groups were compared with a t test. The distributions of the other continuous variables were compared with a Mann–Whitney U test. For the association of binary variables with HJB, ORs with 95% CIs were calculated. For all statistical tests, computer software (SPSS Statistics version 20; IBM Corporation, Somers, NY) was used. Syphilis was found in 2 patients with HJB, tuberculosis in 4 patients with HJB and 1 patient without HJB, M. avium intracellulare complex infection in 1 patient without HJB, Kaposi sarcoma in 5 patients with HJB and 1 patient without HJB, and lymphoma in 1 patient with HJB and 3 patients without HJB. The other results are shown in Table 1. Generally, patients with HJB had more symptomatic HIV infection, higher viral load, lower CD4 and CD8 counts, and shorter duration of combination antiretroviral therapy (cART). For continuous variables, receiver-operating characteristics for the prediction of HJB were made and optimal cutoffs were determined. CD4 count had an area under the receiver-operating characteristics curve of 0.601, CD8 count of 0.642, HIV viral load of 0.580, and cumulative duration of cART in patients with treatment indication of 0.659. The optimal cutoffs were 325 cells per microliter for CD4 count, 870 cells per microliter for CD8 count, detectable (versus nondetectable) for viral load, and 300 days for cumulative duration of cART in patients with treatment indication. CD4 cell count <325 cells per microliter, CD8 cell count <870 cells per microliter, CDC class C (AIDS-defining conditions), and cART duration under 300 days were strong predictors of HJB. A logistic regression model with AIDS-defining condition, CD4 cell count <325 cells per microliter, and cumulative cART duration <300 days in patients with treatment indication as covariates showed CD4 cell count <325 cells per microliter to be the strongest predictor of HJB (adjusted OR: 3.62; 95% CI: 1.45 to 9.04), whereas the other predictors were weakened.

We have shown that HIV-associated hyposplenism is associated with clinical disease stage and may be reversed by successful cART. The main limitations of this study are the retrospective design and the nonquantitative and relatively insensitive test for hyposplenism.

Postmortem splenic histology in patients with AIDS shows prominent germinal center and marginal zone atrophy, whereas the mantle zone remains intact. Furthermore, splenic marginal zone type IgM memory B cells are preferentially depleted from the blood in HIV infection. Two studies found that blood IgM memory B cell count correlates with CD4 cell count, whereas 1 study did not. Postmortem histology of splenic red pulp in patients with AIDS shows macrophage dysfunction. Therefore, we believe hyposplenism, leading to decreased splenic macrophage filter function and decreased IgM response against polysaccharide antigens, contributes to HIV-induced immunodeficiency. Demonstrating hyposplenism may identify those HIV-infected patients with the highest risk of HIV-related infections and, thus, contribute to the decision to start cART.

ACKNOWLEDGMENTS

The authors thank Theo de Jong and Henk Baelde for their detailed information on the examination of blood smears and help in data retrieval, the hematological laboratory for making and assessing the blood smears, and Jaul Noomen and Willemien Dorama for their help with acquiring follow-up blood smears.

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REFERENCES


Unreported Antiretroviral Use by HIV–1–Infected Participants Enrolling in a Prospective Research Study

To the Editors:

The concentration of viral RNA in plasma is the primary risk factor for...
sexual transmission of HIV-1,\textsuperscript{1–3} and reductions in plasma HIV-1 RNA levels because of antiretroviral therapy (ART) result in marked decreases in HIV-1 transmission risk.\textsuperscript{4,5} Results from studies of HIV-1 transmission and disease progression may be more difficult to interpret if a substantial proportion of HIV-1–infected partners have low or undetectable viral loads on ART, and thus, ART use at study enrollment is often an exclusion factor.

Recent reports from clinical trial cohorts of HIV-1 transmission in HIV-1 serodiscordant couples have found that nearly a quarter of HIV–1–infected partners had low enrollment plasma HIV-1 RNA levels (<2000 copies/mL).\textsuperscript{6,7} Low levels of plasma HIV-1 RNA in the HIV–1–infected partners, selected for not having transmitted HIV–1 to their partner for studies of candidate interventions to reduce HIV–1 transmission, may reflect natural host control of viral replication. However, an alternative explanation could be unreported ART use. Distinguishing between these potential sources of low viral load is important for studies seeking to understand the biology of HIV–1 transmission. We tested stored samples from a recent HIV–1 prevention clinical trial to determine the frequency of unreported ART use among HIV–1–infected individuals with low plasma HIV–1 RNA levels.

Between November 2004 and April 2007, we enrolled 3408 heterosexual HIV–1 serodiscordant couples from 7 African countries in a randomized, double-blind, placebo-controlled clinical trial of herpes simplex virus type 2 (HSV-2) suppressive therapy to reduce HIV–1 transmission (Partners in Prevention HSV/HIV Transmission Study), as previously described.\textsuperscript{6} Eligible couples were at least 18 years of age, sexually active, and intending to remain as a couple. All HIV–1–infected partners were HSV-2 seropositive, had CD4 counts of ≥250 cells per microliter (making them ineligible for antiretroviral therapy under national guidelines of the study countries at that time), were not pregnant, and self-reported as not currently taking ART. Quarterly plasma and serum samples were collected for up to 24 months and archived at −80°C for subsequent laboratory testing. HSV-2 suppressive therapy did not reduce HIV–1 transmission within the study partnerships.\textsuperscript{8} At study screening and all follow-up visits, HIV–1–infected participants were asked if they were currently taking ART.

All participants received HIV–1 primary care, referral for ART according to national guidelines and risk reduction counseling, and treatment for sexually transmitted infections during the 24 months of study follow-up. Written informed consent was obtained from all participants. The study protocol was approved by the University of Washington Human Subjects Review Committee and ethical review committees at each of the study sites.

All laboratory testing occurred at the end of study follow-up. Plasma HIV–1 RNA levels were quantified using the COBAS AmpliPrep/COBAS TaqMan real-time HIV–1 RNA assay, version 1.0.

### TABLE 1. Enrollment Characteristics and Type of Antiretroviral (ART) Detected Among HIV–1–Infected Partners

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>≥2000 Copies/mL (N = 2573)</th>
<th>&lt;2000 Copies/mL (N = 443)</th>
<th>Undetectable (N = 355)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender (%)</td>
<td>1650 (64.1)</td>
<td>335 (75.6)</td>
<td>288 (81.1)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>32 (27–39)</td>
<td>31 (26–37)</td>
<td>32 (28–37)</td>
</tr>
<tr>
<td>Education, yrs</td>
<td>8 (6–11)</td>
<td>8 (7–12)</td>
<td>8 (6–11)</td>
</tr>
<tr>
<td>Has monthly income (%)</td>
<td>940 (36.3)</td>
<td>159 (35.9)</td>
<td>120 (33.8)</td>
</tr>
<tr>
<td>Married and/or cohabiting (%)</td>
<td>2340 (90.9)</td>
<td>399 (90.1)</td>
<td>324 (91.3)</td>
</tr>
<tr>
<td>Duration of partnership, yrs</td>
<td>5.3 (2.3–10.4)</td>
<td>4.8 (2.2–9.6)</td>
<td>6.2 (2.6–10.6)</td>
</tr>
<tr>
<td>Number of children</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Sex with outside partner, prior 30 days (%)</td>
<td>92 (3.6)</td>
<td>16 (3.6)</td>
<td>7 (2.0)</td>
</tr>
<tr>
<td>Any unprotected sex, prior 30 days (%)</td>
<td>725 (28.2)</td>
<td>120 (27.1)</td>
<td>117 (33.0)</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count, cells/mm\textsuperscript{3}</td>
<td>458 (345–626)</td>
<td>471 (352–640)</td>
<td>467 (348–650)</td>
</tr>
<tr>
<td>Any sexually transmitted infection (%)</td>
<td>380 (14.8)</td>
<td>56 (12.6)</td>
<td>51 (14.4)</td>
</tr>
<tr>
<td>Antiretroviral (ART) tested</td>
<td>--</td>
<td>N = 430</td>
<td>N = 341</td>
</tr>
<tr>
<td>NRTIs (%)</td>
<td>--</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Abacavir (ABC)</td>
<td>--</td>
<td>0 (0)</td>
<td>9 (2.6)</td>
</tr>
<tr>
<td>Zidovudine (AZT)</td>
<td>--</td>
<td>12 (2.8)</td>
<td>148 (43.4)</td>
</tr>
<tr>
<td>Lamivudine (3 TC)</td>
<td>--</td>
<td>3 (0.7)</td>
<td>43 (12.6)</td>
</tr>
<tr>
<td>Stavudine (D4T)</td>
<td>--</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Didanosine (DDI)</td>
<td>--</td>
<td>11 (2.6)</td>
<td>126 (37.0)</td>
</tr>
<tr>
<td>NNRTIs (%)</td>
<td>--</td>
<td>14 (3.3)</td>
<td>157 (46.0)</td>
</tr>
<tr>
<td>Nevirapine (NVP)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Any ART detected</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*Including Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis.*
In multiple studies of willingness to report information during enrollment, we considered any quantifiable concentration as indicative of ART use.

Low viral load was stratified into 2 groups defined as (1) low detectable (240–2000 copies/mL) or (2) undetectable (<240 copies/mL). We calculated the overall prevalence of unreported ART use at enrollment among HIV-1-infected partners with low plasma HIV-1 RNA (<2000 copies/mL) and in each group. All analyses were conducted using SAS (v.9.2; SAS Institute, Cary, NC).

Among 3371 HIV-1–infected partners who had results for plasma HIV-1 RNA tested at enrollment, 798 (23.7%) had a plasma HIV-1 RNA count of <2000 copies per milliliter, including 443 (13.1%)/798 with low plasma HIV-1 RNA levels (240–2000 copies/mL) and 355 (10.5%)/798 with undetectable RNA (<240 copies/mL). Those with enrollment plasma HIV-1 RNA of <2000 copies per milliliter were more likely to be females compared with those with plasma HIV-1 levels of >2000 copies per milliliter (78.1% vs. 64.1%, P < 0.05); all other characteristics were similar for those with higher versus lower plasma HIV-1 RNA (Table 1).

ART testing was performed on 771 (96.6%) of the persons with <2000 copies per milliliter of plasma HIV-1 RNA where specimens were available. Antiretrovirals were detected in 171 (22.2%)/771: 157 (46.0%)/341 in those with undetectable plasma HIV-1 RNA (<240 copies/mL) and 14 (3.3%)/430 in those with low detectable plasma HIV-1 RNA (240–2000 copies/mL). The most common ARTs detected were lamivudine (20.8%) and nevirapine (17.8%). Most (83.6%) of the 171 participants with detectable ARTs had evidence of multiple drugs, specifically the combinations nevirapine/lamivudine (52.0%), nevirapine/lamivudine/stavudine (21.6%), stavudine/lamivudine (5.3%), and zidovudine/lamivudine with or without nevirapine (4.1%). Differences in ART detection were found among the study sites, but there were no difference in ART detection between men and women.

We have previously reported that nearly a quarter (23.7%) of HIV-1–infected partners in HIV-1 serodiscordant partnerships for the Partners in Prevention HSV/HIV Transmission Study had plasma HIV-1 levels <2000 copies per milliliter at baseline, in the absence of reported use of ART. Our analysis here demonstrates that 22% of those, and nearly half of the subset with undetectable plasma HIV-1, had evidence of unreported ART use. Thus, undetectable plasma HIV-1 RNA is a potential marker of unreported ART use in HIV-1-infected partners. This finding could be significant for studies focused on describing host factors associated with natural viral control because a large proportion of individuals with low plasma HIV-1 levels had pharmacologically and not immunologically induced viral suppression. For example, in genetic studies of elite controllers, the inclusion of subjects with ART-induced viral suppression would undermine the ability of the study to identify any potentially valuable genetic markers.

It is important to note that while unreported ART detection was strongly associated with plasma HIV-1 level (48% for undetectable vs. only 3% for detectable but <2000 copies/mL) the proportion of individuals in the overall study cohort with unreported ART use and detectable ARTs was very small (171/3408; 5%) and equally distributed between the randomization arms in the clinical trial. For these reasons, it is unlikely that unreported ART use would have an important impact on the overall outcomes of this clinical trial or other randomized clinical trials of this kind.

Because ART use was an exclusion criterion in this study, and by definition was not reported, inferences about reasons or circumstances underlying this finding are largely speculative.

Women had a significantly higher proportion of plasma HIV-1 RNA <2000 copies/mL, suggesting that women may have been more likely to have received ART, possibly clinically indicated for prevention of mother-to-child transmission for which nevirapine and lamivudine are included in the recommended regimens and also prominently represented among the ARTs detected in this analysis. However, in our analysis, we did not find significant difference in ART by gender and do not have evidence that women were more likely to have unreported ART detected. Without knowing specific timing of recent doses, we cannot make any determination on whether or not persons with detected ART discontinued drug use before study screening or what drug dose was taken. Nevirapine can be detected in women more than 2 weeks after receiving single-dose nevirapine for prevention of mother-to-child transmission, whereas most nucleoside reverse transcriptase inhibitors such as lamivudine and stavudine can be detected, at most, for a few days after discontinuation.

In resource-limited communities, services and other benefits offered through clinical trial participation could provide an incentive to not disclose ART use, which would have made them ineligible for the trial. Couples enrolled in the Partners in Prevention HSV/HIV Transmission Study did not receive a monetary incentive for participation but received benefits, including free counseling, screening and treatment for sexually transmitted infections, condoms, and travel reimbursement. One previous study of participation in a large-scale HIV-1 prevention trial in South Africa concluded that the level of reimbursement could be a motivating factor for some participants to misreport information during enrollment screening. In multiple studies of willingness to participate in HIV-1 clinical trials in different settings, most participants reported primarily altruistic motivations for participation, although a minority of respondents stated monetary incentives and access to health care as the primary motivator for participation. We recommend further research to understand nondisclosure of ART by participants enrolling in an HIV-1 prevention study.
In summary, we found unreported use of ART to be prevalent among HIV-1–infected individuals with undetectable plasma virus. For randomized control trials of HIV-1 prevention interventions in HIV-1 serodiscordant couples or of novel HIV-1 treatments, assessing viral load may improve the efficiency of the study, by excluding those with low viral loads who would be unlikely to transmit or have a substantial virological response to treatment. More importantly, for observational studies of pathogenesis and transmission, it may be critical to understand the etiology of undetectable viral loads, and thus, particularly important to identify unreported ART use. Studies recruiting HIV-1–infected participants with low levels of plasma HIV-1 RNA should consider laboratory testing for ART.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the contributions of couples who participated in this study and teams at the study sites and at the University of Washington.

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REFERENCES
12. Muro E, Droste JA, Hofstede HT, et al. Nevirapine plasma concentrations are still detectable after more than 2 weeks in the

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Letters to the Editor


