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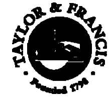

Epstein-Barr virus DNA load in cerebrospinal fluid and plasma of patients with AIDS-related lymphoma

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Detection of Epstein-Barr virus (EBV) DNA in the cerebrospinal fluid (CSF) is associated with acquired immunodeficiency syndrome (AIDS)–related brain lymphoma. Real-time polymerase chain reaction (PCR) was performed to quantify EBV DNA in CSF and plasma from 42 patients with AIDS-related non-Hodgkin's lymphoma (NHL). Twenty patients had primary central nervous system lymphoma (PCNSL) and 22 systemic NHL, including 12 with central nervous system involvement (CNS-NHL). As controls, 16 HIV-infected patients with other CNS disorders were examined. EBV DNA was detected in the CSF from 16/20 (80%) patients with PCNSL, 7/22 (32%) with systemic NHL, 8/12 (67%) with CNS-NHL, and 2/16 (13%) of the controls. The viral EBV DNA levels were significantly higher in the CSF from patients with PCNSL or CNS-NHL compared to patients with systemic NHL or controls. EBV DNA was detected in plasma from 5/16 (31%) patients with PCNSL, 9/16 (56%) with systemic NHL, 4/9 (44%) with CNS-NHL, and 4/15 (27%) controls. No difference in plasma viral load was found between patient groups. From the patients with CNS-NHL, plasma samples drawn prior to CNS involvement contained significantly higher EBV DNA levels than those from systemic NHL patients without subsequent CNS involvement. EBV DNA levels in the CSF, but not in plasma, from patients treated with antiherpes drugs were significantly lower than in untreated patients. High CSF EBV DNA levels were found in HIV-associated brain lymphomas and the viral load can be clinically useful. High plasma EBV DNA levels might predict CNS involvement in systemic NHL. *Journal of NeuroVirology* (2002) **8,** 432–438.

Keywords: CSF; EBV; HIV; lymphoma; plasma; real-time PCR

Introduction

Non-Hodgkinlymphoma (NHL) is a common malignancy in human immunodeficiency virus (HIV)–infected patients and an acquired immunodeficiency syndrome (AIDS)-defining illness. Clinically, AIDS-related NHL occurs as primary central nervous system lymphoma (PCNSL), systemic NHL, often localized to the central nervous system (CNS), or pleural effusion lymphoma (PEL). AIDS-related NHLs are predominantly high-grade B-cell lymphomas that have a rapid clinical progression (Levine, 1992). The use of highly active antiretroviral therapy (HAART) has not reduced the incidence of NHL, except in PCNSL (Rabkin *et al*, 1999; Grulich, 1999). Furthermore, the prolonged survival of HIV-infected patients may lead to an increased incidence of NHL, which remains an important cause of morbidity and mortality. Epstein-Barr virus (EBV) is associated with several lymphoproliferative disorders, including endemic Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, post-transplant lymphoproliferative disease (PTLD), and AIDS-related NHL (Rickinson and Kieff, 1996). It is found in almost all neoplastic CNS tissues (MacMahon *et al*, 1991; Cinque *et al*, 1993) from patients with AIDS-related

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CNS lymphoma, and the detection of EBV DNA in the cerebrospinal fluid (CSF) may be used as a marker of CNS lymphoma (Cinque *et al*, 1993; Arribas *et al*, 1995). EBV has been detected by *in situ* hybridisation in 28% to 66% of AIDS-related systemic NHLs (Ernberg and Altiok, 1989; Shibata *et al*, 1993). An increasing number of studies have shown a possible association between the EBV DNA load in plasma, serum, or peripheral blood mononuclear cells (PBMCs), and the occurrence and/or relapse of PTLD (Kenagy *et al*, 1995; Lucas *et al*, 1998; Kimura *et al*, 1999; Limaye *et al*, 1999; Stevens *et al*, 1999; Niesters *et al*, 2000).

Most of the reported data concerning the polymerase chain reaction (PCR) detection of EBV DNA in CSF samples from patients with AIDS-related NHL are qualitative (Cinque *et al*, 1993; Arribas *et al*, 1995; De Luca *et al*, 1995; Cinque *et al*, 1996), although the relationship between EBV DNA load in CSF samples from patients with PCNSL and the response to treatment has been investigated by means of a semiquantitative PCR (Antinori *et al*, 1999). The EBV DNA load in plasma and PBMCs of patients with AIDS-related NHL has been examined by a limited number of investigators (Laroche *et al*, 1995; Stevens *et al*, 1999) who have suggested that quantification may be clinically relevant.

The aims of this study were to quantify EBV DNA in CSF and plasma samples from patients with AIDS-related NHL by real-time PCR, and to investigate whether these measurements can be used as a indicator of lymphoma.

Results

EBV DNA was detected in CSF from 16 out of 20 patients with PCNSL (80%, mean 5.13 log copies/ml, standard deviation [SD] = 5.53), 7 out of 22 patients with systemic NHL (32%, mean 2.58 log copies/ml, SD = 2.93), 8 out of 12 patients with CNS localization of systemic NHL (CNS-NHL) (67%, mean 5.31 log copies/ml, SD = 5.69) and 2 out of 16 controls (13%, mean 1.42 log copies/ml, SD = 1.86) (Figure 1).

If only patients with histologically or cytologically confirmed diagnosis of brain lymphoma were considered, EBV DNA was present in CSF from 9 out of 13 patients with PCNSL (69%, mean 2.72 log

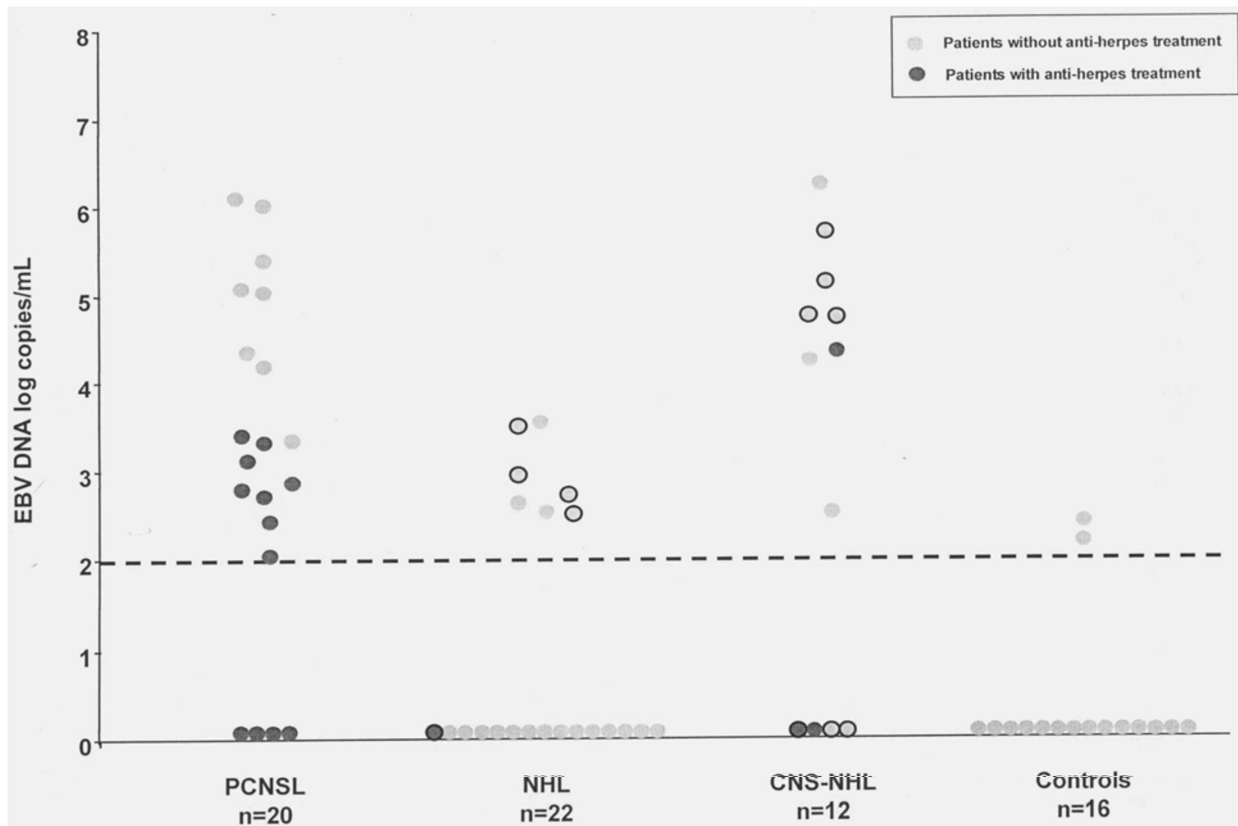

Figure 1 EBV DNA levels in cerebrospinal fluid from patients with HIV-related lymphomas and controls. PCNSL, primary central nervous system lymphoma; NHL, non-Hodgkin lymphoma, CNS-NHL, NHL with CNS involvement. The dashed horizontal line indicated the detection limit of the PCR assay. Black bordered circles indicate the patients who subsequently developed a CNS localization of lymphoma.

copies/ml, SD = 2.17) and in 7 out of 11 patients with CNS-NHL (64%, mean 3.03 log copies/ml, SD = 2.57).

In the whole group, the CSF EBV DNA load was significantly higher in the patients with PCNSL or CNS-NHL than in those with systemic NHL ($P = .006$ and $P = .007$, respectively) or the controls ($P = .01$ for both groups). A large variability in the EBV DNA amount was found in CSF samples from patients with brain lymphoma (range 0.00 to 7.10 log copies/ml).

EBV DNA load correlated inversely with the administration of antiherpesvirus therapy ($P = .001$). The mean EBV DNA load was 1.79 log copies/ml (SD = 1.59) in CSF samples from 14 patients with PCNSL or CNS-NHL receiving anti-herpesvirus drugs, versus the 4.32 log copies/ml (SD = 2.42) in CSF samples from 16 untreated patients ($P = .003$). No association was demonstrated between the viral load and a number of patient variables, including histological type of lymphoma (Burkitt versus Burkitt-like versus diffuse large B-cell lymphoma), clinicoradiological presentation of the brain lesion (focal versus meningeal involvement versus no lesion), presence of malignant cells in the CSF, total CSF white blood cell (WBC) number, CD4+ count, HIV RNA load, chemotherapy administration, and HAART administration (Table 1).

In order to assess the diagnostic value of EBV DNA load in the CSF for brain lymphoma, different EBV DNA load cut-off values were calculated. The best sensitivity (75%) was found using a cut-off value of 2.00 log copies/ml and it was associated with a specificity of 76%. On the other hand, the best specificity (100%) was obtained with a cut-off value of 3.53 log copies/ml, giving a sensitivity of 44%.

Qualitative PCR performed prospectively at the time of the patients evaluation had revealed EBV DNA in 14 out of 20 patients with PCNSL (70%), 2 out of 22 patients with systemic NHL (9%), 7 out of 12 patients with CNS-NHL (58%), and in none of the controls. The sensitivity and the specificity of the qualitative PCR for brain lymphoma diagnosis were 66% and 95%, respectively.

EBV DNA was detected in plasma from 5 out of 16 patients with PCNSL (31%, mean 3.41 log copies/ml, SD = 3.81), 9 out of 16 patients with systemic NHL (56%, mean 5.95 log copies/ml, SD = 5.26), 4 out of 9 patients with CNS-NHL (44%, mean 4.38 copies/ml, SD = 4.78), and 4 out of 15 controls (27%, mean 2.33 copies/ml, SD = 2.67) (Figure 2).

No significant differences in plasma EBV DNA loads were found between the patient groups or between the patients and controls. No association was demonstrated between the viral load and all the patient variables described above for CSF analysis, including antiherpes treatment. From six patients with CNS-NHL, plasma samples collected at onset of systemic NHL were also examined. At the time of systemic NHL diagnosis, EBV DNA was detected in plasma from 5 out of these 6 patients (90%) and in 4 out of 10 NHL patients with no subsequent CNS involvement (40%). Plasma EBV DNA load was higher in the former than in the latter patients (mean 4.43 log copies/ml, SD = 2.42 versus 1.60, SD = 2.23; $P = .04$).

In order to assess the diagnostic value of EBV DNA plasma load for AIDS-related lymphoma, patients with NHL, CNS-NHL, or PCNSL were compared to controls. The best sensitivity (44%) was found using a cut-off value of 2.00 log copies/ml (specificity

Table 1 Non-Hodgkin lymphoma and HIV infection variables in patients and controls

	PCNSL (n = *20*)	*NHL* (n = *22*)	*CNS-NHL* (n = *12*)	*Controls* (n = *16*)
Histology of lymphoma*				
Burkitt	1	5	0	n.a.
Burkitt-like	1	0	0	n.a.
Diffuse large B-cell lymphoma	11	17	7	n.a.
Clinicoradiological presentation of lymphoma				
Focal lesion(s)	20	0	4	n.a.
Meningeal involvement	0	0	9	n.a.
CSF malignant cells	1	0	9	n.a.
CSF WBC§	1 (1–17)	1 (1–30)	10 (1–220)	4 (1–41)
Blood CD4§	50 (3–50)	70 (50–512)	82 (50–966)	65 (50–270)
Chemotherapy	0	0	10	0
Antiretroviral therapy				
HAART	2	1	1	1
Mono- or dual therapy	5	0	3	1
Antiherpes treatment				
Ganciclovir induction	1	0	0	0
Ganciclovir maintenance	9	0	2	0
Aciclovir induction	1	0	0	0
Aciclovir maintenance	0	0	1	0

Note. The number in the table refers to the number of patients if not otherwise indicated. PCNSL, primary central nervous system lymphoma; NHL, non-Hodgkin lymphoma; CNS-NHL, NHL with CNS involvement; CSF, cerebrospinal fluid; WBC, white blood cells; HAART, highly active antiretroviral therapy; n.a., not applicable.
*According to the Revised European-American Lymphoma (R.E.A.L) classification (Harris *et al*, 1994), §Median number/μl (range).

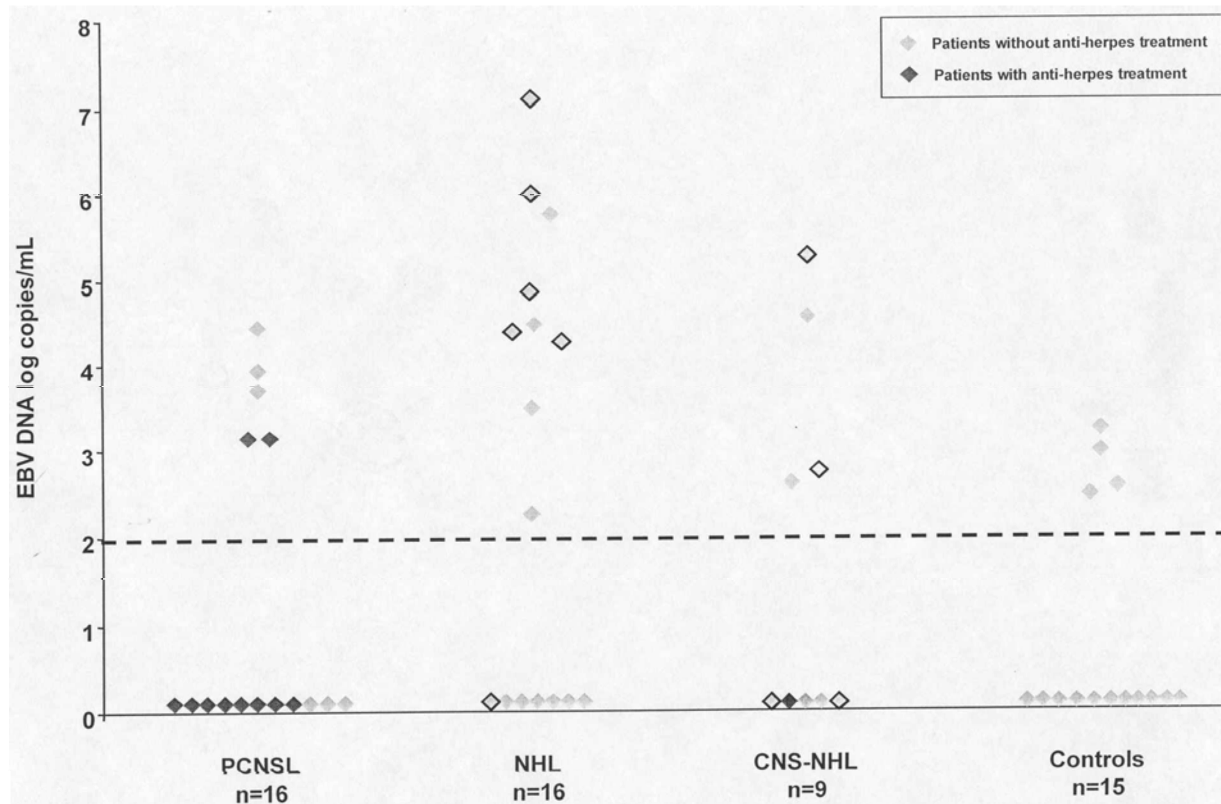

Figure 2 EBV DNA levels in plasma from patients with HIV-related lymphomas and controls. PCNSL, primary central nervous system lymphoma; NHL, non-Hodgkin lymphoma; CNS-NHL, NHL with CNS involvement. The dashed horizontal line indicated the detection limit of the PCR assay. Black bordered squares indicate the patients who subsequently developed a CNS localization of lymphoma.

69%). A specificity of 100% was obtained with a cut-off value of 3.23 log copies/ml (sensitivity 37%).

In each patient group, there was no significant difference in the CSF EBV DNA load between patients with EBV DNA–positive or –negative plasma. EBV DNA levels were significantly higher in the CSF than in the plasma of the patients with PCNSL and CNS-NHL.

Discussion

The EBV DNA load was measured by real-time PCR in CSF and plasma samples drawn from patients with AIDS-related NHL. A large variability of EBV DNA load was observed in both CSF and plasma samples. The highest CSF load was found in the patients with brain lymphoma, whereas the highest plasma values were found in the patients with systemic NHL, especially in those with subsequent brain localization. EBV DNA was detected in the CSF samples of 80% of the patients with PCNSL and 67% of those with CNS-NHL. These findings confirm the previously reported correlation between the presence of EBV DNA in CSF and brain lymphomas in HIV-infected patients (Cinque *et al*, 1993; Arribas *et al*, 1995). Although amounts of EBV DNA have previously been estimated in CSF samples from HIV-infected patients with PCNSL by a semiquantitative PCR assay (Antinori *et al*, 1999), the viral load has never been investigated, to the best of our knowledge, by a quantitative PCR. In this study, the real-time PCR showed a wide distribution of CSF EBV DNA levels of 7 logs and disclosed significantly higher CSF viral loads in the patients with brain lymphomas than in those with systemic NHL or controls. Furthermore, the mean EBV DNA amount in the CSF was higher in the patients with CNS-NHL than in those with PCNSL. This might reflect a greater degree of blood brain barrier damage in patients with CNS-NHL, which is supported by their high number of WBCs and proteins in the CSF, and the meningeal contrast enhancement frequently observed by neuroimaging. This could indicate that the EBV DNA in the CSF might have derived from either circulating EBV-carrying B or tumoral cells. However, the lack of significant correlation between the EBV DNA load and CSF WBC count within the group of patients with CNS-NHL or PCNSL suggests that the viral DNA might also have originated intrathecally from an active infection.

In this study, an inverse correlation was observed between CSF EBV DNA load and administration of

antiherpesvirus drugs. It is known that EBV lytic replication is susceptible to a number of antiherpesvirus agents and recent studies have suggested the possibility of using antiviral drugs to treat EBV-induced lymphoproliferative disorders or even EBV-positive NHL (Fong *et al*, 2000; Raez *et al*, 1999; Schmidt *et al*, 2000). Six out of 7 patients (86%) with brain lymphoma and EBV DNA–negative CSF were actually receiving intravenous antiherpesvirus drugs. Furthermore, among patients with brain lymphoma in whom CSF was EBV DNA positive, the viral load was significantly lower in treated than in untreated patients. Because only EBV lytic replication is susceptible to antiviral drugs, these observations seem to indicate that, in brain lymphomas, EBV is present not only in the latent form but also that active viral replication might actually occur.

As already reported in previous studies (Cinque *et al*, 1993; Arribas *et al*, 1995), EBV DNA was found in the CSF from a number of patients without brain lymphoma. In the present study, EBV DNA was detected in 7 out of 22 patients (32%) with systemic NHL, including 4 patients who subsequently developed CNS localization and 1 patient with neurological symptoms at the time of systemic NHL diagnosis, but in whom CNS localization was not proven by our criteria. It is possible that EBV DNA reflected the presence of a subclinical CNS lymphoma in these cases. In addition, EBV DNA was found in 2 out of 16 (13%) controls, both with cryptococcal meningitis, suggesting meningeal infiltration of EBV-positive lymphocytes. When EBV DNA load cut-off values were used to analyze the CSF results, the best diagnostic sensitivity was found using the cut-off of 2 log copies/ml, equal to the detection limit of the assay, whereas 100% specificity was obtained using a cut-off value of 3.53 log copies/ml. Values between 2.00 and 3.53 log copies/ml were observed in 45% of patients with brain lymphoma, but the great majority of these (89%) were receiving antiherpesvirus drugs. Therefore, although a high CSF EBV DNA load is highly suggestive of CNS lymphoma, low or absent EBV DNA levels are still consistent with this diagnosis, especially in patients receiving antiviral drugs. On the other hand, “false positive” results, with values ranging between 2.00 and 3.53 log copies/ml, were observed in 32% of patients with NHL and no CNS involvement. However, most of these patients presented a later CNS localization, thus indicating that the finding of low EBV DNA values in the CSF of NHL patients with no overt neurological disease should prompt a careful clinical and neuroradiological investigation.

Diagnostic sensitivity of real-time PCR was also analyzed by excluding patients in whom the diagnosis of brain lymphoma was obtained on a clinical basis, in order to avoid the possible bias introduced by using EBV DNA detection in CSF by qualitative PCR as inclusion criterium. As expected, the rate of EBV DNA detection was slightly lower in this group of patients with only histologically or cytologically diagnosed PCNSL or CNS-NHL. Hovewer, it is important to consider, in this smaller patient group, the increased weight of the seven patients in whom CSF was EBV DNA negative and who were receiving antiherpes drugs at the time of sampling.

All of the CSF samples were also tested using a qualitative PCR (Cinque *et al*, 1993) at the time of patient evaluation. The results of this qualitative assay were concordant with those obtained by real-time PCR, except for the eight CSF samples that were real-time PCR positive and qualitative PCR negative. The amount of EBV DNA in these discordant samples was low, confirming that the analytical sensitivity of the real-time PCR was higher than that of qualitative PCR. By using the qualitative assay, higher sensitivity figures were previously obtained in the CSF of patients observed between 1983 and 1993 (Cinque *et al*, 1993). This observation might reflect the still limited use of anti-herpes drugs at that time, especially ganciclovir, which became largely available in the clinical practice only in the 1990s, and thus support the effect of antiherpes drugs on the EBV DNA load.

A limited number of studies have suggested a clinical relevance for plasma or serum EBV DNA quantification in patients with AIDS-related NHL (Laroche *et al*, 1995; Stevens *et al*, 1999). In the present study, EBV DNA was found in plasma from 44% of patients with lymphoma, with or without CNS localization, and in 27% of the controls, indicating that EBV DNA in plasma is not a reliable diagnostic marker of NHL in AIDS patients. In contrast to the CSF findings, no effect of antiherpes drugs was observed on EBV DNA plasma levels, but the number of plasma samples from treated patients was too small to drawn any conclusion (11 treated versus 30 untreated patients). Of note, EBV DNA levels were higher in the patients with systemic NHL than in the others. Moreover, EBV DNA load was significantly higher in the samples from NHL patients with subsequent CNS involvement than in those without and controls. This findings suggest that the virus might reach the CNS from the peripheral blood and it is in line with recently published data indicating that the presence of EBV in HIV-related lymphomas is predictive of CNS localization (Cingolani *et al*, 2000). In conclusion, the detection of EBV DNA in the CSF was confirmed to be a useful tool for diagnosis of brain lymphoma in AIDS patients. Quantitative evaluation of EBV DNA load could be of help in the interpretation of EBV DNA–positive results. Although high CSF viral load are highly predictive for CNS localization of lymphoma, low values should be interpreted cautiously, due to the possibility of carry over from blood, presence of subclinical lymphoma, as well as the effect of antiherpes drugs. Detection of

high amounts of EBV DNA in plasma of patients with systemic NHL might predict subsequent CNS involvement.

Patients and methods

Patients and samples

Forty-two HIV-infected patients with NHL admitted to San Raffaele Hospital, Milan (Italy), between 1995 and 1999 were retrospectively examined. Twenty had PCNSL and 22 systemic NHL, including 12 with CNS-NHL. Control CSF and plasma samples were analyzed from 16 HIV-infected patients with other CNS disorders, including toxoplasmosis ($n = 7$), AIDS-related dementia ($n = 5$), and cryptococcal meningitis ($n = 4$). CSF and plasma samples were collected from the patients for diagnostic purposes and after obtaining informed consent.

A final diagnosis of PCNSL was made by post-mortem histopathological examination in seven patients, stereotactic brain biopsy in five, and both procedures in one (Table 1). A clinical diagnosis of PCNSL was made in seven patients on the basis of the presence of focal contrast-enhancing brain lesions at computed tomography (CT) or magnetic resonance imaging (MRI), a lack of response to antitoxoplasmic treatment, the qualitative PCR detection of EBV DNA in CSF and an increased thallium-201 uptake at single-positron emission computed tomography (SPECT).

Systemic NHL was diagnosed by histopathological examination in all of the 22 patients, and was confirmed at autopsy in 11 (Table 1). Twelve out of these 22 patients showed CNS involvement. Meningeal involvement was diagnosed by CSF cytology in nine patients and confirmed by autopsy in five of them. Focal CNS lesions were observed in the four remaining patients and were diagnosed by brain biopsy in one patient and at autopsy in the other three. The above-mentioned clinical criteria for PCNSL diagnosis were used for the diagnosis of CNS involvement in one patients. CSF and plasma samples from the 12 CNS-NHL patients were drawn at the time of CNS involvement and at the time of systemic NHL diagnosis (mean 71 days before CNS-NHL, range 30 to 270). At the time of sampling, 14 patients (12 PCNSL, 2 NHL-CNS) were also receiving antiherpes drugs for the treatment of concomitant CMV or varicella-zoster disease (Table 1).

Nucleic acid extraction

DNA was extracted from 50 μl CSF or plasma using the QIAamp Blood Kit (QIAGEN, Chatswoth, California, USA) according to the manufacturer's instructions. The extracted DNA was finally eluted in 50 μl of water. To rule out any contamination, calf serum was subjected to extraction every five samples.

Real-time PCR

Real-time PCR was used to detect EBV DNA in the CSF and plasma according to a previously described method (Enbom *et al*, 2001). The PCR primers and probe were selected in the LMP-1 gene, where the sequence of types A and B is conserved. The primers used were the EBV-LMP1 forward primer, 5′-AAGGTCAAAGAACAAGGCCAAG-3′, and the EBV-LMP1 reverse 5′-GCATCGGAGTCGG-3′. The fluorogenic probe (PE-Applied Biosystems, Cheshire, UK) was synthesized using a FAM reporter molecule attached to the 5′ end and a TAMRA quencher linked to the 3′ end (5′-AGGAGCGTGTCCCCGTGGAGG-3′). A standard curve was prepared using a serial dilution of DNA from Namalva cells, each of which contains two copies of EBV genomes. The standard curve was constructed using 10^6, 10^5, 10^4, and 10^3 EBV copies/ml, with each standard being run in triplicate. A negative water control and a positive control (10^5 copies per ml) were included in triplicate for each run. Of each clinical sample, five aliquots were analyzed each time, including two aliquots to which a positive control was added to control for inhibitory substances in the samples. The amplification and detection were performed using an ABI Prism 7700 Sequence Detection System (PE-Applied Biosystems). To each well 5-μl sample and 20-μl PCR mixture, consisting of 12.5 μl Universal Mastermix (PE-Applied Biosystem), 900 nM each primer and 175 nM probe were added. Cycling parameters were 50°C for 2 min, 95°C for for 10 min, 50 cycles of 95°C for 15 s, and 60°C for 1 min. A threshold cycle value (Ct) was calculated for each sample by determining the point at which the fluorescence exceeds the threshold limit chosen for the specific plate. The standard curve was created automatically with ABI 7700 Sequence Detection System software by plotting the Ct values against each standard of known concentration. The EBV DNA copy number was calculated as the mean of the values obtained in the three sample aliquots without the inhibition control. The analytical sensitivity of the identification of EBV was estimated as being 100 genomes/ml.

CSF examination

At the time of patient evaluation, the CSF samples were analyzed for glucose and protein concentrations, and cell counts and cryptococcal polysaccharide antigen and cultures were performed for bacteria, mycobacteria, and fungi. All of the CSF samples were cytologically examined. The presence of EBV, herpes simplex viruses 1 and 2, varicella-zoster virus, cytomegalovirus and JC virus was assessed by nested DNA PCRs (Cinque *et al*, 1996).

Statistical analysis

The Mann-Whitney test was used to compare the CSF and plasma EBV DNA loads in the different patient groups. Differences between the DNA loads in

the paired CSF/plasma samples or between paired samples collected at the different time points were analyzed using Wilcoxon's paired signed-rank test. Spearman's rank correlation test was used to analyze the correlation between the number of CSF WBCs and the number of copies of EBV DNA. Diagnostic sensitivity and specificity were calculated according to the Bayes' formula.

References

Antinori A, Cingolani A, De Luca A, Gaidano GL, Ammassari A, Larocca LM, Ortona L (1999). Epstein-Barr virus in monitoring the response to therapy of acquired immunodeficiency syndrome-related primary central nervous system lymphoma. *Ann Neurol* **45:** 259–261.

Arribas JR, Clifford DB, Fichtenbaum CJ, Roberts RL, Powderly WG, Storch GA (1995). Detection of Epstein-Barr virus DNA in cerebrospinal fluid for diagnosis of AIDS-related central nervous system lymphoma. *J Clin Microbiol* **33:** 1580–1583.

Cingolani A, Gastaldi R, Fassone L, Piercorti F, Giancola ML, Martini M, De Luca A, Ammassari A, Mazzone C, Pescarmora E, Gaidano GL, Larocca LM, Antinori A (2000). Epstein-Barr infection is predictive of CNS involvement in systemic AIDS-related non-Hodgkin's lymphomas. *J Clin Oncol* **18:** 3325–3330.

Cinque P, Brytting M, Vago L, Castagna A, Paravicini C, Zanchetta N, D'Arminio Monforte A, Wahren B, Lazzarin A, Linde (1993). Epstein-Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. *Lancet* **342:** 398–401.

Cinque P, Vago L, Dahl H, Brytting M, Terreni MR, Fornara C, Racca S, Castagna A, D'Arminio Monforte A, Wahren B, Lazzarin A, Linde A (1996). Polymerase chain reaction on cerebrospinal fluid for diagnosis of virus-associated opportunistic diseases of the central nervous system in HIV-infected patients. *AIDS* **10:** 951–958.

De Luca A, Antinori A, Cingolani A, Larocca LM, Linzalone A, Ammassari A, Scerrati M, Roselli R, Tamburini E, Ortona L (1995). Evaluation of cerebrospinal fluid EBV-DNA and IL-10 as markers for in vivo diagnosis of AIDS-related primary central nervous system lymphoma. *Br J Haematol* **90:** 844–849.

Enbom M, Strand A, Falk KI, Linde A (2001). Detection of EBV, but not HHV-8, DNA in cervical secretions from Swedish women by real-time PCR. *Sex Transm Dis* **28:** 300–306.

Ernberg I, Altiok E (1989). The role of Epstein-Barr virus in lymphomas of HIV-carriers. *APMIS Suppl* **8:** 58–61.

Fong IW, Ho J, Lo B, Fong MW (2000). Value of chronic administration of acyclovir and similar agents for protecting against AIDS-related lymphoma: Case control and historical cohort studies. *Clin Infect Dis* **30:** 757–761.

Grulich AE (1999). AIDS-associated non-Hodgkin's lymphoma in the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr Hum Retrovirol* **21:** S27–S30.

Kenagy DN, Schlesinger Y, Weck K, Ritter JN, Gaudreault-Keener MM, Storch GA (1995). Epstein-Barr virus DNA in peripheral blood leukocytes of patients with post-transplant lymphoproliferative disease. *Transplant* **60:** 547.

Kimura H, Morita M, Yabuta Y, Kuzushima K, Kato K, Kojima S, Matsuyama T, Morishima T (1999). Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. *J Clin Microbiol* **37:** 132–136.

Laroche C, Drouet EB, Brousset P, Pain C, Boibieux A, Biron F, Icart J, Denoyel GA, Niveleau A (1995). Measurement by polymerase chain reaction of the Epstein-Barr virus load in infectious mononucleosis and AIDS-related non-Hodgkin's lymphomas. *J Med Virol* **46:** 66–74.

Levine AM (1992). Acquired immunodeficiency syndrome-related lymphoma. *Blood* **80:** 8–20.

Limaye AP, Huang ML, Atienza EE, Ferrenberg JM, Corey L (1999). Detection of Epstein-Barr virus DNA in sera from transplant recipients with lymphoproliferative disorders. *J Clin Microbiol* **37:** 1113–1116.

Lucas KG, Burton RL, Zimmerman SE, Wang J, Cornetta KG, Robertson KA, Lee CH, Emmanuel DJ (1998). Semi-quantitative Epstein-Barr virus (EBV) polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. *Blood* **91:** 3654–3661.

MacMahon EM, Glass JD, Hayward SD, Mann RB, Scott-Becker P, Charache P, McArthur JC, Ambinder RF (1991). Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet* **338:** 969–974.

Niesters HGM, van Esser J, Fries E, Wolthers KC, Cornelissen J, Osterhaus ADME (2000). Development of a real-time quantitative assay for detection of Epstein-Barr virus. *J Clin Microbiol* **38:** 712–715.

Rabkin CS, Marcia AT, Huang J, Von Roenn JH (1999). Kaposi's sarcoma and non-Hodgkin's lymphoma incidence trends in AIDS clinical trial group study participants. *J Acquir Immune Defic Syndr Hum Retrovirol* **21:** S31–S33.

Raez L, Cabral L, Cai JP, Landy H, Sfakianakis G, Byrne GE, Hurley J, Sarpella E, Jahaweera D, Harrington WJ, Jr (1999). Treatment of AIDS-related primary central nervous system lymphoma with zidovudine, ganciclovir and interleukin 2. *AIDS Res Hum Retroviruses* **15:** 713–719.

Rickinson AB, Kieff E (1996). Epstein-Barr virus. In: *Fields virology*, Fields BN (ed.). Philadelphia, PA: Lippincott-Raven, pp. 2397–2446.

Schmidt W, Anagnostopoulos I, Scherubl H (2000). Virostatic therapy for advanced lymphoproliferation associated with the Epstein-Barr virus in an HIV-infected patients (letter). *N Engl J Med* **342:** 440–441.

Shibata D, Weiss LM, Hernandez AM, Nathwani BN, Bernstein L, Levine AM (1993). Epstein-Barr virus-associated non-Hodgkin's lymphoma in patients infected with the human immunodeficiency virus. *Blood* **81:** 2102–2109.

Stevens SJC, Vervoot MBHJ, van den Brule AJC, Meenhorst PL, Meijer JLM, Middelhorp JM (1999). Monitoring of Epstein-Barr virus DNA load in peripheral blood by quantitative competitive PCR. *J Clin Microbiol* **37:** 2852–2857.