Epstein-Barr Viral Load in Cerebrospinal Fluid as a Diagnostic Marker of Central Nervous System Involvement of AIDS-related Lymphoma

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Abstract

Objective AIDS-related lymphoma (ARL) often involves the central nervous system (CNS). Although the diagnostic value of Epstein-Barr virus (EBV)-DNA in cerebrospinal fluid (CSF) in detecting HIV-positive primary CNS lymphoma (PCNSL) has been established, its usefulness for identifying CNS involvement of systemic ARL remains elusive. In this study, we evaluated the utility of the EBV-DNA load in CSF in identifying CNS involvement in patients with systemic ARL.

Methods We retrospectively reviewed the clinical and pathological data of consecutive ARL patients managed at our clinic between January 1998 and June 2012. Sixty-two patients with ARL, including eight PCNSL patients and 52 systemic ARL patients, and 63 controls underwent CSF EBV-DNA load evaluations before receiving chemotherapy. ARL-related CNS involvement was defined as any lesion diagnosed histologically or radiologically as a lymphoma in the brain, meninges, spine, cranial nerves or oculus.

Results A cut off value of 200 copies/mL predicted the presence of CNS lesions with a sensitivity of 70% and a specificity of 85% in both the PCNSL and systemic ARL patients, while a sensitivity of 75% and a specificity of 93% were obtained for systemic ARL. A cut off value of 2,000 (3.30 log) copies/mL provided the best specificity (100%), with a sensitivity of 50%.

Conclusion Our results support the clinical utility of evaluating the quantitative EBV-DNA load in the CSF for the diagnosis of CNS involvement of systemic ARL as well as PCNSL.

Key words: AIDS-related lymphoma, Epstein-Barr virus

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Introduction

Although the incidence of AIDS-related lymphoma (ARL) has decreased following the advent of highly active antiretroviral therapy (HAART), the morbidity and mortality associated with this complication remain significant due to the aggressive clinical course and high frequency of extranodal localization especially in the central nervous system (CNS) (1-3). Since the majority of patients with ARLs are diagnosed at the advanced stage of HIV infection, making the differential diagnosis of CNS lesions from other oppor-

tunistic diseases is crucial for the management of ARL.

Epstein-Barr virus (EBV) can cause various lymphoproliferative disorders in immunocompromised patients and the detection of EBV-DNA in the cerebrospinal fluid (CSF) is a well-established diagnostic tool for identifying primary CNS lymphoma (PCNSL) in HIV-infected individuals (3-10). However, the diagnostic value of detecting EBV-DNA in CNS involvement of systemic ARL remains to be elucidated. In this study, we retrospectively evaluated the value of EBV-DNA in the diagnosis of CNS lesions of ARL, both PCNSL and systemic ARL.

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Table. Characteristics of the Participating Patients

	PCNSL (n=8)	Systemic ARL		non-ARL control subjects (n=63)	p value
		CNS involvement (+) (n=12)	CNS involvement (-) (n=42)		
Male sex, n	8	10	41	60	0.981
Age, median years (range)	38 (28-53)	52 (27-67)	37 (25-63)	38 (22-70)	0.160
Histology					
DLBCL	3	6	16	-	
Burkitt	0	4	16	-	
Others	2	1	10	-	
Not specified	3	1	0	-	
EBER-positive, % (n/total n)	40 (2/5)	40 (4/10)	58.3 (21/36)	-	0.999
CD4 count, median cells/mm ³ (range)	18 (2-79)	83 (3-652)	117 (3-824)	57 (1-450)	0.006
Plasma HIV viral load, median log copies/mL (range)	5.8 (4.5-6.0)	4.7 (1.6-7.1)	4.7 (1.6-7.5)	4.6 (1.7-6.3)	0.081
Plasma EBV-DNA-positive, % (n/total n)	66.7 (4/6)	63.6 (7/11)	58.3 (21/36)	NA	0.999
CSF EBV-DNA-positive, % (n/total n)	62.5 (5/8)	75.0 (9/12)	7.1 (3/42)	20.6 (13/63)	0.035

PCNSL: primary CNS lymphoma, ARL: AIDS-related lymphoma, DLBCL: diffuse large B-cell lymphoma, EBER: EBV-encoded small RNAs, NA: not assessed. CSF: cerebrospinal fluid. The Kruskal-Wallis test was used for comparisons of continuous variables and the chi-square test was used for comparisons of the categorical data.

Materials and Methods

We reviewed the clinical and pathological data of consecutive cases of ARL managed at the AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo between January 1998 and June 2012. CNS involvement of systemic ARL was defined as any lesion histopathologically or radiologically diagnosed as a lymphoma in the brain, meninges, spine, cranial nerves or oculus on either initial diagnosis or recurrence. HIV-infected patients with other opportunistic infections and meningeal or parenchymal brain lesions during the same period were enrolled in the control group for the analysis. Patients who did not have available CSF samples were excluded.

Real-time polymerase chain reaction (RT-PCR) was used to quantify EBV-DNA in CSF samples obtained before chemotherapy and stored at -80°C, using a method previously described (11). Briefly, DNA was extracted using a QIA Symphony Virus/Bacteria Mini kit (Qiagen, Valencia, CA), and the *BNRF1* gene was amplified with the following primers: forward [5'-CCAGTGCTGTGATCGAGCATCT] and reverse [5'-CTGTGACAACTGCTGCATTC] and TaqMan probe [5'-(FAM)-TCTGCTGTGTTTCTGTCTCACCTACCG G-(TAMRA)-3']. The cutoff level for detection was 200 copies/mL.

In patients with available results of *in situ* hybridization (ISH) assay of EBV-encoded small RNAs (EBERs), which were performed on paraffin tissue sections using a cocktail of fluorescein-isothiocyanate-labeled oligonucleotides complementary to the two EBERs (types 1 and 2), as previously described (12, 13), we assessed the correlation between the results of EBER and the CNS localization of lymphoma.

Before the analysis, the levels of EBV-DNA were log-transformed and samples with undetectable EBV-DNA were considered to contain 0.0 copies/mL. For continuous variables, the Mann-Whitney U-test was used to compare two

groups, while the Kruskal-Wallis test was applied to compare three or more groups. Categorical data were examined using the chi-square test. Differences were considered to be significant at $p < 0.05$. The statistical analyses were performed using the SPSS-II software package for Windows, version 17.0J (SPSS Japan Inc, Tokyo, Japan).

Results

During the study period, 76 patients were diagnosed with ARL, including eight patients with PCNSL and 68 patients with systemic ARL. One patient developed ARL twice (diffuse large B-cell lymphoma and plasmablastic lymphoma) within a several year interval and was considered to represent two systemic ARL cases. The frequency of CNS involvement in the systemic ARL patients was 22.1% (15/68). Of the 76 patients with ARL, 62 had available CSF samples and were assigned to the analysis [PCNSL n=8, systemic ARL with CNS involvement (ARL-CNS(+), n=12) and systemic ARL without CNS involvement (ARL-CNS(-), n=42)] (Table). The 63 control subjects with definitive diagnoses of other CNS opportunistic infections during the study period consisted of 18 patients with cryptococcal meningitis, 16 patients with toxoplasmosis, 12 with progressive multifocal leukoencephalopathy (PML), five patients with cytomegalovirus (CMV) encephalitis, three patients with tuberculous meningitis, three patients with neurosyphilis, three patients with Varicella-zoster virus meningitis, two patients with HIV encephalitis, one patient with aseptic meningitis due to acute retroviral syndrome and one patient with CNS candidiasis. Three subjects in the control group had multiple opportunistic infections. There were no significant differences in sex, age or HIV viral load between the two groups. The median CD4 count in the PCNSL group was significantly lower than that observed in the group with systemic ARL with CNS involvement; however, the CD4 counts of the other groups were comparable.

Figure 1. The EBV-DNA loads in the cerebrospinal fluid (CSF) of the patients with AIDS-related lymphoma and the control subjects. PCNSL: primary CNS lymphoma, ARL-CNS (+): systemic AIDS-related lymphoma with CNS involvement, ARL-CNS (-): systemic AIDS-related lymphoma without CNS involvement, NS: not significant. The Mann-Whitney U- test (†) and the Kruskal-Wallis test (‡) were used to compare to the EBV-DNA loads in the CSF. Individual values are plotted, and the horizontal bars represent the median values. The dotted horizontal line indicates the detection limit of the EBV-DNA load assay.

The proportion of patients positive for EBV-DNA in the CSF (with a detection limit of 200 copies/mL) was 62.5% in the PCNSL, 75.0% in the ARL-CNS(+), 7.1% in the ARL-CNS(-) and 20.6% in the control group. The median (range) EBV-DNA loads in the CSF of the above groups were 4.30 (0-5.30), 2.53 (0-4.48), 0.00 (0-2.30) and 0.00 (0.00-3.00) log copies/mL, respectively (Fig. 1). Both the rate of EBV-DNA-positive cases (Table) and the median EBV-DNA load in the CSF (Fig. 1) were significantly higher in the PCNSL and ARL-CNS(+) groups compared with those observed in the ARL-CNS(-) and control groups; however, these values were not different between the PCNSL and ARL-CNS(+) groups or between the ARL-CNS(-) and control groups. Neither the detection of EBV-DNA in plasma nor histological evidence of EBER in tissue were found to be correlated with the CNS localization of lymphoma (Table). Among nine EBER-negative ARL-CNS(+) cases, CSF EBV-DNA was positive in the five patients who were positive for plasma EBV-DNA, while the remaining four patients were negative for both CSF and plasma EBV-DNA. Six EBER-positive ARL-CNS(+) cases included four patients with positive CSF EBV-DNA and negative plasma EBV-DNA, and one patients with positive and one patients with negative EBV-DNA in both the CSF and plasma. The concordant rate of EBV-DNA detection in the CSF and plasma was 100% in the EBER-negative in

Figure 2. Receiver operating characteristic (ROC) curve for the cutoff values of the EBV-DNA load in the cerebrospinal fluid for the diagnosis of CNS involvement of systemic AIDS-related lymphoma. The dotted line is the reference line. The area under the ROC curve was 0.856 (95% confidence interval, 0.690-1.000). A cutoff value of 200 copies/mL had a sensitivity of 75% and a specificity of 93%.

ARL-CNS(+) cases and 33% in the EBER-positive in ARL-CNS(+) cases.

With regard to the diagnostic value of the quantitative EBV-DNA load in the CSF, a cut off value of 200 copies/mL provided a sensitivity of 70% and a specificity of 85% for the CNS localization of all ARLs, including the cases of PCNSL and systemic ARL and provided a higher sensitivity of 75% and a specificity of 93% in the systemic ARL cases. A cut off value of 300 copies/mL exhibited a similar sensitivity of 65% and a higher specificity of 94%; however the best specificity (100%) was noted using a cut off value of 2,000 copies/mL, with a sensitivity of 50%. The area under the receiver operating characteristic (ROC) curve in the diagnosis of CNS localization of ARL was 0.816 for all ARLs and 0.856 for systemic ARLs (Fig. 2). Among the EBER-positive ARLs, a cut off value of 200 copies/mL provided a sensitivity of 83.3% and a specificity of 90.4% in the diagnosis of CNS involvement and provided a sensitivity of 55.6% and a specificity of 100% in the EBER-negative ARL cases.

Discussion

The present study demonstrated the usefulness of measuring the EBV-DNA load in the CSF for diagnosing CNS lesions of ARL, regardless of the type of localization of lymphoma, and the presence of PCNSL or CNS involvement of systemic ARL. Although the diagnostic value of EBV-DNA for HIV-positive PCNSL is well-documented (3-10, 18), evidence showing its usefulness for identifying CNS lesions of systemic ARL is limited (3-10). Since the prevalence (21.7%) of CNS involvement in patients with systemic ARL

is considerably higher (3) than that of non-HIV lymphoma patients (2-7%) (14-16), our results might support the clinical utility of evaluating EBV-PCR with CSF in the management of patients with HIV-positive systemic ARL.

In our study, quantitative EBV-PCR in the CSF with a cut off value of 200 (2.30 log) copies/mL had a sensitivity of 70% and a specificity of 85% for the identification of lymphoma in CNS, while a cut off value of 300 copies/mL provided a similar sensitivity of 65% and a higher specificity of 94%. A previous study that assessed the diagnostic value of quantitative EBV-DNA assays in the CSF for identifying both systemic ARL and PCNSL (10) reported a sensitivity of 75% and specificity of 76% using a cut off value of 100 copies/mL, while the best specificity (100%) was obtained using a cut off value of 3.53 log (3,388) copies/mL. Although our study used a slightly higher detection limit and had a higher specificity and lower sensitivity, the results of the two studies are comparable. In addition, a similar sensitivity (75%) and a higher specificity (93%) were obtained using the cut off value of 200 copies/mL for identifying CNS involvement in systemic ARL than from among all ARLs. Overall, a cut off value of 100-300 seems to be beneficial for identifying CNS lesions of ARL.

In the present study, the prevalence of CSF EBV-DNA in the PCNSL group (62.5%) and the EBER expression (40%) were relatively lower than those reported previously for AIDS-related PCNSL patients (80-100%) (3-10, 18). One possible reason for the low prevalence was the undetectable CSF EBV-DNA load in two patients who had been occasionally treated with anti-herpetic therapy before and during the treatment of PCNSL, including acyclovir for genital herpes in one patient and gancyclovir for CMV retinitis in the other (17). A history of anti-herpetic therapy should be considered when interpreting the results of EBV-PCR. In addition, most previous reports on the high rate of the EBER expression in patients with AIDS-related PCNSL were conducted before or in the early HAART era (18), enrolling severely immunocompromised patients. Since the EBER expression is rare in immunocompetent PCNSL patients (19), our results of low EBER positivity indicate changes in the characteristics of ARL among HIV patients with relatively preserved immunity in the HAART era.

In this study, we found five patients with ARL-CNS(+) who were positive for EBV-DNA in the CSF but negative for the EBER expression in tissue. Notably, among all of the patients with EBER negative ARL-CNS(+), CSF EBV-DNA was detected only when plasma EBV-DNA was detectable, thus suggesting that plasma EBV-DNA transudation into CSF through the blood-brain barrier (BBB) is damaged by CNS involvement of ARL. The presence of plasma EBV-DNA among ARL patients is thought to reflect EBV replication, not in lymphoma tissue, but in other lymphatic tissues such as tonsil endothelial cells, under immunosuppression (20, 21). Although increased EBV activation may lead to ARL development, the increase in the EBV-DNA load in plasma and the EBER expression in tissue are not fully syn-

chronized (20, 21). This may explain our finding of EBER-negative but CSF EBV-DNA positive ARL. Since our study is retrospective, the residue of specimens for EBER ISH was unavailable in 25% of the patients with CNS involvement. Further studies are needed to understand the role of CSF EBV-DNA measurement in the context of EBER-negative ARL.

Conclusion

The EBV-DNA load in the CSF is a marker of CNS involvement of ARL, with 200 copies/mL being a cut off level for the diagnosis of PCNSL and the identification of CNS involvement in patients with systemic ARL. Identifying EBV-DNA may help to differentiate the CNS lesions of ARL from other disorders.

The authors state that they have no Conflict of Interest (COI).

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