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ORIGINAL COMMUNICATION

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A comparision of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients

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Abstract Twenty patients with AIDS who had intracranial lesions underwent both brain biopsy and cerebrospinal fluid (CSF) examination to compare histological diagnosis with the polymerase chain reaction (CSF-PCR) for the identification of infectious agents. CSF-PCR was performed for herpes simplex virus, varicella zoster virus, cytomegalovirus (CMV), JC virus (JCV), Epstein-Barr virus (EBV), *Toxoplasma gondii* and *Mycobacterium tuberculosis*. A definitive diagnosis was obtained by brain biopsy in 14 patients (2 with astrocytoma, 12 with brain infection). CSF-PCR was positive for EBV DNA in 3 of 3 cases of primary cerebral lymphoma, positive for JCV DNA in 6 of 7 biopsy-proven (and one autopsy-proven) cases of progressive multifocal leukoencephalopathy (PML). CSF-PCR was positive for CMV DNA in one biopsy-proven and one autopsy-proven case of CMV encephalitis (the former also had PML) and positive for *M. tuberculosis* DNA in one case of tuberculous encephalitis. None of the five toxoplasmic encephalitis cases (one definite, four presumptive) were *T. gondii* DNA positive. There was close correlation between histology and CSF-PCR for CMV encephalitis, PML and PCL. Antitoxoplasma therapy affected the sensitivity of both histological and CSF-PCR methods.

Key words AIDS · Brain biopsy · Cerebral spinal fluid · Polymerase chain reaction

Introduction

Central nervous system (CNS) opportunistic diseases are a major cause of morbidity in AIDS patients. A presumptive diagnosis may be made for toxoplasmic encephalitis (TE) only when it responds to specific treatment, but the lack of improvement after therapy does not rule out the disease [3, 7]. For the diagnosis of other CNS opportunistic diseases, histological examination of brain biopsy specimens is required, acccording to the Centers for Disease Control [3]. However, this procedure is invasive and calls for the availability of a neurosurgery department; moreover, in 5–33% of cases, no diagnosis can be made because of sampling problems; diffuse encephalitis can only rarely be diagnosed [4, 10].

Recently, cerebrospinal fluid polymerase chain reaction (CSF-PCR) has been shown to be a sensitive and specific method for detecting the genomic sequences of many opportunistic agents [1, 8, 15], although the clinical significance of these findings in the management of HIV-infected patients has yet to be further defined.

The aims of this prospective study were to compare the results of CSF-PCR with pathological diagnoses made using brain biopsy, and to evaluate the clinical impact of these two procedures on the management of HIV-infected patients with neurological complaints.

Patients and methods

All of the patients were hospitalized in the infectious diseases units of L Sacco and S Raffaele Hospitals (Milan, Italy) between Janu-

ary 1992 and December 1993. The criteria for brain biopsy were the presence of neurological complaints and neuroradiological evidence of intracranial lesions, a Karnofsky index > 50 and written informed consent.

Prior antitoxoplasmic treatment was given for at least 14 days in patients with multiple hyperdense lesions; the subjects who did not respond to such therapy were considered candidates for brain biopsy, which was performed by means of a stereotactic procedure using a Talaraich coordinate frame suitably adapted for computed tomography (CT). The target lesion was detected by means of CT, using a double-dose of contrast medium. In each case, at least three serial specimens were taken from the same area. Patients were monitored for the first 24 h after surgery, and CT was repeated within the first 6 h.

The biopsy fragments were snap-frozen (one fragment), fixed in glutaraldehyde for ultrastructural examination (one fragment) or fixed in neutral buffered formalin and paraffin-embedded. In selected cases, one fragment was used for cultures. Histological examination was performed on haematoxylin-eosin stained sections; special stains were also used to demonstrate the presence of opportunistic agents (PAS, Giemsa, Grocott-Gomori, Ziehl-Neelsen).

Immunohistochemistry was performed using the double indirect immunoperoxidase method on both the paraffin-embedded and frozen specimens, by using mono- and polyclonal antibodies directed against opportunistic agents (*Cytomegalovirus* (CMV), *Pneumocystis carinii*, herpes simplex virus -1 and -2, Dako SpA, Milan, Italy; *Toxoplasma gondii*, BioGenex Las, San Ramon, CA, USA; Anti *SV40* large T-antigen, Oncogene Science Inc., Manhasset, N.Y., USA); HIV antigens (anti-HIV -gp41- Genetic System, Seattle, Wash., USA); and leukocyte and glial cell antigens.

In situ hybridization (DNA probes JCV, CMV, Enzo Biochem Co., N.Y., USA; EBER-EBV, Dako SpA, Milan, Italy) was performed to confirm the diagnoses of progressive multifocal leukoencephalopathy (PML) and CMV infections, as well as to demonstrate the presence of Epstein-Barr (EBV) EBER sequences in primary cerebral lymphoma (PCL).

The cerebrospinal fluid (CSF) samples were all obtained between 10 days before and 10 days after stereotactic brain biopsy. Before CSF was taken, each patient was checked for the presence of high intracranial pressure by means of fundoscopy and, when possible, by CT. If the intracranial pressure was high, the patient was excluded from the examination.

Both the CSF untreated samples and supernatant fraction (obtained by centrifugation at 1500 g for 10 min) were stored at –20°C and thawed just before polymerase chain reaction (PCR) assay.

PCR was performed on CSF 10-ml aliquots, previously heated at 95°C for 10 min. Each sample was analysed by means of eight different nested PCR assays for the detection of DNA sequences from herpes simplex virus (HSV-1 and HSV-2) [5], varicella zoster virus [13], CMV [5], EBV [6], JC virus [2], and *T. gondii* [15]. Nested PCR for *Mycobacterium tuberculosis* DNA was performed by using the outer primers described by Portillo et al. [8] and, as inner primers, a fragment of the *mpt*-40 region (Gori A et al., unpublished material). All of the assays were made in duplicate on each sample, and each experiment had a DNA-free control. Strict procedures were adopted to avoid contamination [9].

We also examined at least ten sections from the frontoparietal lobes, deep grey matter, cerebellum, brainstem and spinal cord of autopsied patients. Further sections were obtained from any macroscopic lesion found at autopsy. The histopathological diagnoses were made as described above.

Results

Brain biopsy was performed in only 20 out of 247 (8%) HIV-infected patients with neurological complaints and neuroradiological evidence of intracranial lesions; of the others, 97 (39%) responded to antitoxoplasmic therapy, 114 (46%) were not considered for biopsy because of their severe clinical condition, 13 refused and 3 had lesions at risk for haemorrhagic complications.

In 14 of the 20 biopsied patients, neurological complaints occurred without a previous diagnosis of AIDS; in the remaining 6 (30%), AIDS had been diagnosed a median of 14 months before (range: 6–39 months). The median CD4+ cell count at the onset of neurological symptoms was 41/μl (range: 7–436/μl). Serum IgG antibodies for *T. gondii* were present in 13/20 cases (65%). Presenting symptoms included: seizures (6 cases); hemiparesis (4 cases); cranial nerve palsies (4 cases); distal limb weakness (3 cases); headache (2 cases); hemianopsia (1 case).

CT revealed hyperdense enhancing lesions in ten cases; hypodense non-enhancing lesions in nine; and mixed, hyper- and hypodense lesions in one. The preferential sites of involvement were the frontoparieto-occipital areas (14 cases) and basal ganglia (5 cases). Nineteen patients underwent magnetic resonance imaging which, in two cases, revealed multiple lesions where CT had shown only single one. Brain biopsy was performed a mean of 2 months after symptom onset (range: 1–4 months). Prebiopsy antitoxoplasmic therapy was given in 14 cases (pyrimethamine 50 mg + sulphadiazine 6–8 g or clindamycin 2400–4800 mg/day) for a mean of 50 days (range: 7–83 days). Six patients did not receive any antitoxoplasmic therapy because they had no suggestive lesions. No death occurred as a result of the biopsy procedure. In one case, severe haemorrhage led to transient left hemiparesis. In Table 1 demographic, immunological, clinical and therapeutical characteristics of each of the 20 patients are summarized.

PML was diagnosed in seven cases (35%), one of which occurred in association with another opportunistic disease (see below). TE was histologically diagnosed in one case (5%) and confirmed by immunohistochemistry; in four other cases (20%) we could formulate only presumptive diagnoses based on histological features suggestive of TE (i.e. necrosis with inflammatory macrophagic reaction, calcification, and/or microglial nodules without giant cells) in the absence of any other opportunistic agent. When performed (two cases), autopsy confirmed the TE diagnosis. PCL was diagnosed in three cases (15%); astrocytoma (grades I and II) in 2 cases (10%), both with asymptomatic HIV infection and mild immunodepression (CD4+ cell counts of 270 and 436/ml); tuberculous encephalitis in one case (5%); and CMV disease, together with PML, in another (5%). The specimens from two patients were not diagnostic.

In the cases with enhancing lesions (10/20), TE-related lesions were the most frequent finding (4/10). PCL accounted for three of the diagnoses, the lesion being always single, localized at the basal ganglia, with a nodular pattern and perilesional oedema. Astrocytoma was the diagnosis in two cases; tuberculous encephalitis was diagnosed in one case with a single lesion.

Table 1 Demographic, immunological and clinical characteristics of the 20 patients who underwent CSF examination and brain biopsy (*PCL* primitive cerebral lymphoma, *TE* toxoplasmic encephalitis, *PML* progressive multifocal leukoencephalopathy, *TB* tuberculous encephalitis, *CMV* cytomegalovirus encephalitis)

Patient	Age/sex (years)	Prior AIDS	CD4+[a] (counts/μl)	Prior therapy	Symptoms to biopsy (months)	CSF[b] to biopsy (days)	Histology of biopsy specimen
1	31/m	No	10	Yes	3	– 3	PCL
2	28/m	No	270	Yes	2	– 5	Astrocytoma grade II
3	39/m	No	41	Yes	2	– 7	PML
4	31/m	No	52	Yes	2	– 5	Not diagnostic
5	29/m	No	2	No	1	+ 4	PML
6	36/m	No	64	No	3	– 10	PML
7	29/m	Yes	15	Yes	2	– 6	TB
8	29/m	Yes	50	Yes	1	– 5	PCL
9	29/f	Yes	50	Yes	1	– 6	PML
10	32/f	No	10	Yes	4	– 10	Presumptive TE
11	32/f	No	10	Yes	2	– 3	Presumptive TE
12	30/m	No	26	No	4	– 5	PML
13	31/f	No	34	Yes	4	– 3	Not diagnostic
14	31/f	Yes	13	Yes	2	– 4	Presumptive TE
15	30/f	Yes	9	No	2	– 5	PML + CMV enceph
16	39/f	No	68	No	4	– 3	PML + HIV-E
17	34/m	No	436	Yes	4	– 8	Astrocytoma grade I
18	38/m	No	14	No	1	– 6	TE
19	30/m	No	48	Yes	3	– 4	Presumptive TE
20	33/m	Yes	15	Yes	3	+ 10	PCL

[a] CD4+ counts at the onset of neurological symptoms
[b] Days from CSF to biopsy; – = CSF taken before biopsy, + = CSF taken after biopsy

Table 2 Correlation between histological diagnoses and positivity for different genomes from cerebrospinal fluid (CSF) in 20 patients who underwent stereotactic brain biopsy (*PCL* primitive cerebral lymphoma, *TE* toxoplasmic encephalitis, *PML* progressive multifocal leukoencephalopathy, *CMV* cytomegalovirus)

Histological diagnoses from brain biopsy	*N*	PCR on CSF[a]	*N* autopsy	Histological diagnosis from autopsy
Astrocytoma	2	All negative	–	–
PCL†	3	3 EBV-DNA pos	3	1 PCL + TE + HIV-encephalitis 2 PCL
TE	5	1 CMV-DNA pos	2	1 TE, 1 TE + CMV encephalitis
PML†	6	5 JCV-DNA pos	2	2 PML
CMV encephalitis + PML	1	CMV-DNA pos, JCV-DNA pos	1	1 CMV + PML
Tuberculous encephalitis	1	1 TB-DNA pos	–	–
Not diagnostic	2	1 JCV-DNA pos	1	1 PML

[a] HSV, VZV, EBV, JCV, CMV, *T. gondii*, *M. tuberculosis*
† 1 case with associated HIV encephalitis

In the cases with hypodense non-enhancing lesions (9/20), PML was the most frequent disease (6/9 cases); a presumptive diagnosis of TE was made in 1/9 cases (confirmed at autopsy); in 2 patients, we failed to obtain a histological diagnosis.

In one case with mixed lesions, PML and CMV encephalitis were found in the same specimen.

The results of CSF-PCR for the different genomes are compared with the histological diagnoses in Table 2. PCR specificity was 100% for all the tested genomes. JCV-DNA was found in 6/7 cases of biopsy-proven PML and in 1/1 case with only autopsy-proven PML. CSF was negative for *T. gondii*-DNA in all proven and suspected cases of TE. EBV-DNA was found in all three cases of PCL. Negative results for all the genomes were obtained in the two astrocytoma cases, as well as in one of the two undiagnosed cases. CMV-DNA was found in two cases, one with biopsy-proven and the other with autopsy-proven CMV encephalitis. CSF was positive for TB-DNA in one case of tuberculous encephalitis.

At the time of writing, 18/20 patients have died; the median survival was 62 days (range:10–480 days). The achievement of diagnosis *in vitam* led to changes in therapeutic approach in 12/20 cases (60%). The patients with astrocytoma and 2/3 patients with lymphoma underwent radiotherapy (whole-brain irradiation with 4000 rads, administered over 3 weeks in 270-rad fractions). Survival duration was 270 and 450 days in the two patients with astrocytoma (the latter being still alive), and 150, 60 and 10 days in the patients with lymphoma (the third patient did not undergo radiotherapy). An average of two cycles of arabinosyl-citosine (ARA-C; 2 mg/kg i.v. from day 1 to day 5 every 28 days) was administered to 5/6 patients with PML; mean survival time was 60 days. The patient with tuberculous encephalitis underwent acute specific therapy (survival 60 days). The patient with CMV encephalitis and PML was given acute anti-CMV therapy (survival 10 days). All five patients with a diagnosis of TE continued antitoxoplasmic therapy; the patient whose CSF was positive for CMV-DNA was also given acute anti-CMV therapy. The mean survival of the TE patients was 330 days.

Discussion

Several retrospective studies suggest that CSF-PCR may be a sensitive and specific method for identifying the opportunistic agents causing CNS lesions in AIDS patients [2, 5, 6, 12]. In this prospective study, we compared the results of CSF-PCR with brain biopsy histological diagnoses. The following observations can be made.

1. Brain biopsy was feasible in only 20/150 (13%) patients not responding to antitoxoplasmic therapy, thus confirming that this procedure is difficult to use in the clinical care of advanced AIDS patients.

2. As in the retrospective studies [5, 6, 12], all the nested-PCR assays showed a very high specificity.

3. CSF-PCR was very sensitive for viral-associated CNS diseases; PCR for CMV, EBV and JCV had a sensitivity for CMV encephalitis, PML and PCL of respectively 100%, 100% and 83%. Furthermore, one case with an autopsy diagnosis of PML and one with CMV encephalitis had been diagnosed by CSF-PCR only.

4. The diagnosis of toxoplasmic encephalitis was the main question left unanswered, since both biopsy and CSF-PCR are poorly sensitive in previously treated patients. A definitive histological diagnosis is based on the identification of cysts or tachyzoites of *T. gondii*; however, their number can be lowered by even a brief course of therapy [11] and recognizing them in necrotic samples can be very difficult. Hence, TE was diagnosed by using restrictive criteria (i.e. immunohistochemical positivity) in only one case. In four other cases, we could formulate only a presumptive histological diagnosis of TE, confirmed in the two autopsied patients. In all cases (including the only patient whose TE was confirmed by immunohistochemistry) PCR was negative for *T. gondii*. The sensitivity of PCR seems to be related to therapy duration, as has been already reported [12, 14].

5. The therapeutic approach was modified in the majority of our cases; however, with the exception of the TE patients, their median survival duration remained very low.

In conclusion, this study demonstrates a close correlation between brain biopsy histological diagnosis and CSF-PCR in patients with CMV encephalitis, PML and PCL. The diagnosis of TE by both biopsy and PCR is greatly affected by specific therapy, and prospective studies are required to evaluate the sensitivity of PCR in early CSF samples.

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