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Clinical significance of Epstein-Barr virus DNA quantification in the cerebrospinal fluid: villain, accomplice, or innocent bystander?

Klinički značaj kvantifikacije DNA virusa Epstein–Barr u cerebrospinalnoj tekućini: zlikovac, suučesnik ili nedužni promatrač?

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Summary

Objective: Clinical significance of Epstein-Barr (EBV) virus DNA in the cerebrospinal fluid (CSF) is not fully understood. Our aim was to evaluate clinical implications of detectable EBV DNA in the cerebrospinal fluid (CSF) of immunocompetent and immunocompromised patients in the context of their final diagnosis.

Methods: This was a single-center, retrospective cohort study that included all consecutively hospitalized adult patients whose CSF samples were referred for PCR-based detection of EBV DNA in the period between January 2008 and December 2019. The patients were classified as having encephalitis according to the International Encephalitis Consortium case definition.

Results: EBV DNA was detectable in 54/646 CSF samples (8.4%) from 37 patients. Of 34 patients included in this analysis, 15 patients were diagnosed with an immunocompromising condition including advanced HIV disease (n=12), solid organ transplantation (n=2) and chronic lymphocytic leukemia (n=1). The most frequent clinical diagnosis in HIV-infected patients included biopsy-proven primary CNS lymphoma (PCNSL, n=5) and toxoplasmosis (n=3). Six of 15 immunocompromised patients died (including 4/5 with CNSL). The immunocompetent group (n=19) included patients with viral CNS infections (n=6), EBV encephalitis (n=5), postinfectious meningoencephalitis (n=1), autoimmune encephalitis (n=1), neurotuberculosis (n=1) and five patients with uncertain etiology. Four patients died during hospitalization (all with unknown etiology). Patients with EBV encephalitis were treated with acyclovir (n=4) and corticosteroids (n=5) and they all improved.

Conclusions: CSF EBV-DNA is of highest clinical significance in patients with advanced HIV disease and primary CNS lymphoma, but it requires careful interpretation in immunocompetent adults, particularly those with CNS co-infections.

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Sažetak

Cilj: Klinički značaj DNA virusa Epstein–Barr (EBV) u cerebrospinalnoj tekućini još uvijek nije u potpunosti razjašnjen. Cilj ovog istraživanja bio je procijeniti kliničke implikacije detektabilne EBV DNA u cerebrospinalnoj tekućini imunokompetentnih i imunokompromitiranih bolesnika u kontekstu njihove konačne dijagnoze.

Metode: Provedena je jednocentrična, retrospektivna kohortna studija, koja je obuhvatila sve uzastopno hospitalizirane odrasle bolesnike čiji su uzorci cerebrospinalne tekućine bili upućeni na PCR detekciju EBV DNA u razdoblju od siječnja 2008. do prosinca 2019. godine. Bolesnici su klasificirani kao oboljeli od encefalitisa prema definiciji Međunarodnog konzorcija za encefalitis.

Rezultati: EBV DNA je detektirana u 54 od 646 uzoraka cerebrospinalne tekućine (8,4%) kod 37 bolesnika. Od 34 bolesnika uključenih u analizu, 15 je bilo imunokompromitirano, s uznapredovalom HIV infekcijom (n=12), transplantacijom solidnog organa (n=2) i kroničnom limfocitnom leukemijom (n=1). Najčešće dijagnoze među HIV-pozitivnim bolesnicima bile su biopsijom potvrđen primarni limfom središnjeg živčanog sustava (PCNSL, n=5) i toksoplazmoza (n=3). Šest od 15 imunokompromitiranih bolesnika je preminulo, uključujući četiri od pet bolesnika s PCNSL-om.

Skupina imunokompetentnih bolesnika (n=19) uključivala je bolesnike s virusnim infekcijama središnjeg živčanog sustava (n=6), EBV encefalitisom (n=5), postinfekcijskim meningoencefalitisom (n=1), autoimunim

encefalitisom (n=1), neurotuberkulozom (n=1) te pet bolesnika s nejasnom etiologijom. Četiri bolesnika preminula su tijekom hospitalizacije (svi s nepoznatom etiologijom). Bolesnici s EBV encefalitisom liječeni su aciklovirom (n=4) i kortikosteroidima (n=5) te su svi pokazali kliničko poboljšanje.

Zaključak: Detekcija EBV DNA u cerebrospinalnoj tekućini ima najveći klinički značaj u bolesnika s uznapredovalom HIV infekcijom i primarnim limfomom središnjeg živčanog sustava, no zahtijeva opreznu interpretaciju kod imunokompetentnih odraslih osoba, osobito uz prisutnost koinfekcija središnjeg živčanog sustava.

Introduction

Epstein-Barr (EBV) virus is a widely disseminated herpesvirus infecting more than 90% of adult population.[1] After primary infection in childhood, which is usually asymptomatic or presents with syndrome of infectious mononucleosis, latent, lifelong infection develops. In contrast to other herpesviruses, reactivation of EBV is considered clinically significant only in immunocompromised patients, usually associated with lymphoproliferative disorders.[1]

EBV can cause various neurological disorders, mainly in pediatric population, often in the absence of symptoms of acute infectious mononucleosis, which can lead to severe neurological disease. Indeed, the majority of data on neurological complications of EBV infection are from pediatric population.[2,3]

In immunocompromised patients, the finding of EBV DNA has been historically considered as a marker of lymphoproliferative disease.[4] In contrast, there are only several case reports or small case series describing EBV CNS infection in immunocompetent adults.[5-11] Furthermore, due to the availability of PCR diagnostics, EBV DNA has been more frequently detected in CSF samples, often as a co-pathogen or in other neurological conditions.[12-15]

Therefore, the role of Epstein-Barr (EBV) virus in central nervous system (CNS) infections and the clinical significance of EBV DNA in the cerebrospinal fluid (CSF) are not fully understood.

The aim of this study was to investigate clinical implications of positive CSF EBV DNA PCR results in the context of the patients' final diagnosis.

Materials and Methods

Study design and population

This was a retrospective cohort study that included all consecutively hospitalized adult patients whose CSF samples were subjected to PCR-based detection of EBV DNA from January 2008 to December 2019 at the University Hospital for Infectious Diseases, Zagreb, Croatia (UHID). Included were patients diagnosed with syndrome of encephalitis and excluded those for whom sufficient medical records were not available. The study conformed to the ethical guidelines of the Declaration of Helsinki and approved by the UHID Ethics Committee (August 30th 2019, approval number 1-1247-3-2019).

Data collection and definitions

Demographic data, comorbidities, clinical presentation, selected laboratory data (including CSF white blood cell count, glucose and proteins), clinical evaluation, treatment, complications, mortality, and functional status at hospital discharge were collected from patients' charts.

Patients were classified as having encephalitis according to the International Encephalitis Consortium case definition.[16] Individuals were considered immunocompetent if they had no immunocompromising disease and did not receive immunosuppressive drugs. CNS lymphoma (CNSL) was defined as typical histopathologic or neuroimaging findings without alternative diagnoses. Patients were considered to have alternative diagnosis if other pathogen was detected accompanied with characteristic clinical presentation.

EBV DNA quantification in the CSF and serum was performed by using a standardized real-time PCR assay (LightCycler EBV quantification kit, Roche Diagnostics, Mannheim, Germany). Results of EBV serology including IgM and IgG antibodies to viral capsid antigen, IgG antibodies to early antigen-diffuse (EA-D) and EBV nuclear antigen (EBNA) were analyzed to assess whether the patients had primary infection or reactivation.

Statistical analysis

Clinical characteristics, laboratory and demographic data were evaluated and descriptively presented as frequencies (%) and medians with interquartile ranges (IQR). We used Fisher's exact test and the Mann-Whitney U test to compare the two groups. All tests were two-tailed; a p value < 0.05 was considered statistically significant. Statistical analyses were performed using the GraphPad Prism Software version 9.1.1. (San Diego, California, USA).

Results

Of 646 CSF samples tested for EBV by real-time PCR, a total of 54 CSF samples (8.4%) from 37 patients

were positive for EBV DNA. Medical records were incomplete for three patients who were excluded. Therefore, 34 patients were included in our cohort and their demographic and clinical features are shown in Table 1 and Table 2. A total of 15 patients were diagnosed with an immunocompromising condition, including human immunodeficiency virus type 1 infection (HIV-1; n=12), solid organ transplantation (n=2), and hematological malignancy (chronic lymphocytic leukemia, CLL, during R-CHOP therapy). The remaining 19 patients did not have any evident immunocompromising condition.

CSF EBV DNA in immunocompromised adults

A total of 12 patients had advanced HIV disease (11 males, median age 43.5, IQR 35-48.7 years) with low CD4+ T-cell counts (median 23, IQR 27-49 cells/μL) and a median viral load of 372,500 HIV-1 RNA copies/mL (IQR 125,794-829,250 copies/mL) (Table 1). All patients had CSF pleocytosis with a median of 273 WBC / mm^3 (IQR 17-738) with predominance of mononuclear cells (median 100%, IQR 95-100%) and proteinorrachia (665 mg/dL, IQR 480-1343). Regarding clinical presentation, seven patients presented with altered mental status, eight with headache and four with seizures.

Clinical diagnoses in HIV-infected patients included: biopsy-proven primary CNS lymphoma (PCNSL, n=5; EBV DNA of 99,325 copies/mL, IQR 7,112-201,125), toxoplasmosis (n=3, including 1 suspected CNSL; EBV DNA 226,500 copies/mL, IQR 174,750-226,500), cryptococcal meningitis (n=1), tuberculous meningitis (n=2) and two patients with HIV-encephalopathy (including one patient with CMV coinfection). The mortality was high in this group, six patients died (four with CNSL, one with toxoplasmosis, one with advanced HIV encephalopathy).

Of two solid-organ transplant recipients, one had invasive CNS aspergillosis (EBV DNA 5,700 copies/mL) and the other EBV reactivation (EBV DNA

Table 1. Selected demographic, laboratory and clinical parameters in immunocompromised patients with detectable Epstein-Barr virus DNA in the cerebrospinal fluid

Tablica 1. Odabrani demografski, laboratorijski i klinički parametri u imunokompromitiranih bolesnika s detektabilnom DNA virusa Epstein-Barr u cerebrospinalnoj tekućini

Pt	Age	Gender	Underlying condition and the number of CD4+ T-cells	CSF – cells/μL	CSF – protein mg/L	CSF EBV DNA, copies/mL	Co-pathogen	Definite diagnosis	Outcome
1	37	M	HIV / CD4+ 49	2,784	410	236,000	-	CNSL	Fatal
2	44	M	HIV / CD4+ 50	24	640	149,500	*T. gondii*	Toxoplasmosis	Fatal
3	58	M	HIV / CD4+ 20	15	350	30,000	CMV	HIV encephalopathy	Fatal
4	25	M	HIV / CD4+ 16	12	480	9,150	-	CNSL	Fatal
5	36	M	HIV / CD4+ 43	15	690	21,500	-	HIV encephalopathy	Improved
6	46	M	HIV / CD4+ 23	1,260	12,800	38,650	*M. tuberculosis*	Tuberculosis	Improved
7	30	M	HIV / CD4+ 8	1,440	2,009	11,350	*C. neoformans*	Cryptococcosis	Improved
8	48	F	HIV / CD4+ 24	390	690	253,000	*T. gondii*	Toxoplasmosis	Improved
9	32	M	HIV / CD4+ 333	156	1,121	1,000	-	CNSL	Fatal
10	53	M	HIV / CD4+ 60	18	480	55,000	-	CNSL	Fatal
11	51	M	HIV / CD4+ 17	564	2,320	189,500	-	CNSL	Improved
12	43	M	HIV / CD4+ 18	492	540	200,000	*T. gondii*	Toxoplasmosis	Improved
13	18	M	SOT - liver	80	360	402,000	-	Encephalitis	Improved
14	34	M	SOT - heart	2304	273	5,700	*A. fumigatus*	Aspergillosis	Fatal
15	56	F	CLL - CHOP	8	550	186,500	-	EBV encephalitis	Improved

- *Toxoplasma gondii* (*T. gondii*), cytomegalovirus (CMV), *Mycobacterium tuberculosis* (*M. tuberculosis*), *Cryptococcus neoformans* (*C. neoformans*), *Aspergillus fumigatus* (*A. fumigatus*), Epstein-Barr virus (EBV), CNS lymphoma (CNSL)

Table 2. Selected demographic, laboratory and clinical parameters in immunocompentent adult patients with detectable Epstein-Barr virus DNA in the cerebrospinal fluid

Tablica 2. Odabrani demografski, laboratorijski i klinički parametri u imunokompetentnih odraslih bolesnika s detektabilnom DNA virusa Epstein-Barr u cerebrospinalnoj tekućini

Pt	Age	Gender	CSF – cells/μL	CSF – protein mg/L	CSF EBV DNA, copies/mL	Co-pathogen	MR findings	Definite diagnosis	Outcome
16	26	F	775	5,900	402,000	-	Symmetrically enlarged ventricular choroid plexuses with enhancement	EBV encephalitis	Improved
17	24	F	48	170	5,5000	-	Multifocal white matter changes	EBV encephalitis	Improved
18	25	M	240	310	26,200	Enterovirus	Leptomeningeal enhancement	Enteroviral encephalitis	Improved
19	16	M	1,280	1,805	5,700	-	Temporal enhancing lesions	Unknown	Fatal
20	21	M	50	690	5,700	-	Multiple demyelinating lesions in white matter	EBV encephalomyelitis	Improved
21	24	M	3,100	6,160	27,000	-	Multiple demyelinating lesions in white matter	Autoimune encephalitis	Improved
22	35	M	1,540	550	6,550	Enterovirus	Normal	Enteroviral encephalitis	Improved
23	44	M	5	1,550	23,000	-	Normal	Unknown	Improved
24	45	M	2,320	430	6300	-	Multiple demyelinating lesions in white matter	Postinefectious encephalitis	Improved
25	50	F	293	2,510	8900	HSV-1	Right temporal enhancing lesions	HSV encephalitis	Improved
26	53	M	304	861	950,000	-	-	Unknown	Fatal
27	64	M	1,100	1,560	15,050	-	Confluent demyelinating lesions	Encephalitis	Fatal
28	68	M	1,035	2,520	5,980	*M. tuberculosis*	Hydrocephalus	Tuberculosis	Improved
29	68	F	300	924	7,550	HSV-1	Right temporal enhancing lesions	HSV encephalitis	Stable
30	70	M	1,464	430	19,000	VZV	Diffuse cerebral edema	VZV encephalitis	Improved
31	72	F	1,420	1,100	5,540	VZV	-	VZV encephalitis	Stable
32	56	F	10	650	160,500	-	Confluent hyperintensity of cerebral white matter	EBV encephalitis	Improved
33	36	M	1,304	473	6,700	-	-	Unknown	Fatal
34	38	M	180	390	10,2000	-	Bilateral increased trigeminal and facial nerve intensity T2	EBV encephaloneuritis	Improved

- Herpes simplex virus type 1 (HSV-1), *Mycobacterium tuberculosis* (*M. tuberculosis*), varicella zoster virus (VZV), Epstein-Barr virus (EBV)

402,000 copies/mL). One patient with CLL who was treated with R-CHOP developed severe EBV encephalitis (EBV DNA 186,500 copies/mL) during treatment, and this was only patient in immunocompromised group in whom other diagnosis was not established.

CSF EBV DNA in immunocompetent adults

The immunocompetent group included 19 patients (13 males; age 44 years, IQR 25-60) (Table 2). The most frequent clinical symptom was fever (16 patients, 84%), followed by headache (14, 74%) and

altered mental status (13, 68%) with median Glasgow coma score of 11 (IQR 9-14). Eight patients (42%) had other significant neurological deficit (three tetraparesis, two hemiparesis, one ataxia, one facial nerve palsy) and seven (37%) new onset seizure. CSF examination was abnormal in all patients; all of them had pleocytosis (median of 905 cells/mm^3, with predominance of mononuclear cells (90%, IQR 62-96%). Proteinorrachia was observed in all but two patients, with a median protein concentration of 775 mg/L (IQR 440-1743 mg/L). CSF glucose was decreased in only two patients (one with autoimmune encephalitis, one with postinfectious encephalitis).

Viral etiology was established in six patients and included HSV-1 (n=2), VZV (n=2) and enteroviruses (n=2). One patient was diagnosed with postinfectious meningoencephalitis (ME), one with autoimmune encephalitis (anti-MAG) while neurotuberculosis was detected in one patient. In five patients the etiology remained uncertain (in two patients CNS lymphoma was suspected, but both of them died before the diagnosis was confirmed).

A total of five patients were diagnosed with EBV encephalitis. EBV viral load in the CSF varied between 5,540 and 950,000 DNA copies/mL (median of 15,050, IQR 6,425-41,000 EBV DNA copies/mL). EBV DNA was tested in the blood in ten patients and three of them were positive, all with high viral loads (Pt 16 – 354,000 copies/mL; Pt 32 – 31,000 copies/mL; Pt34 – 605,000 copies/mL) and all were diagnosed with EBV CNS infection.

Patients with viral ME had lower CSF EBV DNA (8,225 copies/mL, IQR 6,298-20,800) than patients with unknown etiology (15,050 copies/mL, IQR 6,200-486,500) or EBV encephalitis (102,000 copies/mL IQR 30,350-281,250).

Results of EBV serology were available for 14 patients; in 11 they showed past infection, in two the serological profile was consistent with reactivation (EA and EBNA positive) and in one patient an acute infection was observed (VCA IgM, EA positive, EBNA negative).

Patients with EBV encephalitis were treated with acyclovir (n=4) and corticosteroids (n=5).

Magnetic resonance neuroimaging was performed in 16 patients, in two were normal, and in 14 abnormal with main findings shown in Table 2. Typical MR findings included leptomeningeal enhancement and parenchymal lesions compatible with encephalitis (single or multiple areas of increased T2 signal, with gadolinium enhancement and associated edema or suspected demyelization), as presented in Table 2. A total of four patients died during hospitalization, all of them with unknown etiology (in contrast to patients diagnosed with EBV infection who all improved and were discharged from hospital).

Discussion

The presence of EBV DNA in the CSF, both as a single finding and in the context of co-infections, requires careful interpretation regarding the clinical significance in both immunocompromised and immunocompetent patients. According to our results, detection/quantification of EBV DNA in the CSF as a single etiological agent has the highest clinical significance in HIV-infected patients with CNSL, while its significance in immunocompetent patients is less clear, especially in the setting where EBV was considered as a co-pathogen, irrespective of high CSF EBV DNA levels.

The majority of immunocompromised patients included in this analysis had advanced HIV disease with CD4 T-cell counts below 60 cells/μL, suggesting an association between severe T-cell immune deficiency and the EBV DNA reactivation. As expected, CNSL was the most frequent clinical diagnosis in this group. This is in concordance with the literature reporting poor prognosis of HIV-related CNSL (for review see Brandsma et al, 2018).[17] Although a definite diagnosis of CNSL requires histopathologic confirmation, CSF EBV DNA quantification is part of supporting differential diagnostics.[17] Predictive value of EBV DNA detection in the CSF for the diagnosis of HIV-related CNSL has been evaluated in several case series with limited patient numbers.[18-19] The studies obtained conflicting results on the positive predictive value of EBV DNA (67% vs. 29%), possibly due to the different methodological approaches, analytical sensitivities and specificities of EBV DNA molecular assays. Importantly, in 7 of 12 HIV patients we found alternative diagnosis despite high CSF EBV DNA viral load, and only one immunocompromised patient (with an underlying lymphoproliferative disease) was diagnosed with EBV encephalitis in our small cohort. Martelius et al. analyzed the clinical significance of CSF EBV DNA positivity in an unselected patient material from a single tertiary care hospital in Helsinki and found that EBV was the only pathogen in 17 of 32 immunocompromised patients, suggesting an important role of EBV as a cause of encephalitis in stem cell transplant recipients.[12] However, authors found that 25% of patients with detectable EBV DNA in the CSF had co-infection. The spectrum of clinically significant co-infections in half of our immunocompromised patients included *Toxoplasma gondii*, *Cryptococcus ne-*

oformans, *Aspergillus fumigatus* and *Mycobacterium tuberculosis*, thus highlighting the need for comprehensive diagnostic approach and search for alternative diagnosis in immunocompromised patients.

Although neurological complications associated with EBV infection in immunocompetent adults are rare and usually self-limited, clinically severe and even fatal EBV CNS infection in immunocompetent adults have only been described in seven case reports so far.[5-11] Di Ascenção et al. described the first case of meningoencephalitis with frontal bilateral hemorrhage with detectable CSF EBV DNA in an immunocompetent 58-year-old man with a favorable outcome.[8] Koning et al. described an encephalitis developing during acute EBV infection in an immunocompetent 21-year-old female presenting with leptomeningeal enhancement along the cerebellar folia.[9] Recently, Maiese et al. described a case of fatal EBV infection characterized by meningoencephalitis, myocarditis and interstitial nephritis in an immunocompetent 74-year-old man diagnosed postmortem based on the presence of EBV DNA in the CSF as well as immunohistochemical detection of EBV antigens in brain tissue.[12] By using next-generation sequencing, Huang et al. diagnosed a fatal EBV brainstem hemorrhagic encephalitis in an immunocompetent adult.[11] From these reports we can assume that CNS EBV infections have a diverse clinical, radiological and laboratory features, complicating the clinical decisions management. In this study, we report additional five immunocompetent patients with a final diagnosis of EBV encephalitis with the spectrum of their clinical, laboratory and neuroradiological findings.

The availability of standardized EBV DNA quantification assays as well as the increasing use of multiplex-based approach to molecular detection of herpesviruses increased the rate of positivity in CSF samples submitted for routine diagnostics. However, clinical significance of EBV DNA real-time PCR results in the CSF needs to be interpreted with caution. Currently, there is no definitive way to determine whether EBV is the causative agent of encephalitis syndrome in an individual patient, and the diagnosis is based on careful exclusion of other pathogens and conditions, which is often challenging. Although clinically validated thresholds for EBV DNA quantification in the CSF are not currently available, it should be emphasized that the viral loads in the CSF of immunocompetent patients diagnosed with EBV encephalitis were consistently higher compared with patients subsequently diagnosed with viral CNS infections or in those with unknown etiology. Further studies are needed to more objectively evaluate the clinical significance of EBV viral load in the CSF in order to assist clinical decision protocols.

Several recent laboratory-based studies analyzed the frequency of EBV DNA findings in the CSF samples collected for routine clinical diagnostics in heterogeneous groups of both pediatric and adult patients presenting to clinical care with CNS symptoms.[13-15] Recently, Lee et al. analyzed the clinical significance of EBV DNA detection in the CSF of immunocompetent patients in 780 samples tested over a 10-year period at the Pusan National University Hospital, R. of Korea.[14] A total of 5.4% of samples were positive for EBV DNA with 33 patients subsequently classified as CNS infection. The most frequent co-pathogens identified in addition to EBV included VZV, CMV, HSV-1/2, *Streptococcus pneumoniae* and *Enterococcus faecalis*. Importantly, no differences in age, gender, laboratory findings (except higher CSF proteinorrachia in the co-infected group), results of brain imaging studies, clinical manifestations or prognosis were found in EBV-only group (n=23) vs. patients with co-infections (n=10), similarly as in our study.

The main limitation of our study arises from its retrospective design. Similar as in previous studies, EBV DNA was detected in CSF with commercial tests which do not distinguish between reactivation/infection or contamination due to the pleocytosis; antibody index was available for the majority of patients; therefore, intrathecal antibody production could not be evaluated. Nevertheless, we report a relatively large cohort of patients with positive CSF EBV DNA and their clinical, laboratory and neuroradiological characteristics.

Conclusion

The results of our study emphasize that the interpretation of the clinical significance of EBV DNA in the CSF needs to be specifically evaluated individually with consideration for the role of co-pathogens.

Highlights:

- clinical significance of EBV DNA in the CSF requires careful interpretation in both immunocompromised and immunocompetent patients.
- detection/quantification of EBV DNA in the CSF as a single etiological agent has the highest clinical significance in patients with advanced HIV disease and primary CNS lymphoma
- clinical significance in the CSF in immunocompetent adults is less clear, especially in the setting where EBV was considered as a co-pathogen, irrespective of high CSF EBV DNA levels.

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The authors declare that they have no conflict of interest.

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