	36 (20.11)	18 (22.50)	18 (18.18)	0.514	0.474
Elevated IL-6	97 (54.19)	55 (68.75)	42 (42.42)	12.352	<0.001
Elevated IL-10	44 (24.58)	27 (33.75)	17 (17.17)	6.560	0.010

CHD, coronary heart disease; CRP, C-reactive protein; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; Ig, immunoglobulin; IGT, impaired glucose tolerance; IL, interleukin; LDH, lactic dehydrogenase; mRS, Modified Rankin Scale; NK cell, natural killer cell.

Results

Clinical Characteristics of the Patients

Of the 200 patients enrolled in the study, 21 were excluded because they either did not meet the inclusion criteria (19 patients) or were lost to follow-up (2 patients) (Fig. 1). Finally, 179 patients were included in the analysis. The median age of the included patients was 37 (26, 53) years, and 67.60% of them were males. The median mRS score at admission was 2 (1, 4). The diagnoses of patients admitted to the NICU were diverse: 30.73% were diagnosed with immune-mediated CNS disorders, 17.32% with CNS-LPD, 16.76% with virus encephalitis or meningitis, 9.50% with bacterial meningitis/brain abscess, 7.26% with tuberculous meningitis, 6.70% with fungal meningitis, and 11.73% with other conditions. The clinical characteristics of the patients are summarized in Table 1.

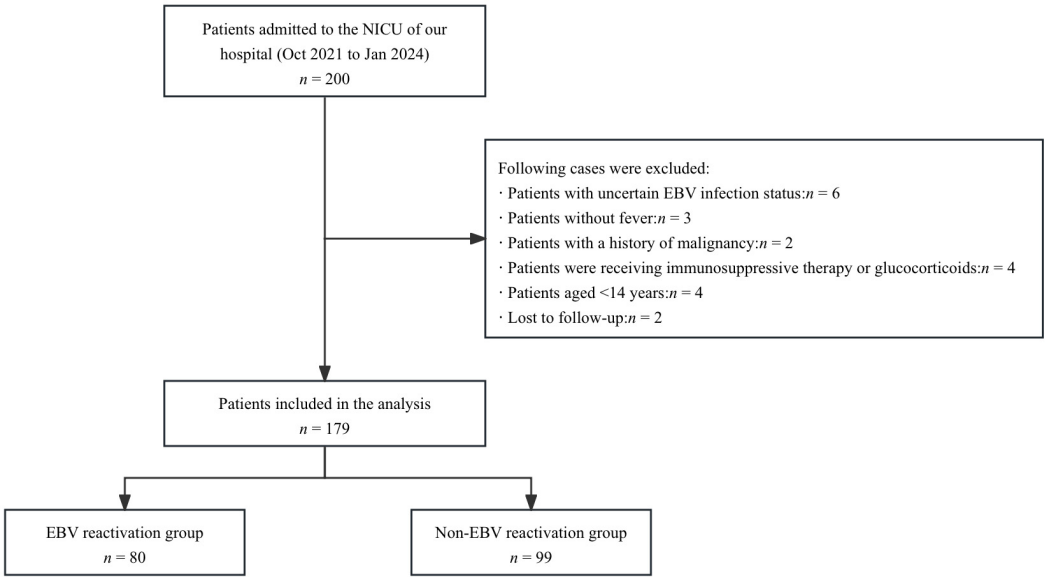

Fig. 1. Flowchart of patient screening and enrollment. NICU, neurointensive care units; EBV, Epstein-Barr virus.

Viral Reactivation

In this study, 80 patients (44.69%) had EBV reactivation. EBV serology was positive in 59 patients; EBV DNA was detected in the blood or CSF samples of 46 patients; and EBV was detected using mNGS in 69 patients. Compared to traditional methods, such as serology and PCR, mNGS is more sensitive in diagnosing infectious diseases of the CNS. And previous studies have reported that the sensitivity of mNGS is about 73%–83%, while the sensitivity of traditional methods is less than 50% (Feng et al, 2023; Wilson et al, 2019; Xing et al, 2020; Zhang et al, 2020). Thirty-seven patients had EBV reactivation upon admission. Forty-three patients had EBV reactivation within two weeks of admission. There were no significant differences between the two groups in gender, age, mRS score, or medical history at admission ($p > 0.05$). However, more patients with EBV reactivation demon-

Table 2. Comparison of blood and CSF tests at admission and two weeks after admission in 179 patients.

	At admission	Two weeks after admission	χ^2	*p*-value
Serological test, *n* (%)				
Elevated CRP	50 (27.93)	47 (26.26)	0.127	0.721
Elevated IL-6	78 (43.58)	55 (30.73)	6.329	0.012
Elevated IL-10	6 (3.35)	2 (1.12)	-	0.284
Elevated IgG	33 (18.44)	30 (16.76)	0.173	0.677
Elevated IgA	11 (6.15)	6 (3.35)	1.544	0.214
Elevated IgM	3 (1.68)	0 (0)	-	0.248
CSF involvement, *n* (%)				
Elevated intracranial pressure	93 (51.96)	40 (22.35)	33.605	<0.001
Elevated leukocyte count	101 (56.42)	88 (49.16)	1.894	0.169
Elevated protein concentration	123 (68.72)	109 (60.89)	2.400	0.121
Decreased glucose levels	44 (24.58)	26 (14.53)	5.754	0.016
Reduced chloride levels	36 (20.11)	12 (6.70)	13.858	<0.001
Elevated IL-6	97 (54.19)	72 (40.22)	7.005	0.008
Elevated IL-10	44 (24.58)	31 (17.32)	2.851	0.091

strated elevated lactic dehydrogenase (LDH) (32.50% versus 16.16%, $p = 0.010$), C-reactive protein (CRP) (36.25% versus 21.21%, $p = 0.026$), IgG (26.25% versus 12.12%, $p = 0.015$), CSF leukocyte counts (67.50% versus 47.47%, $p = 0.007$), interleukin-6 (IL-6) (68.75% versus 42.42%, $p < 0.001$), and interleukin-10 (IL-10) (33.75% versus 17.17%, $p = 0.010$) (Table 1).

In addition, comparison of blood and CSF examinations at admission and two weeks after admission revealed significantly fewer individuals with elevated serum interleukin (IL)-6 (43.58% versus 30.73%, $p = 0.012$), elevated CSF pressure (51.96% versus 22.35%, $p < 0.001$), decreased glucose (24.58% versus 14.53%, $p = 0.016$), reduced chloride levels (20.11% versus 6.70%, $p < 0.001$), and elevated IL-6 levels (54.19% versus 40.22%, $p = 0.008$) (Table 2).

Mortality and Morbidity

The 6-month mortality rate for patients with EBV reactivation was 7.50% (6/80), compared to 3.03% (3/99) for patients without EBV reactivation ($p = 0.302$, Table 3). The 6-month poor prognosis rate for patients with EBV viral reactivation was 42.50% (34/80) versus 26.26% (26/99) for patients without EBV reactivation ($p = 0.022$, Table 3). Thirty-four cases in the EBV reactivation group showed poor functional prognosis, including 16 patients with varying degrees of motor dysfunction, 7 patients required continuous care due to varying degrees of impaired consciousness, 5 patients were unable to live independently due to cognitive dysfunction, and 6 patients died. However, no significant difference in the 6-month prognosis rate was observed in patients with EBV reactivation at two different time points: at admission and during the second week of admission (43.24% versus 41.86%, $p = 1.000$). The Kaplan-Meier analysis demonstrated no significant difference in survival between patients with and without EBV reactivation ($p = 0.17$) (Fig. 2).

Table 3. Morbidity and mortality of patients with or without EBV reactivation.

	EBV reactivation group (n = 80)	Non-EBV reactivation group (n = 99)	χ^2	p-value
Death at 6 months, n (%)	6 (7.50)	3 (3.03)	-	0.302
Poor functional outcomes at 6 months, n (%)	34 (42.50)	26 (26.26)	5.235	0.022
Requiring ventilator, n (%)	21 (26.25)	20 (20.20)	0.917	0.338
Using catecholamine treatment, n (%)	11 (13.75)	6 (6.06)	3.044	0.081
Diagnosis, n (%)			41.726	<0.001
Viral encephalitis or meningitis	8 (10.00)	22 (22.22)		
Bacterial meningitis/brain abscess	9 (11.25)	8 (8.08)		
Tuberculous meningitis	5 (6.25)	8 (8.08)		
Fungal meningitis	5 (6.25)	7 (7.07)		
Immune-mediated CNS disorders	20 (25.00)	35 (35.35)		
CNS-LPD	29 (36.25)	2 (2.02)		
Others	4 (5.00)	17 (17.17)		

CNS-LPD, central nervous system lymphoproliferative disorder.

Additionally, we found that the proportion of patients requiring ventilator and catecholamine therapy was not statistically different between the two groups ($p > 0.05$, Table 3).

In addition, there were significant differences in the disease composition between patients with and without EBV reactivation ($p < 0.001$): a greater proportion of patients in the EBV reactivation group were diagnosed with CNS-LPD, whereas a greater proportion of patients in the non-EBV reactivation group were diagnosed with viral CNS infections, and the proportion of immune-mediated CNS disorder did not differ significantly between the two groups. The majority of the patients with CNS-LPD had EBV reactivation (29/31, 93.55%), and 20 of 55 patients with immune-mediated CNS disease had EBV reactivation (36.36%). One-third of patients with EBV reactivation had concomitant infection with other pathogens (27/80, 33.75%), and 27 of 72 patients with CNS infection had EBV reactivation (37.50%) (Table 3).

Discussion

To our knowledge, this is the first prospective study to evaluate the impact of EBV reactivation in immunocompetent patients in the NICU with a follow-up of six months. The main finding of this study was that patients with EBV reactivation have poorer functional prognosis. However, EBV reactivation was not associated with increased mortality rate. Moreover, we observed that patients with EBV reactivation had elevated serum levels of LDH, CRP, IgG, CSF leukocyte counts, IL-6, and IL-10 compared to those without reactivation. Furthermore, CNS-LPD was more commonly observed in patients with EBV reactivation.

Few studies have examined EBV reactivation in CNS diseases. Musukuma-Chifulo et al (2023) found that the prevalence of EBV DNA in the CSF of adults living with HIV and presenting neurological symptoms in Zambia was 28.9%. Sim-

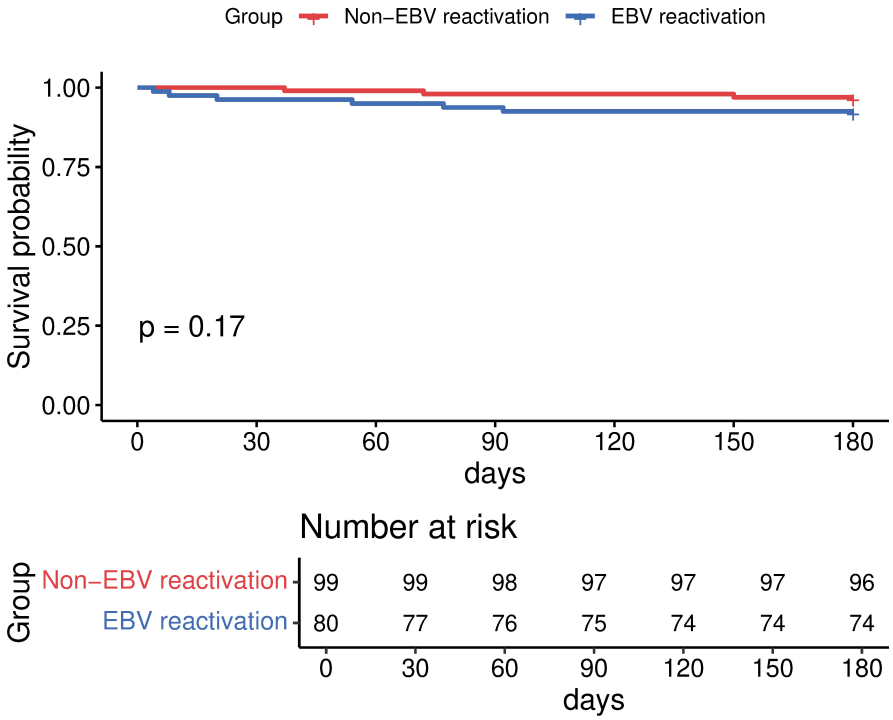

Fig. 2. Kaplan-Meier survival analyses based on patients with and without EBV reactivation.

ilarly, Lupia et al (2020) reported that EBV DNA was detected in the CSF of 25.1% of the patients with viremia. In this study, all patients tested carried latent EBV infection, and the prevalence of EBV reactivation was 44.69%. Possible explanations for the discrepancy in EBV detection rate compared to other studies include the following: First, we used both blood and CSF samples for EBV serology, EBV DNA quantification, and mNGS to screen for EBV reactivation, a strategy that may increase the positive rate of the test; and second, neurocritically ill patients may have underlying immune dysfunction that contributes to EBV reactivation.

There are limited data on the prognostic impact of EBV reactivation in neurocritically ill or noncritical patients, and studies on its prognostic impact in patients in the intensive care unit (ICU) have exhibited mixed results. In a multicenter prospective study, Guiouillier et al (2024) found no significant difference in mortality between patients with and without EBV reactivation. However, survival was significantly lower in patients with EBV reactivation present at the time of admission compared to those without. Goh et al (2020) found a trend toward higher 28-day mortality in sepsis patients with EBV reactivation in the ICU, although the difference was statistically nonsignificant. Simonnet et al (2021) also found no association between mortality and EBV reactivation in critically ill patients. Conversely, He et al (2017) reported a significant increase in mortality among ICU patients with ARDS who carried EBV reactivation, compared to those without. Our study did not demonstrate a significant difference in mortality between patients with and without EBV reactivation. However, we found that patients with EBV reactivation exhibited a worse 6-month functional prognosis. The mRS is primarily used to assess the ability to perform daily activities and the degree of disability in patients with stroke, and in this study, it was used as an index to assess the functional prognosis of the

patients, so as to provide a clearer understanding of the prognosis of the patients. Therefore, larger multicenter prospective studies are needed to further clarify the correlation between EBV reactivation and prognosis in neurocritically ill patients.

In addition, we observed an association between EBV reactivation and systemic inflammation, as evidenced by the elevated blood CRP, IgG, CSF IL-6, and IL-10 levels. Similar results have been reported in previous studies; for instance, Lehner et al (2020) reported higher levels of IL-6 and CRP in COVID-19 patients with EBV reactivation in a retrospective study, and Peluso et al (2023) found that EBV reactivation was associated with elevated IL-10 levels. This association may be correlated with the effects of EBV reactivation on the immune system, which has been found to induce deleterious effects though deoxyuridine triphosphate nucleotide hydrolase (dUTPase) *in vitro*, as well as immune dysregulation and expression of IL-6 and IL-10 in peripheral blood mononuclear cells (PBMCs) (Glaser et al, 2006; Guiouillier et al, 2024). These cytokines have been implicated in the immune effects of EBV and tumorigenesis (Huang et al, 2017). A study by Chen et al (2016) found that the levels of IL-10 were highly elevated in the CSF of patients with primary central nervous system lymphoma, a sign of the tumor cell proliferation. Therefore, the high expression of inflammatory indicators in patients with EBV reactivation observed in this study may be associated with the occurrence of immune-mediated CNS disorders and CNS-LPD. Lymphoproliferative disorders are among the most common diseases that are caused by EBV reactivation. The clinicopathological spectrum ranges from lymphoproliferation with minimal malignant potential to malignant proliferation with an aggressive clinical course (Quintanilla-Martinez et al, 2023). In the current study, 25.00% and 36.25% of the patients with EBV reactivation were diagnosed with immune-mediated CNS disorders and CNS-LPD, respectively. Besides, 36.36% (20/55) of the patients with immune-mediated CNS disorders and 93.55% (29/31) of the patients with CNS-LPD had EBV reactivation. However, the mechanisms underlying EBV reactivation in immune-mediated CNS disorders and CNS-LPD remain obscure and thus require further investigations.

EBV reactivation is commonly detected in patients with other CNS pathogenic infections, such as herpes simplex virus (HSV), varicella zoster virus (VZV) and cytomegalovirus (CMV). In a retrospective study, Lee et al (2021) found that among 42 immunocompetent patients with EBV reactivation, 23.81% had EBV detected along with other CNS pathogens. In our study, 33.75% (27/80) of patients with EBV reactivation had concurrent infections with other CNS pathogens, and 37.50% (27/72) of CNS infections had EBV reactivation. EBV reactivation is associated with a wide spectrum of neurological complications; however, its pathogenic role remains unclear, as serum or CSF EBV positivity does not necessarily indicate that EBV is the causative agent. Consequently, positive EBV results should be interpreted with caution, and other potential diseases should be carefully excluded.

Moreover, previous studies have demonstrated that EBV reactivation is associated with prolonged hospital stay, increased ventilator time, prolonged catecholamine therapy, ARDS, infections, and septic shock in patients receiving care in the ICU (Goh et al, 2020; Simonnet et al, 2021). However, these associations were

not observed in this study. Possible reasons for this discrepancy include the fact that all our patients were from the NICU, and their reasons for admission differed significantly from those of patients in the ICU. Furthermore, neurocritically ill patients in our center demonstrated a higher prevalence of CNS infections (40.22%), and the median age of our patients was lower compared to those in other studies, which may have influenced the results.

Several limitations of this study should be acknowledged. First, only a subset of neurocritically ill patients, primarily middle-aged individuals, were selected from a single center; therefore, the results obtained may not fully represent the broader population of neurocritically ill patients. Second, we may have underestimated the frequency of EBV reactivation because we only screened for it at admission and during the second week of admission. Third, our sample had a low mortality rate after six months of follow-up, indicating that a larger sample size and a longer follow-up period may be necessary to validate our findings.

Conclusion

EBV reactivation is common in immunocompetent patients with neurocritical illnesses. Although it is not associated with increased mortality, it is linked to a poorer functional prognosis. Additionally, EBV reactivation is associated with systemic inflammation, and patients with EBV reactivation are more likely to develop CNS-LPD.

Key Points

- Epstein-Barr virus (EBV) reactivation is frequent among immunocompetent, neurocritically ill patients.
- EBV reactivation is associated with a poorer functional prognosis in immunocompetent, neurocritically ill patients but not with mortality.
- EBV reactivation is associated with elevated serum CRP, IgG, LDH, CSF leukocyte count, IL-6 and IL-10.
- The unique clinical characteristics and functional prognosis of neurocritically ill patients with EBV reactivation highlight the need for new strategies for prevention and management of EBV reactivation.

Availability of Data and Materials

The data included in this study are available from the corresponding author on reasonable request.

Author Contributions

JTZ, YW, YM, MH and FY designed the research study. XX, JHZ, XY and RL performed the research, data collection and analysis. XX wrote the first draft. All authors contributed to revising the manuscript critically for important intellectual

content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Chinese PLA General Hospital (Approval ID: S2022-314-01). All procedures followed the ethical principles outlined in the Declaration of Helsinki, and informed consent was obtained from the patients or legal guardians of all the participants.

Acknowledgement

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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