Impact of Early Antiretroviral Therapy on the Performance of HIV Rapid Tests and HIV Incidence Assays

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Background: Antiretroviral therapy (ART) can downregulate antibody responses to HIV infection. We evaluated the impact of early vs. delayed ART on the performance of HIV diagnostic and incidence assays.

Methods: Samples were obtained from 207 participants in the HPTN 052 trial, who were stably suppressed on ART for ≥4 years [Malawi sites; pre-ART CD4 cell count 350–550 cells/mm^3 (early ART arm, N = 180) or <250 cells/mm^3 or an AIDS-defining illness (delayed ART arm, N = 27)]. Samples were tested with 2 HIV rapid tests and 2 HIV incidence assays; selected samples were also tested with two fourth-generation immunoassays and a Western blot (WB) assay. A pre-ART sample was analyzed if the follow-up sample had a false-negative or weakly-reactive rapid test result, or had an incidence assay result indicative of recent infection (false-recent result).

Results: Ten (4.8%) samples had a nonreactive or weakly-reactive rapid test result (7/180 early ART arm, 3/27 delayed ART arm, $P = 0.13$); one sample had nonreactive fourth-generation assay results and 3 had indeterminate WBs. Forty (18.9%) samples had a false-recent incidence assay result; 16 (7.8%) had false-recent results with both incidence assays. Baseline samples had stronger rapid test and WB bands, higher fourth-generation assay signal-to-cutoff values, and fewer HIV incidence assay results indicative of recent infection.

Conclusions: False-negative/weakly-reactive HIV rapid tests and false-recent HIV incidence assay results were observed in virally-suppressed individuals, regardless of pre-ART CD4 cell count. Downregulation of the antibody response to HIV infection in the setting of ART may impact population-level surveys of HIV prevalence and incidence.

Key Words: HPTN 052, early ART, HIV rapid tests, HIV incidence assays, suppressive ART

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INTRODUCTION

Anti-HIV antibodies appear shortly after HIV infection and usually increase over the first 6–12 months of infection.[1] The Limiting Antigen Avidity assay[2,3] (LAg-avidity assay) and other serologic assays have been developed for cross-sectional HIV incidence estimation.[4,5] These assays use characteristics of the anti-HIV antibody response to identify individuals with recent HIV infection. Many factors impact the performance of serologic incidence assays, including low CD4 cell count, viral suppression, and antiretroviral therapy (ART).[6–10] The package insert for the LAg assay recommends against using the assay for individuals who are virally suppressed or are on ART.[11] ART can impact the performance of HIV rapid tests,[12–15] presumably by downregulating anti-HIV antibody production. Undisclosed ART has been reported in research and clinical settings.[16–19] Individuals on ART who choose not to disclose their HIV infection status may have false-negative HIV tests.[14,20,21] False-negative HIV rapid tests and false-recent incidence assays could also compromise population-level surveys of HIV infection.[22] In these settings, false-negative rapid tests may underestimate HIV prevalence, and false-recent incidence assays may overestimate HIV incidence.

Historically, ART was only initiated after the CD4 cell count declined. ART is now recommended for all HIV-infected individuals, regardless of CD4 cell count.[23,24] This reflects recent findings that demonstrate benefits of early ART for HIV treatment and prevention.[25,26] Previous studies evaluating the impact of ART on HIV rapid tests and HIV incidence assays were performed in populations and settings where ART was started at lower CD4 cell counts. In this study, we evaluated the impact of ART on anti-HIV antibody responses in a cohort that includes individuals who initiated ART at higher CD4 cell counts (350–550 cells/mm^3).

METHODS

Study Cohort

Samples were obtained from adults enrolled in HIV Prevention Trials Network (HPTN) 052 (NCT00074581).[26,27] HPTN 052 was a multinational, Phase 3, randomized, controlled clinical trial that evaluated the impact of early ART on HIV transmission in serodiscordant couples.[26,27] HIV-infected (index) participants had a CD4 cell count between 350 and 550 cells/mm^3 at enrollment and were randomized to start ART at enrollment (early ART arm) or after their CD4 cell count was $<$250 cells/mm^3 on 2 consecutive study visits or they developed an AIDS-defining illness (delayed ART arm). Viral load testing was performed at study sites. Viral suppression was defined as having 2 consecutive viral load measurements $\leq$400 copies/mL.[26,27] In May 2011, ART was offered to all index participants, regardless of CD4 cell count.[27]

This substudy included index participants from Blantyre and Lilongwe, Malawi who initiated ART before May 2011 and were virally suppressed on ART for $\geq$4 years (viral load $<$400 copies/mL at all visits once viral suppression was achieved). These 2 study sites enrolled the largest number of participants in HPTN 052 and provided a sample set from participants with a single prevalent HIV subtype. Samples were tested retrospectively at the HPTN Laboratory Center (Baltimore, MD).

HIV Screening Tests

Samples collected $\geq$4 years on ART (follow-up) were tested with 2 HIV rapid tests: the OraQuick ADVANCE Rapid HIV-1/2 Antibody Test and the Uni-Gold Recombigen HIV-1/2 Test (Supplemental Digital Content, Figure 1, http://links.lww.com/QAI/B19). Dilution panels were used to guide interpretation of rapid test results (Supplemental Digital Content, Figure 2, http://links.lww.com/QAI/B19). Additional testing was performed for participants who had a nonreactive/weakly-reactive rapid test result at follow-up. This included HIV rapid testing at pre-ART (baseline) and interim study visits; baseline and follow-up samples were also tested using 2 fourth-generation immunoassays and a Western blot assay (WB, Supplemental Digital Content, Figure 1, http://links.lww.com/QAI/B19).

HIV Incidence Tests

Follow-up samples were tested with the LAg-avidity assay [HIV-1 Limiting Antigen (LAg)-Avidity EIA] and the Bio-Rad avidity assay.[28] Testing was repeated for samples with an OD-n $<$2.0 to confirm test results. Samples with results below the following cutoffs were classified as assay positive (indicating recent infection or a false-recent test result): LAg-avidity assay: normalized optical density (OD-n) $<$1.5; Bio-Rad avidity assay: avidity index (AI) $<$80%. If a sample had a result below the cutoff for either assay, the corresponding baseline sample was analyzed.

Statistical Methods

The Fisher exact test or χ^2 test was used to compare the proportion of samples in the early ART vs. delayed ART study arms that had a nonreactive/weakly-reactive HIV rapid test result or a false-recent HIV incidence assay result.

Ethical Considerations

Written informed consent was obtained from participants in their native language before enrollment. The trial was approved by institutional review boards/Ethics Committees at each participating institution.

RESULTS

In HPTN 052, 277 of 481 serodiscordant couples enrolled at study sites in Malawi initiated ART by May 2011; samples were available for 207 (74.7%) of the 277 index participants who were virally suppressed for $\geq$4 years before the study ended in May 2015 [early ART arm (n = 180); delayed ART arm (n = 27)]. The median time between ART initiation and collection of the follow-up sample was 5 years (range: 4–9 years) in the early ART arm and 5 years (range: 4–8 years) in the delayed ART arm.

Follow-up samples were tested with 2 HIV rapid tests. One sample had a nonreactive result and 9 (4.3%) samples had weakly-reactive results (Table 1). The frequency of nonreactive/weakly-reactive rapid test results was similar in the 2 study arms [early ART: 7/180 (3.9%), delayed ART: 3/27 (11.1%), $P = 0.13$]. In 4/10 cases, samples had nonreactive/weakly-reactive results with both rapid tests. Additional testing was performed for these 10 cases (Supplemental Digital Content, Figure 1, http://links.lww.com/QAI/B19). In 5/10 cases, weakly-reactive rapid test results were observed after only 1 year on ART (Supplemental Digital Content, Table, http://links.lww.com/QAI/B19). In addition, one follow-up sample had nonreactive results with 2 fourth-generation assays and 3 follow-up samples had indeterminate WB results (Table 1). Baseline samples were reactive/positive with all assays in all 10 cases; none of the baseline samples had weakly-reactive rapid test results.

HIV incidence assay results were obtained for 206/207 follow-up samples. Forty (19.4%) samples had a positive (false-recent) result with one or both incidence assays (Table 2). Nine of the 16 samples that had positive results with both incidence assays also had weakly-reactive HIV rapid test results. The proportion of samples that had positive results with both incidence assays was similar in the 2 study arms [early ART: 14/179 (7.8%); 2/27 delayed ART (7.4%), $P = 1.0$, Table 2]. Baseline samples were tested for 39/40 cases that had positive assay results at follow-up. Fifteen (38.4%) of the 39 baseline samples had a positive result with one or both assays (Table 2).

DISCUSSION

We assessed the impact of ART on the performance of HIV screening assays and HIV incidence assays by testing samples from adults enrolled in HPTN 052 who were virally suppressed on ART for at least 4 years. False-negative (nonreactive) or weakly-reactive HIV rapid test results were obtained for 4.8% of the 207 samples tested. The proportion of samples with nonreactive/weakly-reactive HIV rapid tests was similar in the early and delayed ART study arms; however, we may not have detected a difference between study arms because the number of participants in the delayed ART arm was small (N = 27).

Most of the samples with weakly-reactive test results had a very faint or extremely faint band that could easily be missed if the test were read in a nonlaboratory testing environment, such as a mobile van, or in an area that was not well lit. A study from Zambia found that the accuracy of

TABLE 1. HIV Screening Test Results for Participants Who Had a Nonreactive or Weakly-reactive Rapid Test Result After ≥4 Years on Suppressive ART*

	Study Arm	Sample Type	Time on ART, yr	Rapid OraQuick	Rapid Uni-Gold	Fourth-gen Abbott (S/CO)†	Fourth-gen Bio-Rad	Western Blot‡
1	Early	Baseline	0	R	R	R (520.1)	R	POS
		Follow-up	4.9	R**	R	R (114.4)	R	POS§
2	Early	Baseline	0	R	R	R (481.9)	R	POS
		Follow-up	4.9	R**	R	R (181.3)	R	IND§
3	Early	Baseline	0	R	R	R (392.2)	R	POS
		Follow-up	8.9	R***	R*	R (60.2)	R	POS
4	Early	Baseline	0	R	R	R (375.3)	R	POS
		Follow-up	5.0	R***	R*	R (24.8)	R	POS§
5	Early	Baseline	0	R	R	R (175.6)	R	POS
		Follow-up	4.9	R***	R**	R (19.6)	R	POS§
6	Early	Baseline	0	R	R	R (52.3)	R	POS
		Follow-up	4.9	R***	R	R (66.1)	R	POS
7	Early	Baseline	0	R	R	R (38.2)	R	POS
		Follow-up	4.0	R***	R	R (70.5)	R	IND§
8	Delayed	Baseline	0	R	R	R (682.5)	R	POS
		Follow-up	4.6	R**	R	R (357.0)	R	POS§
9	Delayed	Baseline	0	R	R	R (419.5)	R	POS§
		Follow-up	5.1	R*	R	R (130.1)	R	IND§
10	Delayed	Baseline	0	R	R	R (645.9)	R	POS
		Follow-up	4.4	NR	NR	NR (0.1)	NR	IND§

*Tests included: 2 HIV rapid tests [the OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (Orasure Technologies, Inc., Bethlehem, PA) and the Uni-Gold Recombigen HIV-1/2 Test (Trinity Biotech, Wicklow, Ireland)], 2 fourth-generation immunoassays [the ARCHITECT HIV Ag/Ab Combo assay (Abbott Laboratories, Abbott Park, IL) and the GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories, Redmond, WA), and the GS HIV-1 Western blot (Bio-Rad Laboratories)].

†For the 7 cases in the early ART arm, the median S/CO ratio was 375 (range: 38–520) for the baseline samples and 66 (range: 20–181) for the follow-up samples. For the 3 cases in the delayed ART arm, the median S/CO ratio was 646 (range: 419–682) for the baseline samples and 130 (range: 0.1–357) for the follow-up samples.

‡All cases had one or more Western blot bands that were weaker in strength at the follow-up visit compared with the baseline visit.

§Cases with one or more Western blot bands absent. Absent bands included: case 1: p18; case 2: p31, p18; case 4: gp41, p18; case 5: gp120, gp41, p18; case 7: p40; case 8: p18; case 9: p18; and case 10: gp160, gp120, p65, gp41, p40, p31, p24, p18.

ART, antiretroviral therapy; gen, generation; IND, indeterminate; NR, nonreactive; POS, positive; R***, weakly-reactive with an extremely faint band; R**, weakly-reactive with a very faint band; R, reactive, R*, weakly-reactive with a faint band; S/CO, signal-to-cutoff ratio.

TABLE 2. Frequency of Follow-up Samples From Individuals on Long-term Suppressive ART That Had HIV Incidence Assay Results Below the Assay Cutoffs*

	LAg-Avidity Assay Only (OD-N <1.5), N (%) Positive	Bio-Rad Avidity Assay Only (AI <80%), N (%) Positive	Both Assays, N (%) Positive
All, N = 206	12 (5.8)†	12 (5.8)‡	16 (7.8)§
Early ART arm, N = 179	10 (5.6)	10 (5.6)	14 (7.8)
Delayed ART arm, N = 27	2 (7.4)	2 (7.4)	2 (7.4)

*A positive assay result indicates a result below the assay cutoff. The assay cutoff used for the LAg-avidity assay (Sedia Biosciences Corportation, Portland) was 1.5 normalized optical density units (OD-n). The assay cutoff used for the Bio-Rad avidity assay (based on the Genetic Systems HIV Combo Ag/Ab EIA; Bio-Rad) was avidity index (AI) <80%.

†At baseline, none of the samples had a positive LAg-avidity result; one sample had a positive Bio-Rad avidity result.

‡At baseline, none of the samples had a positive LAg-avidity result; 5 samples had a positive Bio-Rad avidity result.

§At baseline, one sample had a positive LAg-avidity result only, 5 samples had a positive Bio-Rad avidity result only, and 3 samples had positive results for both assays.

AI, avidity index; ART, antiretroviral; LAg, limiting antigen; N, number.

HIV rapid tests was higher when testing was performed by trained laboratory personnel compared with nonlaboratory personnel.[29] In this report, all testing was performed by experienced laboratory personnel under ideal testing conditions; the frequency of false-negative results in the setting of long-term ART suppression may be higher in other settings. In a previous study of children and adolescents who were virally suppressed on ART, the Uni-Gold rapid test performed better than the OraQuick rapid test.[30] In this report, the frequency of weakly-reactive HIV rapid tests was higher with the OraQuick test than the Uni-Gold test (9/207 vs. 3/207); however, this difference was not statistically significant. The 2 rapid tests have different target antigens and differ in other ways that could impact the sensitivity for detecting infection in individuals with long-term viral suppression.

Positive HIV incidence assay results indicating low antibody avidity were obtained for 19.4% of the follow-up samples tested. Because these samples were collected at least 4 years after ART initiation in participants who were known to be HIV infected at study enrollment, these were classified as false-recent test results. The proportion of samples with false-recent test results was similar in the early and delayed ART arms. HIV incidence assays are often used in multiassay algorithms for HIV incidence estimation.[31] A two-assay algorithm that includes the LAg-avidity and Bio-Rad avidity assays was validated for cross-sectional HIV incidence estimation.[3] Sixteen (7.8%) follow-up samples in this study had false-recent results with both of these assays and would be incorrectly classified as recently infected using the 2-assay algorithm. All samples tested in this report would have been correctly classified as "non-recent" using a multiassay algorithm that includes HIV viral load (ie, where samples with low viral loads are classified as nonrecent), because all were from virally-suppressed individuals. However, use of viral load as a biomarker of recent infection may be problematic in settings implementing universal ART, where ART may be started in individuals with recent HIV infection.[23] In some settings, low or undetectable HIV viral loads are also observed in a high proportion of individuals with recent infection who are not on ART.[32] The performance of HIV screening tests and incidence assays may also be lower in settings where ART is started very early in infection; false-negative serologic test results and high false-recent rates with incidence assays have been observed in individuals who started ART during acute infection[33] or within 6 months of infection.[6] In this study, some samples with false-recent incidence test results had strongly-reactive HIV rapid tests. The results obtained for these assays could reflect differences in target antigens, or other differences between the test methods. HIV rapid tests are designed to maximize sensitivity for antibody detection. In contrast, avidity assays developed for cross-sectional HIV incidence testing are designed to weaken or partially disrupt antibody binding to target antigens. For this reason, a decrease in antibody reactivity may be more readily detected using avidity-based incidence assays, compared with HIV rapid tests.

This study documents a decrease in HIV-specific antibodies with long-term suppressive ART. Baseline samples had stronger HIV rapid test bands than follow-up samples; in 5/10 cases weakly-reactive rapid test results were observed only 1 year after ART initiation. In 3/10 cases, indeterminate WB results were obtained after long-term suppressive ART. In addition, the median S/CO ratio for the fourth-generation ARCHITECT assay was 6-fold lower at follow-up compared with baseline. These results are consistent with a recent study showing slow but persistent antibody loss in HIV-infected children and adolescents on long-term suppressive ART.[30] In contrast, in chronically-infected adults, the strength of third-generation enzyme immunoassay test results was similar at baseline and after 5 years of suppressive ART; the WB banding patterns and band intensities were also similar before and after ART in that study.[34]

In this report, nonreactive/weakly-reactive HIV rapid tests and false-recent HIV incidence assays were observed in virally-suppressed individuals who started ART at both high and low CD4 cell counts. This indicates that downregulation of the serologic response to HIV infection in the setting of suppressive ART also occurs in individuals who start ART earlier in infection. Although HIV-infected individuals who are virally suppressed on ART are not usually tested for HIV infection in clinical settings, they may be tested for HIV infection in population surveys or clinical trials. Individuals may enroll in research studies or seek health care without disclosing knowledge of HIV status or ART use. In this setting, they may falsely believe that their initial HIV-test results were incorrect or that they were cured if HIV tests are negative. Education that addresses the possible impact of ART on HIV diagnostic tests is important in clinical and

research settings. In addition, it is important to recognize that increasing availability of ART and increasing use of ART early in infection may impact clinical trials and population surveys where HIV prevalence and incidence are key outcomes.

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