Detection of Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus DNA in CSF from persons infected with HIV who had neurological disease

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Abstract

***Objectives*—To determine the frequency and clinical relevance of Epstein-Barr virus (EBV) and Kaposi's sarcoma associated herpesvirus (KSHV) DNA detection in the CSF from patients infected with HIV.**

***Methods*—Cerebrospinal fluid was obtained prospectively from 115 consecutive patients infected with HIV undergoing diagnostic lumbar puncture for investigation of neurological disease. Amplification of DNA was performed using a nested polymerase chain reaction (PCR) for detection of EBV internal repeat and KSHV minor capsid sequences.**

***Results*—EBV DNA was detected in the CSF supernatant of 18 patients. This included all patients with primary CNS lymphoma (seven patients) or a combination of systemic and CNS lymphoma (two patients). By contrast EBV DNA was not detected in the CSF supernatant of any patient with systemic, but not CNS, lymphoma (10 patients). EBV DNA was also detected in the supernatant of nine further patients without a diagnosis of lymphoma at the time of lumbar puncture, two of whom subsequently developed CNS lymphoma. No EBV DNA was detected in CSF supernatant from the remaining 87 samples (two of these patients subsequently developed lymphoma). KSHV DNA was detected in the CSF of two patients, one had systemic (but not CNS) lymphoma and the other did not have lymphoma.**

***Conclusion*—A diagnosis of CNS lymphoma is strongly associated with the presence of EBV DNA in CSF. In the absence of clinical and radiological features of CNS lymphoma, the presence of detectable CSF EBV DNA may predict subsequent tumour development. KSHV DNA is rarely detected in CSF in this patient group and shows no correlation with lymphoma or other neurological disease.**

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Epstein-Barr virus (EBV) has been associated with some lymphoproliferative disorders in the immunocompromised patient.[1] In particular EBV DNA has been detected in tumour tissue of virtually all AIDS related CNS lymphoma[2 3] and in about 50% of patients with AIDS related systemic lymphoma.[4] Histological confirmation of the diagnosis of primary CNS lymphoma in a patient infected with HIV may be difficult; some patients may be considered too unwell to undergo brain biopsy and in others the lesions may be located at an anatomically inaccessible site. Attempts have therefore been made to identify other markers of primary CNS lymphoma. Retrospective studies have shown that 83%-97% of patients with HIV with a primary CNS lymphoma have detectable EBV DNA in CSF.[5 6] Although this provides clinically useful information and may obviate the need for brain biopsy, questions have been raised about the relevance of a positive result in the absence of neuroradiological features of lymphoma.[7]

Kaposi's sarcoma is a common AIDS defining illness in patients with HIV. Recently DNA from a novel herpesvirus, now known as Kaposi's sarcoma associated herpesvirus (KSHV), has been detected in Kaposi's sarcoma tumour tissue[8] and tumour tissue taken from patients with body cavity based lymphomas.[9] The full range of clinical disease associated with this newly described herpesvirus remains, however, to be defined.

The aim of this prospective study was to determine the frequency and clinical relevance of EBV and KSHV DNA detection in CSF taken from patients with HIV undergoing diagnostic lumbar puncture for investigation of neurological disease and to correlate these results with the final clinical diagnosis in this group of patients.

Patients and methods

PATIENTS

Cerebrospinal fluid was obtained prospectively from 115 consecutive patients with HIV (108 men and seven women) admitted to the HIV/AIDS Inpatient Unit, University College London Hospitals between March 1993 to November 1995. All patients were admitted for investigation of neurological disease. Six patients had more than one CSF sample taken (two samples in five patients and three samples in one). This study was performed within the guidelines of the Middlesex Hospital clinical investigations panel.

Patients were divided into two groups on the basis of whether, at the time of lumbar

Table 1 Correlation between CSF polymerase chain reaction (PCR) results for the detection of Esptein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) DNA and clinical manifestations in 115 patients infected with HIV

Diagnosis	Patients (n)	EBV DNA in CSF (supernatant)		KSHV DNA in CSF (pellet)	
		Detected	Not Detected	Detected	Not Detected
Lymphoma:					
Cerebral	7	7	0	0	5
Cerebral and systemic	2	2	0	0	1
Systemic	10	0	10	1	6
Total	19	9	10	1	12
Other neurological conditions:					
HADC	27	1	26	0	9
Cryptococcal meningitis	17	0	17	0	1
CMV encephalitis (one also had CMV polyradiculopathy)	13	2	11	0	3
CMV retinitis (two also had CMV polyradiculopathy)	7	3	4	1	2
Self limiting headache	6	1	5	0	3
Toxoplasmosis	3	1	2	0	1
Mononeuneuritis multiplex	2	0	2	0	0
Transient ischaemic attack	2	0	2	0	0
Sepsis, or confusional state	2	0	2	0	1
Isolated cranial nerve palsy	2	0	2	0	0
Acute retinal necrosis	2	0	2	0	0
Disseminated MAI infection	2	0	2	0	0
TB meningitis	1	1	0	0	1
Myelopathy	1	0	1	0	1
Miscellaneous conditions	9	0	9	0	0
Total	96	9	87	1	22

HADC = HIV associated dementia complex; CMV = cytomegalovirus; MAI = *Mycobacterium avium-intracellulare*; TB = *Mycobacterium tuberculosis*.
Miscellaneous conditions includes a single case each of sinusitis, prolapsed intervertebral disc, postviral encephalitis, idiopathic epilepsy, viral encephalitis (aetiology undetermined), disseminated Hodgkin's disease, disseminated extraneural cryptococcosis, and cerebellar astrocytoma.

puncture, they had lymphoma (either CNS, systemic, or a combination of both) or an alternative diagnosis (table 1). In 17 patients the diagnosis of lymphoma was made by histological analysis of tissue obtained at biopsy (11 patients; two also had postmortem confirmation) or postmortem (five patients). In a further three patients, all of whom had refused brain biopsy, the diagnosis was made on the basis of typical appearances on MRI[10] together with typical thallium-201 scan appearances (one patient).[11]

Protein and glucose concentrations in CSF were determined and the presence or absence of a CSF pleocytosis was noted. The CSF was stained histochemically and cultured for bacteria, mycobacteria, and fungi and assayed for antibodies to *Treponema pallidum* and *Cryptococcus neoformans* antigen. An aliquot of CSF was analysed for the presence of EBV DNA. In a subset of 36 patients sufficient CSF remained to enable analysis for the presence of KSHV DNA to be performed.

NESTED POLYMERASE CHAIN REACTION AMPLIFICATION

The nested polymerase chain reaction (PCR) method and oligonucleotide primers used for the detection of EBV internal repeat and KSHV minor capsid sequences have been described.[12 13] For EBV the CSF samples were pelleted (15 000 g, 5 minutes); the supernatant was boiled for 10 minutes and cooled on ice, and then 10 µl was added directly to the first round mixture. We have shown previously that centrifugation at 15 000 g is sufficient to avoid cellular contamination of the CSF as human DNA sequences (pyruvate dehydrogenase) cannot be detected using a sensitive nested PCR.[14] In 24 patients, seven of whom had systemic (but not CNS) lymphoma, and 17 of whom had an alternative diagnosis, the CSF cell pellet was also analysed by nested PCR amplification for EBV DNA. For KSHV DNA detection (on a subset of 36 patients) both CSF cell pellet and supernatant liquid were analysed. The supernatant was processed as for EBV DNA detection, DNA was extracted from the cell pellet using 20 µl extraction buffer containing 10 mM TRIS, 5 mM KC1, 2.5 mM $MgC1_2$, 0.5% NP40, 0.5% Tween 20, and 50 µg/ml proteinase K and incubated for 2 hours at 56°C. Extracted DNA (10 µl) was added to the first round of PCR.

Any obviously contaminated blood samples were treated as described above, but were also examined for inhibition of nested PCR by titration of dilutions of the positive control in aliquots of these samples. The sensitivity of each PCR was assessed using tissue culture derived virus (EBV) and purified KSHV DNA (prepared in house), each of which was diluted in pooled CSF that did not contain detectable herpesvirus DNA. It is estimated that 10 or less copies of EBV and two to five copies of KSHV DNA could be detected after two rounds of nested PCR. Reaction conditions and cycling indices were unchanged from those previously described[12 13] and the products of PCR were examined by ethidium bromide gel electrophoresis. Appropriate positive and negative controls were included in each PCR run: pooled herpesvirus negative CSF, distilled water, and the equivalent of 10 and 100 copies of EBV and KSHV DNA.

End point titration was used to determine EBV viral load in a subset of eight patients, four with a primary CNS lymphoma, one with a

Table 2 Alternative diagnoses, follow up, and outcome in nine patients with no lymphoma at the time of EBV DNA detection in CSF supernatant

Patient No	*Alternative clinical diagnosis*	*Cranial MRI*	*Follow up (weeks)*	*Necropsy*	*Cause of death*
1	CMV retinitis, cutaneous K.S	No focal lesion	107	Yes	Primary CNS lymphoma
2	CMV retinitis and CMV radioculopathy	No focal lesion	19	Yes	Primary CNS lymphoma
3	HADC	No focal lesion	62	No	HADC, HIV wasting
4	Cerebral toxoplasmosis	Multiple ring enhancing lesions	60	No	HIV wasting
5	Self limiting headache	No focal lesion	13	No	HIV wasting, disseminated KS
6	CMV retinitis and CMV encephalitis	No focal lesion	4	No	CMV encephalitis HIV wasting
7	Tuberculous meningitis	No focal lesion	3	No	Tuberculous meningitis
8	CMV retinitis CMV encephalitis	No focal lesion	2	No	CMV encephalitis
9	CMV retinitis confusion due to pneumonia	No focal lesion	1	No	Bacterial pneumonia, disseminated KS, HIV wasting

CMV=cytomegalovirus; HADC=HIV associated dementia complex; KS=Kaposi's sarcoma.

combination of systemic and CNS lymphoma, and three with alternative neurological diagnoses.

STATISTICAL ANALYSIS

Results were compared using a two tailed Fisher's exact test. Values with $p<0.05$ was considered significant.

Results

EBV DNA DETECTION IN CSF SUPERNATANT

Epstein-Barr virus DNA was detected in the CSF supernatant of 18 patients. This included all patients with primary CNS lymphoma (seven patients) and all those with both CNS and systemic lymphoma (two patients). By contrast EBV DNA was not detected in the CSF of any patient with systemic but not CNS lymphoma (10 patients) (table 1).

Nine patients without a diagnosis of lymphoma at the time of lumbar puncture had detectable EBV DNA in the CSF supernatant, two of whom subsequently developed CNS lymphoma confirmed at necropsy 19 and 107 weeks later (table 2). The remaining seven patients with detectable EBV DNA in CSF supernatant but without a diagnosis of CNS lymphoma at the time of lumbar puncture all died between 1 and 62 weeks after their original lumbar puncture—none developed clinical or radiological features of lymphoma before death (table 2). Of the 96 patients without detectable EBV DNA in CSF supernatant and with alternative diagnoses (table 1), two subsequently developed CNS lymphoma, 13 and 40 weeks later, diagnosed on the basis of typical MRI and thallium-201 appearances and failure of response to antitoxoplasma therapy. Necropsy in a further 14 of these 96 patients confirmed the absence of CNS and systemic lymphoma.

REPEAT EPISODES

Six patients had repeat CSF sampling. One patient had CNS lymphoma with blast cells and EBV DNA in CSF supernatant. After cranial radiotherapy he presented again 20 weeks later with cytomegalovirus polyradiculopathy; Cytomegalovirus and EBV DNA were detected in CSF supernatant. Necropsy confirmed CNS lymphoma. Another patient (No 3, table 2) had HIV associated dementia complex (HADC), EBV DNA was detected in CSF supernatant. He underwent repeat lumbar puncture 4 and 16 weeks later because of progressive decline in cognitive function, the CSF supernatant on neither occasion contained detectable EBV DNA. Two further patients had cryptococcal meningitis; they were sampled again by lumber puncture 47 and 26 weeks later when the diagnosis was respectively relapse of cryptococcal meningitis and cytomegalovirus retinitis with septicaemia. The fifth patient had HADC; he presented again 32 weeks later with cerebral toxoplasmosis, which responded to specific treatment. The final patient had acute retinal necrosis due to varicella zoster virus infection; he presented again 27 weeks later with a toxic confusional state. EBV DNA was not detected in CSF supernatant from any of the samples from these four patients.

EBV DNA DETECTION IN CSF CELL PELLET

Three patients had detectable EBV DNA in the cellular pellet but not the CSF supernatant. One subsequently developed CNS and systemic lymphoma, confirmed at necropsy 15 weeks later, one had systemic lymphoma at the time of lumbar puncture and one remains alive at 133 weeks with severe HADC but no clinical evidence of lymphoma.

KSHV DNA DETECTION

KSHV DNA was not detected in the CSF supernatant from any of the 36 samples (13 patients with lymphoma and 23 with alternative diagnoses, table 1). However, KSHV DNA was detected in the CSF cellular pellet from two patients, one with systemic lymphoma but without CNS lymphoma and no evidence of Kaposi's sarcoma and the other with cytomegalovirus retinitis, no lymphoma, but widespread cutaneous Kaposi's sarcoma. This second patient also had detectable EBV DNA in the CSF supernatant and subsequently developed CNS lymphoma (patient 1, table 2).

Counts for CD4 lymphocytes were available in all patients; The median CD4 count for patients with lymphoma was 0.06×10^9/l; normal range $0.35–2.2\times10^9$/l) and for those with alternative diagnoses it was 0.02×10^9/l (range $0–0.31\times10^9$/l). In all but one patient with lymphoma the white blood cell count in CSF ranged from 0–20 cells/mm^3 (12 patients had no white cells in the CSF). There was evidence of a CSF pleocytosis in one patient (264 cells mm^3); this person had a concomitant cytomegalovirus polyradiculopathy.

There was a significant association between detectable EBV DNA in CSF supernatant and

Table 3 End point titration with nested PCR for detection of EBV DNA in CSF supernatant of patients with lymphoma and other diagnoses

Diagnosis	*Detectable copies of EBV DNA/ µl CSF supernatant*
Cerebral lymphoma	10
Cerebral lymphoma	1000
Cerebral lymphoma	1000
Cerebral lymphoma	10000
Cerebral and systemic lymphoma	1000
Cerebral toxoplasmosis*	<10
Self limiting headache†	10
CMV retinitis/CMV encephalitis‡	10000

*Patient 4; †patient 5; ‡patient 6; as shown in table 2.

the diagnosis of primary CNS lymphoma or a combination of CNS and systemic lymphoma compared with patients with systemic (but not CNS) lymphoma ($p<0.0001$; two tailed Fisher's exact test).

Semiquantification of CSF EBV DNA was carried out on a subset of eight patients using end point titration. The results are shown in table 3. There was no apparent difference in the viral load of patients with and without lymphoma. Of the three patients who did not have lymphoma, the highest EBV DNA load was seen in a patient (No 6, table 2) with cytomegalovirus retinitis and encephalitis who died 4 weeks later from progressive encephalitis and HIV wasting syndrome, without clinical evidence of lymphoma.

Discussion

In this prospective study 115 patients with HIV were investigated for the presence of detectable EBV DNA in CSF. Patients were classified into those with a diagnosis of lymphoma and those with an alternative diagnosis. There was a significant association between detectable EBV DNA in the CSF supernatant and both primary CNS lymphoma and cerebral involvement by disseminated systemic lymphoma. By contrast EBV DNA was not detected in CSF supernatant of patients with disseminated systemic lymphoma who did not have CNS involvement.

A study of 83 patients with HIV who had CSF obtained within 180 days of death and necropsy showed that all 17 cases of primary CNS lymphoma had detectable EBV DNA using nested PCR and EBV DNA was detected in only one of 68 patients with an alternative diagnosis.[6] No EBV DNA was detected in the CSF of two patients with a disseminated systemic lymphoma and with CNS involvement, a finding which contrasts with ours. In a further study of 500 patients infected with HIV 36 had primary CNS lymphoma confirmed by necropsy; 35 of these had detectable CSF EBV DNA.[7] These studies, together with ours, confirm that detection of EBV DNA in CSF is strongly associated with primary CNS lymphoma and may obviate the need for brain biopsy in those patients with clinical and radiological features of lymphoma. However, it may not be possible to perform a lumbar puncture in all patients with suspected cerebral lymphoma as the presence of a mass lesion on neuroradiological imaging may be a contraindication. Detection of CSF EBV DNA therefore, forms part of the diagnostic investigation of an immunosuppressed patient with an intracerebral mass lesion—the precise application will depend on the individual patient's clinical and neuroradiological features.

The clinical relevance of detectable EBV DNA in CSF in the absence of features consistent with a diagnosis of CNS lymphoma needs careful evaluation. In this study a minority (nine of 96) of patients without clinical or radiological features of lymphoma had detectable EBV DNA in CSF supernatant; two of these patients subsequently developed lymphoma. This suggests that the detection of EBV DNA in CSF from patients with HIV without lymphoma may predict subsequent tumour development. Such patients should be considered as at possible risk of developing lymphoma and promptly investigated if clinically indicated. However, results must be interpreted with caution as we showed transient detection of EBV DNA in the CSF of one patient. In addition, a further two patients without detectable CSF EBV DNA subsequently developed cerebral lymphoma. The absence of detectable CSF EBV DNA does, however, play a critical part in the evaluation of a focal CNS lesion in an immunosuppressed patient, as this strongly suggests an alternative diagnosis.

As nested PCR is a very sensitive technique, the clinical relevance of a positive result needs careful interpretation. A potential area of concern is contamination of CSF with virus from mononuclear cells in peripheral blood resulting in detectable EBV DNA in CSF arising from EBV DNA contained in these cells. This is important as it has been shown, using nested PCR, that mononuclear cells in peripheral blood from 61% of patients with HIV with low CD4 lymphocyte counts contain detectable EBV DNA.[13] Contamination of CSF by such cells is unlikely in this study as cell free supernatant was examined. In addition, there was a significant association between detectable CSF EBV DNA and a diagnosis of CNS lymphoma. A potential concern about the use of cell free supernatant is a reduction in diagnostic sensitivity. For this reason we examined the CSF cell pellet on a subset of patients. However, all of the patients with CNS lymphoma in this study had detectable EBV DNA in CSF supernatant, indicating the suitability of this CSF fraction for analysis in patients with suspected CNS lymphoma.

We examined the EBV DNA load in CSF in a subset of patients, both with and without lymphoma at the time of lumbar puncture (table 3). Although the number analysed were too small to allow definitive comment, there was no apparent difference in the viral load of patients with and without lymphoma. This finding needs further investigation with the inclusion of larger sample numbers.

As the clinical disease range of infection with KSHV remains to be precisely defined, the possible association of infection with this virus and neurological disease in patients with HIV was examined in a subset of 36 patients. No

association between detectable KSHV DNA and neurological disease—in particular lymphoma—was noted in this small group.

Detection of EBV DNA in the CSF of patients with HIV is strongly associated with primary CNS lymphoma as well as cerebral involvement from disseminated systemic lymphoma. However, EBV DNA may be detected in the CSF of patients without clinical or radiological features of lymphoma—this may predict for subsequent tumour development. No association between detectable KSHV DNA in CSF and neurological disease was noted in this group of patients with HIV.

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