

## HIV-1 Viral Load After Leukodepletion

### To the Editor:

More than 40 million people are infected with HIV, the causative agent of AIDS, and more than 95% of them are in developing countries. The screening of donated blood and education about the danger of unprotected sex were the main factors to herald the explosion of the HIV pandemic. The serology-based screening of donated blood is not 100% risk-free, however. This is because antibodies against HIV are not seen in serum immediately after exposure; it may take up to 8 weeks, the "window period," before they are seen.<sup>1</sup> In this window period, the viremia is high and then drops as the cytotoxic CD8 T lymphocytes develop and an individual viral load set point is reached during chronic infection. Viral set points differ greatly among individuals and are used to predict disease progression.<sup>2</sup>

The danger of HIV being transmitted through blood transfusion during the window period is practical. In the United Kingdom, the statistical projections of potential window period-infected donations suggest an occurrence of approximately 1 in 2.5 million.<sup>3</sup> It is estimated to be higher in South Africa, where it ranges from 1.1 to 3.9 per 100,000 units, with a likely estimate of 2.2 per 100,000 units.<sup>4</sup> The first advance in this regard was the inclusion of P24 viral antigen screening, which has been shown to reduce the diagnostic window of HIV infection by 6 days compared with the use of a "third-generation" anti-HIV enzyme immunoassay alone.<sup>5</sup> The second advance was the screening for viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) in pooled blood samples. The risk of HIV transmission can be further reduced 45% to 72% by nucleic acid amplification technology (NAT) screening.<sup>6</sup> In the United States, NAT has reduced this risk of HIV transmission through blood donation to

approximately 1 in 1,900,000.<sup>7</sup> The technology of RT-PCR is expensive, however, and cannot be used in developing countries, where the largest bulk of HIV incidence is concentrated. For such countries, some researchers have advocated the use of white blood cell (WBC) filters to reduce the likelihood of transmission of infective agents, such as cytomegalovirus (CMV), HIV, and *Leishmania*.<sup>8</sup> The principle of the WBC filtration (leukodepletion) is based on the assumption that HIV and other intracellular infective agents reside in CD4 T lymphocytes and macrophages. Blocking these reservoirs of infective agents from transmission to blood recipients would decrease the load of transfused agents, and this might reduce the infectivity of blood and blood products, especially in window period when conventional serologic tests are not reliable. Cervia et al<sup>9</sup> have done a comprehensive review on leukodepletion and its role in attenuation of infection risks among transfusion recipients.

We have undertaken a study to investigate the amount of HIV-1 viral load reduction after leukocyte filtration. We have initiated this pilot study to evaluate HIV-1-infected WBC filtration using blood samples from patients at Sultan Qaboos University Hospital (SQUH). We have performed the investigation using extracted blood from known HIV-positive patients who are not receiving highly active antiretroviral therapy (HAART). With ethical approval and patient consent, blood donated from each patient was collected using the blood collection pack (PackPure WB, Baxter, Berkshire, UK) system. The system is composed of 2 450-mL packs connected with a flexible screen filter with a polyvinylidene difluoride membrane (1.0  $\mu$ m and 0.65  $\mu$ m). The movement through the filter is gravity dependent. A 5-mL blood sample was taken before filtration from the blood collection pack into an ethylenediaminetetraacetic acid (EDTA) tube for viral load measurement. The blood moved with gravity from the collection pack to another pack set at a lower level through a filter. The pack containing the filtered blood was disconnected from the filter and collection pack. A 5-mL blood sample was removed after filtration from the filtered pack into the EDTA tube for

viral load measurement. Viral load measurements in plasma of the unfiltered and filtered blood samples were run at the same time using the COBAS AMPLICOR (Roche Molecular Diagnostics, Pleasanton, CA). The HIV-1 RT-PCR assay was based on the concentration of RNA at high-speed centrifugation, alcoholic extraction of RNA from the plasma samples followed by reverse transcription using *Thermus thermophilus*, and the eventual PCR assay as per the manufacturer's instructions. The amplicons were detected using an enzyme-linked immunosorbent assay (ELISA) and quantified as per a standard curve. The tests were repeated 3 times for every sample. Two patients met the criteria of having HIV and still not being on HAART. The samples were tested first serologically for HIV-1 by ELISA and then confirmed by Western blot analysis.

We noted a nonsignificant increase of virus load rather than the expected decrease of virus load after leukodepletion (Table 1). The original viral loads for both patients were high 78,000 ( $\pm$ 400) copies/mL and 69,500 ( $\pm$ 200) copies/mL, respectively. There was no decrease in the viral load of both patients after filtration; the viral loads of filtered blood were 79,950 ( $\pm$ 350) copies/mL and 70,000 ( $\pm$ 200) copies/mL, respectively.

In the ultrasensitive PCR assay employed, the variability of the standards used ranges from 63 to 1000 for the lower limit of the assay range and from 5100 to 82,000 for the upper limit of the assay range. This means that the significance of the results is limited to the log phase only. There is still the possibility that the slight increase in the postsamples is attributable to the pressures of

**TABLE 1.** Viral Load Before and After WBC Filtration for Samples A and B

	Viral Load (Copies/mL)
Sample A	
Before filtration	78000 $\pm$ 400
After filtration	79950 $\pm$ 350
Sample B	
Before filtration	69500 $\pm$ 200
After filtration	70000 $\pm$ 200

Each sample was processed 3 times before and after WBC filtration.

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filtration, which could cause leukocyte lyses releasing more viruses.

The leukodepletion procedure is labor-intensive and requires extra time compared with conventional techniques, which may add to delaying the freezing time of donated blood. Leukodepletion is usually achieved by filtration of whole blood or blood components or by certain apheresis techniques without filtration, and most filters provide 3- to 4-log removal of leukocytes.<sup>10,11</sup>

HIV transmission through blood and blood product transfusion is still a real problem, especially in countries with a high rate of infection and with limited resources.<sup>9</sup> A search for alternative methods for the reduction and/or elimination of viral load before blood transfusion is highly needed. Although we report here that leukodepletion does not reduce HIV-1 viral load, we would recommend that other studies with a larger sample size and variable viral load be conducted.

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## HIV RNA Suppression Among HIV-Infected Ugandan Children With Measles

#### To the Editor:

In the course of HIV disease, coincident infections by several agents have been associated with transient increases in plasma HIV RNA levels,<sup>1</sup> but

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only a few, such as scrub typhus (*Orientia tsutsugamushi*),<sup>2</sup> have been associated with transient decreases. Low plasma HIV RNA levels during measles virus infection were first noted by Moss et al<sup>3</sup> in a cross-sectional study of hospitalized Zambian children. When a recent measles outbreak occurred among HIV-infected children participating in a longitudinal observational cohort, we evaluated HIV RNA levels before, during, and after the measles episode. Because measles virus can elicit an immune response without manifesting the classic clinical syndrome,<sup>4</sup> we also sought to determine whether such "subclinical" measles infection caused changes in plasma HIV RNA levels in this cohort. Finally, we evaluated HIV RNA levels among children without measles who received live-attenuated measles vaccine.

From October 2005 through September 2006, 300 HIV-infected children aged 1 to 10 years were enrolled into the Children with HIV and Malaria Project (CHAMP) from a dedicated pediatric HIV clinic at Mulago Hospital, Kampala, Uganda.<sup>5</sup> The guardians of all children provided informed consent. The research was approved by the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, and the University of California, San Francisco Committee on Human Research and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Children with clinical measles were identified from the study clinic from June through December 2006. The World Health Organization (WHO) criteria of fever  $\geq 38.0^\circ\text{C}$  and maculopapular rash with cough, coryza, and/or conjunctivitis were used as our case definition of measles.<sup>6</sup> To identify cases of subclinical measles infection, dried blood spots (DBSs) that had been routinely collected from a convenience sample of 94 participants were screened for measles immunoglobulin M (IgM). To determine if vaccination affected HIV RNA level, the children who had routine laboratory tests coincidentally obtained in the 14-day period following measles vaccination (Sanofi Pasteur, Lyon, France) were retrospectively identified.

Plasma HIV RNA (Roche Ampli-cor Version 1.5 [level of detection of 400 copies/mL] Pleasanton, CA), absolute CD4 cell count, CD4 percent (CD4%),

**TABLE 1.** Plasma Viral Load and CD4 Cell Count Before, During, and After Measles Illness

Patient No.	Plasma HIV RNA Load (log <sub>10</sub> [Copies/mL])				CD4 Count (Cells/μL)			CD4%			TLC (×10 <sup>3</sup> Cells/μL)		
	Before	During	After	Δ	Before	During	After	Before	During	After	Before	During	After
ART-naive													
1	5.9	3.9	3.5*	-2.0	173	85	309	5	4	7	4.4	2.5	4.9
2	5.6	3.7	5.9	-1.9	411	369	437	34	23	33	1.2	1.6	1.4
3	5.1	3.7	5.5	-1.4	835	935	631	35	34	30	2.6	3.5	2.1
4	5.0	3.5	5.7	-1.5	n/a	527	591	n/a	16	27	2.5	3.2	2.5
5	4.7	3.4	4.5	-1.3	1295	749	1513	32	33	28	5.8	4.1	4.8
6	4.6	3.6	4.7	-1.0	633	262	565	27	28	26	2.9	1.1	2.1
7	4.5	3.1	4.7	-1.4	622	353	467	21	21	20	2.8	1.5	2.5
8	4.0	3.6	4.1	-0.5	568	408	445	24	21	29	2.6	1.8	1.7
Receiving ART													
9	5.3	3.7†	2.8	-1.7	621	753	1010	11	21	27	5.7	3.9	2.8
10	4.6	2.8‡	2.6	-1.8	646	300	814	10	11	31	8.5	3.5	3.4
11	3.0	2.8§	n/a	-0.3	1634	334	n/a	20	19	n/a	8.5	1.8	n/a
12	und	und	und	—	628	111	548	27	16	33	2.3	0.8	1.8
13	und	und	und	—	347	100	347	8	7	17	4.9	1.5	2.1
14	und	und	und	—	900	874	1435	31	32	35	3.2	3.4	4.0

\*Began ART after measles diagnosis and 2 weeks before the follow-up measles laboratory tests.

†Began ART 7 days before measles diagnosis.

‡Began ART 1 year before measles diagnosis with variable plasma HIV RNA level since then.

§Began ART 3.5 months before measles diagnosis; died as a result of measles illness.

Before indicates 9 to 73 days before measles diagnosis; During, day of measles diagnosis; After, 9 to 76 days after measles diagnosis; Δ, During - Before.

n/a indicates not available; TLC, total lymphocyte count; und, undetectable (<400 copies/mL).

and total lymphocyte count were determined routinely at 12-week intervals and additionally obtained at the time of clinical presentation. DBSs were tested for measles antibodies using Measles Enzygnost ELISA IgM Kits (Dade Behring, Marburg, Germany); the kit protocols were modified for use with DBSs, as per Riddell et al,<sup>7,8</sup> yielding qualitative results of negative, equivocal, and positive for the presence of antibodies.

For all statistical analyses, the log transformation of plasma HIV RNA (log<sub>10</sub> [copies/mL]) was used. To determine the acute change in plasma viral load, the HIV RNA level during measles was subtracted from the HIV RNA level obtained at the most recent preceding visit; these acute changes in HIV RNA level were then tested against the null hypothesis of no change, using the nonparametric Wilcoxon signed rank test. To determine if this acute change with measles differed from normal variation in HIV RNA level, a list of the changes in HIV RNA level between sequential visits on which participants reported no illness was generated from all available CHAMP data, randomly selecting 1 value per participant. The median changes with measles were then compared with the median changes between these routine visits, using the nonparametric Wilcoxon

2-sample test. To determine if the HIV RNA set point changed as a result of measles illness, the most recent HIV RNA level from before measles illness was subtracted from the first level available after the resolution of illness.

Among the 300 children in the CHAMP cohort, 14 developed clinical measles in the period from June to December 2006. Participants who had measles were similar to other study participants in median age (6.8 vs. 5.7 years), CD4 count (631 vs. 762 cells/μL), and CD4% (23% vs. 23%). The date of presentation and diagnosis ranged from 1 to 7 days after the onset of fever. Every HIV-infected child with detectable HIV RNA experienced a decrease in HIV RNA level during measles illness (Table 1). Among the antiretroviral therapy (ART)-naive children, the median decrease was 1.4 log<sub>10</sub> copies/mL (interquartile range [IQR]: 1.2 to 1.6; *P* = 0.008). A total of 114 sequential routine visits without concurrent illness were identified to measure normal variation in HIV RNA level; the median change of -0.05 log<sub>10</sub> copies/mL was significantly different (*P* < 0.0001) from the changes during acute measles. Of the 6 children receiving ART, 3 had decreases in HIV RNA level during measles but they also had recent ART initiation; the remaining

3 children had undetectable HIV RNA before, during, and after clinical measles.

After resolution of measles, HIV RNA returned to preillness levels in all ART-naive cases. At least 3 HIV RNA levels were available after illness for each participant; in all cases, at least 1 laboratory value was from more than 172 days after diagnosis. The earliest follow-up HIV RNA level was obtained in case 2: 9 days after measles diagnosis, 15 days after the onset of fever, and 8 days after its resolution. There was no significant difference between the first HIV RNA level available from after and the most recent level from before measles illness (*P* > 0.38). Total lymphocyte counts declined significantly by a median of 1.6 × 10<sup>3</sup> cells/μL (IQR: 1.9 to 0.5; *P* = 0.009). The absolute CD4 count likewise declined significantly by a median of 247 cells/μL (IQR: 371 to 42; *P* = 0.013); however, CD4% remained stable, with a median change of 0% (IQR: -1 to 1; *P* = 0.67).

Every child in the cohort who did not develop clinical measles received measles vaccination; in 11 cases, laboratory studies were routinely collected in the 14 days after vaccination. No significant change in HIV RNA level was observed (median of -0.13 log<sub>10</sub> [copies/mL], IQR: -0.26 to 0.14). For 2 children, this was their first vaccination; for 7, it was

their second; and for 2, it was their third; no differences were noted between those subgroups. Among the 94 children who were screened for subclinical infection, no IgM-positive samples were identified.

Although measles infection was associated with low plasma HIV RNA level in a prior report,<sup>3</sup> this is the first study to document a decline in HIV RNA level prospectively in individual children during clinical measles. This enabled us to demonstrate universal suppression of plasma HIV RNA in a small group of children and to quantify the dramatic change, with a median decrease of 1.4 log<sub>10</sub> (copies/mL) among the ART-naïve children. The decreases in HIV RNA level were transient and without effect on the HIV RNA set point. Similar changes were not observed after measles vaccination.

The underlying mechanism by which measles infection affects plasma HIV RNA is unknown. Measles virus is known to cause multiple immunomodulatory changes in infected hosts, including a profound lymphopenia affecting circulating T-cell populations<sup>9</sup> that could result in a loss of host cells for HIV replication. Studies using HIV-infected peripheral blood mononuclear cell (PBNC) cultures have shown that measles virus lowers p24 antigen production independent of cell death, however.<sup>10</sup> Our study provides clinical correlation for this finding; a profound drop in HIV RNA level was seen despite a rise in total lymphocyte count in 3 cases, and a rise in absolute CD4 count was seen in 1 case. Thus, although a loss of host cells may contribute, it seems to not be the sole mechanism for the decline in HIV RNA level.

It is interesting that vaccination with live-attenuated measles was not associated with decreases in HIV RNA level in our study. The attenuated Edmonston B strain of measles, used for vaccination in the 1960s and 1970s, is able to generate significant HIV suppressive effects in PBMCs and ex vivo lymphoid tissue.<sup>10,11</sup> It may be that the same HIV suppressive factors follow vaccination and wild-type infection but that their magnitude is simply too low after vaccination to affect HIV replication. Modern measles vaccines use more attenuated strains of measles that produce fewer side effects, do not elevate interferon (IFN)- $\gamma$  levels, and have been shown to generate lower titers of anti-measles IgM and IgG than wild-type

infection.<sup>12</sup> It would have been interesting to evaluate changes in HIV RNA levels after subclinical infection by wild-type virus, but we did not identify any cases.

Despite dramatic suppression of HIV replication by measles virus in vivo and in laboratory assays, the precise mechanism remains unclear. Further investigation of this powerful interaction between measles virus, the host immune response, and HIV may yield insight into the control of HIV replication and directions for new means of ART.

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## Evolution of Plasma Hepatitis C Virus Load in Patients Coinfected by HIV and Hepatitis C Virus Started on a Protease Inhibitor-Containing Antiretroviral Regimen, Agence Nationale de Recherches sur le SIDA CO8 APROCO-COPILOTE Cohort

### To the Editor:

Hepatitis C virus (HCV) genotype and load are the strongest predictors

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of sustained virologic response in HIV-infected patients treated for HCV infection with peginterferon and ribavirin.<sup>1</sup> A high level of HCV replication could explain the lower virologic response rate to anti-HCV therapy in HIV-coinfected patients. The impact of antiretroviral therapy on HCV load has been evaluated after the introduction of combination antiretroviral therapy (cART). Most studies showed that HCV load did not decrease during the first months of antiretroviral treatment<sup>2-5</sup> and could even increase significantly (at least 0.5 log<sub>10</sub>) in some patients.<sup>4,5</sup> Some authors have suggested that introduction of cART could yield a significant decrease in HCV load after 12 months of successful antiretroviral treatment,<sup>6</sup> leading to HCV clearance in some cases.<sup>7,8</sup> These studies were limited by their small sample size or a short follow-up period (no longer than 1 year), however.

We describe here the change in plasma HCV load during the first 24 months after initiation of a protease inhibitor (PI)-containing antiretroviral regimen in 112 HCV-HIV-coinfected patients enrolled in the Agence Nationale de Recherches sur le SIDA (ANRS) CO8 APROCO-COPILOTE cohort.

The ANRS CO8 APROCO-COPILOTE cohort is a prospective observational cohort in France that describes the effects of PI regimens in the context of routine care. A total of 1281 patients infected with HIV-1 and naive to PIs were enrolled from May 1997 to June 1999 at the time they initiated treatment with a PI-containing regimen. Data were recorded at baseline, after 1 and 4 months of PI therapy, and then every 4 months. Patients were eligible for inclusion in the study if they were HCV infected, defined as having positive serum antibodies to HCV by an enzyme-linked immunoassay (ELISA) third-generation test (Ortho HCV 3.0 ELISA, Monolisa antiHCV; Sanofi Diagnostics Pasteur, Paris, France) and detectable plasma HCV RNA by a sensitive polymerase chain reaction (PCR) technique (Cobas Amplicor HCV 2.0; Roche Diagnostics, Branchburg, NJ). Patients who had received previous anti-HCV treatment without documented virologic response at least 6 months before inclusion could be

included. They were not eligible for analysis if they were receiving anti-HCV treatment during the follow-up period. Plasma samples were evaluated at baseline and after 4, 12, and 24 months of PI therapy in a single laboratory. Samples were stored at -80°C before HCV quantification, which used Versant HCV RNA 3.0 (Bayer Diagnostics, Eragny, France). If the HCV load was lower than the level of detection (3200 copies/mL or 615 IU/mL), HCV clearance was checked using a sensitive test (Cobas TaqMan HCV [sensitivity of 15 IU/mL]; Roche Diagnostics). To take into account intrinsic variability of quantitative assays and intraindividual variations, a decrease or increase in plasma HCV load was defined as a change between baseline and 24 months of >0.5 log<sub>10</sub> (ie, 3-fold).

The following potential determinants of plasma HCV load were considered for statistical analysis: age, gender, HIV transmission group, AIDS stage at

baseline, HCV genotype, type of PI initially prescribed, and duration of PI therapy. The association between potential determinants and plasma HCV load at month 24 was studied by a linear regression model adjusted for plasma HCV load at initiation of PI therapy to take into account potential regression to the mean. Data were analyzed with SAS software, version 8 (SAS Institute, Cary, NC).

Among the 1281 patients included in the cohort, 293 (23%) were HCV-antibody positive; 112 patients had available plasma samples after 24 months of PI therapy and were eligible for the current analysis (Table 1). At baseline, the plasma HCV load did not differ significantly according to age, gender, duration of HIV infection, CD4 T-lymphocyte count, plasma HIV-1 RNA level, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values, and HCV genotype. There was no significant change of median plasma HCV load over time

**TABLE 1.** Characteristics of 112 HIV-HCV-Coinfected Patients Analyzed for the Evolution of Plasma HCV Load Over Time, ANRS CO8 APROCO-COPILOTE Cohort

Characteristic	n (%)
Male gender	87 (78)
Age, median y (range)	36 (33 to 39)
Source of HIV infection	
Intravenous drug use	76 (67.8)
Others	36 (32.2)
CDC HIV-1 infection classification	
A	50 (44.5)
B	38 (33.9)
C	24 (21.4)
PI in antiretroviral regimen	
Indinavir	50 (44)
Nelfinavir	26 (23)
Others	36 (33)
HCV load, median log <sub>10</sub> copies/mL (range)	
Baseline	6.0 (5.6 to 6.5)
Month 4	6.4 (5.8 to 6.6)
Month 12	6.1 (5.6 to 6.4)
Month 24	6.1 (5.6 to 6.5)
CD4 T-lymphocyte count, median cells/ $\mu$ L (range)	
Baseline	269 (157 to 424)
Month 4	329 (215 to 496)
Month 12	403 (266 to 583)
Month 24	460 (324 to 659)
HIV load, median log <sub>10</sub> copies/mL (range)	
Baseline	4.3 (3.6 to 4.9)
Month 4	2.3 (2.3 to 2.7)
Month 12	2.3 (1.7 to 2.7)
Month 24	2.1 (1.7 to 2.8)

Data are number (%) of patients.  
 CDC indicates Centers for Disease Control and Prevention.

(see Table 1). Among the 112 patients, 29 (28%) had a significant increase in plasma HCV load and 22 (20%) had a significant decrease at month 24, whereas 61 (52%) patients had no significant variation in plasma HCV load. Evolution of HIV RNA level and CD4 T-lymphocyte count did not significantly differ between these 3 groups. There was also no difference according to the prescribed PI.

HCV clearance was reported in 4 (4%) of the 112 patients (95% confidence interval [CI]: 1.0 to 8.9). In 3 patients, the initial plasma HCV loads were 6.6, 6.1, and 5.3 log<sub>10</sub>, respectively. HCV RNA was undetectable at month 12 in 1 patient and at month 24 in the 2 others. In the fourth patient, baseline plasma HCV load was lower than the level of quantification but with a positive qualitative measurement. Clearance of HCV RNA was obtained as early as the first point of follow-up (month 4) and was confirmed later on (months 12 and 24). In contrast, among 29 patients with a significant increase in plasma HCV load, 3 had a baseline HCV load lower than the level of quantification with a positive qualitative test result. After initiation of cART, the plasma HCV load increased and remained persistently elevated during the entire follow-up period (5.3 to 6.0 log<sub>10</sub>).

In our study, plasma HCV load did not vary significantly in patients receiving cART-containing PI over a 24 month-period. HCV clearance was obtained in 4% of patients.

We were able to evaluate HCV load changes in a large population of patients during a longer period of follow-up than those of previous studies. We observed that most patients had no significant HCV load change during the 24 months of the study. Among others, HCV load increased or decreased significantly in 28% and 20%, respectively. It is highly plausible that the observed results are linked to the effect of regression to the mean rather than representing a true biologic variation.<sup>9</sup> There was no association between HCV load variation and viroimmunologic parameters linked to HIV disease.

As previously reported,<sup>7,8</sup> we observed HCV clearance in 4% (95% CI: 1.0 to 8.9) of patients. Whether this

represents spontaneous clearance or is linked to antiretroviral therapy cannot be solved. A CD4 cell response against HCV antigens (primarily core antigens) can be observed in peripheral blood mononuclear cells (PBMCs) in HCV-HIV-coinfected patients receiving cART. This response may be restored by cART, explaining the suppression of HCV RNA. Conversely, the HCV load strongly increased in 3 patients with an undetectable HCV load before initiation of cART. To our knowledge, such a phenomenon has never been described, although an increase in HCV load (more than 1 log<sub>10</sub> copies/mL) has been reported.<sup>4</sup> The increased number of cytotoxic T lymphocytes might cause immune-mediated lysis of HCV-infected cells, resulting in intracellular virus release. Alternatively, decreased HIV RNA levels could be associated with reduction in endogenous  $\alpha$ -interferon, favoring an elevated HCV load.<sup>10</sup>

In summary, PI-containing cART is most often not associated with biologically significant changes in HCV load in the first 24 months after its initiation. Strong individual variations can be observed, however, ranging from a high increase in HCV load to HCV clearance. HCV-HIV-coinfected patients should receive specific treatment of their hepatitis C, preferably before starting cART.

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## Early Identification and Care of HIV-Exposed and HIV-Infected Children in Rural Africa

### *The Role of Primary Health Care Centers*

#### **To The Editor:**

Programs for pediatric antiretroviral therapy (ART) traditionally focus on urban tertiary hospitals, but many African children have their first health care encounter at a rural primary health care center. Their access to ART depends on HIV screening and timely referral by primary health care workers (PHCWs). Rural PHCWs, however, are usually among the last to be trained for the prevention of mother-to-child transmission of HIV (PMTCT) and the integrated management of childhood illness (IMCI). Low training coverage rates result in intermittent service provision. These programs pay little attention to HIV screening among siblings of PMTCT babies and regular follow-up of HIV-infected children (older than 5 years) with minor HIV symptoms. Thus, many African children do not have access to HIV care. The urgency of this situation compels one to look for an additional strategy to improve pediatric HIV/AIDS care.

Attention to non-ART HIV care tends to be overshadowed by the attention to ART, but adequate treatment preparation is one of the most important determinants of successful ART.<sup>1</sup> The use of a register to monitor HIV-infected patients on ART is part of the public health approach of the World Health Organization, but there are many benefits from enrolling children long before ART is initiated. A regular follow-up of HIV-exposed children from the age of 3 months on facilitates early HIV testing, timely commencement of cotrimoxazole prophylaxis, and ART preparedness. A “safety net,” through the monitoring of

a limited set of indicators of vulnerability, can accompany such a care program and provide a timely alert for psychosocial problems that could interfere with the child's health care. Despite the fact that much HIV care could be provided by PHCWs, there has so far been little debate and research into their potential role. An observational study in Chimanimani district (Eastern Zimbabwe) tried to evaluate the role of PHCWs by developing a strategy to improve the identification and care of HIV-exposed and HIV-infected children at primary health care centers and analyzing the most important results of 7 months of pilot testing.

The PHCWs participated in the development of the strategy so that it was considered meaningful, feasible, and not too time-consuming for PHCWs; it included their ideas for further simplifications and gave them ownership of the program. The nurses introduced the name CFU, derived from child follow-up, but used only its abbreviated form to avoid stigmatization. Before the project, there were no data about the district's number of HIV-infected children and the pediatric HIV care provided at primary health care centers (including HIV testing and provision of cotrimoxazole prophylaxis), but the care was believed to be negligible. There were no medical doctors employed in the district. Permission for the pilot project was given by Zimbabwean Ministry of Health authorities.

PHCWs enrolled children ranging in age from birth to 16 years who met at least 1 of the following criteria: being born from a mother enrolled in the PMTCT program, being a sibling of a PMTCT baby, being referred for HIV testing after health education, exhibiting clinical symptoms suggestive of HIV, or when the parent was known to be HIV-positive or exhibited symptoms suspicious for HIV. Consent from the legal guardian for enrollment in the program was crucial and independent from consent for HIV testing. Enrolled children were followed on a monthly basis unless transport problems required longer follow-up periods.<sup>2</sup> Children were “officially” discharged from the register only in the case of a referral to a pediatric ART program; a referral to the well-baby clinic (if they tested HIV-negative); or

when investigations in the community concluded that the child had died, emigrated, or was lost. The information from each follow-up visit was recorded in the register in a concise and visual manner to limit the administrative burden (Fig. 1). Reminders helped to offer timely vitamin A, the additional measles vaccine, and an HIV test. The serostatus of the child was monthly reassessed, whereby all children were classified according to the following 4 categories: “tested,” “too young for rapid tests” (<18 months), “lack of testing facilities,” or “no consent for testing yet” (in case of a refusal or when the necessary discussions within the family were not yet finalized). Gradually, over time, the project was implemented in 15 sites: 5 operational testing sites where PMTCT was provided, 6 sites with trained staff that became operational PMTCT sites only after the pilot study, and 4 sites that were not yet included in the national PMTCT program. Each clinic completed a progress report on a monthly basis. This provided a timely alert for the need for a follow-up visit and is in line with other national systems for monitoring and evaluation. Data from the monthly progress reports were compared with the corresponding registers and entered into spreadsheets for a descriptive analysis.

Between October 5, 2005 and April 25, 2006, the strategy allowed initiating HIV care for 112 children. One third of these children were enrolled before the age of 6 months. IMCI training was needed for the identification of symptomatic HIV-infected children. The enrollment of asymptomatic PMTCT babies and their siblings, however, does not require training because it can be done by routinely inquiring about the PMTCT history of the child (eg, on the occasion of a postpartum visit or immunization). Thus, even PHCWs who were not yet trained in PMTCT and IMCI could identify HIV-affected children and apply this strategy.

After 7 months of pilot testing, 4 children had migrated to another catchment area, 7 (6%) had died, and 2 were discharged to the well-baby clinic. Although there was no official loss to follow-up, 8 children (7%) had not been seen for 2 or more consecutive monthly visits. The selected indicators of vulnerability were: being a maternal orphan

The opinions expressed in this article are those of the authors and may not necessarily reflect the opinion of their organizations.

Write the first letter of the month	O	N	D	J <sup>06</sup>	F	M
Write the age of the child at this month	3	4	5	6	7	8
Caregiver	M	M	M	M	M	
Cotrimoxazole						
Infant feeding-practice	MF	MF	H/S	H/S	H/S	
Infant feeding counseling	✓					
Immunization	✓	✓	✓	M1		
Malnutrition - vitamin A						
Write the first letter of the month	N	D	J <sup>06</sup>	F	M	
Write the age of the child at this month	6y					
Caregiver	M	M	M	M	G2	
Cotrimoxazole						
Infant feeding-practice	S	S	S	S	S	
Infant feeding counseling						
Immunization						
Malnutrition - vitamin A	(A)					

**FIGURE 1.** Recording data in the CFU register. In this part of the CFU register, the data from each monthly visit are summarized in the corresponding column and allow deduction of the following information. The first child was enrolled in the month of October (O) at the age of 3 months (3). The mother (M) presented monthly at the clinic. There were no gaps in cotrimoxazole provision (continued blue bar). The child was first on mixed feeding (MF) but later received heat-treated breast milk and solids (H/S). Nutritional counseling was provided in October. Immunizations were given according to the Expanded Programme on Immunizations at the ages of 3, 4 and 5 months, and an additional measles vaccine (M1) was given at 6 months. The child was malnourished until its death in February (red bar). Vitamin A was due in January (reminded by the circle around 6) but was only given in February (circled A). The second child was enrolled in November (N) at the age of 6 years (6y) and was accompanied by the mother (M) each month, except in March, when it was brought by the paternal grandmother (G2). There were no gaps in cotrimoxazole provision (continued blue bar). Nutritional counseling was not yet provided. Vitamin A was due and given in November (circled A). Since March, the child had become malnourished (red bar).

(16 children), being brought to the clinic by 3 or more different caregivers on separate occasions (1 child), and gaps in compliance with clinic appointments. There was anecdotal evidence of successful investigations in the community resulting in nonattendees resuming follow-up, suggesting that some kind of safety net had been established. Thus, 91 (81%) of the registered children were still on monthly follow-up by April 2006.

By the end of the study, there was documented evidence that 92% of the followed children had received their cotrimoxazole supply on a monthly basis. PHCWs reported that the register facilitated the procurement of adequate cotrimoxazole supplies. The study results cannot determine how many children actually ingested the cotrimoxazole

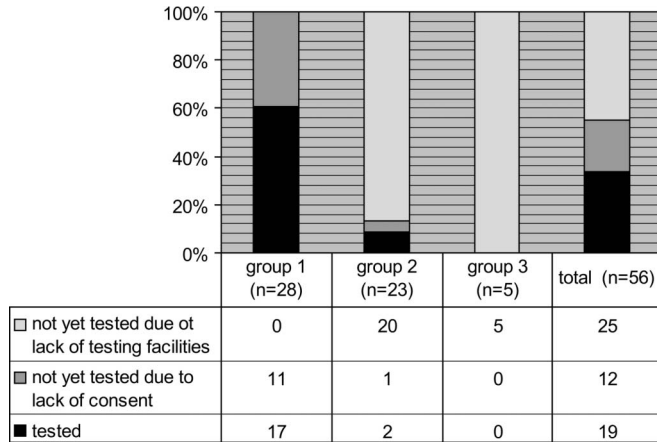
daily but suggest that the implementation of cotrimoxazole guidelines improved. Although several of the suggestions expressed by Zachariah et al<sup>3</sup> seem to be confirmed, the CFU strategy is more integrated, and may thus allow better optimal use of resources allocated to scaling up cotrimoxazole prophylaxis.

Because the project aimed at sustainable improvement of the implementation of national HIV guidelines within the resources of the Ministry of Health, infant diagnostic tests were not introduced at this stage, but the use of rapid tests in older children was promoted. Within 7 months, 34% of all registered children older than 18 months had been tested for HIV, 21% still lacked consent for testing, and 45% were not tested because of the lack of testing facilities.

Among the children enrolled at the 5 operational testing sites, 61% were tested despite gaps in the provision of rapid tests through the national PMTCT program (Fig. 2). The findings emphasize how pediatric HIV screening can also substantially be increased through logistic support and further decentralization of PMTCT programs.

The data allowed assessing the impact of the national PMTCT program on this group of children: a complete PMTCT regimen (nevirapine given to the mother and the infant) was given to 25 (22%) of the enrolled children. This low figure is explained by the delay in the national PMTCT roll-out to this district and by the fact that not only PMTCT babies but also older children were enrolled (eg, through IMCI). For 77 children, no PMTCT was done or the guardian was unable to provide this information. Ten children received an incomplete regimen. In 7 of these 10 cases, the nevirapine was given to the baby but not to the mother. Further investigations should clarify whether this phenomenon is caused by mothers' simply forgetting to take nevirapine, late enrollment in the PMTCT program as a result of home deliveries (BCG immunizations possibly functioning as a safety net for PMTCT), or resistance against counseling mothers during labor. HIV transmission rates among babies with incomplete PMTCT regimens have not been equally studied but may plead for an alternative PMTCT regimen. Also, lost opportunities for PMTCT in mothers who seroconverted after a negative routine HIV test result early in pregnancy become apparent only through an analysis of HIV test results in infants.<sup>4</sup> Thus, more attention to the evaluation of PMTCT interventions from the side of the infant is urgently needed.

The number of enrolled children in need of ART cannot be obtained from the registers and could only be estimated by the percent of malnourished children (29%). PHCWs can diagnose malnutrition but cannot necessarily differentiate HIV-related malnutrition from other types of malnutrition. More research is necessary to investigate how PHCWs with limited training in pediatric HIV clinical staging and little or no access to CD4 cell counts could most



**FIGURE 2.** HIV testing in registered children who were older than 18 months by April 2006. HIV testing was conducted among the 56 registered children who were older than 18 months by April 2006. The candidates for rapid tests included 28 of the 74 children enrolled at the 5 operational PMTCT sites (group 1), 23 of the 30 children enrolled at 6 sites with trained staff that became operational PMTCT sites only after the pilot study (group 2), and 5 of the 8 children enrolled at the 4 small sites where nobody was trained in PMTCT or IMCI.

effectively identify children who need referral to ART clinics, where the demand for pediatric ART usually exceeds its availability. Low weight for age could possibly be such a simple indicator.<sup>5</sup>

Despite the many limitations and short duration of this pilot project, the results demonstrate that it is possible to achieve greater involvement of PHCWs in pediatric HIV/AIDS care than is currently achieved and emphasize the critical need for more debate about indicators of primary pediatric HIV care in rural Africa and how the follow-up at primary health care centers can be better used for improved non-ART HIV care and ART preparedness in children.

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## Awareness of HIV Prevention Strategies Under Development

### Word on the Street

#### To the Editor:

A number of biomedical interventions to prevent HIV acquisition and transmission are currently being tested in the United States for safety and, for some, efficacy. Three strategies being tested among men who have sex with men (MSM) include preventive HIV vaccines,<sup>1</sup> suppression of herpes simplex virus type 2 (HSV-2) infection,<sup>2</sup> and use of antiretrovirals for prevention of infection.<sup>3</sup>

The first approach, preventive HIV vaccines, has been under development in the United States since 1988, and phase 1 and 2 trials are ongoing. Two efficacy trials completed to date have not demonstrated efficacy of test vaccines.<sup>4,5</sup> The second approach, suppression of HSV-2 infection with acyclovir, is based on evidence that HSV-2 infection increases the risk of HIV infection at least 2-fold.<sup>6,7</sup> A recently completed trial testing the efficacy of suppressive therapy with acyclovir in preventing HIV acquisition among MSM in the United States and Peru and women in Africa did not show efficacy of this prevention strategy.<sup>8</sup> Lastly, recommendations for nonoccupational postexposure prophylaxis (nPEP) using antiretrovirals were published by the Centers for Disease Control and Prevention (CDC) in 2005, although clinicians and health departments have

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recommended use of nPEP since 1998.<sup>9</sup> One study of pre-exposure prophylaxis (PrEP) among MSM is underway in the United States and is examining the safety of PrEP, biologically and with regard to potential increases in risk behaviors.<sup>3</sup>

Information on awareness of potential interventions within at-risk communities can be useful in preparing for the dissemination of effective approaches in the future. Furthermore, there is concern that some strategies, such as PrEP, are already being used within at-risk populations before they are proven to be effective.<sup>3</sup> Information on the level of awareness of these potential prevention strategies in the MSM community is limited. Using data from the New York City HIV Behavioral Surveillance Study, we assessed the awareness of HIV vaccines and of the association between herpes and HIV in a venue-based community sample of MSM. We also inquired about the use of nPEP and PrEP in this study sample, as a measure of awareness of this strategy.

The National HIV Behavioral Surveillance survey in New York City (NHBS-NYC) was part of a national survey conducted in 17 cities.<sup>10</sup> This cross-sectional survey, using time-space sampling methods,<sup>11</sup> was designed to estimate the frequency of risk behaviors among MSM who attend public venues. HIV antibody testing was also included in 5 cities. The methods for the NHBS-NYC have been described previously.<sup>12</sup> For the NHBS-NYC, HIV antibody testing was also included, although not offered to the first 101 participants because of late institutional review board (IRB) approval of the testing component.

The questionnaire collected data on demographics, history of HIV antibody testing, and sexual risk behaviors and drug and alcohol use in the prior 12 months. For awareness of preventive HIV vaccines, men were asked "Have you heard about vaccines that could be used to prevent getting infected with HIV?" and where they had heard about HIV vaccines. For the HIV-herpes association, participants were asked "If someone has genital herpes, do you think that their risk of getting HIV is the same, greater or less than someone without genital herpes?" The men were also asked if they ever had a blood test for herpes. For nPEP or PrEP, the men were asked

"Have you ever used anti-HIV medications to prevent HIV infection either before or after a high-risk sexual or drug use exposure?" The study was approved by the IRBs of the participating institutions.

These analyses were based on the 503 men recruited in 2004 to 2005 who reported having had a male partner (MSM) in the previous 12 months. Approximately half (51.1%) of the men were younger than 30 years of age, 27.4% were Latino, and 23.3% were African American. Approximately half of the sample (53.7%) was recruited at bars and 11.3% at dance clubs, with the remaining men recruited at street locations, retail businesses, cafes/restaurants, events, social organizations, parks, and gyms. Most men (74.2%) had at least some college education. Of the 402 men offered HIV antibody testing, 349 accepted testing and 18.3% were found to be infected. In the previous 12 months, 53.1% of men reported having unprotected anal intercourse: 32.4% with main partners and 42.2% with nonmain partners.

Overall, 46.2% of men had heard of preventive HIV vaccines. Of those, 33.6% had heard about HIV vaccines from local newspapers, 25.8% from television reports, 22.3% from the Internet, 21.4% from local magazines, and 6.1% from community presentations. Close to one third (32.8%) of men had heard about HIV vaccines from multiple places. The youngest men (aged 18 to 24 years) were less likely to have heard about HIV vaccines compared with older men (30.0% vs. 53.6%;  $P < 0.0001$ ), as were less educated men (high school degree or less) compared with more educated men (35.4% vs. 50.4%;  $P = 0.003$ ) and HIV-uninfected men compared with HIV-infected men (44.9% vs. 64.1%;  $P = 0.006$ ). Men who reported unprotected insertive anal intercourse with a main partner were significantly more likely to be aware than men who did not (57.5% vs. 44.6%;  $P = 0.041$ ). Awareness of HIV vaccines was not related to race/ethnicity, venue of recruitment, sexual identity, other sexual risk behaviors, or use of specific drugs.

Overall, 59.0% of men knew that infection with genital herpes increases the risk of HIV infection. One third (33.7%) of men thought that the risk was the same whether or not someone had genital herpes, 5.6% did not know, and

1.6% thought that the risk was lower compared with the risk in someone without genital herpes. Approximately half (47.3%) of the men had ever been tested for herpes infection. Men who had previously been tested for herpes were not significantly more likely to know that herpes increases the risk of HIV compared with men who had never been tested for herpes (62.7% vs. 55.7%;  $P = 0.11$ ). Men who were younger (18 to 24 years of age) were less likely to know of the increased risk of HIV associated with herpes compared with older men (51.3% vs. 62.4%;  $P = 0.022$ ), as were less educated men (high school degree or less) (51.5% vs. 61.7%;  $P = 0.043$ ). No other demographic, sexual risk behavior, or drug use was associated with knowledge of the herpes-HIV association. Only 10 (2.0%) men reported that they had ever used nPEP or PrEP.

The NHBS-NYC survey provided an opportunity to assess the awareness of potential prevention strategies within the MSM community in New York City. The men were recruited at public venues and included only those who chose to participate; thus, they do not necessarily represent all MSM in New York City. Furthermore, the questionnaires were interviewer administered; thus, some behaviors may have been underreported.

Almost half of these MSM were aware of an important strategy being tested in New York City and around the world—preventive HIV vaccines. This is of particular interest, because recruitment for a large HIV vaccine efficacy trial was going on during the same time as the NHBS-NYC survey. Because of the wording of the question, we were not able to distinguish men who believe, incorrectly, that a licensed HIV vaccine exists and is available from men who were aware of HIV vaccines under development. Thus, the proportion aware of HIV vaccines under development may have been overestimated. HIV-infected men were significantly more likely to be aware of HIV vaccines for prevention of HIV infection than HIV-uninfected men. Although it is important to include all persons in building support for HIV vaccine trials, the results emphasize the need to continue and expand community education about HIV vaccine trials among HIV-uninfected men to increase participation of eligible MSM.

Only 59% of these MSM knew that infection with genital herpes increases the risk of HIV infection. Furthermore, less than half of the men had ever been tested for herpes, and those tested were not significantly more likely to know about the herpes-HIV association. Previous studies have shown that symptoms of genital ulcers are neither sensitive nor specific for HSV-2 infection and that the increase in HIV infection is present for those who have HSV-2 infection, regardless of symptoms.<sup>7</sup> Thus, this raises another important area for community education efforts to underscore the importance for MSM to take precautions against acquiring infection with HSV-2, to increase the knowledge of the herpes-HIV association, and to test for HSV-2 infection when indicated. Community outreach efforts for HIV vaccines and herpes need to focus on younger men and less educated men, 2 groups with particularly low levels of knowledge.

As has been observed in another survey,<sup>13</sup> few men reported using nPEP or PrEP. For nPEP, we do not know if this is a result of lack of awareness of this intervention or other factors, such as high cost or personal preferences. For PrEP, this is not a proven intervention; thus, it was not expected that many men would have reported PrEP use. If PrEP is shown to be effective in reducing HIV acquisition, it would be important to monitor trends in use and any subsequent changes in HIV risk behaviors.

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