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Comparison of the QUANTIPLEX HIV-1 RNA 2.0 Assay with the AMPLICOR HIV-1 MONITOR 1.0 Assay for Quantitation of Levels of Human Immunodeficiency Virus Type 1 RNA in Plasma of Patients Receiving Stavudine-Didanosine Combination Therapy

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We compared the QUANTIPLEX HIV-1 RNA 2.0 assay with the AMPLICOR HIV-1 MONITOR 1.0 assay for quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma in the Stadi trail, which evaluated a stavudine plus didanosine combination therapy in 52 patients. HIV-1 RNA baseline values measured with AMPLICOR HIV-1 MONITOR 1.0 were significantly higher than those measured with QUANTIPLEX HIV-1 RNA 2.0, and decreases in HIV-1 RNA levels from baseline were also found to be significantly higher when measured with the AMPLICOR HIV-1 MONITOR 1.0 assay. The frequency of HIV-1 RNA levels below the lower limit of quantitation was significantly higher with QUANTIPLEX HIV-1 RNA 2.0 than with AMPLICOR HIV-1 MONITOR 1.0. Reanalysis of these results by an ultrasensitive procedure of AMPLICOR HIV-1 MONITOR 1.0 or by a modified version of the test that included additional primers adapted for non-B HIV-1 clades yielded greater differences between the QUANTIPLEX HIV-1 RNA 2.0 assay and the AMPLICOR HIV-1 MONITOR 1.0 assay. Our results indicate that a valid comparison of the virological efficacies obtained with different antiretroviral drug regimens requires the use of the same viral load quantitation procedure; further standardization between the different HIV-1 RNA quantitation kits is therefore needed.

Human immunodeficiency virus type 1 (HIV-1) RNA levels in plasma (viral load) can now be considered the most relevant indicator to predict disease progression and to assess the efficacy of antiretroviral therapies (3, 4, 7, 9, 10). Therefore, the virological evaluation of new drug regimens in clinical trials is based mainly on the utilization of this marker. Different methods, such as reverse transcription-coupled PCR, branched DNA, or nucleic acid sequence-based amplification, which are suitable for HIV-1 RNA quantitation, have been made available in commercial kits (8, 11, 19). These commercial assays have demonstrated equal reliability, but they present different lower limits of detection and dynamic ranges (5, 15, 17). Moreover, differences in absolute HIV-1 RNA concentration determined by the different assays can be observed (5, 13). It has also been shown that HIV-1 genetic diversity has an influence on HIV-1 RNA level determination, since RNAs extracted from different HIV-1 clade strains are not equally quantitated by the different methods (5, 13). Therefore, the virological data obtained in clinical trials of patients receiving antiretroviral therapies could depend on the choice of the HIV-1 RNA quantitation procedure. To evaluate this effect, we compared the QUANTIPLEX HIV-1 RNA 2.0 assay (Chiron Diagnostics, Cergy-Pontoise, France) with the AMPLICOR HIV-1 MONITOR 1.0 assay (Roche Diagnostic Systems, Neuilly, France) for HIV-1 RNA quantitation in the Stadi trial, a pilot study of a stavudine plus didanosine combination therapy in which didanosine was administered once daily (16).

Fifty-two patients were included in this study. Quantitation of HIV-1 RNA in plasma was performed in samples collected on weeks −2 (preentry), 0 (entry), 8, 24, and 48. The HIV-1 RNA baseline value was the mean of the values obtained for the samples collected at preentry and entry. HIV-1 RNA quantitation was performed in accordance with the manufacturers' instructions. Samples with values lower than the detection limit as determined by the AMPLICOR HIV-1 MONITOR 1.0 assay were reanalyzed by the ultrasensitive procedure (12). The lower limit of quantitation of the QUANTIPLEX HIV-1 RNA 2.0 assay was 500 HIV-1 RNA copies/ml (2.70 log HIV-1 RNA copies/ml). The lower limit of quantitation of the AMPLICOR HIV-1 MONITOR 1.0 assay using the standard or the ultrasensitive procedure was established for the individual plasma samples by calculating the amount of HIV-1 RNA obtained by setting the absorbance value of the test at 0.20. When results were below the value of the lower limit of quantitation, this value was used for analytical purposes. HIV-1 was isolated from patients' peripheral blood mononuclear cells collected at entry following standard lymphocyte coculture (6). HIV-1 subtyping was performed by the heteroduplex mobility assay on the HIV-1 proviral DNA extracted from cultured cells as previously described (5). Forty-six HIV-1 isolates belonged to clade B, five belonged to clade A, and one belonged to clade C.

As shown in Table 1, HIV-1 RNA levels at baseline determined with the QUANTIPLEX HIV-1 RNA 2.0 assay were significantly lower than those determined with the AMPLICOR

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TABLE 1. Comparison of results obtained with the QUANTIPLEX HIV-1 RNA 2.0 assay and the AMPLICOR HIV-1 MONITOR 1.0 assay for quantitation of HIV-1 RNA in plasma from patients included in the Stadi pilot study

Assay	Log HIV-1 RNA copies/ml [mean ± SD (range)] at:			Change from baseline [mean ± SD (range)] at:		
	Baseline (n = 52)	Week 8 (n = 48)	Week 24 (n = 45)	Week 48 (n = 30)	Week 8 (n = 48)	Week 24 (n = 45)	Week 48 (n = 30)
QUANTIPLEX	4.49 ± 0.60 (3.33–5.81)	2.93 ± 0.55 (<2.70–5.22)	3.03 ± 0.64 (<2.70–5.66)	2.89 ± 0.38 (<2.70–4.07)	−1.51 ± 0.51 (−0.46–−2.50)	−1.39 ± 0.65 (0.09–−2.86)	−1.49 ± 0.69 (−0.31–−3.82)
AMPLICOR	4.92 ± 0.74 (2.68–6.23)	2.94 ± 0.86 (<1.93–5.67)	3.02 ± 0.98 (<1.91–5.85)	2.88 ± 0.74 (<2.00–4.30)	−1.93 ± 0.72 (−0.30–−3.44)	−1.85 ± 0.91 (0.26–−3.48)	−1.93 ± 0.88 (−0.33–−3.68)
AMPLICOR (ultrasensitive procedure)[a]	4.92 ± 0.74 (2.68–6.23)	2.75 ± 1.04 (<1.30–5.67)	2.80 ± 1.21 (<1.15–5.85)	2.61 ± 1.03 (<0.95–4.30)	−2.12 ± 0.82 (−0.30–−4.09)	−2.07 ± 1.02 (0.26–−4.10)	−2.17 ± 1.06 (−0.33–−4.31)

[a] The ultrasensitive procedure was performed when the HIV-1 RNA levels were below the lower limit of quantitation of the AMPLICOR HIV-1 MONITOR 1.0 standard procedure.

TABLE 2. Frequency (%) of HIV-1 RNA levels below the lower limit of quantitation

Time (n)	% of HIV-1 RNA levels below the lower limit of quantitation		
	QUANTIPLEX assay	AMPLICOR assay	AMPLICOR assay (ultrasensitive procedure)[a]
W8 (48)	75	29	8
W24 (45)	62	36	13
W48 (30)	73	33	17

[a] The ultrasensitive procedure was performed when the HIV-1 RNA levels were below the lower limit of quantitation of the AMPLICOR HIV-1 MONITOR 1.0 standard procedure.

HIV-1 MONITOR 1.0 assay (Student's t test, $P < 0.0001$). We also observed a difference in HIV-1 RNA level changes from baseline since the HIV-1 RNA decreases determined by the standard or ultrasensitive procedures of the AMPLICOR HIV-1 MONITOR 1.0 assay or the QUANTIPLEX HIV-1 RNA 2.0 assay were significantly different (analysis of variance, $P < 0.0001$). The difference between the HIV-1 RNA changes from baseline determined by the QUANTIPLEX HIV-1 RNA 2.0 assay and the standard ($P = 0.0001$) or ultrasensitive ($P < 0.0001$) AMPLICOR HIV-1 MONITOR 1.0 procedures was highly significant. The difference between the HIV-1 RNA changes from baseline determined by the standard or the ultrasensitive AMPLICOR HIV-1 MONITOR 1.0 procedures reached the limit level of significance ($P = 0.05$). Differences between the QUANTIPLEX HIV-1 RNA 2.0 assay and the AMPLICOR HIV-1 MONITOR 1.0 assay resulted on the one hand from the lower limit of quantitation of the AMPLICOR HIV-1 MONITOR 1.0 assay and on the other hand from the higher HIV-1 RNA baseline values measured with AMPLICOR HIV-1 MONITOR 1.0. In contrast, the observed differences between the standard and the ultrasensitive AMPLICOR HIV-1 MONITOR 1.0 procedures resulted only from a decrease in the lower limit of quantitation of the test.

As shown in Table 2, the frequency of values below the lower limit of quantitation was significantly higher with the QUANTIPLEX HIV-1 RNA 2.0 assay than with the AMPLICOR HIV-1 MONITOR 1.0 assay; this frequency was also significantly higher with the AMPLICOR HIV-1 MONITOR 1.0 standard procedure than with the ultrasensitive procedure (χ^2, $P < 0.001$).

It has been shown that HIV-1 RNA levels can be underestimated by the AMPLICOR HIV-1 MONITOR 1.0 assay in plasma samples collected from patients infected with non-B clade strains, mainly in those infected with clade A strains (5, 13). For two patients (patients 10 and 31) infected with clade A strains, HIV-1 RNA levels measured with the QUANTIPLEX HIV-1 RNA 2.0 assay were much higher (>1.0 log HIV-1 RNA copies/ml) than those measured with the AMPLICOR HIV-1 MONITOR 1.0 assay. Samples collected from the six patients infected with non-B HIV-1 clade strains were therefore reanalyzed by a modified version of the AMPLICOR HIV-1 MONITOR 1.0 assay (Table 3). In this version, a pair of modified primers was added into the PCR mix; these modified primers have the same length and bind to the same primer binding sites as the primers SK462 and SK431 that are already present in the test kit (2, 14). As shown in Table 3, HIV-1 RNA levels measured with this modified version in plasma from patients 10 and 31 were dramatically increased in comparison with the levels measured in the absence of additional primers. Moreover, for these two patients, HIV-1 RNA could be quantitated by the modified version in the samples found below the

TABLE 3. Comparison of the QUANTIPLEX HIV-1 RNA 2.0 assay with the AMPLICOR HIV-1 MONITOR 1.0 assay for quantitation of RNA from HIV-1 non-B clades in plasma

Patient no.	HIV-1 clade	Week	Log HIV-1 RNA copies/ml		
			QUANTIPLEX assay	AMPLICOR assay	Modified AMPLICOR assay
10	A	0	4.65	3.55	5.25
		8	<2.70	<1.15	2.53
17	A	0	3.41	3.72	3.69
		8	<2.70	1.57	1.57
		24	<2.70	1.30	1.61
		48	<2.70	2.27	2.27
31	A	0	4.32	2.68	5.05
		8	<2.70	<1.40	2.05
		24	<2.70	<1.20	2.19
		48	<2.70	<1.26	2.11
33	A	0	3.33	3.95	3.66
		8	<2.70	2.34	2.32
		24	<2.70	2.17	2.08
		48	<2.70	2.13	2.70
39	C	0	4.34	5.25	4.95
		8	<2.70	2.15	2.32
		24	<2.70	<1.15	<1.04
		48	<2.70	<0.95	<1.72
49	A	0	4.19	4.58	4.49
		8	<2.70	2.76	2.69
		24	3.26	1.82	1.90
		48	<2.70	2.42	2.08

lower limit of quantitation in the absence of additional primers. Therefore, the use of the modified version of the AMPLICOR HIV-1 MONITOR 1.0 assay led to an increase in the mean baseline value (4.98 ± 0.66 log HIV-1 RNA copies/ml versus 4.92 ± 0.74 log HIV-1 RNA copies/ml) and a decrease in the frequency of results below the lower limit of quantitation (4% versus 8% at week 8, 11% versus 13% at week 24, and 13% versus 17% at week 48).

Taken together, these results confirm that the choice of the HIV-1 RNA quantitation procedure has an influence on the virological results obtained in antiretroviral clinical trials since differences could be observed in the absolute HIV-1 RNA copy number, the HIV-1 RNA changes from baseline and the frequency of results below the limit of quantitation. Inclusion of patients infected with HIV-1 strains belonging to non-B HIV-1 clades could also have an effect on the virological results. However, this effect can now be eliminated by including additional primers in the AMPLICOR HIV-1 MONITOR 1.0 assay or by using the currently available AMPLICOR HIV-1 MONITOR 1.5 assay (18).

In spite of the differences observed between the two assays, we cannot recommend the use of one of them in particular. Indeed, it has been reported that the different commercially available assays are equally reliable for HIV-1 RNA quantitation (5, 15, 17), and it has been shown that the use of a common external standard could eliminate differences among absolute HIV-1 copy number estimates made with the commercial assays (1). The use of the ultrasensitive procedure, which allows the quantitation of small amounts of HIV-1 RNA, confers an advantage to the AMPLICOR HIV-1 MONITOR 1.0 assay. However, the availability of a third-generation QUANTIPLEX assay with a detection limit lowered to 50 HIV-1 RNA copies/ml could abolish the differences between the two assays for determining relative HIV-1 RNA changes or frequency of values below the limit of quantitation.

In summary, the results obtained in the present study indicate that a valid comparison of the virological efficacies obtained with different antiretroviral drug regimens requires the use of the same viral load quantitation procedure; further standardization between the different HIV-1 RNA quantitation kits is therefore needed.

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