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Reduced Rate of Diagnostic Positive Detection of JC Virus DNA in Cerebrospinal Fluid in Cases of Suspected Progressive Multifocal Leukoencephalopathy in the Era of Potent Antiretroviral Therapy

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Fifty-nine human immunodeficiency virus (HIV)-infected patients with suspected progressive multifocal leukoencephalopathy and 224 controls were tested for JC virus (JCV) DNA in cerebrospinal fluid by PCR. The diagnostic positive detection rate dropped from 89.5% (95% confidence intervals of 75.5 to 103.5%) in the pre-highly active antiretroviral therapy (HAART) era to 57.5% (95% confidence intervals of 42.1 to 72.9%) in the HAART era; the specificity remained unchanged. Predictors of failure to detect JCV DNA were exposure to HAART at disease onset and higher CD4 counts.

JC virus (JCV) is the polyomavirus causing progressive multifocal leukoencephalopathy (PML) (5), a demyelinating disorder of the central nervous system (CNS) that affects 5 to 8% of AIDS patients (3). The disease is characterized by disseminated development of demyelination plaques in the cerebral white matter and adjacent areas. At the rim of these foci, virus accumulates to high concentrations in oligodendrocytes causing their destruction by cytolysis (6). Before the advent of highly active antiretroviral therapy (HAART), PML was characterized by an extremely poor disease prognosis (10). The definitive diagnosis of PML is based on the concomitant presence of a compatible clinical and neuroimaging picture and characteristic histopathologic features with JC virus detection in the brain tissue (11, 12). However, in vivo brain biopsy is an invasive method with recognized risks and high costs. Therefore, several authors developed and validated less-invasive diagnostic methods based on the amplification of JCV target DNA from cerebrospinal fluid (CSF) by PCR (9, 16). The different reports showed a variable efficiency of PCR-based assays with diagnostic positive detection rate ranging from 17 to 90% and specificity from 92 to 100% (13, 15). After the introduction of HAART, disease prognosis has improved in association with an induced clearance of JCV DNA from the CSF (8, 9). The aim of the present study was to analyze whether, in the HAART era, the diagnostic positive detection rate of JCV DNA detection in CSF has changed and to investigate predictors of JCV DNA detection in the CSF of human immunodeficiency virus (HIV)-infected patients with suspected PML.

We analyzed all HIV type 1-positive patients undergoing lumbar puncture for diagnosis of central nervous system (CNS) disorders at the Clinical Infectious Diseases Department of the Catholic University in Rome between January 1992 and December 1995 (pre-HAART era) and between September 1996 and December 2002 (HAART era). Uniform criteria for defining "suspected PML" were used throughout the study; these consisted of the concomitant presence of compatible clinical and brain magnetic resonance imaging features, as well as the exclusion of alternative diagnoses by the search of other agents associated with CNS disorders in the CSF and by clinical follow-up (1, 7). All patients (suspected PML and other disorders) were tested for JCV DNA in CSF prospectively, for diagnostic purposes, by a qualitative nested PCR with a detection limit of 1,600 JCV DNA copies/ml, as previously described (9). In case of negative results, the JCV DNA assay was repeated on a new CSF sample collected after 1 to 4 weeks. A total of 436 CSF samples from 283 patients were analyzed: 184 samples from 102 patients (19 with suspected PML) from the pre-HAART era and 252 samples from 181 patients (40 with suspected PML) belonging to the HAART era. The baseline characteristics of the suspected PML patients are shown in Table 1. The control group included CSF samples from 83 patients of the pre-HAART era and 141 subjects of the HAART era with various disorders: HIV encephalopathy (17 pre-HAART era, 63 HAART era), toxoplasmic encephalitis (16 pre-HAART era, 22 HAART era), primary brain lymphoma (19 pre-HAART era, 6 HAART era), cryptococcal meningitis (12 pre-HAART era, 3 HAART era), systemic non-Hodgkin's lymphoma (13 pre-HAART era, 8 HAART era), cytomegalovirus encephalitis (12 HAART era), tubercular meningitis (3 pre-HAART era, 6 HAART era), other CNS diseases (3 pre-HAART era, 14 HAART era), and other systemic diseases (7 HAART era). The proportion of suspected PML cases among all the HIV-infected patients undergoing diagnostic lumbar puncture did not differ significantly between eras (19 of 102 [18.6%] in the pre-HAART era and 40 of 181 [22.1%] in the HAART era; $P = 0.54$ as determined by the Fisher exact test).

In the pre-HAART era, 17 of 19 suspected PML and 1 of 83

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TABLE 1. Features of suspected PML patients and controls[a]

Variable	Suspected PML (n = 59)		Controls (n = 224)
	Pre-HAART era (n = 19)	HAART era (n = 40)	
Mean age (yr) ± SD	38 ± 7	39 ± 7	37 ± 7
Sex (% female)	10.5	20.0	26.8
IDU (%)	68.4	52.5	50.0
Prior CDC class C (%)	36.8	50.0	41.5
CD4 (mean no. of cells/μl ± SD)	51 ± 49	104 ± 134	48 ± 55
Plasma HIV RNA median (IQR) (95% CI) (only for HAART era)	ND	4.78 (3.47–5.53)	5.14 (4.23–5.66)
NRTI therapy at onset (%)	57.9	0	14.6
HAART at onset (%)	0	42.5	39.0

[a] Suspected PML was defined by the concomitant presence of compatible clinical and brain magnetic resonance imaging features, the exclusion of alternative diagnoses by PCR assays in the CSF, and clinical follow-up (1, 8). IDU, intravenous drug user; CDC, Centers for Disease Control; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; ND, not detected.

controls tested positive; in the HAART era, 23 of 40 suspected PML and 0 of 141 controls tested positive. Positive detection rate, specificity, and positive and negative predictive values of the test in the two eras are summarized in Table 2. In the HAART era, a significant reduction in the diagnostic positive detection rate and in the predictive value of the negative test were observed. At univariate analysis, factors that predicted a failure to detect JCV DNA in CSF in the 59 suspected PML patients (pre-HAART era + HAART era) were: HAART exposure at onset of the disease (odds ratio [OR], 0.19; 95% confidence interval [CI], 0.06 to 0.66; $P = 0.008$), CD4 counts higher than 100 cells/μl (OR, 0.16; 95% CI, 0.04 to 0.60; $P = 0.006$), HAART era versus pre-HAART era (OR, 0.16; 95% CI, 0.03 to 0.81; $P = 0.024$), plasma HIV RNA levels (available in 36 patients of the HAART era only) (for 1-log increases, OR, 0.46; 95% CI, 0.23 to 0.93; $P = 0.026$). In a multivariable logistic regression, only CD4 counts above 100 cells/μl were significantly associated with a reduced odds of JCV DNA detection after we adjusted for era and HAART exposure at disease onset.

Noteworthy, in the subset of patients from the HAART era, the quantitation of CSF JCV DNA (by semiquantitative nested PCR) (9) showed lower concentrations in PML patients treated with HAART before disease onset (mean JCV DNA in CSF 3.26 versus 3.99 $\log_{10}$ copies/ml; $P = 0.003$).

The results of the present study show that JCV DNA detection in CSF by PCR has reduced its diagnostic positive detection rate during the HAART era. This is in agreement with previous reports showing an increased prevalence of undiagnosed PML cases, even after CSF investigation by multiple lumbar punctures (2). The mechanism through which combination antiretroviral therapy may act on JCV burden is not known. It might be hypothesized that it inhibits the entry into brain of JCV-infected B lymphocytes, usually facilitated during HIV infection, as well as the transactivation of JCV by the HIV tat protein (4). We show that immune reconstitution, represented here by CD4 counts above 100 cells/μl is an independent predictor of failure to detect JCV DNA in CSF of PML patients. Therefore, a larger proportion of suspected PML patients showing higher CD4 counts in the HAART era might be at least a partial explanation for the observed reduction of the diagnostic positive detection rate.

The emergence of an increasing number of patients with undiagnosed PML in the HAART era stresses the need for an improvement of the PCR aiming at a better positive detection rate for the detection of JCV in CSF by keeping specificity unchanged. Indeed, some patients with reduced JC viral replication could have shown a negative result with the PCR assay used in the present study simply because the JC viral DNA copy number in their CSF was below its detection limit. If an improved sensitivity cannot be achieved, brain biopsy should be reconsidered as an option in case of PML suspect and repeatedly negative CSF assays: histological findings may also have an impact on therapeutic decisions (14).

TABLE 2. Diagnostic accuracy of nested PCR detection of JCV DNA on CSF samples of repeated lumbar punctures for diagnosis of PML in HIV-infected patients according to study era[a]

Parameter	Results for:		P
	Pre-HAART era	HAART era	
Positive detection rate[b]	17/19 (89.5 [75.5–103.5])	23/40 (57.5 [42.1–72.9])	0.014
Specificity[c] (%)	82/83 (98.8 [96.4–101.2])	141/141 (100)	NS
Negative predictive value (%)	98	89	0.03
Positive predictive value (%)	95	100	NS

[a] Pre-HAART era, January 1992 to December 1995; HAART era, September 1996 to December 2002. JCV^+ and JCV^- denote positive and negative JCV DNA assay results in CSF, respectively. NS, not significant.
[b] Calculated as the number of JCV^+/suspected PML patients (% [95% CI]).
[c] Calculated as the number of JCV^-/HIV^+ controls (% [95% CI]).

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