MAJOR ARTICLE

Use of a Multifaceted Approach to Analyze HIV Incidence in a Cohort Study of Women in the United States: HIV Prevention Trials Network 064 Study

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(See the major article by Laeyendecker et al, on pages 232–9 and the editorial commentary by Mastro, on pages 204–6.)

Background. Reliable methods for estimating the incidence of human immunodeficiency virus (HIV) infection are needed to monitor the epidemic, identify at-risk populations, and evaluate HIV prevention strategies. We used a multifaceted approach to estimate HIV incidence in the HIV Prevention Trials Network (HPTN) 064 study.

Methods. The HPTN 064 study enrolled 2067 HIV-seronegative women and 32 HIV-seropositive women with no prior HIV infection diagnosis. Women were followed for up to 12 months. HIV incidence estimates were based on (1) detection of acute HIV infection, (2) documentation of HIV seroconversion, and (3) detection of recent HIV infection, using a multiassay algorithm (MAA).

Results. Two women had acute HIV infection at enrollment, 4 seroconverted, and 2 were identified as recently infected at enrollment using the MAA. The annual HIV incidence estimate based on acute infection at enrollment (2.52% [95% confidence interval {CI}, .17%–9.33%], using a 14-day window period) was higher than the estimate based on seroconversion (0.24% [95% CI, .07%–.62%]; $P = .027$). Incidence estimates obtained using the MAA at enrollment and at the end of study were 0.25% (95% CI, .03%–.93%) and 0.13% (95% CI, .006%–.76%), respectively.

Conclusions. We detected a high frequency of acute infection at enrollment. Cross-sectional HIV incidence estimates obtained using the MAA were similar to the longitudinal estimate based on HIV seroconversion.

Clinical Trials Registration. NCT00995176.

Keywords. HIV-1; women; United States; incidence.

Accurate methods for estimating the incidence of human immunodeficiency virus (HIV) infection are needed to monitor the HIV epidemic, to identify populations at high risk of HIV acquisition, and to evaluate the effectiveness of HIV prevention strategies [1, 2]. In most HIV prevention studies, HIV incidence is assessed by enrolling an HIV-uninfected cohort and following the cohort over time for HIV seroconversion. This approach is costly and labor-intensive, and it is subject to bias related to study enrollment and

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retention [3, 4]. HIV incidence can also be estimated by detecting acute HIV infections, using assays that detect HIV antigen or HIV RNA. A disadvantage of that approach is that the early stage of HIV infection is very short [5]. Several serologic assays have been developed for detecting recent HIV infection in HIV-seropositive individuals. Unfortunately, most of those assays lack the precision needed for accurate estimation of HIV incidence [6, 7].

We recently described a multiassay approach for cross-sectional HIV incidence determination that combines results of 2 serologic assays (the BED capture enzyme immunoassay [BED-CEIA] [8] and an antibody avidity assay [9]) with 2 nonserologic biomarkers (HIV load and $CD4^+$ T-cell count) [10]. In clade B populations, this multiassay algorithm (MAA) has a window period of 141 days (95% confidence interval [CI], 94–150 days) and has a very low rate of false-recent misclassification (ie, classification of individuals with long-standing infection as recently infected) [10]. In a clinical cohort study, the cross-sectional HIV incidence estimate obtained using the MAA was nearly identical to the estimate based on a longitudinal assessment of HIV seroconversion during study follow-up [10].

In this study, we analyzed samples from women in the United States who were enrolled in the HIV Prevention Trials Network (HPTN) 064 study (NCT00995176) [11]. We estimated HIV incidence on the basis of HIV seroconversion and also estimated HIV incidence at enrollment and at the end of the study, using cross-sectional methods based on detection of acute HIV infection and detection of recent HIV infection using the MAA described above.

Table 1. Summary of Enrollment and Follow-Up of Women in the HIV Prevention Trials Network (HPTN) 064 Study

		Last Study Visit		
Subset	Overall (no.)	Enrollment	6 mo[a]	12 mo
All enrolled women	2099	119	455	1525
HIV status at enrollment				
HIV seronegative, uninfected[b]	2065	114[c]	447	1504
HIV seronegative, infected[d,e]	2	1	0	1
HIV seropositive[d,f]	32	4	8	20

Abbreviation: HIV, human immunodeficiency virus.

[a] Overall, 1980 women completed 6 months of follow-up in HPTN 064. This included 406 women who were scheduled to complete only 6 months of follow-up, 49 women who were scheduled to complete 12 months of follow-up but were lost to follow-up after the 6-month visit, and 1525 women who completed 12 months of follow-up. A total of 455 women had their last study visit at 6 months.

[b] These women had nonreactive HIV rapid test results at enrollment with no other evidence of HIV infection at enrollment.

[c] One hundred fifteen women were identified as HIV uninfected on the basis of HIV rapid testing performed at study sites. Of those, 111 had nonreactive HIV Combo test results and nonreactive Aptima HIV RNA test results, confirming that they were HIV uninfected at study enrollment; 3 women did not have samples available for evaluation with the HIV Combo test; and 1 had acute infection at enrollment.

[d] These women were not included in the analysis of HIV seroconversion during study follow-up (method B).

[e] Two women had nonreactive HIV rapid test results at enrollment but were subsequently determined to have had acute HIV infection at that visit. One woman seroconverted at the 6-month visit; the other woman was lost to follow-up after enrollment.

[f] These women had reactive HIV rapid test results and positive Western blot results at study enrollment.

METHODS

Source of Samples Used for Analysis

The HPTN 064 study enrolled 2099 women (age, 18–44 years) who reported no prior HIV diagnosis and were from areas with high poverty and high HIV prevalence. Screening and enrollment criteria and study procedures are described elsewhere [11]. Eligible women had had recent unprotected sex, and either the woman or her male sex partner(s) had at least 1 other risk factor for HIV infection. Women were systematically approached for prescreening when they entered a predetermined recruitment area. Recruitment venues were not advertised, and women who contacted study sites asking about study entry were referred to existing HIV testing sites. At study visits, women received HIV testing, HIV prevention counseling and condoms, and they were referred for support services if these were requested or perceived as necessary by study personnel.

At enrollment, 2067 women had nonreactive HIV rapid test results (OraQuick Advance Rapid HIV-1/2 Antibody Test, OraSure Technologies, Bethlehem, PA), and 32 women had reactive HIV rapid test results with confirmed HIV infection (Table 1) [11]. HIV rapid testing was repeated 6 and 12 months after enrollment. If the result of the rapid test was reactive, the $CD4^+$ T-cell count was determined, and a Western blot was performed; the Western blot was repeated using a second sample if the first result was indeterminate or positive. $CD4^+$ T-cell counting was not performed at subsequent study visits if HIV infection was confirmed.

This research was performed in compliance with the Helsinki Declaration. The HPTN 064 study was approved by ethical review committees at each of the study sites and collaborating institutions.

Laboratory Methods

Quality assurance testing was performed to confirm results of HIV testing performed at study sites. This included testing (1) all available samples from women who seroconverted during the study, (2) matched enrollment and follow-up samples from an equal number of women who did not seroconvert, (3) randomly selected enrollment samples from women identified

as HIV uninfected at enrollment (50 from each study site), and (4) all enrollment samples from women identified as HIV infected at enrollment. Assays used for quality assurance testing included the Vitros EIA Human Immunodeficiency Virus Type 1 and/or 2 (HIV-1/2) Antibody Detection in Human Serum and Plasma (hereafter, the Vitros EIA test; Johnson & Johnson, Pencoed, United Kingdom), the Genetics System HIV-1 Western blot (BioRad Laboratories, Redmond, WA), and the Aptima HIV-1 RNA Qualitative Assay (hereafter, the Aptima HIV RNA test; Gen-Probe, San Diego, CA).

Other tests used for analysis of HIV incidence included the Architect HIV Ag/Ab Combo assay (hereafter, the HIV Combo test; List: 2P36; Abbott Diagnostics, Wiesbaden, Germany), the BED-CEIA (Calypte Biomedical Corporation, Lake Oswego, OR) [8], and an avidity assay based on the Genetic Systems HIV-1/HIV-2+O EIA [9]. The BED-CEIA measures the proportion of immunoglobulin G that is HIV specific; the avidity assay measures the avidity of antibodies for HIV target antigens. HIV load was measured using the Amplicor HIV-1 Monitor Test, version 1.5 (hereafter, the Roche HIV RNA test; Roche Diagnostics, Indianapolis, IN); selected samples were also tested using the Abbott Real*Time* HIV-1 assay (hereafter, the Abbott HIV RNA test; Abbott Molecular, Des Plaines, IL). $CD4^+$ T-cell count testing was performed at study sites.

Women were classified as having acute infection if they had a nonreactive HIV rapid test result with a reactive HIV Combo test result (confirmed with an HIV RNA test) or detectable HIV RNA, as well as either a nonreactive Vitros EIA test result or a reactive Vitros EIA test result with a negative or indeterminate Western blot result. When using the MAA, recent infection was defined as having all of the following test results: a BED-CEIA result of <1.0 normalized optical density units (OD-n), an avidity index of <80%, a $CD4^+$ T-cell count of >200 cells/mm^3, and an HIV load of >400 copies/mL [10].

Statistical Analysis

Calculation of HIV Incidence Estimates: Methods A, B, and C

Three methods were used to assess HIV incidence. For method A, the annual HIV incidence estimate was based on detection of acute HIV infection and was calculated as follows: [(number of women classified as having acute infection) × (100)]/[(number of HIV-uninfected women) × (window period in years)]. Two window periods were used for these assessments. The first window period was 14 days (0.038 years); this reflects the time between HIV RNA detection and detection of HIV infection using a third-generation HIV rapid test [12]. The second window period was 26 days (0.071 years); this reflects the time between HIV RNA detection and development of a positive Western blot result [13].

In method B, the incidence estimate was based on HIV seroconversion during follow-up and was calculated as follows: [number of seroconversion events]/[number of person-years].

In method C, the incidence estimate was based on detection of recent infections using the MAA and was calculated as described for method A, using a window period for the MAA of 0.38 years (141 days [95% CI, 94–150 days]) [10].

Calculation of CIs

For methods A and B, standard "exact" Poisson-based CIs were computed as described elsewhere [14]. For method C, exact CIs, which account for the uncertainty in the window period, were calculated [3]. CIs were not available for method A, so it was not possible to account for uncertainty in the window period when computing the CIs for incidence with this method.

Comparison of Incidence Estimates Obtained Using Different Methods

We also report a *P* value comparing HIV incidence based on acute infection (method A) to HIV incidence based on HIV seroconversion during follow-up (method B), using an exact, randomization-based test. Briefly, each woman who was HIV seronegative at enrollment was assumed to have contributed 14/365.25 or 26/365.25 (depending on the assumed acute window period) person-years of acute infection time and her observed person-years of longitudinal follow-up. The (6) observed HIV infections were then randomly distributed among the women-periods (acute infection or HIV seroconversion), with probability proportional to the duration of the period, subject to the constraint that each woman could only have 1 infection. The number of infections that was assigned to the acute period was then counted. This procedure was repeated 100 000 times to determine the distribution of the number of acute infections under the null hypothesis. The *P* value was taken as the proportion of the simulations in which the number of acute infections equaled or exceeded the observed number (2).

RESULTS

We used 3 different approaches to assess HIV incidence among women enrolled in the HPTN 064 study (see Methods).

Method A: Analysis of Acute HIV Infection at Enrollment

Among the 2067 women identified as HIV seronegative at enrollment, 1949 had at least 1 seronegative follow-up visit and were assumed to have been HIV uninfected at enrollment. Three women (subjects 1, 3, and 4) had confirmed HIV infection at their first follow-up visit (documented HIV seroconversion; Table 2). At enrollment, 2 of these 3 women (subjects 3 and 4) had nonreactive results obtained using 2 assays that are cleared by the US Food and Drug Administration for detection of acute HIV infection: a fourth-generation HIV

Table 2. Test Results From Analysis of HIV Incidence, Using Different Test Methods

Method	Study Subject	Visit	BED-CEIA (OD-n)	Avidity (%)	Assessment
Method A	1	Enr	NA	NA	Acute infection at enrollment[a]
		6 mo after Enr	0.25	53.12	(Seroconverted at 6 mo)
		12 mo after Enr	0.46	94.76	
	2	Enr	NA	NA	Acute infection at enrollment[b]
					(Lost to follow-up after enrollment)
Method B	3	**<6 mo after Enr**	0.28	17.46	Seroconverter
		6 mo after Enr	0.64	31.78	
		12 mo after Enr	1.19	98.63	
	4	**6 mo after Enr**	0.26	13.32	Seroconverter[c]
		12 mo after Enr	0.46	60.41	
	5	**12 mo after Enr**	0.25	9.32	Seroconverter
	6	**12 mo after Enr**	1.0	11.22	Seroconverter
Method C	7	Enr	0.54	79.39	Recent infection at enrollment[d]
		6 mo after Enr	1.40	98.78	
	8	Enr	0.30	19.23	Recent infection at enrollment[e]
		6 mo after Enr	1.27	90.16	
		12 mo after Enr	1.21	94.46	
Standard BED-CEIA alone[f]	9	Enr	0.70	99.30	False-recent misclassification by BED-CEIA
		6 mo after Enr	0.72	99.87	
		12 mo after Enr	0.72	95.53	
	10	Enr	0.37	94.96	False-recent misclassification by BED-CEIA
		6 mo after Enr	0.30	102.87	
		12 mo after Enr	0.30	99.36	
	11[g]	Enr	0.55	95.30	False-recent misclassification by BED-CEIA

Test results are shown for 11 women (subjects 1–11) enrolled in the HIV Prevention Trials Network 064 study who were classified as having acute infection at enrollment (method A), HIV seroconversion (method B), or recent HIV infection (method C), or who appear to have been misclassified as recently infected by the standard BED-CEIA alone. Assessment indicates the final assessment of HIV status, which was based on all available test results. Bold text indicates the visit at which HIV seroconversion was documented.

Abbreviations: BED-CEIA, BED capture immunoassay; Enr, enrollment; mo, months; OD-n, normalized optical density units; S/CO, signal/cutoff ratio.

[a] At enrollment, subject 1 had a reactive Aptima HIV RNA test result (S/CO, 19.52) and a viral load of 2030 HIV RNA copies/mL (Roche HIV RNA test); results of the HIV Combo test and the Vitros test were nonreactive. These test results are consistent with Fiebig stage I infection [20].

[b] At enrollment, subject 2 had a reactive HIV Combo test result (S/CO, 19.88), a reactive Aptima HIV RNA test result (S/CO, 7.53), a reactive Vitros EIA result, and an indeterminate Western blot result. These test results are consistent with Fiebig stage IV infection [20]. This woman was lost to follow-up after the enrollment; a second sample was not available to confirm her HIV status.

[c] Subject 4 had undetectable HIV RNA at seroconversion (<50 copies/mL by the Roche HIV RNA test and <40 copies/mL by the Abbott HIV RNA test); therefore, she would have been classified by the multiassay algorithm as not recently infected. The other 3 women who seroconverted in the study were all classified by the multiassay algorithm as recently infected at the seroconversion visit.

[d] Subject 7 was characterized as recently infected at enrollment using the multiassay algorithm (BED-CEIA result, 0.54 OD-n; avidity index, 79.39%; $CD4^+$ T-cell count, 933 cells/mm^3, and HIV load, 6369 copies/mL); she was also characterized as recently infected at enrollment by the standard BED-CEIA alone (with a cutoff of <0.8 OD-n).

[e] Subject 8 was characterized as recently infected at enrollment by the multiassay algorithm (BED-CEIA result, 0.30 OD-n; avidity index, 19.23%; $CD4^+$ T-cell count, 1005 cells/mm^3; and HIV load, 819 copies/mL); she was also characterized as recently infected at enrollment using either the standard BED-CEIA alone (with a cutoff of <0.8 OD-n) or the standard avidity assay alone (with a cutoff of avidity index of <40%).

[f] Subjects 9–11 were classified as recently infected by the standard BED-CEIA alone (with a cutoff of <0.8 OD-n), but not by the multiassay algorithm or the standard avidity assay alone.

[g] Subject 11 had a low viral load (<50 copies/mL by the Roche HIV RNA test and <44 copies/mL by the Abbott HIV RNA test).

antigen/antibody test (the HIV Combo test) and the Aptima HIV RNA test. These 2 women (subjects 3 and 4) were considered to have been HIV uninfected at enrollment (Table 2). The third woman (subject 1) had a reactive Aptima HIV RNA test result and a viral load of 2030 copies/mL; results of the HIV Combo test and the Vitros test were nonreactive. This woman was classified as having acute HIV infection at enrollment (Table 2). The remaining 115 women who were identified as HIV seronegative at enrollment did not return for subsequent study visits. Enrollment samples were available for

Table 3. HIV Incidence Estimates Obtained Using Four Different Analytic Methods

Method	Type of Analysis	Outcome Measure	HIV Status at Enrollment[a]	Assessment Period/Time	Women Analyzed (no.)	Events, No.	Estimated Window Period (d)	Estimated HIV Incidence (%; 95% CI)
A	Cross-sectional	Acute HIV infection[b]	Seronegative	Enrollment	2064	2	14	2.52 (.17–9.33)
							26	1.36 (.091–5.02)
B	Longitudinal	HIV seroconversion	Seronegative	Follow-up	1951[c]	4	NA	0.24 (.07–.62)
C	Cross-sectional	Recent HIV infection (multiassay algorithm)	Seropositive[d]	Enrollment	32 HIV+, 2067 HIV–	2	141	0.25 (.03–.93)
				End of study	33 HIV+, 1947 HIV–	1	141	0.13 (.006–.76)

Abbreviations: CI, confidence interval; NA, not applicable; d, days.

[a] Based on testing performed at study sites.

[b] Annual incidence estimates based on acute infection were calculated using 2 window periods: a window period of 14 days, based on the time between detection of HIV RNA and detection of HIV infection at study sites using a third-generation HIV rapid test [12], and a window period of 26 days, based on the time between HIV RNA detection and a positive Western blot [13].

[c] 1638.8 person-years of follow-up.

[d] Samples from HIV-seropositive women (HIV+) were evaluated using the multiassay algorithm; the number of women who were HIV uninfected at enrollment and at follow-up (HIV–) were included in the calculation of HIV incidence. Overall, 38 HIV-infected women were identified in the study (32 were HIV seropositive at enrollment, 2 had acute infection at enrollment, and 4 seroconverted), of whom 5 were lost-to-follow up after enrollment (Table 1) and were not included in the cross-sectional estimate of HIV incidence at the end of study.

112 women; those samples were tested using the HIV Combo test and the Aptima HIV RNA test. Samples from 111 women had nonreactive results with both assays; those women were considered to be HIV uninfected at enrollment. The remaining woman (subject 2) had reactive results with the HIV Combo test, the HIV Aptima HIV RNA test, and the Vitros EIA test, and had an indeterminate Western blot result; the viral load was undetectable (<50 copies/mL, by the Roche HIV RNA test). This woman was classified as having acute HIV infection at enrollment (Table 2) and was lost to follow-up after enrollment. Overall, 2 women (subjects 1 and 2) had acute HIV infection at enrollment among 2064 women at risk (Table 2). HIV incidence immediately prior to enrollment was 2.52%/year (95% CI, .17%–9.33%/year), using a 14-day window period, and 1.36%/year (95% CI, .091%–5.02%/year), using a 26-day window period.

Method B: Analysis of HIV Seroconversion During Follow-Up

Among 2067 women identified as HIV seronegative at enrollment on the basis of testing performed at study sites, 2 had acute HIV infection at enrollment (see above), and 2065 were HIV uninfected. Of those, 114 women were lost to follow-up after the enrollment visit and did not contribute to the analysis of HIV seroconversion. Of the 1951 women who continued in the study, 400 completed 6 months of planned follow-up, 1504 completed 12 months of follow-up, and 47 were lost to follow-up after 6 months (overall, there were 1638.8 person-years of follow-up; Table 1). Four women (subjects 3–6) who were HIV uninfected at enrollment seroconverted during the study (Table 2). On the basis of this analysis, HIV incidence in the cohort was 0.24%/year (95% CI, .07%–.62%/year; Table 3). This incidence estimate was significantly lower than the estimate that was based on analysis of acute HIV infection at enrollment when a 14-day window was used for acute infection (2.52%/year; $P = .027$), but the estimate was not significantly lower when a 26-day window was used (1.36%/year; $P = .078$).

We also tested women for acute HIV infection at their last study visit (Table 1). Among 1947 women who had a nonreactive HIV rapid test result at their last follow-up visit, 1913 (442 at 6 months and 1471 at 12 months) had a sample available for analysis. Three samples had reactive HIV Combo test results (signal/cutoff ratio [S/CO], 1.02, 2.41, and 7.11; a S/CO >1 is classified as reactive). One sample had a nonreactive repeat HIV Combo test result (S/CO, 0.83), and 2 had repeat reactive test results (S/CO, 2.35 and 6.89). However, all 3 samples had nonreactive test results for the Aptima HIV RNA test, the Abbott HIV RNA test, and the Vitros EIA; these samples were considered to have false-reactive HIV Combo test results. Therefore, we did not detect any women who had acute infection at study exit.

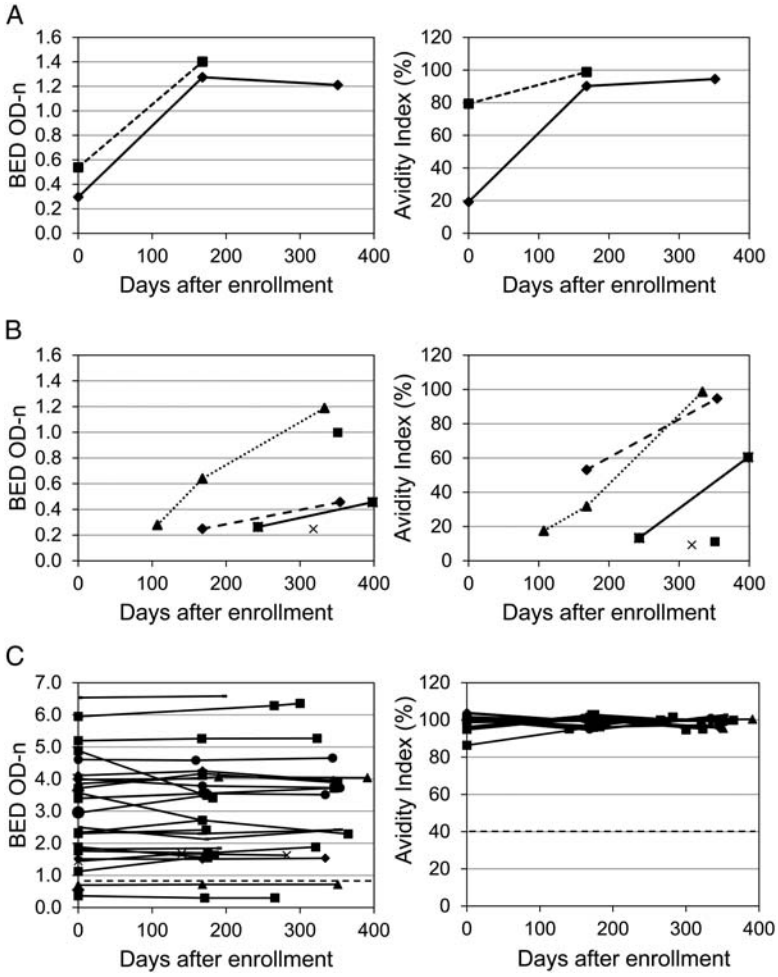

Figure 1. BED capture enzyme immunoassay (BED-CEIA) and avidity test results. The plots show results from the BED-CEIA (normalized optical density units [OD-n]) and the avidity assay (avidity index, %) for women at different study visits (plotted as days after enrollment). *A*, Results for 2 women (subjects 7 and 8) who received a new diagnosis of human immunodeficiency virus (HIV) infection at the time of study enrollment and were identified as recently infected at enrollment using the multiassay algorithm (MAA; Table 2). *B*, Results for five women who seroconverted during the study, of whom 1 (subject 1) had acute infection at study entry and 4 (subjects 3–6) were HIV uninfected at study entry (Table 2). *C*, Results for 30 women who received a new diagnosis of HIV infection at the time of study enrollment and were identified by the MAA as not recently infected at enrollment. The dashed lines indicate the cutoffs for the standard BED-CEIA (<0.8 OD-n) and the standard avidity assay (<40%); higher cutoffs are used in the MAA for both assays (<1.0 for the BED-CEIA and <80% for the avidity assay). Note that 2 women had BED-CEIA results that decreased over time; in both cases, the values remained greater than the cutoff for the standard BED-CEIA (>0.8 OD-n) and greater than the cutoff that is used in the MAA for the BED-CEIA (>1.0 OD-n). Both of those women had high HIV loads at all visits tested (range, 25 810 to >10 000 copies/mL). One of the women had a very low $CD4^+$ T-cell count at study enrollment (11 cells/μL), which could have explained the decline in her serologic response to HIV infection over time, as measured by the BED-CEIA.

Method C: Analysis of Recent HIV Infection Using the MAA

We used the MAA to test whether any of the 32 women who had reactive HIV rapid test results at enrollment with confirmed HIV infection were likely to have been recently infected when they entered the study (see Methods). On the basis of the MAA, enrollment samples from 2 of the 32 women (subjects 7 and 8) had test results consistent with recent infection (Table 2). Furthermore, for both women, BED-CEIA and avidity test values increased over time, consistent with antibody maturation following recent HIV infection (Figure 1*A* and Table 2). The increases in BED-CEIA and avidity values that we observed for those 2 women were similar to increases in those values observed over time in 2 women (subjects 3 and 4) who acquired HIV infection during the study and in 1 woman (subject 1) who had acute HIV infection at enrollment with seroconversion at 6 months (Figure 1*B* and Table 2); 2 other women (subjects 5 and 6) first tested positive for HIV infection at 12 months and did not have subsequent samples available for analysis (Table 2). In contrast, BED-CEIA and avidity values were generally stable over time among women

who were HIV infected at enrollment but who did not meet the criteria for recent infection using the MAA (Figure 1*C* and 1*D*). The MAA has a window period of 141 days (95% CI, 94–150 days) in adults with subtype B HIV infection [10]. On the basis of cross-sectional analysis of recent infection at enrollment (conducted using the MAA), HIV incidence was 0.25%/year (95% CI, .03%–.93%/year; Table 3).

We compared results obtained with the MAA with results obtained using either the BED-CEIA alone or the avidity assay alone; for this analysis, recency was defined using the standard cutoffs for each assay (BED-CEIA, <0.8 OD-n; avidity index, <40%). Five women had BED-CEIA values below the standard assay cutoff at enrollment. This included the 2 women (subjects 7 and 8) who were identified by the MAA as recently infected and 3 additional women (subjects 9–11; Table 2). Those 3 women had avidity values over the standard avidity assay cutoff of 40% and over the avidity assay cutoff of 80% that is used in the MAA. Two of the 3 women had samples available from subsequent study visits (the third woman was lost-to follow-up after enrollment); for those 2 women, results from the BED-CEIA remained below the standard assay cutoff of <0.8 OD-n throughout follow-up (Figure 1*C*), providing evidence that they were misclassified as recently infected at the enrollment using the standard BED-CEIA alone. One of these women (subject 11) had a low HIV load at enrollment (Table 2), which may have been related to BED-CEIA misclassification. Only 1 of 32 women tested (subject 8, who was 1 of 2 women identified by the MAA as recently infected) had an avidity test result below the standard assay cutoff of 40% (Table 2).

We also used the MAA to estimate HIV incidence at the last study visit. At that visit (which occurred 6 or 12 months after enrollment), 1947 women were HIV uninfected, and 33 were HIV infected (28 were HIV seropositive at enrollment [4 other HIV-seropositive women were lost to follow-up], 1 had acute infection at enrollment [1 other woman with acute infection at enrollment was lost to follow-up], and 4 acquired HIV infection during the study). One woman (subject 5, who was 1 of 4 seroconverters) was classified by the MAA as recently infected at the 12-month visit (Table 2). This finding is consistent with the window period of the MAA (141 days). Two seroconverters acquired HIV infection before the 6-month visit and would therefore have been infected for >6 months by the 12-month visit. The other 2 seroconverters acquired HIV infection between the 6- and 12-month visits and could therefore have been infected for up to 6 months by the 12-month visit; one of those 2 women was classified by the MAA as recently infected at 12 months, as noted above. On the basis of cross-sectional analysis of recent infection at the last study visit (conducted using the MAA), HIV incidence was 0.13%/year (95% CI, .006%–.76%/year; Table 3). This estimate was not significantly different from the MAA-derived incidence estimate at enrollment ($P = 1.0$) or the incidence estimate based on longitudinal analysis of HIV seroconversion ($P = 1.0$).

DISCUSSION

This study used a multifaceted approach to estimate HIV incidence in a cohort of women in the United States. This novel approach provided a more comprehensive assessment of HIV incidence in the study cohort than an assessment based solely on HIV seroconversion. In this cohort, the HIV incidence estimate based on detection of acute HIV infection at study enrollment was higher than the incidence estimate based on longitudinal assessment of HIV seroconversion; this difference was statistically significant when a window period of 14 days was used for acute HIV infection. We used 2 tests to screen for acute HIV infection: an unpooled HIV RNA test and the HIV Combo test. The HIV Combo test is designed for high-throughput testing and is well suited for detection of acute HIV infection in large cross-sectional studies. Because longitudinal data were available for most women in the HPTN 064 study, we performed HIV Combo testing at enrollment only for women who were lost to follow-up after enrollment; samples from these women were also screened for acute HIV infection by use of a nonpooled HIV RNA test. At the last study visit, women were screened for acute HIV infection by means of the HIV Combo test only. Studies based on cultured viruses suggest that the HIV Combo test will detect samples with >30 000 HIV RNA copies/mL [15]. In previous studies, samples from individuals with acute HIV infection (HIV RNA positive and HIV antibody negative) that were not detected as reactive with the HIV Combo test had viral loads ranging from 725–21 548 copies/mL [16–18]. Therefore, it is possible that some women in the HPTN 064 study may have had acute HIV infection with low viral loads at the last study visit and may not have been identified as having acute HIV infection. Another limitation of this study is that only 2 cases of acute infection were detected; in one case, the participant was lost to follow-up after enrollment and a second sample was not available to confirm her HIV status.

The MAA described in this report has a very low rate of false-recent misclassification in populations with clade B HIV infection [10]. This reflects 2 features of the MAA design. First, samples classified using the MAA as indicative of recent infection must test "recent" using both the BED-CEIA and the avidity assay (note that the MAA uses increased assay cutoffs for both assays). These assays include different target antigens and measure different characteristics of the humoral response to HIV infection. Second, samples classified as indicative of recent infection must have HIV load and $CD4^+$ T-cell count results that rule out viral suppression or advanced HIV disease as a potential cause of false-recent misclassification [19, 20]. In

contrast to the MAA, many samples from individuals with long-standing HIV infection are misclassified by the standard BED-CEIA as indicative of recent infection [10, 19]. In the HPTN 064 study cohort, when the standard BED-CEIA was used alone, 2.5 times as many women were identified as recently infected at enrollment, compared with the MAA (5 women vs 2 women). However, results from other testing (eg, avidity assay test results and assessment of BED-CEIA results over time) indicated that the 3 women who were identified as recently infected solely on the basis of findings of the standard BED-CEIA were misclassified and were not recently infected. The standard avidity assay has a lower rate of false-recent misclassification than the BED-CEIA in populations with clade B HIV infection, but this rate still exceeds that of the MAA [21]. In the HPTN 064 study cohort, the standard avidity assay identified only 1 woman as recently infected at study enrollment (1 of 2 women who were identified by the MAA). Further studies are needed to compare the performance of the MAA and standard avidity assay in other populations and other settings. One limitation of the MAA is the requirement for $CD4^+$ T-cell count data. Because it is impractical and costly to cryopreserve samples for retrospective $CD4^+$ T-cell count testing, $CD4^+$ T-cell counts must be determined for all HIV-infected individuals (eg, at the time of HIV rapid testing). We are evaluating alternative testing algorithms for cross-sectional HIV incidence determination that do not require $CD4^+$ T-cell count data [22].

In this cohort, the HIV incidence estimate obtained using a traditional, longitudinal assessment of HIV seroconversion was similar to the estimate obtained by cross-sectional analysis of recent infection at the last study visit. However, the number of incident infections in this cohort was relatively low (2 women had acute infection at enrollment, 4 had HIV seroconversion during follow-up, 2 had recent infection at enrollment, and 1 had recent infection at the last study visit). In a previous study of the HIV Network for Prevention Trials 001 study cohort, the HIV incidence estimates obtained from longitudinal follow-up and the MAA were nearly identical [10]; the number of incident infections was much higher in that cohort (90 seroconversion events and 30 recent infections). These findings support use of the MAA for cross-sectional analysis of HIV incidence in clade B epidemics. Further studies are needed to assess the performance of the MAA in different settings (eg, in population surveys, cohort studies, and clinical trials) and in populations infected with different HIV strains.

Notes

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