The effect of opportunistic illness on HIV RNA viral load and CD4+ T cell count among HIV-positive adults taking antiretroviral therapy

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Abstract

Introduction: HIV RNA viral load (VL) has been shown to increase during opportunistic illnesses (OIs), suggesting active HIV replication in response to infection among patients not taking antiretroviral therapy (ART). We assessed the effects of OIs on HIV RNA VL and CD4+ T cell counts among patients on ART with initially suppressed VL.

Methods: Between 2003 and 2007, we enrolled and followed 1094 HIV-1-infected adults who initiated ART and had quarterly blood draws for VL and CD4+ T cell count. In VL/CD4+ T cell measurement intervals following undetectable VL, we compared the elevation in VL to detectable levels and CD4+ T cell count changes between intervals when participants had episodes of OIs and intervals when they did not have OIs.

Results: VL was more likely to be detectable if participants had OIs in the prior three months compared to when they did not (OR = 4.00 (95% CI = 1.9–8.6)). The CD4+ T cell counts declined 24.1 cells/µL per three months in intervals where the participants had OIs compared to an increase of 21.3 cells/µL per three months in intervals where they did not have OIs (adjusted difference in the rate of CD4+ T cell count change of 61.7 cells/µL per three months (95% CI = 13.7–109.7), P value = 0.012). The rate of CD4+ T cell count increase was 25.6 cells/µL per three months (95% CI = 11.6–39.6) higher for females compared to males (p value = < 0.001), 1.4 cells/µL per three months lower per one year increase in age (p value = 0.046) and 4 cells/µL per three months lower per 10 cells/µL increase in the starting CD4+ T cell count value (p value = < 0.001).

Conclusion: Episodes of opportunistic infections among patients taking ART with undetectable VL were associated with elevation of HIV RNA VL to detectable levels and decline in CD4+ T cell counts.

Clinical Trial Number: NCT00119093.

Keywords: ART; CD4+ T cell lymphocyte count; HIV RNA viral load; HIV; opportunistic infections; Uganda.
ALT < 5 times upper limit of normal, a calculated creatinine clearance ≥ 25 mL/min, and a Karnofsky Score > 40%. The Cockcroft-Gault formula was used to estimate creatinine clearance. The first-line ART regimen was stavudine, lamivudine and nevirapine (or efavirenz for those taking concurrent rifampicin), and all participants received cotrimoxazole prophylaxis. Pre-packaged ART, cotrimoxazole and other medicines were replaced using a weekly storage container, and pill counts conducted at the study clinic by a pharmacist.

All participants provided written informed consent in their preferred language and were free to withdraw their participation in the study at anytime without losing access to free ART from TASO. The study was approved by Uganda National Council of Science and Technology, the Institutional Review Boards of the Uganda Virus Research Institute, and the Centers for Disease Control and Prevention (CDC). Funding was provided by the U.S. Department of Health and Human Services/CDC through the Emergency Plan for AIDS Relief.

Data collection methods
Trained lay field officers visited clients’ homes weekly to deliver medications and collect data regarding drug adherence, potential symptoms of drug toxicity or death of a household member in the preceding seven days [11]. Participants were weighed monthly during home visits, and these weights and body mass index scores were systematically provided to clinicians. After enrolment, no routine clinic visits were scheduled, but participants were encouraged to come to the clinic or hospital if they were ill, and were transported to the clinic for assessment if they had specifically defined symptoms or severe illness during a home visit.

CD4+ T cell counts and HIV RNA VL were measured at three-monthly intervals. Field workers completed weekly client monitoring forms that included information on client symptoms, problems with taking medication or other information which might impact participant health. All diagnoses of OIs were presented at the weekly medical case conference and reviewed by the medical team as a whole. For diagnosis of an OI, we considered all WHO Stage 3 or 4 illnesses and we also included malaria. While not technically an opportunistic illness, which usually refers to WHO Stage 3 or 4 illnesses, malaria is one of the most common causes of illness and death among HIV-positive individuals in endemic areas of sub-Saharan Africa. As well malaria infections have been shown to affect HIV VL in HIV-positive individuals not on ART [2].

Diagnosis and measurement methods
Cryptococcal disease was diagnosed by compatible symptoms and serum cryptococcal antigen testing (Crypto-LA, Wampole Laboratories, Cranberry, NJ). Pulmonary tuberculosis (TB) was defined as two sputum smears positive for acid-fast bacilli using microscopy or negative sputum smears with a chest radiograph compatible with TB and a lack of response to a two-week trial of antibiotic therapy. Extra-pulmonary TB was diagnosed by clinical presentation and infrequently by lymph node biopsy and pathological confirmation. Diagnoses of Kaposi’s sarcoma and cervical cancer were based on biopsy results. Pneumocystis jirovecii pneumonia was diagnosed clinically using chest radiography, clinical presentation and a response to cotrimoxazole treatment. All illnesses were diagnosed by physicians at the study clinic in Tororo and reviewed at the weekly medical case conferences.

We measured HIV VLs using Cobas Amplicor HIV-1 Monitor version 1.5 (Roche, Branchburg, NJ) and enumerated CD4+ T cell count using TriTEST reagents following an in-house dual platform protocol and MultiSET and Attractors software using an FACSscan or FACSCalibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ).

We estimated adherence using pill count data stored in a computerized pharmacy database as: number of pills delivered minus number of pills returned divided by number of pills delivered.

Data analysis methods
Data were entered using Epi Info (CDC, Atlanta, GA) and analyzed using SAS (SAS Institute, Cary, NC).

To assess the effect of OIs on HIV RNA VL and CD4+ T cell count among participants with suppressed HIV RNA VL, we compared the elevation in HIV RNA VL to detectable levels and CD4+ T cell count changes between measurement intervals when participants had episodes of OIs and intervals when they did not have OIs. We based our analysis on quarterly measurement intervals that started with undetectable HIV RNA VL (≤ 50 copies/mL). We conducted analysis with two outcome measures, i.e., detectable HIV RNA VL (> 50 copies/mL) in measurements that followed a previous quarterly measurement with undetectable HIV RNA VL, and rate of CD4+ T cell count change within measurement intervals. For the analysis of the effect of OIs on detectable HIV RNA VL, we used a logistic regression model using a log-link function. For the analysis of rate of change in CD4+ T cell count, we used a linear regression model. In both models, generalized estimating equation method with an exchangeable correlation structure was used to take into account the dependence of observations due to repeated measures for the same individuals. Covariates that were considered in the models were gender, duration on ART at the start of the interval, age, BMI, ART adherence and first-line ART regimen.

For participants who had an OI following undetectable HIV RNA VL, we plotted the subsequent changes in CD4+ T cell count over time, starting from the time of the undetectable HIV RNA VL preceding the OI. For comparison, we also plotted a similar graph for those who never had OIs, starting from the time of their first undetectable HIV RNA VL.

Results
Of the 1094 antiretroviral-naïve clients who were started on ART, 47 had no follow up HIV RNA VL or CD4+ T cell count measurements after initiating ART and were excluded from the analysis. Of the remaining 1047 participants, 73% were female, the median age was 38 years, the median CD4+ T cell count at enrolment was 131 cells/µL (IQR = 72–196) and median HIV RNA VL at enrolment was 207,787 copies/mL (IQR = 69,100–492,000) (Table 1).

The median interval between lab measurements was 91 days, and the 1047 people included in the analysis had
Table 1. Baseline characteristics of HIV-positive adults who were enrolled between May 2003 and April 2007

| Characteristic                             | N = 1047 | %   |
|--------------------------------------------|----------|-----|
| Gender                                     |          |     |
| Female                                     | 767      | 73.3|
| Male                                       | 280      | 26.7|
| Education level                            |          |     |
| None                                       | 562      | 54.7|
| Primary                                    | 218      | 21.2|
| Post-primary                               | 248      | 24.1|
| Missing                                    | 19       |     |
| Median age in years (IQR)                  | 38 (32–43)|   |
| Baseline CD4+ T cell count in cells/μL     |          |     |
| < 50                                       | 194      | 18.5|
| 50–200                                     | 622      | 59.4|
| > 200                                      | 231      | 22.1|
| Median (IQR)                               | 131 (70–196)|  |
| Baseline viral load (copies/mL)            |          |     |
| < 1000                                     | 8        | 0.8 |
| 1000–9999                                  | 42       | 4.0 |
| 10,000–99,999                              | 298      | 28.5|
| ≥ 100,000                                  | 699      | 66.8|
| Median (IQR)                               | 207,394 (69,100–492,000)|   |
| Body mass index (kg/m²)                    |          |     |
| < 18.5                                     | 299      | 29.4|
| 18.5–24.9                                  | 661      | 65.1|
| 25–29.9                                    | 46       | 4.5 |
| ≥ 30                                       | 10       | 1.0 |
| Missing                                    | 31       |     |
| Median (IQR)                               | 19.8 (18.2–21.5)|   |
| First-line ART regimen                     |          |     |
| Efavirenz + lamivudine + stavudine          | 33       | 3.2 |
| Nevirapine + lamivudine + stavudine         | 1014     | 96.8|

Table 2. Association between opportunistic illness and HIV RNA viral load elevation to detectable levels

| Variable                                      | Female OR (95% CI) | p    | Male OR (95% CI) | p    | All OR (95% CI) | p    |
|------------------------------------------------|--------------------|------|------------------|------|----------------|------|
| Had OI in the previous three months           | 1.53 (0.19–12.65)  | 0.693| 4.87 (2.13–11.16)| <0.001| 3.82 (1.73–8.39)| 0.001|
| CD4+ T cell count at start of interval ('00) | 1.11 (1.02–1.20)   | 0.020| 1.03 (0.96–1.10) | 0.419| 1.06 (1.01–1.12)| 0.032|
| Duration on ART at start of interval (in months) | 0.98 (0.96–1.01) | 0.143| 0.98 (0.97–1.00) | 0.029| 0.98 (0.97–0.99) | 0.005|
| Age (in years)                               | 0.98 (0.96–1.00)  | 0.126| 1.01 (0.99–1.02) | 0.484| 1.00 (0.98–1.01) | 0.559|
| BMI at baseline                              | 1.05 (0.97–1.15)  | 0.246| 0.96 (0.93–1.01) | 0.090| 0.98 (0.95–1.02) | 0.289|
| Adherence preceding the interval             | 0.71 (0.58–0.88)  | 0.001| 0.87 (0.74–1.03) | 0.108| 0.81 (0.71–0.92) | 0.001|
| First-line ART regimen                       |                    |      |                  |      |                 |      |
| Efavirenz + lamivudine + stavudine           | 1.01 (0.33–3.10)   | 0.979| 1.13 (0.56–2.28) | 0.740| 1.07 (0.60–1.92) | 0.818|
| Nevirapine + lamivudine + stavudine          | 1.00               | ref  | 1.00             | ref  | 1.00             | ref  |
| Female                                       | 0.70 (0.54–0.91)   | 0.007|                  |      |                 |      |
IQR = 1304–82,800 copies/mL) compared to those who had not had an OI (126 copies/mL; IQR = 68–399 copies/mL).

**Effect of OIs on CD4+ T cell counts**

Table 3 shows models for the rate of CD4+ T cell count change per three months. CD4+ T cell count decreased at a rate of 24.1 cells/µL per three months in intervals when participants had OIs, compared to an increase of 21.3 cells/µL per three months in intervals when they had no episodes of OIs. After controlling for age, baseline BMI, CD4+ T cell count at the start of the interval, duration on ART, first-line ART regimen and ART adherence, the adjusted difference in the rate of CD4+ T cell count change was 60.7 cells/µL per three months (95% CI = 12.6–108.8; \( p = 0.013 \)), comparing intervals when participants had OIs to intervals when they did not have OIs. Other factors that were significantly associated with the rate of CD4+ T cell count change were previous three-month CD4+ T cell count, duration on ART and gender. After controlling for OIs, age, CD4+ T cell count at the start of the interval and BMI, the rate of CD4+ T cell count increase was on average 25.7 cells/µL per three months (95% CI = 11.6–39.7) higher for females compared to males (\( p = <0.001 \)). The rate of increase was negatively associated with older age (1.5 cells/µL per three months lower per one year increase in age; \( p = 0.044 \)) and CD4+ T cell count values at the start of the interval (0.4 cells/µL per three months lower per unit increase in CD4+ T cell count at start of the interval, \( p = <0.001 \)).

Figure 1 shows changes in CD4+ T cell count over time since the undetectable HIV RNA VL preceding the OI or since first detectable HIV RNA VL for those without OIs. The graph showed a decline in CD4+ T cell count in the three-month period following an episode of OI. Though the CD4+ T cell count appears to improve thereafter, the CD4+ T cell counts of those who were observed beyond one and a half years appear to continue declining among participants who had an OI.

An analysis for effects of OIs on changes in CD4+ T cell count percent showed CD4+ T cell count percent to decrease at a rate of 0.04 percentage points per three months in intervals when participants had OIs, compared to an increase of 0.8 percentage points per three months in intervals when they had no episodes of OIs. However, the adjusted difference was not statistically significant (adjusted difference = 1.77; 95%CI = 0.93–4.48; \( p = 0.198 \)).

**Discussion**

In this analysis, we found an association between having had an opportunistic illness and short-and long-term effects on HIV RNA VL and CD4+ T cell count among people taking ART. Participants who had an OI following an assessment in which their HIV RNA VL was undetectable had four times the odds of having a detectable HIV RNA VL in the following three-month assessment compared to when there was no episode of an OI (OR = 3.8; 95% CI = 1.7–8.4). In addition, participants had a mean decline of 24.1 CD4+ T cells/µL per three months in intervals during which they had episodes of OIs compared to a mean increase of 21.3 CD4+ T cells/µL per three months in intervals during which they had no episodes of OIs. We also observed that participants who had episodes of OIs tended to have declines in CD4+ T cell count in the long run (figure 1).

Though our results on elevation of VL were based on a small number of OIs and elevated VLs, our findings are similar to what has been observed among HIV-positive

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**Table 3. Association between opportunistic illness and change in CD4+ T cell count per three months**

| Variable                        | Female                  | Male                           | All                        |
|---------------------------------|-------------------------|--------------------------------|---------------------------|
|                                 | Coefficient (95% CI)    | \( p \)                        | Coefficient (95% CI)       | \( p \)                   | Coefficient (95% CI)    | \( p \)                   |
| Had OI in the previous three    | –59.0 (–125.6 to 7.5)   | 0.082                         | –60.6 (–119.6 to –1.6)     | 0.044                    | –60.7 (–108.8 to –12.6) | 0.013                    |
| months                          |                         |                                |                           |                         |                           |                           |
| CD4+ T cell count at start of   | –0.9 (–1.0 to –0.7)     | <0.001                        | –0.4 (–0.5 to –0.3)        | <0.001                   | –0.4 (–0.5 to –0.3)      | <0.001                    |
| interval                        |                         |                                |                           |                         |                           |                           |
| Duration on ART at start of     | 3.3 (2.4 to 4.2)        | <0.001                        | 3.9 (0.9 to 6.8)           | 0.010                    | 3.4 (1.2 to 5.6)         | 0.003                    |
| interval (in months)            |                         |                                |                           |                         |                           |                           |
| Age (in years)                  | –2.0 (–3.9 to –0.2)     | 0.033                         | –1.6 (–3.6 to 0.4)         | 0.112                    | –1.5 (–2.9 to –0.0)      | 0.044                    |
| BMI at baseline                 | 8.6 (0.8 to 16.4)       | 0.031                         | 1.5 (–0.8 to 3.9)          | 0.199                    | 1.8 (–0.3 to 4.0)        | 0.098                    |
| Adherence preceding the         | –5.2 (–19.0 to 8.6)     | 0.463                         | 9.9 (–0.3 to 20.1)         | 0.056                    | 7.0 (–1.1 to 15.1)       | 0.088                    |
| interval                        |                         |                                |                           |                         |                           |                           |
| First-line ART regimen          |                         |                                |                           |                         |                           |                           |
| Efavirenz + lamivudine +        | –9.8 (–71.3 to 51.8)    | 0.756                         | –23.0 (–51.7 to 5.6)       | 0.115                    | –18.5 (–40.2 to 3.1)     | 0.093                    |
| stavudine                       |                         |                                |                           |                         |                           |                           |
| Nevirapine + lamivudine +       | ref                     |                                | ref                       | ref                     | ref                       | ref                       |
| stavudine                       |                         |                                |                           |                         |                           |                           |
| Female                          |                         |                                | 25.7 (11.6 to 39.7)        | <0.001                   |                           |                           |

Ekwaru JP et al. *Journal of the International AIDS Society* 2013, 16:17355
http://www.jiasociety.org/index.php/jias/article/view/17355 | http://dx.doi.org/10.7448/IAS.16.1.17355
In conclusion, our findings show that episodes of OIs among HIV-infected adults with suppressed VL while taking ART were associated with elevations in VL and reduced improvement in CD4+ T cell counts. Prevention of OIs is therefore important even among patients on ART who have attained suppressed VLs.

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Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JPE analyzed the data and wrote the paper. JM and DMM participated in the design and management of study implementation, reviewed and edited the manuscript. JC and WW implemented study procedures, reviewed and edited the manuscript. SM reviewed and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgements
The authors would like to thank the participants in the HBAC project, the CDC-Uganda staff who cared for the participants, collected and compiled the data for analysis. We also acknowledge the support of the Ugandan Ministry of Health and The AIDS Support Organization (TASO).

Funding: Funding for the study was provided by the U.S. Department of Health and Human Services/CDC through the Emergency Plan for AIDS Relief. John Paul Ekwaru was also supported by the Fogarty AIDS International Training and Research Program (1-D43-TW00803) at the University of California, Berkeley.

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