Comparative sensitivity of automated (Abbott M2000) and manual plasma HIV-1 RNA PCR assays for the detection of persistent viremia after long-term antiretroviral therapy

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ABSTRACT

Background: The ability of automated, FDA-cleared plasma HIV-1 RNA assays to detect low-level viremia, compared to manual, highly sensitive research-only methods, is not well-defined. We therefore tested paired plasma samples from people with HIV-1 (PWH) on long-term antiretroviral therapy (ART) with both the Abbott M2000 RealTime HIV-1 Viral Load assay (Abbott) and a quantitative reverse transcriptase (RT)-initiated PCR assay that has a reported 95% detection limit of 1 HIV-1 RNA copy/ml (single copy assay, SCA).

Methods: Plasma samples from 309 participants in the AIDS Clinical Trials Group study A5321 were tested by both Abbott and SCA. Participants were mostly men (82%). All were on stable ART for a median of 7 years with HIV-1 RNA <40 copies/mL by Abbott. Pooled plasma from each donor was divided and tested. Abbott results were reported as target detected <40 copies/mL but not quantifiable (target detected <40) or target not detected (TND), and SCA results were classified as HIV-1 RNA detected or not detected.

Results: By Abbott, 17% (51/309) of sample results were target detected <40, whereas 83% (258/309) were TND. Of the samples that were target detected <40 by Abbott, 73% (37/51) had HIV-1 RNA detected by SCA. By contrast, 43% of samples that were TND by Abbott (110/258) had HIV-1 RNA detected by SCA (p < 0.001).

Conclusion: Plasma samples from PWH with HIV-1 RNA detected but <40 copies/ml by the automated Abbott M2000 assay are likely (73% of 51 samples) to have HIV-1 RNA detected by an optimized manual assay with single copy sensitivity. An Abbott HIV-1 RNA result of target not detected did not exclude low-level viremia: 43% of 258 samples had HIV-1 RNA detected by the single copy assay. These findings indicate that the Abbott M2000 assay cannot exclude the persistence of viremia on ART and thus may have less utility, compared to a manual single copy assay, for assessing the impact of experimental interventions designed to eliminate low-level viremia as a step towards achieving ART-free HIV-1 remission.

1. Introduction

People with HIV-1 (PWH) on long-term, clinically effective antiretroviral therapy (ART) often have low-level viremia, measured as plasma HIV-1 RNA. The ability of automated, FDA-cleared plasma HIV-1 RNA assays to detect low-level viremia compared to research-use single copy HIV-1 RNA assays (SCA) is unclear. Most commercial testing platforms report HIV-1 RNA levels that are detectable and quantifiable, detectable but too low to quantify, or undetectable. Often, little attention is given to the difference between results that are undetectable versus those that are detectable but not quantifiable. Both results indicate virologic suppression on ART, but closer examination could provide insight into the persistence of viremia.

The AIDS Clinical Trials Group (ACTG) study A5321 monitors the
decay of HIV-1 reservoirs in a large cohort of participants on long-term, suppressive ART.\textsuperscript{3-7} We determined the feasibility of using the high-throughput Abbott RealTime HIV-1 Viral Load assay as a tool to screen for changes in low-level viremia by directly comparing it with SCA using plasma samples from A5321 participants. Additionally, we assessed potential correlations between different plasma HIV-1 RNA readouts of the Abbott assay with clinical or virological parameters before ART (pre-ART) and during ART (on ART).

2. Materials and methods

\textit{Participants} We performed cross-sectional evaluation of 309 adults who initiated ART during chronic HIV-1 infection and had sustained virologic suppression.\textsuperscript{8} Participant characteristics were assessed both pre-ART and at the time of virologic measurement on ART, and adherence was assessed over multiple study visits based on self-report of missed ART doses over the four days prior to a study visit using the ACTG adherence questionnaire.\textsuperscript{9}

\textit{Virologic Assays} SCA was performed as published\textsuperscript{10} but briefly, 5 mL of plasma was centrifuged for 15 min at room temperature to remove cellular debris, then 2hr at 21,000 g to pellet HIV-1 virions. Nucleic acid was precipitated with isopropanol, washed with ethanol, dried, and resuspended in 5 mM Tris buffered with RNAsin and DTT. The reported sensitivity of SCA was 95% detection of one HIV-1 RNA copy/ml in a 5 mL sample. The Abbott RealTime HIV-1 Viral Load assay was performed according to the manufacturer’s recommendations for EDTA plasma on the Abbott M2000 RealTime System.

SCA results were classified categorically as HIV-1 RNA “detected” or “not detected” using the lowest detection limit of one HIV-1 RNA copy in a 5 mL sample (0.4 copies/ml). Abbott RealTime HIV-1 Viral Load assay results were reported as target not detected (TND), target detected but not quantifiable at <40 copies/ml (target detected <40). All Abbott results were either TND or target detected <40. Comparisons were made with HIV-1 DNA and cell-associated HIV-1 RNA results per million CD4\textsuperscript{+} T cells that were performed as described previously.\textsuperscript{11,12} Statistical comparisons used Wilcoxon rank sum or Fisher’s exact tests.

\textit{Ethics Statement} The institutional review boards at the participating site institutions approved the study. All participants provided written informed consent for their participation in the study.

3. Results

The 309 A5321 participants were predominantly male (82%) on ART for a median of 7.3 years at sample collection with a median CD4\textsuperscript{+} T cell count of 681 cell/mm\textsuperscript{3}. All Abbott results were either TND (83%) or <40 target detected (17%). Of the 17% of samples that were <40 target detected, 73% had detectable HIV-1 RNA by SCA at a median of 1.8 copies/mL. Of the samples that were TND by Abbott, a significantly lower but substantial fraction (43%) had HIV-1 RNA detected by SCA at a median of <0.4 copies/ml (Fig. 1; p < 0.001).

We next examined the categorical associations of Abbott results target detected <40 or TND with various pre-ART and on-ART virologic and immunologic measures. There were no significant associations between Abbott results and pre-ART HIV-1 DNA, pre-ART plasma HIV-1 RNA, pre-ART CD4\textsuperscript{+} T cell count, or pre-ART CD4/CD8 ratio. While not statistically significant, on-ART cell-associated HIV-1 RNA was modestly correlated with plasma HIV-1 RNA target detected <40 (p = 0.06). Additionally, women were more likely to be TND by Abbott than men (91% vs 82%), consistent with general trends in sex differences in long-term suppressed viremia.\textsuperscript{13-15} There were no significant associations between categorical Abbott results and duration of ART, HIV-1 DNA, CD4\textsuperscript{+} T cell count, or body mass index on ART (Table 1).

4. Discussion

Plasma samples from PWH on long-term ART tested by the automated Abbott M2000 commercial assay with a result of target detected <40 copies/ml (i.e., not quantifiable) are more likely to have HIV-1 RNA detected by an optimized manual PCR assay with single copy sensitivity than samples that were TND. A prior study described a similar correlation between HIV-1 RNA values that were detected but not quantifiable below the limit of quantification (20 copies/mL) using the Roche COBAS AmpliPrep/COBAS TaqMan system and SCA.\textsuperscript{16} Commercial assay results below the limit of quantification, but “target detected”, have been associated with higher likelihood of subsequent virologic failure on ART than a target not detected result. For example, a previous study (n = 326) showed that compared to donors with undetectable HIV-1 RNA, those with at least three consecutively detected, but not quantified, HIV-1 RNA values (<20 copies/mL) experienced higher risk of virologic failure (HR, 3.81).\textsuperscript{17} Moreover, in a UK study, donors were stratified by HIV-1 RNA 40–49 copies/mL, <40 copies/mL with RNA detected, and <40 copies/mL with no RNA detected. The study found that compared to individuals with no detectable HIV-1 RNA, having viremia of 40–49 copies/mL increased the risk of HIV-1 rebound to >50 copies/mL by 4.67-fold, while having detectable RNA at <40 copies/mL increased the risk by 1.97-fold. The risk of rebound to >400 copies/mL was increased by 6.91-fold and 2.88-fold, respectively.\textsuperscript{18}

Together, these findings reinforce that there are important differences between HIV-1 RNA results that are undetectable vs. those that are detectable but not quantifiable by commercial assays, and that our comparison between automated commercial and optimized manual assays shows that plasma HIV-1 RNA levels are different in samples with target detected vs. target not detected results determined by automated commercial assay.
In the current study, we found no statistically significant associations between Abbott M2000 results of TND or target detected < 40 with pre- or post-ART participant or other virologic measures. By contrast, in a prior report on this same cohort and plasma samples, we found that SCA was positively correlated with pre-ART HIV-1 RNA (r = 0.20; p < 0.001) and negatively with pre-ART CD4\(^+\) T cell count (r = 0.12, p < 0.05). In addition, males were more likely than females to have detectable plasma HIV-1 RNA by SCA (52% vs 29%, p = 0.003), even after adjusting for age, pre-ART HIV-1 RNA and CD4\(^+\) T cell count, years on ART and BMI (p = 0.004). Higher BMI was associated with higher SCA levels (r = 0.12, p < 0.04) after adjustment for age, sex, pre-ART HIV-1 RNA and CD4\(^+\) T cell count, and years on ART.\(^ {11,19}\) These same associations were not found using Abbott HIV-1 RNA results. Although Abbott results of target detected < 40 were associated with a high probability of detecting HIV-1 RNA by SCA (73%), almost half (43%) of samples that were TND by Abbott were still positive for HIV-1 RNA by SCA. This reinforces the greater sensitivity of optimized manual PCR (SCA) and helps to explain the stronger association of SCA results with other key clinical and virologic parameters described above.\(^ {12,20}\)

These findings from the current study indicate that the Abbott M2000 assay cannot exclude the persistence of viremia on ART and thus may have less utility, compared to an optimized manual PCR assay, for assessing the impact of experimental interventions designed to eliminate low-level viremia. Nevertheless, commercial HIV-1 RNA platforms could provide an early indication of reduction in low-level viremia when evaluated in large-scale clinical trials of interventions to eliminate...
persistent viremia on ART. Specifically, a binary detectable/non-detectable readout could be used by an automated platform to screen for reductions in low-level viremia. Longitudinal samples with target detected <40 vs TND results could be further tested by SCA to assess the extent of HIV-1 RNA reduction. With careful study design, incorporating commercial assays could reduce dependency on SCA for interventional trials aimed at clearing viremia. Indeed, a study of a putative latency-reversing agent romidepsin showed no effect on target detected vs TND by the Abbott assay, which was confirmed by SCA. It has recently been reported that testing multiple replicates of the same sample on an automated platform can lower the limit of quantification of an automated testing platform. This approach can provide single copy quantification with higher throughput, but multiple replicate testing can be costly. Although single replicate automated assays have lower sensitivity than multiple replicates or optimized manual assays, they could provide initial insight into whether low-level viremia has been influenced by an experimental intervention without performing higher sensitivity assays on all samples.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

1. Chen W, Markowitz M, Perelson AS, Leonard JM, Ho DD, Neumann AU. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995; 373(6510):123–126.
2. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science. 1996;271(5255):1506–1508.
3. Markowitz M, Vesanen M, Perelson AS, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature. 1997;387(6629):188–191.
4. Dornadula G, Zhang H, Valleri A, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. JAMA. 1999;282(17):1627–1632.
5. Gandhi M, Gandhi RT, Stefanescu A, et al. Cumulative antiretroviral exposure measured in hair is not associated with measures of HIV persistence or inflammation among individuals on suppressive ART. J Infect Dis. 2018;218(2):234–238.
6. Gandhi RT, McMahon DK, Bosch RJ, et al. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. PLoS Pathog. 2017;13(4), e1006285.
7. Thomas AS, Jones RL, Gandhi RT, et al. T-cell responses targeting HIV Nef uniquely correlate with infected cell frequencies after long-term antiretroviral therapy. PLoS Pathog. 2017;13(9), e1006629.
8. Smurzynski M, Collier AC, Kolekar SL, et al. AIDS clinical trials group longitudinal linked randomized trials (ALLRT): rationale, design, and baseline characteristics. HIV Clin Trials. 2008;9(4):269–282.
9. Reynolds NR, Sun J, Nagaraja HN, Gifford AL, Wu AW, Chesney MA. Optimizing measurement of self-reported adherence with the ACTG Adherence Questionnaire: a cross-protocol analysis. J Acquir Immune Defic Syndr. 2007;46(4):402–409.
10. Tosiano MA, Jacobs JL, Shutt RA, Cyktor JC, Mellors JW. A simpler and more sensitive single-copy HIV-1 RNA assay for quantification of persistent HIV-1 viremia in individuals on suppressive antiretroviral therapy. J Clin Microbiol. 2019;57(3).
11. Cyktor JC, Bosch RJ, Mar H, et al. Male sex and obesity are associated with residual plasma HIV-1 viremia in persons on long-term antiretroviral therapy. J Infect Dis. 2020.
12. Hong F, Aga E, Cillo AR, et al. Novel assays for measurement of total cell-associated HIV-1 DNA and RNA. J Clin Microbiol. 2016;54(4):902–911.
13. Scully EP. Sex differences in HIV infection. Curr HIV AIDS Rep. 2018;15(2):136–146.
14. Napravnik S, Poole C, Thomas JC, Eron Jr JJ. Gender difference in HIV RNA levels: a meta-analysis of published studies. J Acquir Immune Defic Syndr. 2002;31(1):11–19.
15. Addo MM, Allfied M. Sex-based differences in HIV type 1 pathogenesis. J Infect Dis. 2014;209(Suppl 3):S86–S92.
16. Margot N, Koontz D, McCallister S, Mellors JW, Callebaut C. Measurement of plasma HIV-1 RNA below the limit of quantification (<20 copies/mL) of commercial assays with the integrase HIV RNA single-copy assay. J Acquir Immune Defic Syndr. 2008;49(4):269–272.
17. Perelson AS, Neumann AU, Markowitz M, et al. Measured in hair is not associated with measures of HIV persistence or inflammation among individuals on suppressive ART. J Infect Dis. 2018;218(2):234–238.
18. Reynolds NR, Sun J, Nagaraja HN, Gifford AL, Wu AW, Chesney MA. Optimizing measurement of self-reported adherence with the ACTG Adherence Questionnaire: a cross-protocol analysis. J Acquir Immune Defic Syndr. 2007;46(4):402–409.
19. Bohm R, Agarwal S, Pohle A, et al. Quantification of persistent HIV-1 viremia in individuals on suppressive ART. J Infect Dis. 2020.
20. Reynolds NR, Sun J, Nagaraja HN, Gifford AL, Wu AW, Chesney MA. Optimizing measurement of self-reported adherence with the ACTG Adherence Questionnaire: a cross-protocol analysis. J Acquir Immune Defic Syndr. 2007;46(4):402–409.
21. Jacobs JL, Tosiano MA, Koontz DL, et al. Automated, multi-replicate quantification of persistent HIV-1 viremia in individuals on antiretroviral therapy. J Clin Microbiol. 2020.