Effect of HIV-1 Genetic Diversity on HIV-1 RNA Quantification in Plasma: Comparative Evaluation of Three Commercial Assays

To the Editor: The rapid rate of HIV-1 replication in vivo (10^9 virions per day), coupled with the poor fidelity of reverse transcription of the HIV-1 genome, results in the production of new virus variants (1). This makes it easy to understand how multiple clades of HIV-1 have emerged throughout the world and explains the development of genetic diversity even within clades (2).

Numerous reports describe assays designed for the detection or quantification of HIV-1 genomic RNA in plasma. One such assay is based on a coupled reverse transcription–polymerase chain reaction (RT-PCR) process in which specific oligonucleotides sequence primers are used in conjunction with enzymes to amplify the number of HIV-1 genomes in a specimen (3). Another approach involves the nucleic acid sequence–based amplification (NASBA) procedure, which is founded on isothermal amplification of an HIV-1 RNA target sequence by simultaneous enzymatic activity of three enzymes (4). A third procedure involves direct detection of the genomic nucleic sequences by a series of signal amplification processes using a technology called branched DNA (bDNA)(5). These assay methods have been developed into commercial kits (RT-PCR: Amplicor Monitor by Roche Diagnostics Systems, Neuilly, France; NASBA: HIV-1 RNA QT assay by Organon Teknika, Fresnes, France; bDNA: Quantiplex HIV-1 RNA by Chiron Corporation, Cergy Pontoise, France) and are being used more frequently in the routine clinical management of patients infected with HIV-1.

To assess the impact of genetic variability on HIV-1 RNA quantification, we evaluated these commercial kits by using a panel of HIV-1 isolates representing clades A to H. Group O HIV-1 strains were not tested, because the different assays are exclusively recommended for quantitation of RNA for group M HIV-1 strains. These isolates were expanded in culture. Virus was collected by ultracentrifugation and resuspended in HIV-seronegative plasma. To standardize the quantities of virus to similar levels in each preparation, the p24 antigen was determined (Coultronics, Margency, France) and the volume adjusted so that each specimen contained approximately 10 pg of p24 antigen per ml.

HIV-1 RNA was quantitated by the three assays according to the manufacturers instructions. Each specimen was tested in duplicate by each procedure, and results were expressed as the mean value. Results obtained by the two determinations were concordant (i.e., log difference <0.5 log) for all specimens. As presented in Table 1, disparate results were obtained according to the strains tested. We observed that clade A strains were not detected by RT-PCR and that clade G strains were not detected by NASBA. However, the copy number detected by RT-PCR in one clade E (CM235) and in one clade F (163.3070) was much lower than the copy number detected by bDNA and NASBA (log difference >0.5). All the HIV-1 clades could be detected and quantitated by bDNA, although for clades B and D (UG270), the HIV-1 RNA levels measured by bDNA were lower than those obtained by RT-PCR and NASBA.

These results demonstrate that quantification of HIV-1 RNA is highly influenced by the HIV-1 genome sequence. The level of detection of clade A strains by the Amplicor qualitative and quantitative assays has been previously reported (6,7). Our results confirm these findings and indicate that the NASBA procedure is also influenced by the HIV-1 genomic diversity. In contrast, HIV-1 RNA quantification with the Quantiplex bDNA assay demonstrated minor influence by genetic variability, presumably because of the large number of probe sequences designed into the assay (5).

All three tests can be used for the quantification of RNA from clade B HIV-1 strains, which are predominant in European and American countries. However, in the case of an HIV-1 RNA level lower than the detection limit obtained with RT-PCR or NASBA in an untreated, HIV-1–infected patient, the clinician should consider verifying the RNA level by another procedure; this observation should also lead to the characterization of the HIV-1 clade.

TABLE 1. *Effect of the HIV-1 clade on quantitation of HIV-1 RNA by RT-PCR, bDNA, and NASBA*

Subtype	HIV-1 strain	HIV-1 RNA copies per ml of HIV-seronegative plasma		
		RT-PCR[a]	bDNA	NASBA
A	DJ258	<400	111.500	100.000
A	DJ263	<400	79.800	60.000
B	SF2	225.500	38.000	240.000
B	III-B	54.000	17.000	360.000
C	ZAM18	78.300	70.000	66.000
C	ZAM20	178.800	125.800	420.000
D	UG270	179.800	29.200	170.000
D	UG274	320.000	41.400	32.300
E	CM241	18.800	72.800	35.000
E	CM235	4.700	52.000	15.000
F	163.3069	36.200	94.000	57.000
F	162.3070	2.800	78.100	26.000
G	G98	254.700	269.000	<400
G	LBV21	184.500	295.000	<400
H	VI557	950.000	587.000	125.000

[a] RT-PCR, coupled reverse transcription–polymerase chain reaction amplification process (Amplicor Monitor by Roche Diagnostics Systems, Neuilly, France); bDNA, branched DNA amplification process (Quantiplex HIV-1 RNA by Chiron Corporation, Cergy Pontoise, France); NASBA, nucleic acid sequence–based amplification procedure (HIV-1 RNA QT assay by Organon Teknika, Fresnes, France).

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Mycobacterial Acute Lumbosacral Polyradiculopathy as the Initial Manifestation of AIDS

To the Editor: Acute lumbosacral polyradiculopathy (ALP) is a well characterized syndrome in AIDS and is most frequently caused by cytomegalovirus (CMV) (1). Mycobacterial infection of the central nervous system may occasionally manifest as spinal radiculomyelitis (2,3), with clinical features that can be indistinguishable from those of ALP. As far as we know, tuberculosis has not been reported as an etiologic agent of ALP in AIDS patients, although focal spinal mycobacterial meningomyelitis has been reported (4). We present a patient who developed a mycobacterial ALP as the initial manifestation of AIDS.

A previously healthy 49-year-old man developed progressive paraparesis, headache, cognitive inattention, and somnolence over 15 days. Two days before admission, he had fever and urinary incontinence. General examination showed fever of 38°C and folliculitis in the face, neck, and legs. The bladder was palpable and required catheterization. Neurologic examination disclosed a drowsy and confused patient with neck stiffness, marked paraparesis, and absence of ankle jerks. Plantar responses were flexor. Straight-leg raising evoked severe pain. Sensory examination findings were normal. Cerebrospinal fluid (CSF) contained 228×10^6 leukocytes/L (i.e., 90% neutrophils, no malignant cells), 1.17 g/L of protein, and 1.66 mmol/L of glucose (i.e., blood glucose level of 5 mmol/L). Cryptococcal antigen, VDRL, and bacterial, mycobacterial, fungal, and viral cultures were negative. Polymerase chain reaction (PCR) test results for CMV, herpes simplex, and varicella-zoster virus were negative. Serologic test results for HIV were positive. The CD4 lymphocyte count was 200×10^6 cells/L. The test result for CMV antigenemia was negative, and that for the tuberculin skin test was positive. Chest radiographic findings were normal. Magnetic resonance imaging (MRI) of the brain disclosed a left thalamic infarct. An Unenhanced MRI showed the thoracic and lumbosacral spine were normal. The electrophysiologic study demonstrated a predominantly motor, severe axonal polyradiculopathy in the legs.

Empiric treatment with cefotaxime, isoniazid, pyrazinamide, and rifampin was started soon after admission. On day 7, a skin biopsy of a lesion of the neck disclosed granulomatous inflammation with acid-fast bacilli, and cefotaxime was discontinued. The patient's mental status improved significantly, but the paraparesis evolved to a flaccid, areflexic paraplegia. The CSF contained 78×10^6 lymphocytes, 0.97 g/L of protein, and 2.4 mmol/L of glucose (i.e., blood glucose of 4.4 mmol/L). Two months later, the areflexic paraplegia remained unchanged, and the CSF parameters were normal. He died 6 months after admission of a generalized wasting syndrome.

This patient presented with an ALP and neutrophilic meningitis associated with disseminated mycobacterial disease. Although mycobacterial species could not be identified, it was most likely *Mycobacterium tuberculosis,* because this case fulfills the Ahuja (5) diagnostic criteria for highly probable tuberculous meningitis. CMV infection seems unlikely, because his CD4 count was 200×10^6 cells/L and because the test for viral antigenemia and PCR detection of virus in CSF were negative. Tuberculous leptomeningitis is thought to be frequently associated with involvement of the spinal cord and nerve roots (2,6). No clinical features nor imaging data supported a myelitis involvement in our patient, who instead demonstrated meningoradicular affection.

For these reasons, we believe tuberculosis should be included in the differential diagnosis of ALP in AIDS, because neither the clinical features nor spinal fluid findings differentiate a mycobacterial from a CMV cause. Empiric treatment with antituberculous drugs should be considered, especially in patients with a low probability for CMV infection and in countries were tuberculosis is prevalent. Prompt treatment is imperative to preserve neurologic function.

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