False-Positivity of HIV-2 Immunoblots in a Cohort of Elite Suppressors Infected With HIV-1

To the Editor:

Infection with HIV-2, the second causative etiology for AIDS, is mainly present in West Africa, with a slow spread to other continents. Compared with HIV-1 infection, the course of HIV-2 infection is associated with slower progression to AIDS, most likely attributable to lower plasma viral loads observed in these patients.[1] In dual-infected patients, control of HIV-1 may be associated with the ability to respond to HIV-2 *gag* epitopes and to maintain HIV-specific CD4 T-cell responses.[2]

We recently tested a small group of long-term nonprogressors with HIV-1 infection (elite suppressors) to rule out coinfection with HIV-2. Three of 13 patients underwent testing with an HIV-2 enzyme immunoassay (EIA), and all 13 had HIV-2 Immunoblots performed at VircoMed Laboratories/Laboratory Corporation of America (Minnetonka, MN). One of the 13 patients had a significant HIV-2 risk factor with a previous sexual partner from West Africa of unknown HIV status.

Using the commercially available assays, specimens were considered positive for the HIV-2 Immunoblot test if the gp36 band (*env*) was present. All patients tested positive with the HIV-2 EIA and the HIV-2 Immunoblot (of whom 2 were weakly positive). When HIV-2 qualitative polymerase chain reaction (PCR) tests were performed, all patients were negative, thus ruling out coinfection with HIV-2.

Although previous studies have reported high sensitivity (91% to 100%) and specificity (81% to 100%) for HIV-2 antibody testing,[3] this may become more challenging in coinfected patients because of cross-reactivity. The 2 viruses have similar morphology and cell tropism, with homology exhibited in the conserved genes, such as the core proteins (*gag*) and reverse transcriptase (*pol*), and in other less conserved envelope (*env*) genes.[3,4] Although qualitative PCR testing is available at reference laboratories, quantitative viral loads are not available except in research facilities.

Given the high false-positivity rate of the commercially available assays because of cross-reactivity with HIV-1, serologic testing for HIV-2 infection should be used only in patients with negative HIV-1 Western blot test results in areas in which HIV-2 is not endemic. In cases when dual infection is suspected, our recommendation would be to proceed directly to HIV-2 PCR testing.

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The Impact of Primary Prophylaxis for Cryptococcosis on Fluconazole Resistance in *Candida* Species

To the Editor:

Primary anticryptococcal prophylaxis in advanced HIV infection is generally not recommended, given no evidence for improved survival, associated excess costs, and the potential risk for emerging drug resistance.[1] In Thailand, however, the prevalence of cryptococcosis is high, primary fluconazole prophylaxis has been associated with reduced invasive fungal infections and mortality, and primary cryptococcal prophylaxis is recommended in national HIV treatment guidelines.[2,3] Although primary prophylaxis has not been shown to select for fluconazole resistance among patients who subsequently developed cryptococcal meningitis,[4] the impact of primary anticryptococcal prophylaxis on the emergence of fluconazole resistance in *Candida* species among patients with HIV infection has not been evaluated.

Thammasart University is a 450-bed tertiary care referral hospital in central Thailand. Infectious diseases consultative services and care formally began in January 2003, with an annual increase in the number of HIV-related admissions from the year 2002 (N = 125) to the year 2006 (N = 405). Most HIV-related admissions were for the evaluation of fever; 45% of cases had CD4 counts <100 cells/μL.[5] Since 2003, primary anticryptococcal prophylaxis has been routinely prescribed to patients with advanced HIV infection (CD4 counts <100 cells/μL), as recommended by the Thai National HIV Treatment Guideline.[3] We conducted a drug utilization and antimicrobial resistance correlation analysis of inpatient antifungal consumption and the prevalence of fluconazole-resistant *Candida* spp. among hospitalized adults with HIV infection for the calendar years 2002 to 2006. The rate of inpatients' antifungal use was recorded as the total number of grams of drug, converted into defined daily doses (DDDs) per 1000 patient-days, in accordance with the recommendations of the World Health Organization.[6] Oral and parenteral drug expenditures were included for analysis. Antimicrobial susceptibility patterns of *Candida* spp., and the proportion of resistant isolates, were derived from existing data in the Microbiology Department. Correlations between antifungal consumption and resistance were assessed by Pearson correlation analyses.

There was an incremental increase in oral fluconazole consumption (from 6 to 35 DDDs per 1000 patient-days)

TABLE 1. Antifungal Consumptions and Incidence of Inpatient *Candida* spp. From Year 2002 Through 2006

	Antifungal DDDs per 1000 Patient-Days*				
Antifungal Agents	**2002 (N = 125)**	**2003 (N = 246)**	**2004 (N = 306)**	**2005 (N = 350)**	**2006 (N = 405)**
Oral agent					
Fluconazole	6.0	12.0	19.0	25.0	35
Itraconazole	5.4	5.0	4.0	6.0	6.0
Intravenous agent					
Amphotericin B	2.3	2.4	2.2	1.5	2.1
Fluconazole	4.8	6.0	5.6	5.5	5.8
Itraconazole	0	0	0	0.5	0.4
Other†	0	0	0.14	0.14	0.21
Microorganisms					
Candida albicans	95%	82%	71%	61%	54%
Fluconazole-resistant *Candida* spp.‡	2%	14%	20%	25%	33%

*Persons in routine university-based HIV care at Thammasart Hospital.
†Includes echinocandins and voriconazole.
‡Includes *C. glabrata*, *C. kreseii*, and other *Candida* species that have antimicrobial susceptibility indicating fluconazole resistance.

during the years 2002 to 2006, whereas the consumption of oral itraconazole and other parenteral antifungal agents (eg, fluconazole, amphotericin B) remained unchanged (Table 1). Notably, 86% of oral fluconazole prescriptions were for primary anticryptococcal prophylaxis, and 36% of the patients prescribed oral fluconazole had CD4 counts >100 cells/μL for at least 6 months. During the same 5-year period that the prevalence of fluconazole-resistant *Candida* spp. increased from 2% to 33%, detection of *Candida albicans* significantly declined from 95% to 54% (see Table 1). A significant correlation between oral fluconazole consumption and fluconazole-resistant *Candida* spp. ($r = 0.71$, $P < 0.001$) was noted. No correlation was evident for fluconazole-resistant *Candida* spp. and other antifungal drugs.

Our study findings have significant implications for clinical and laboratory practices. The incremental increase in oral fluconazole consumption was temporally attributed to the national HIV prevention policy for opportunistic infections. Notably, this increase in fluconazole use correlated with increased fluconazole-resistant *Candida* spp. Our findings suggest that systematic surveillance for fluconazole-resistant *Candida* spp. is judicious in countries where primary anticryptococcal prophylaxis is given to persons with advanced HIV infection. In addition, our findings imply potential benefit from interventions that minimize inappropriate antifungal use in developing countries. Focused educational interventions aimed at improving guideline compliance with prophylaxis against opportunistic infections are needed.

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Delayed Recognition of HIV Infection in Malnourished Children Is Associated With Poor Clinical Outcome in Low HIV Prevalence Settings

Preliminary Observations

To the Editor:

Protein-energy malnutrition (PEM) and late HIV disease, both common features in sub-Saharan Africa, have similar features.[1–4] Consequently, distinguishing the 2 conditions clinically is difficult.[5] Several studies have shown that HIV is common in malnourished children, especially children with marasmus and marasmic-kwashiorkor.[4,6] The World Health Organization (WHO) recommends that severely malnourished children should not be tested routinely for HIV on the premise that knowledge of HIV status does not play any role in the management of the child, except to diagnose interstitial lymphocytic pneumonia, and that a positive test result might cause the nursing staff to neglect the child.[7] Some reports have indicated that mortality among malnourished HIV-positive children was significantly higher than for malnourished HIV-negative children,[4,8] although other studies have not found a similar significant difference between HIV-positive and HIV-negative malnourished children.[6,9,10] In a study,[9]

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HIV-positive malnourished children had nearly twice the odds of dying as HIV-negative malnourished children. It has been suggested that early identification in HIV disease may present a critical window of opportunity to intervene effectively.[2,11] In the years after the WHO recommendation on routine HIV testing of malnourished children, management of HIV in resource-poor settings has improved and HIV awareness has increased dramatically. This recommendation not to routinely screen for HIV in malnourished children therefore might require some modifications to fit the present context.

In the absence of routine screening of malnourished children for HIV, the level of suspicion of the health care staff becomes important in identifying children who might be at higher risk of HIV. In high HIV prevalence areas, because of the high index of suspicion for HIV, there is an increased chance of picking up HIV infection coexisting with PEM. In low HIV prevalence settings, the index of suspicion is low, increasing the chances that HIV might not be considered in the diagnostic workup. This is even more likely in areas in which resources are limited and infrastructure is lacking. In such settings, a delayed or missed diagnosis of HIV-PEM comorbidity could increase mortality from malnutrition.[4] All available information on HIV-PEM interaction comes from high-prevalence settings. Similar information from low HIV prevalence resource-poor settings is scarce.

We carried out a retrospective case-control study as a preliminary assessment to investigate the effect of HIV on clinical outcome in children managed at a nutritional supplementation center in a low HIV prevalence setting in rural Gambia to provide information that should guide further research in this area. Routine screening for HIV is not carried out at the center; screening is conducted when there is poor response to management, diagnosis of tuberculosis (TB), and confirmed HIV in 1 or both parents. We compared the clinical outcome in malnourished children with confirmed HIV with that in malnourished children of negative or unknown serostatus. For each case, 4 gender- and age-matched controls were chosen. The outcome measures were duration of hospitalization and mortality.

There were 286 admissions, with an HIV prevalence of 2.10%. Table 1 describes the baseline characteristics of the cases and controls. All the anthropometric indices (weight-for-height z score [WHZ], weight-for-age z score [WAZ], height-for age z score [HAZ], and body mass index [BMI]) were significantly poorer in the HIV-positive group than in the controls. The HIV-positive group stayed longer on admission than the HIV-negative controls ($P < 0.001$). The association remained strong after controlling for baseline anthropometric indices (WHZ, WAZ, HAZ, and BMI; β-coefficient = 5.59 days; $P = 0.002$). Two deaths occurred in the HIV-positive group and none in the controls (Fisher exact P value = 0.034). Among the HIV-positive group, the average duration from admission to when a decision to screen for HIV was made was 20.8 days (range: 7 to 40 days, SD = 15.4 days). Children who were screened earlier (within 2 weeks) had a shorter stay at the center compared with children who were screened later (after 2 weeks) (duration of stay: 5.6 weeks vs. 11.2 weeks; $t = 2.63$; $P = 0.078$). The 2 deaths occurred among those in whom the decision to screen for HIV was made later in their management.

The results from this preliminary study suggest that in low HIV prevalence settings, delayed diagnosis of HIV in malnourished children is associated with a poorer outcome. This is similar to the finding by other workers from high HIV prevalence settings with larger population samples.[4,12] We have previously shown that comorbidities were common among children seen in primary health care settings, many of whom present with overlapping symptoms and signs such that it becomes difficult to define their relative contributions to childhood morbidity and mortality properly (CVN, West African College of Physicians [WACP] thesis, 2007). The presence of multiple comorbidities therefore might account for the difference between these findings and those that reported no significant difference.

Although our small numbers of HIV-positive children makes extrapolating our results to other low HIV prevalence settings difficult, the study suggests that delayed diagnosis of HIV infection in severely malnourished children is associated with poor clinical outcome. In low-prevalence resource-poor settings in which such diagnosis is likely to be missed, clinical guidelines outlining the indications for HIV screening in malnourished children might improve clinical outcome.

TABLE 1. Characteristics of HIV-Positive Serostatus (Cases) and HIV-Negative or Unknown Serostatus (Controls)

Parameter	Cases	Controls	*t* Test	*P*
n	6	24		
Age, mo (mean ± SEM)	18.9 ± 4.9	17.9 ± 2.0	−0.20	0.84
Height-for-age z score (mean ± SEM)	−3.4 ± 0.9	−2.1 ± 0.3	1.77	0.09
Weight-for-age z score (mean ± SEM)	−4.9 ± 0.4	−3.5 ± 0.1	3.93	0.0007
Weight-for-height z score (mean ± SEM)	−3.9 ± 0.4	−2.8 ± 0.2	−2.91	0.008
BMI, kg/m^2 (mean ± SEM)	10.8 ± 0.4	12.6 ± 0.2	3.43	0.002
Duration of admission, wk (mean ± SEM)	9.2 ± 1.5	2.9 ± 0.2	−7.15	< 0.001
Hemoglobin concentration, g/L (mean ± SEM)	94.4 ± 4.3	97 ± 3.5	0.35	0.73

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Tumor Necrosis Factor-α, Interleukin-10, and α-Defensins in Plasma and Breast Milk of HIV-Infected Highly Active Antiretroviral Therapy–Treated and Untreated Pregnant Women in Mozambique

To the Editor:

The risk of HIV transmission through breast milk has been shown to be dependent on the size of the inoculum,[1] the amount of cell-free and cell-associated HIV,[2] the presence of coinfections[3] and of antiviral substances such as defensins,[4] and the local specific immune response to HIV.[5] Other yet undefined factors may be involved in determining the rate of mother-to-child transmission, however, and further studies on the analysis of breast milk components could help in clarifying the mechanisms that could favor or prevent transmission during the lactation period. In the present study, we evaluated the concentrations of tumor necrosis factor-α (TNFα), interleukin-10 (IL-10) and α-defensins in plasma and breast milk of highly active antiretroviral therapy (HAART)–treated (group A, n = 20) and untreated (group B, n = 34) HIV-positive mothers in Maputo, Mozambique. The design of the study has been extensively described elsewhere.[6] Group A comprised pregnant women attending the Ante Natal Clinic in Matola and enrolled in the Drug Resource Enhancement Against AIDS and Malnutrition (DREAM) program who received antiretroviral regimens (generic formulation of zidovudine [or stavudine if hemoglobin [Hb] <8 g/dL] plus lamivudine and nevirapine) from 28 weeks of gestational age until 1 month postpartum. Group B women included mothers who were tested for HIV at delivery; if positive, they were asked to participate in the study. Women from both groups did not breast-feed their infants. Breast milk was expressed manually 5 times a day for 1 week, and 1-week breast milk samples were selected for testing. At the same time, blood samples were also collected and clinical examinations were performed to recognize the presence of mastitis or breast inflammation. TNFα and IL-10 were detected in plasma and skim milk samples by enzyme-linked immunosorbent assay (ELISA; Biosource Hu-TNF-α US and Hu-IL-10 US, respectively; BioSource International, Camarillo, CA). Concentrations of α-defensins were determined using a commercial ELISA kit recognizing human alpha defensins (HNP) 1 to 3 (hemoglobin [Hbt] Human HNP 1 to 3; Hycult Biotechnology, Uden, The Netherlands). All statistical analyses were performed using SPSS for Windows version 13.0 (SPSS, Chicago, IL).

Twelve (60%) HAART-receiving women had detectable HIV RNA in plasma versus 100% of untreated mothers ($P < 0.001$); HIV RNA in breast milk was lower than the limit of detection in 45% of treated mothers, whereas it was detectable in 91.2% of untreated mothers ($P = 0.005$). The concentrations of HIV RNA in plasma and breast milk were significantly lower in HAART-treated women ($P < 0.001$), who also had better preservation of their peripheral blood CD4 cell count ($P = 0.002$) with respect to untreated mothers. HIV DNA in breast milk cells was detectable in 30% and 47.1% of group A and B women, respectively.

In untreated women, significantly higher levels of TNFα, IL-10, and α-defensins were detected in breast milk with respect to plasma ($P < 0.001$, $P = 0.035$, and $P = 0.001$, respectively), whereas in HAART-treated women, similar levels of IL-10 and α-defensins were found in the 2 maternal districts ($P = 0.309$ and $P = 0.445$, respectively). Only TNFα concentration in breast milk significantly increased in comparison to plasma levels in both groups of mothers ($P < 0.001$; Fig. 1A). An analysis was also performed including samples of both groups to determine the correlation between TNFα, IL-10, and α-defensins and viral replication levels in plasma and in breast milk. TNFα positively correlated with HIV RNA viral load in plasma ($r = 0.338$, $P = 0.012$). Conversely, in plasma, no correlations were detected between HIV RNA and IL-10 ($r = 0.232$, $P = 0.091$) or α-defensins ($r = 0.050$, $P = 0.724$). In breast milk, we found a strong correlation between HIV RNA viral load and TNFα, IL-10, and α-defensins (see Fig. 1B).

This study suggests that cytokine production in plasma and in breast milk is independent and that HAART therapy influences breast milk cytokine profiles. In fact, naive mothers showed breast milk levels of TNFα, IL-10, and α-defensins from 2- to 6-fold higher than in plasma, whereas in HAART-treated mothers, only breast milk TNFα levels were significantly higher than the plasma levels. The high levels of these soluble factors in breast milk, where HIV RNA copies/mL are lower than in plasma, suggest that their concentrations are unlikely to be simply a reflection of those found in the plasma and support the hypothesis that breast milk cells productively release factors in response to stimuli, such as HIV replication. In this view, differently from plasma, TNFα, IL-10, and α-defensins in breast milk highly correlated with HIV RNA viral load in both groups of women. An active role of breast milk cells has been demonstrated by spontaneous release of IL-1β and TNFα[7] and by the fact that cellular components of breast milk are able to produce substantial quantities of many known cytokines in

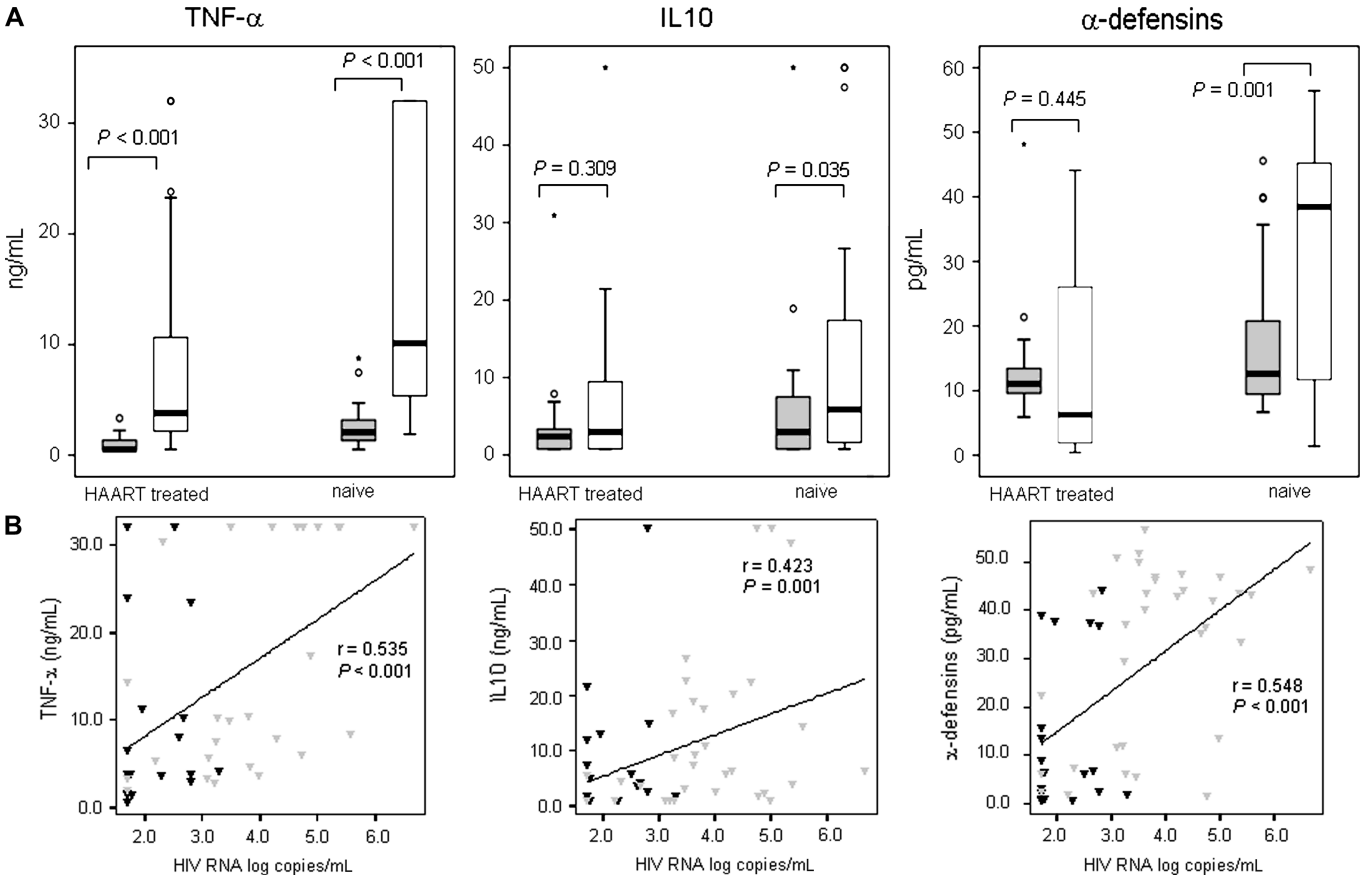

FIGURE 1. A, TNFα, IL-10, and α-defensin concentrations found in plasma (▒) and breast milk (□) samples collected 7 days after delivery in HAART-treated (group A) and naive (group B) mothers. Values are reported in pg/mL (TNFα and IL-10) and ng/mL (α-defensins). B, Correlations between soluble factor levels and HIV RNA viral load in breast milk. Correlations were investigated by using the Spearman correlation test. Gray and black triangles indicate group A and group B values, respectively. $P < 0.05$ was considered significant.

response to various stimuli.[8] Regarding α-defensins, their role in milk is controversial,[5,9] but higher α-defensin concentrations found in the breast milk could be involved in processes reducing infectivity of the virus in breast milk or increasing infant resistance; their exact role in mother-to-child transmission needs to be explored in large clinical studies. A limitation of our study is that we could not ascertain whether the elevated concentrations of cytokines or soluble factors could be important in influencing postnatal transmission because these mothers did not breast-feed. Nevertheless, our study provides new data on the breast milk compartment of HIV-infected mothers and supports the hypothesis of a compartmentalization between plasma and breast milk and of a functional role of breast milk cells in an adaptive and innate immune response to HIV infection.

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