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Short communication

Performance evaluation of the COBAS/TaqMan HIV-1 v2.0 in HIV-1 positive patients with low viral load: A comparative study

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A B S T R A C T

HIV-1 viral load determination is a crucial step for monitoring the efficacy of highly active antiretroviral therapy (HAART) and predicts disease progression. Real-time PCR based assays are available for monitoring the viral load. They differ in sensitivity, genomic target region and dynamic range. In this study, the performance of the Roche Cobas Taqman HIV-1 v2.0 was evaluated on plasma samples from HIV-1 positive patients in parallel with the Abbott RealTime HIV-1 assay in a routine diagnostic setting. Overall, there was a good agreement between the two assays. However, some samples detected by the Abbott RealTime HIV-1 assay but below the limit of quantitation of the assay were found negative result when tested with the Roche Cobas Taqman HIV-1 v2.0. It is conceivable that signal anomalies or background noise may affect the lower-end precision of the Abbott RealTime HIV-1 assay. Based on these results, it is concluded that it is not recommended to switch platform during longitudinal viral load monitoring of HIV-1 positive patients.

1. Introduction

Quantitation of HIV-RNA is a critical step to monitor highly active antiretroviral therapy (HAART) and predict disease progression (Mellors et al., 1997). Viral load levels below the limit of quantitation usually reflect adherence to treatment and efficacy of HAART (Gross et al., 2001; Bagchi et al., 2007). On the contrary, quantifiable HIV-1 RNA may suggest poor treatment adherence or virological failure. In elite controllers, individuals who control spontaneously viral replication without antiretroviral drugs, low levels of viremia (down to 2 copies/ml) have been detected by ultrasensitive methods and were related to higher HIV-1-specific antibody responses and low levels of CD4+ (Pereyra et al., 2009). Understanding the meaning of very low viral load may be relevant for patient management (Di Mascio et al., 2004).

Currently, several commercial assays are available for quantitation of plasma HIV-1 RNA. They differ in sensitivity, dynamic range, target region, and amplification method (Peter and Sevall, 2004). Real-time PCR is the method used most widely and it offers a series of advantages over the conventional molecular methods: such as (i) increased analytical sensitivity, (ii) faster results, (iii) reduced risk of contamination, and (iv) wider dynamic range.

The extensive genetic variability of HIV-1 strains circulating world-wide has an important impact on the management of this infection, from the identification of infected persons to viral load determination and monitoring of treatment. There is currently no assay able to quantify the whole spectrum of circulating HIV-1 strains. Differences in primers/probe design, target region, technology used may be responsible for underestimation of the viral load or failure of detection with direct implications for clinical management and detection of treatment failure (Peeters et al., 2010).

Amplification of multiple targets of HIV-1 genome may improve the accuracy of viral load determination. In this study, plasma samples from HIV-1 positive patients were tested by the Abbott RealTime HIV-1 assay in comparison with the Roche Cobas Taqman HIV-1 v2.0 (Roche Diagnostics, Branchburg, NJ, USA), which uses a multiplex real-time PCR approach to amplify the *gag* and LTR regions within the HIV-1 genome. The goal of the study was to verify whether the Roche multiplex real-time approach improves the accuracy of viral load determination especially in samples with low or not quantitated viremia (<40 copies/ml) as measured by the Abbott RealTime HIV-1 assay.

2. Materials and methods

2.1. Study group

The study was carried out on 109 plasma samples from 59 HIV-1 positive patients (25 women and 34 men) admitted at the

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Table 1
Range of HIV-1 viral load in the three groups of patients examined.

	Abbott RealTimeHIV-1 (copies/ml)		Cobas Taqman HIV-1 v2.0 (copies/ml)		N. viral subtypes
	N. samples	Viral load range	N. samples	Viral load range	
Group I	28	40–100	3	TND	2, B; 1, F
			4	<20 detected	2, NA; 2, B
			6	20–40	2, CRF02_AG; 2, F; 1, B; 1, G 2,
			6	40–100	NA; 3, B; 1, G
			9	>100[a]	2, G; 6, B; 1, CRF01_AE
Group II	12	>100	0	TND	
			0	<40	
			3	40–100	2, B; 1, CRF02_AG
			9	>100	2, NA; 6, B; 1, C
Group III	69	<40	17	TND	14, B; 2, NA; 1, G
			22	<20 detected	15, B; 2, G; 1, NA; 1, C; 1, F; 2, CRF02_AG
			14	20–40	10, B; 1, F; 1, CRF02_AG, 2, NA;
			11	40–100	10, B; 1, G
			5	>100[b]	5, B

TND: target not detected; NA: not available.
[a] Viral load >100 copies/ml detected by Cobas Taqman HIV-1 v2.0 in group I.
[b] Viral load >100 copies/ml detected by Cobas Taqman HIV-1 v2.0 in group III.

Infectious Diseases ward of the Polyclinic Tor Vergata. Of these patients, four were infected by the recombinant form CRF02_AG, one by CRF01_AE, five by subtype G, four by subtype F, one by subtype C, and 37 by subtype B. No information on the viral subtype was available for 7 patients. CD4+ count was also performed as part of the routine investigation.

2.2. HIV-1 RNA extraction and amplification

Two aliquots of 1 ml each of plasma were collected from each patient and tested independently by the Roche and Abbott systems. In the case of Roche assay, the COBAS Ampliprep instrument was used for automated specimen processing and the Cobas TaqMan48 for the automated amplification and detection (Roche Molecular System, Inc., Branchburg, NJ, USA). Samples tested by the Abbott assay were run on the *m*2000 system, a platform capable of automated RNA extraction and PCR set-up, followed by amplification/detection (Abbott Molecular Inc., Des Plaines, IL, USA).

The Cobas Ampliprep/Cobas Taqman HIV-1 v2.0 can quantitate HIV-1 RNA over the range of 20–10,000,000 copies/ml, while the Abbott HIV-1 RealTime test quantitates over a range of 40–10,000,000 copies/ml.

When the samples were discordant qualitatively (positive/negative), an "in-house" nested PCR targeting a 220 bp fragment of the V3 region within the *gp120* gene was performed. The sensitivity of the method is 10 copies/ml (data not shown). Primers sequences are available upon request. Viral RNA was reverse transcribed using the SuperScript® One-Step RT-PCR System (Invitrogen, Milan, Italy) according to the following thermal profile: 1 cycle at 50 °C, 30 min, then 1 cycle at 94 °C, 2 min, 40 cycles at 95 °C, 30 s, 51 °C, 30 s, 72 °C, 50 s; with a final extension step at 72 °C, for 10 min. In the second round PCR the conditions were: 1 cycle at 93 °C, 12 min; 40 cycles at 95°, 30 s, 52 °C, 30 s, 72 °C, 40 s; followed by 1 cycle at 72 °C, 10 min. Precautions were taken to avoid contamination and controls were included in each PCR run. The amplicons were checked on a 2% agarose gel under UV light.

2.3. Statistical analysis

Linear regression and correlation analysis were employed to determine assays relationship. The method of Bland–Altman was applied to assess the agreement between the two assays.

3. Results

Of the 109 plasma samples tested by the Abbott HIV-1 RealTime assay, 28 had a viral load between 40 and 100 copies/ml (group I), 12 >100 copies/ml (group II), while 69 were positive but below the limit of quantitation of the assay (<40 copies/ml, group III). When the same samples of the group I were tested by Cobas TaqMan HIV-1 v.2.0, 3 were negative (target not detected), 4 were <20 copies/ml (detected), 6 between 20 and 40 copies/ml, 6 between 40 and 100 copies/ml, and 9 above 100 copies/ml. In the group II, 3 samples had a viremia between 40 and 100 copies/ml, and 9 >100 copies/ml. Finally, in the group III, where all 69 samples tested by the Abbott assay were <40 copies/ml, 17 were undetected, 22 were positive but <20 copies/ml, 14 had a viremia between 20 and 40 copies/ml, 11 between 40 and 100, and 5 >100 copies/ml.

In the group I, three patients had a subtype G, two a subtype F, two a CRF02_AG recombinant form, one CFR02_AE recombinant form, two an unknown genotype, and 22 were infected by subtype B.

In the group II, one patient had subtype C, one subtype CRF02_AG, two an unknown genotype and eight a subtype B. Group III, three patients were infected with the recombinant form CFR02_AG, two with subtype F, two with subtype G, one with

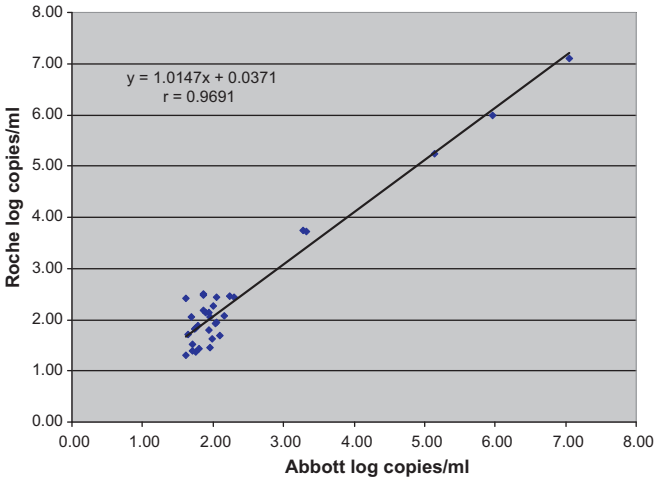

Fig. 1. Correlation of viral load results obtained using Abbott HIV-1 RealTime PCR and Cobas TaqMan HIV-1 v2.0. The linear regression trend is shown.

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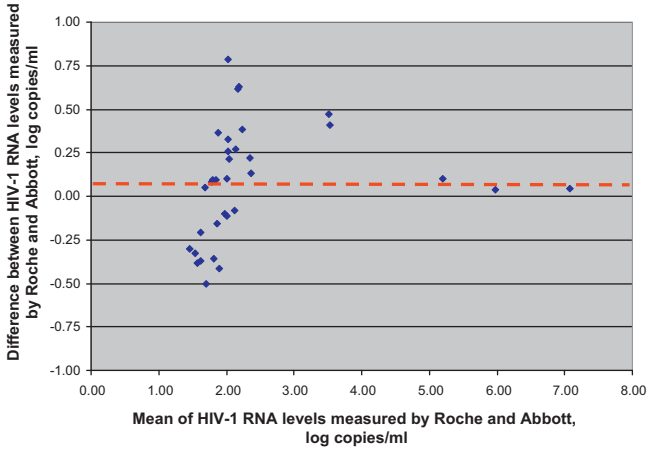

Fig. 2. Bland–Altman analysis of the samples tested by Abbott HIV-1 RealTime PCR and Cobas TaqMan HIV-1 v2.0.

subtype C, and 29 with subtype B. Based on the viral sub-types distribution within the three groups of patients examined, discordant results do not seem to be related to different viral subtypes (Table 1).

Linear regression analysis carried out on the 33 samples quantified by both systems showed a good correlation ($r = 0.9691$) between the two assays as shown in Fig. 1, although this result could be biased by the low number of samples with high viremia tested. Bland–Altman analysis showed a good agreement between the two assays (Fig. 2).

Of the 17 discordant samples, negative by the Roche assay but detected (<40 copies/ml) by the Abbott test, extra plasma of 9 samples was available for further testing. The nested PCR targeting the V3 region of the *gp120* gene was negative on all samples tested, confirming the result observed with the Roche assay.

4. Conclusions

Accurate determination of HIV-1 viral load is crucial for evaluating the response to treatment and the adherence to therapy. Achieving low levels of viremia during antiretroviral treatment predicts a sustained virological response. For this reason, samples with low viral load were selected and tested in parallel by two commercial kits: the Cobas TaqMan HIV-1 v.2.0 and the Abbott RealTime HIV-1 assay. Both assays are based on real-time PCR with a wide dynamic range and high sensitivity. Overall, a good agreement was found between the two test systems when considering the samples quantified within the dynamic range of both tests, although this result is partly biased by the low number of samples with high viremia tested. Bland–Altman analysis showed a good agreement between the two methods. Of the samples tested, it is worth noting that 30 samples not quantitated by the Abbott RealTime HIV-1 assay were quantitated by the Roche Cobas TaqMan HIV-1 v.2.0 (Table 1). The multiplex PCR approach of the Roche assay with the amplification of two viral regions (LTR and *gag* gene) instead of the amplification of one viral region (*pol* gene) as with Abbott may explain this difference. The apparent under-quantitation of HIV-1 RNA levels observed in 14 samples (9 group I and 5 group II) with the Abbott assay could also be attributed to the issue of primer and probe binding polymorphisms as a result of genetic diversity within different viral subtypes. The amplification of two viral targets in one reaction could increase the accuracy of the viral load determination and the spectrum of quantifiable HIV-1 isolates.

Recent studies addressing the performance of the Roche and Abbott HIV-1 real-time assays have detected an increasing number of positive samples with viral load below the limit of quantitation of both assays (Wirden et al., 2009; Sloma et al., 2009). Similar results were reported in studies comparing the Cobas TaqMan HIV-1 v.2.0 assay with the Cobas Amplicor assay (Lima et al., 2009; Gatanaga et al., 2009) where several samples were detected but not quantified by both assays. The clinical meaning of such findings is still unclear. Some studies showed that these episodic low-level viremias do not necessarily reflect the appearance of drug-resistant strains or virological failure (Manavi, 2008; Smit et al., 2009), while others hypothesized that these viremic blips are linked to altered specimen-processing procedures (Rebeiro et al., 2008). Similar results were obtained in this study where several samples were below the limit of quantitation by both assays irrespective of the viral subtype tested.

Finally, 17 samples positive by the Abbott assay but below the limit of quantitation of the assay gave a negative result when tested by Roche assay. This negative results were confirmed by an "in-house nested" PCR when plasma was available for further testing. Since all patients examined in this study were HIV-1 positive, artefacts due to cross-hybridization of primers/probe can be excluded. It is conceivable that in some circumstances signal anomalies or background noise may affect the lower-end precision of the Abbott RealTime HIV-1 assay (Shain and Clemens, 2008).

In the light of these findings, virological follow-up should be performed using the same assay and viremic blips should be interpreted with caution before changing therapy. A strict follow-up may indicate whether there is a virological relapse or blip with no impact on the ongoing antiviral therapy. Amplification of multiple viral targets by real-time PCR represents an important step forward for a virus with high genetic variability such as HIV-1. It should improve the reliability and accuracy of the virological follow-up.

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