Short Communication: Prospective Comparison of Qualitative Versus Quantitative Polymerase Chain Reaction for Monitoring Virologic Treatment Failure in HIV-Infected Patients

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Abstract

Less costly but still accurate methods for monitoring HIV treatment response are needed. We prospectively evaluated if a qualitative polymerase chain reaction (PCR) amplification assay for virologic monitoring could maintain accuracy while reducing costs in Seoul, South Korea. We conducted the first prospective study comparing a qualitative PCR amplification of HIV-1 reverse transcriptase (RT) versus a commercial real time PCR assay (i.e., viral load) for virologic monitoring of 150 patients receiving antiretroviral therapy (ART) between November 2011 and August 2012 at an urban hospital in Seoul, South Korea. A total of 215 blood plasma samples from 150 patients receiving ART for more than 6 months were evaluated. Using the individual viral load assay, 12 of 215 (5.6%) plasma samples had more than 500 HIV RNA copies/ml. The qualitative PCR amplification assay detected individual samples with ≥ 500 HIV RNA copies/ml with 100% sensitivity. The specificities of the qualitative PCR amplification of the HIV-1 RT assay were 94.1%, 93.6%, and 93.2% compared to the real time PCR at 500, 1,000, and 5,000 threshold of HIV RNA copies/ml, respectively, and \$24,940 USD would have been saved for 150 patients during 10 months. The qualitative PCR amplification of the HIV-1 RT assay might be a useful approach to effectively monitor patients receiving ART and save resources.

BY 2007 ABOUT 3 MILLION HIV-infected people were receiving antiretroviral therapy (ART).¹ The current clinical guidelines for monitoring for ART failure is to perform viral loads every 3–6 months, and this monitoring strategy has proven to be cost effective in resource-wealthy settings.² However, because of capability and cost, the use of viral loads to monitor for virologic failure during ART is not recommended or performed in most resource-limited settings (RLS).^{3–5} In efforts to save resources, some have proposed detailed historic, hematologic, immunologic, and clinical monitoring instead,^{6,7} but previous studies have demonstrated that measures that do not monitor for active viral replication are not sufficient to detect virologic failure and can foster the spread of drug resistance.^{7,8}

Recently, a qualitative method based on polymerase chain reaction (PCR) amplification of HIV-1 reverse transcriptase (RT) was proposed as a way to reduce costs of virologic monitoring of

ART.^{9–11} Here, we prospectively compared this qualitative assay to standard commercial methods in Seoul, South Korea.

HIV-1-infected Korean patients who had been continuously receiving ART, consisting of three or more antiretroviral drugs for at least 6 months at a 2,000-bed university-affiliated tertiary care referral hospital, consented to participate in the study. We prospectively enrolled 150 HIV-infected patients from November 2011 to August 2012. The Institutional Review Board of Severance Hospital approved the protocol.

To develop the qualitative PCR amplification of HIV-1 RT, the PCR protocol used the heminested primers in the HIV-1 RT coding region, which are specific to regions that are conserved across HIV-1 subtypes as previously described.⁹ The cost per assay was \$20 USD.

Viral load measurements were conducted on a single blood plasma sample that underwent a single thaw prior to testing.

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TABLE 1. PATIENT DEMOGRAPHIC DATA

Characteristics	Values
Age, mean in years (range)	48 (21–81)
Male sex, <i>n</i> (%)	137 (91.3)
HAART regimen	
PI based, <i>n</i> (%)	86 (57.4)
NNRTI based, <i>n</i> (%)	48 (32.0)
II based, <i>n</i> (%)	6 (4.0)
PI and II based, <i>n</i> (%)	5 (3.3)
NNRTI and II based, <i>n</i> (%)	3 (2.0)
NRTI only, <i>n</i> (%)	2 (1.3)
HAART duration (days)	1,898 (56–6,830)
CD4 cell counts at sample acquisition	
Median CD4 cell count, median cells/mm ³ (range)	486 (5–1,252)
<200 cells/mm ³ , <i>n</i> (%)	19 (8.8)
200–350 cells/mm ³ , <i>n</i> (%)	41 (19.1)
350–500 cells/mm ³ , <i>n</i> (%)	53 (24.7)
>500 cells/mm ³ , <i>n</i> (%)	102 (47.4)
Lowest CD4 cell count, median cells/mm ³ (range)	191 (2–716)
Viral load	
<20 copies/ml, <i>n</i> (%)	167 (77.7)
20–100 copies/ml, <i>n</i> (%)	28 (13.0)
101–500 copies/ml, <i>n</i> (%)	8 (3.7)
501–1,000 copies/ml, <i>n</i> (%)	1 (0.5)
>1,000 copies/ml, <i>n</i> (%)	11 (5.1)

HAART, highly active antiretroviral therapy; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; II, integrase inhibitor.

The Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, version 2.0 (Roche Molecular Systems, Pleasanton, CA) procedure was performed according to the manufacturer's instructions. Reported values for the upper and lower limits of quantitation were 10,000,000 copies/ml and 20 copies/ml, respectively. The cost per assay was \$136 USD.

All variables are expressed as the mean (range) or number (percent), unless otherwise indicated. We determined the positive and negative predictive values, sensitivity, and specificity for the qualitative PCR amplification of HIV-1 RT to detect varying levels of viremia (500, 1,000, and 5,000 copies /ml) according to commercial quantitative viral load measurements of same samples.

Between November 2011 and August 2012, 215 blood plasma samples from 150 patients receiving ART were evaluated. Table 1 shows the baseline characteristics of patients. The majority (91.3%) of the patients were male, with a

mean age of 48 years (range 21–81 years). The median CD4⁺ T cell count was 486 cells/mm³ (range 5–1,252 cells/mm³). Using the commercial viral load assay, 48 of 215 (22.3%) plasma samples contained more than 20 HIV RNA copies/ml, 12 of 215 (5.6%) plasma samples had more than 500 HIV RNA copies/ml, and 11 (5.1%) had viral loads of 1,000 copies/ml or greater.

The qualitative PCR amplification of HIV-1 RT was performed on 215 blood plasma samples. Table 2 shows diagnostic accuracy of the assay for detecting virologic failure in HIV patients, as compared to the commercial viral load platform. Of 24 qualitative PCR-positive samples, 12 samples had more than 500 HIV RNA copies/ml. Seven of 24 positive results had viral loads between 100 and 500 HIV RNA copies/ml, and the rest showed low level viremia from 40 to 100 HIV RNA copies/ml. The qualitative assay detected all samples with ≥ 500 HIV RNA copies/ml with 100% sensitivity. The specificities of the qualitative assay were >93% for thresholds of HIV RNA copies/ml between 500 and 5,000 HIV RNA copies/ml.

The per-assay costs of the quantitative and qualitative PCR amplification assays in South Korea were \$136 USD and \$20 USD, respectively. Based on per-assay costs, the qualitative PCR amplification assay would have saved \$24,940 USD for 150 patients during 10 months.

Simple assay costs remain a large barrier to the implementation of virologic monitoring of patients receiving ART,^{2,4,12} even though viral load testing has been shown to be superior to CD4⁺ T cell count and clinical monitoring for detecting ART failure.^{8,13} In most resource-limited settings, viral load monitoring is either not performed or not recommended because of prohibitively high costs.^{3,5,8} To improve HIV care, more cost-effective approaches to monitor for virologic failure are needed.

One limitation of this study was that the qualitative PCR assay was a nested PCR with a series of manual steps, which might increase the risk PCR contamination and amplifying very low level HIV RNA (<20 copies/ml), and thus can lead to false positive results. Such false positive results may lead to costly, unnecessary changes in therapy. However, in most RLS, viral load monitoring is either not performed or not recommended because of the prohibitively high costs of the commercial real time PCR assay. The inability to detect early virologic failure could lead to an accumulation of resistant mutations and the selection of viruses with broad cross resistance to antiretroviral drugs. An increasing number of patients carrying drug-resistant virus will inevitably lead to a spread of these resistant strains in the population. This problem would also be quite costly to individual patients, to

TABLE 2. DIAGNOSTIC ACCURACY OF QUALITATIVE POLYMERASE CHAIN REACTION AMPLIFICATION FOR THE DETECTION OF VARYING LEVELS OF VIROLOGIC FAILURE IN HIV PATIENTS

Characteristics	Threshold of real time PCR (HIV RNA copies/ml)		
	500	1,000	5,000
PPV, %	50.0 (12/24)	45.8 (11/24)	41.7 (10/24)
NPV, %	100.0 (191/191)	100.0 (191/191)	100.0 (191/191)
Sensitivity, %	100.0 (12/12)	100.0 (11/11)	100.0 (10/10)
Specificity, %	94.1 (191/203)	93.6 (191/204)	93.2 (191/205)

PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value.

the public, and to the healthcare system. Therefore, this qualitative PCR assay with low costs and high sensitivity could be valuable for public health, although there is high risk of false positive results by PCR contamination.

In an effort to reduce the costs of virologic monitoring, this prospective study evaluated whether a previously described qualitative PCR assay⁹ could be used in a clinical setting. Compared with a quantitative assay (Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, version 2.0), the qualitative assay was both highly sensitive and specific. Additionally, the qualitative assay was considerably less costly than the commercial assay (USD \$20 versus \$136 per sample tested). In addition, since the qualitative method amplifies the HIV-1 RT region and almost all ART regimens contain some if not all RT inhibitors, especially in resource-limited settings, the direct sequencing of the PCR product could provide genotypic resistance information⁹ that could guide the next ART regimen choices. Taken together, this qualitative assay seems to be a highly viable method for virologic monitoring of ART.

Acknowledgments

This work was supported by the National Research Foundation of Korea grant funded by the Korean Government (NRF-2013R1A1A2005412 and NRF-2011-220-E00015), a grant from the Chronic Infectious Disease Cohort (4800-4859-304-260) from Korea Centers for Disease Control and Prevention, the BioNano Health-Guard Research Center funded by the Ministry of Science, ICT & Future Planning (MSIP) of Korea as Global Frontier Project (grant H-GUARD_2013M3A6B2078953), the Department of Veterans Affairs, and grants from the National Institutes of AI100665 (Smith-Training), AI080353 (Smith-Pooled Testing), DA034978 (Smith-Avant Garde), and AI36214 (Richman-CFAR).

Author Disclosure Statement

No competing financial interests exist.

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