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Prognostic Value of HIV-1 RNA on CD4 Trajectories and Disease Progression Among Antiretroviral-Naive HIV-Infected Adults in Botswana: A Joint Modeling Analysis

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Abstract

Although HIV-1 RNA levels are measured at the time of initial diagnosis, the results are not used for the clinical follow-up of the patients. This study evaluates the prognostic value of the baseline HIV-1 RNA levels (above or below 10,000 copies/ml) on rate of disease progression, among antiretroviral therapy (ART)-naive patients in Botswana. A prospective cohort of 436 HIV-infected ART-naive adults with baseline CD4 > 400 cells/mm^3 were followed quarterly for 5 years in an urban clinic in Botswana. Baseline HIV-1 RNA levels and longitudinal CD4$^+$ T-cell count data were analyzed, using mixed-effects regression jointly modeled with the times to a composite endpoint defined by AIDS-defining clinical conditions or death. During 1,547 person-years (PYs) follow-up time, 106 individuals became eligible for ART initiation (incidence rate: 0.07 PYs) and 6 participants died of AIDS-related illness. There were 203 (47%) individuals with baseline HIV-1 RNA <10,000 copies/ml and 233 (53%) individuals with baseline RNA >10,000 copies/ml. The slope of the predicted CD4 trajectory for individuals with baseline HIV-1 RNA >10,000 copies/ml is 30% steeper than that for those with baseline RNA <10,000. The hazard of reaching the composite endpoint for the individuals with baseline HIV-1 RNA >10,000 copies/ml was 2.3 (95% confidence interval: 1.5–3.0) times higher than that for those with baseline HIV-1 RNA <10,000 copies/ml. CD4 decline in individuals with HIV-1 RNA >10,000 copies/ml is much faster than that in those with RNA <10,000. The elevated HIV-1 RNA can be used as a marker to identify individuals at risk of faster disease progression.

Introduction

The monitoring of HIV-infected patients is based on measurements of plasma HIV-1 RNA load (viral load) and CD4$^+$ T-cell count (CD4) in the blood. The association between levels of these two markers in HIV-infected individuals has been extensively demonstrated.[1–3] However, because monitoring of routine CD4 is considerably more feasible and cost-effective than that of viral load, CD4 has been considered a hallmark of disease progression in HIV-infected people[4]; hence, it is one of the most important criteria for the initiation of antiretroviral therapy (ART) since the beginning of HIV/AIDS epidemic. WHO guidelines have recommended using viral load testing only as the preferred approach to monitoring the success of ART and diagnosing treatment failure, along with CD4 and clinical monitoring.[5] Although viral load is measured at the time of initial diagnosis, the results are not used for the clinical follow-up of the patients.[5]

Previous studies demonstrated prognostic values of viral load in HIV-1 subtype B settings,[2,3] using time-to-event approach. The purpose of this study is to determine the prognostic value of viral load in HIV-1 subtype C infection in southern Africa using a joint modeling approach.

Materials and Methods

Ethics statement

Informed written consent was obtained from all participants, and the study was approved by Harvard School of Public Health's Institutional Review Board and the Health Research Development Committee in Botswana.

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Study population

We established a clinical cohort of 442 HIV-infected ART-naive individuals in Gaborone, Botswana, in 2005. The study aimed to observe HIV disease progression among individuals infected with HIV-1 subtype C who did not qualify for ART according to Botswana national guidelines ($CD4^+$ T-cell count ≥200 per mm^3 and a WHO clinical stage I or II) at the time of enrollment. During the longitudinal follow-up, participants visited clinics quarterly, including 1 month after enrollment. The baseline plasma HIV-1 RNA was obtained from all the participants at enrollment. The objectives of the study were (i) to determine the kinetics of HIV-1 subtype C disease progression (ii) to estimate the rate of $CD4^+$ T-cell count decline, and (iii) to analyze the time to first HIV-associated or AIDS-defining condition or death in persons with initial $CD4^+$ T-cell count >400 per mm^3. Observations spanned from April 12, 2005 to November 30, 2009.

In January 2002, the national antiretroviral (ARV) treatment program was launched in Botswana. According to the national guidelines at that time, individuals infected with HIV became eligible for treatment if they had symptomatic disease (WHO adult stage IV and advanced stage III disease) irrespective of the $CD4^+$ T-cell count, or when the $CD4^+$ T-cell count fell below 200 per mm^3. The revised guidelines in 2008 changed the threshold for treatment initiation to 250 CD4 cells/mm^3. Therefore, the endpoint of the study was ART eligibility according to the national guidelines or death. The inclusion criteria were HIV-positive adults with $CD4^+$ T-cell count ≥400 per mm^3 at least 90 days before the enrollment. The dates of seroconversion were unknown. The baseline viral load in this study is assumed to reflect the viral set point in the asymptomatic stage. To exclude the possibility that some of these participants may have been enrolled during the acute infection period, however, we computed the observed change in plasma HIV-1 RNA levels during the first 6 and 12 months after the baseline observation and targeted for detailed review those persons found to have experienced a decrease in HIV-1 RNA of 1.0 log or greater during the first 6 months. Since all participants were unexposed to ART, a significant decrease in plasma HIV RNA level during the early observation period was considered to be a possible indicator of recent infection. Six participants were considered to represent probable early HIV infection and were excluded from further analysis.

For this analysis, we considered the last $CD4^+$ T-cell counts measured for the patient before transfer to the national treatment program. The composite outcome event was AIDS-defining clinical conditions or death.

Statistical analysis

Factors between groups (above or below 10,000 copies HIV-1 RNA at enrollment) were compared using the chi-squared test for categorical variables and the Mann–Whitney *U* test for continuous variables. Summary results are expressed as medians (with 25th and 75th quartiles) or percentages. Kaplan–Meier survival curves for estimated event-free time were generated for the two groups and compared with the log-rank test.

To assess the effect of the baseline viral loads on AIDS-free survival, taking into account the repeatedly measured $CD4^+$ T-cell count data, we employ a joint modeling approach. Comparing with the approach using a Cox proportional hazard model with CD4 data as time-varying covariates, the joint modeling approach permits the assessment of viral load on CD4 trajectories over time and also provides more efficient estimates of the effects of baseline viral load on time-to-event outcomes.[6]

Table 1. Baseline Patients' Characteristics Included in the Analysis

Viral load category	*Statistics*	*Age*	*Baseline CD4*	*Viral load (log 10)*	*Female*[a] *(%)*
RNA <10,000, $N=203$	Mean	33	565	3.2	88
	SD	8.3	190	0.49	
	25th percentile	27	430	2.6	
	50th percentile	31	534	3.4	
	75th percentile	37	670	3.7	
	Minimum	20	217	2.6	
	Maximum	63	1,196	4	
RNA >10,000, $N=233$	Mean	34	464	4.7	76
	SD	8.4	156	0.47	
	25th percentile	27	349	4.3	
	50th percentile	32	442	4.6	
	75th percentile	40	535	5	
	Minimum	19	216	4	
	Maximum	57	1,047	5.9	
Total, $N=436$	Mean	33	511	4	82
	SD	8.4	180	0.86	
	25th percentile	27	385	3.4	
	50th percentile	32	475	4.1	
	75th percentile	39	602	4.7	
	Minimum	19	216	2.6	
	Maximum	63	1,196	5.9	

[a]Indicates percentage of women in each category.
SD, standard deviation.

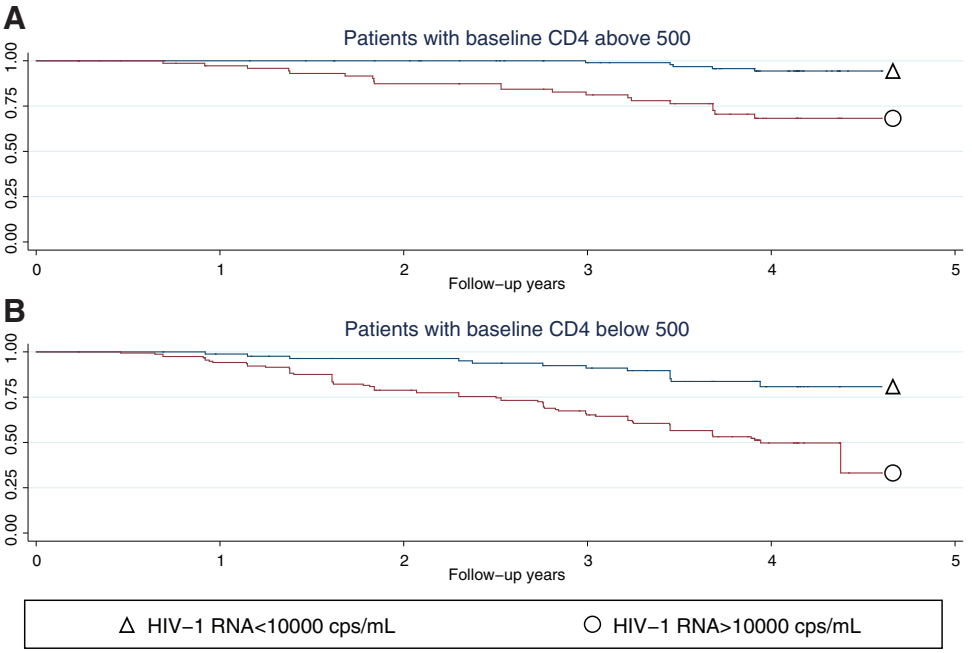


FIG. 1. Kaplan–Meier survival curves, time to composite endpoint (AIDS-defining clinical conditions or death) stratified by baseline viral load (**A**). Patients with baseline CD4 > 500 (**B**), patients with baseline CD4 < 500. Color images available online at www.liebertpub.com/aid

We fitted a series of joint longitudinal–survival models with several options for the longitudinal and survival submodels and their combination to find the best fit, using the likelihood ratio test. The models were adjusted for age and gender. We modeled the longitudinal outcome using random effects models as they permit the investigation of mean change of CD4 while allowing for between-individual variability around population average parameters. In the longitudinal model, we took the natural logarithm of CD4 for a better goodness of fit. To model the hazard of our composite time-to-event outcome, we used a Weibull model. To implement the model, we used the user-written stjm command in Stata 14.0.[7] In this joint model, to calculate an overall log-hazard ratio for the effect of viral load on the outcome, we used the following formula:

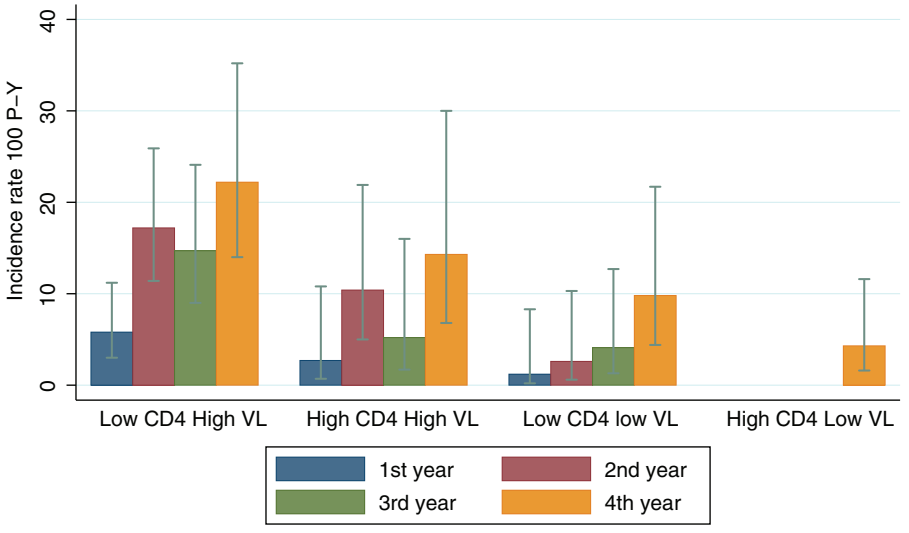
$$\gamma\alpha + \beta,$$

where α is the effect of the baseline viral load on the longitudinal CD4, β is the effect of baseline viral load on the time to

TABLE 2. KAPLAN–MEIER ADJUSTED FAILURE FUNCTION

Follow-up time (years)	*RNA <10,000*	*RNA >10,000*
1	0.007	0.059
2	0.023	0.221
3	0.064	0.353
4	0.167	0.518

Failure function adjusted for baseline CD4.



High CD4>500; Low CD4<500; high viral load>10,000; low viral load<10,000
Error bars represent 95% confidence interval

FIG. 2. Incidence rate of the composite endpoint (AIDS-defining clinical conditions or death) by baseline viral load and CD4 categories for 4 years of follow-up. Color images available online at www.liebertpub.com/aid

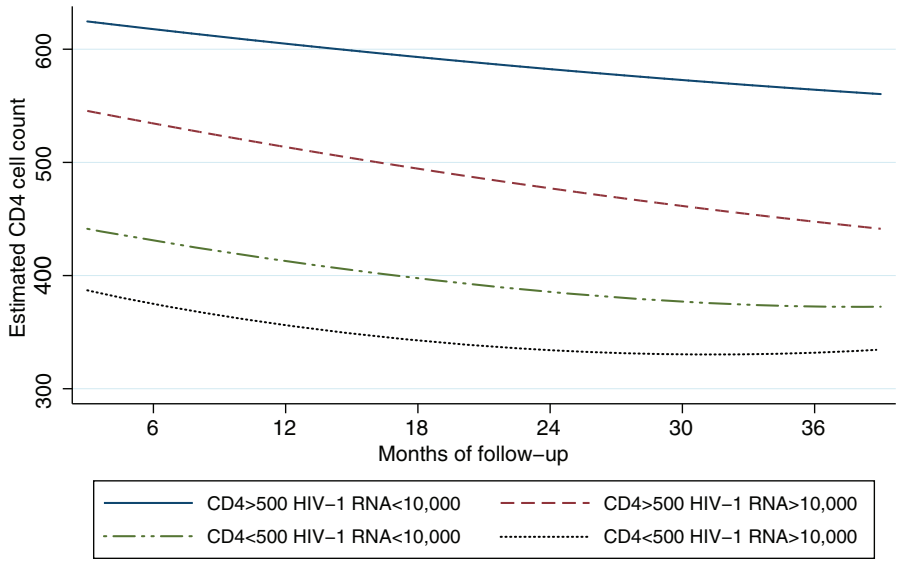


FIG. 3. Predicted CD4-trajectory curves by baseline viral load and CD4 categories for 4 years of follow-up, using joint modeling of longitudinal and survival data. Color images available online at www.liebertpub.com/aid

event, and γ is the effect of the longitudinal CD4 on the time to event.[6] The overall effect was calculated using Stata's nlcom (nonlinear combinations of coefficients) command.

Results

A total of 436 participants were followed up for 5 years. Table 1 contains descriptive statistics for the patients included in the analysis. During 1,547 person-years (PYs) follow-up time, 102 individuals had AIDS-defining clinical conditions (incidence rate (IR): 0.07 PYs) and 6 participants died of AIDS-related illness. There were 203 (46.6%) individuals with baseline HIV-1 RNA <10,000 copies/ml [of whom 87 individuals (43%) had baseline CD4 < 500 cells/mm^3 and 116 individuals (57%) had baseline CD4 > 500] and 233 individuals (53%) with baseline HIV-1 RNA >10,000 copies/ml [of whom 156 individuals (67%) had baseline CD4 < 500 cells/mm^3 and 77 individuals (33%) had baseline CD4 > 500].

There were 356 women (82%) and 80 men (18%) in the cohort. Median age of the participants was 33 years old [interquartile range (IQR): 27–39], with the youngest participant being 19 years old and the oldest being 63 years old. Median follow-up was 48 months (IQR: 36–54). The shortest follow-up time was 3 months and the longest follow-up time was 60 months. Out of 102 (23.4%) participants who became eligible for ART during the study, only 18 (17.7%) individuals had baseline HIV-1 RNA <10,000 copies/ml; at the end of the follow-up period, 280 (64.2%) participants were transferred to the national ART program for further follow-up. There were 48 (11.0%) lost-to-follow-up participants.

Median baseline viral load was at 12,500 copies/ml (IQR: 2,600–45,000). In about 55 (12.6%) of the samples, baseline viral load was below 400 (the quantification limit of the assay). The correlation between baseline viral load and CD4 was weak (Spearman's $r = -0.23$) but statistically significant ($p < .001$).

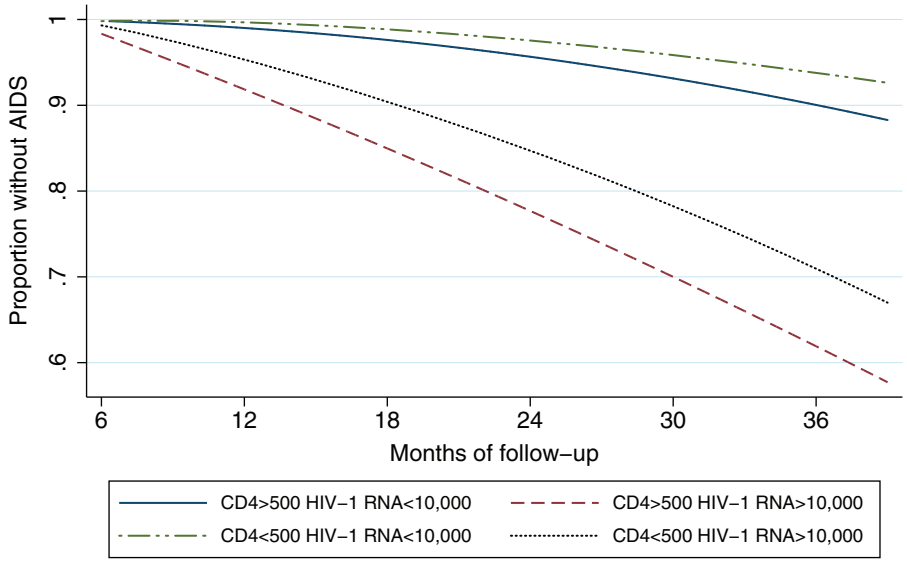


FIG. 4. Predicted AIDS-free survival probability curves by baseline viral load and CD4 categories for 4 years of follow-up, using joint modeling of longitudinal and survival data. Color images available online at www.liebertpub.com/aid

For the purpose of this analysis, the participants were divided into four categories: (1) ''HIV-1 RNA <10,000, CD4 > 500''; (2) ''HIV-1 RNA >10,000, CD4 > 500''; (3) ''HIV-1 RNA >10,000, CD4 < 500''; and (4) ''HIV-1 RNA <10,000, CD4 < 500.'' The large number of the participants, 156 (35.8%) individuals, were in category 3, ''HIV-1 RNA >10,000, CD4 < 500,'' followed by category 1, ''HIV-1 RNA <10,000, CD4 > 500'' with 116 individuals (26.6%). There were 87 (20%) individuals with HIV-1 RNA <10,000 copies/ml in the low CD4 category (400 < CD4 < 500 cell count), whereas 77 (17.7%) participants had HIV-1 RNA >10,000 copies/ml in the highest CD4 category (>500 cell count). Highest incidence of AIDS or death was in category 3, ''HIV-1 RNA >10,000, CD4 < 500'' (156 participants, 45%), as compared to only 5 participants (4%) in category 1, ''HIV-1 RNA <10,000, CD4 > 500.'' Log-rank test, stratified by baseline CD4, showed that the individuals with HIV-1 RNA >10,000 copies/ml are significantly different in terms of AIDS-free survival than those with low viral loads ($p < .0001$).

The relationships between baseline viral load and baseline CD4 and progression to AIDS or death were examined with Kaplan–Meier survival curves and log-rank tests (Fig. 1). Kaplan–Meier estimates of the proportion of individuals who progressed to either AIDS or death, stratified by baseline viral load and CD4, revealed that baseline viral load provided excellent discrimination of time to AIDS or death (Mantel–Haenszel test, $p < .001$). Table 2 shows Kaplan–Meier estimates stratified by baseline CD4 of the probability of developing the composite outcome (AIDS or death) for 5 years of follow-up. In each year of follow-up, the probability of reaching the endpoint for HIV-infected individuals with HIV-1 RNA >10,000 copies/ml is much higher than that for those with HIV-1 RNA <10,000, adjusted for baseline CD4.

Additional evidence of the importance of baseline viral load in influencing prognosis is shown in Figure 2 and illustrates AIDS or death incidence rate in different categories. It shows that AIDS or death incidence rate in high viral load groups is higher than in low viral load groups. For example, the AIDS or death incidence rate in the first year of follow-up for the participants in category 4, ''HIV-1 RNA <10,000, CD4 < 500,'' is 1.2 PYs, which is lower than that for the participants in category 2, ''HIV-1 RNA >10,000, CD4 > 500,'' at 2.7 PYs. This gap widens in the second year, from 2.6 PYs in category 4 to 10.6 PYs in category 2.

Using joint modeling, we observed a statistically significant effect of viral load on AIDS-free survival. We found a statistically significant direct effect of the baseline viral load on CD4 count.* Controlling for age, and gender, viral load is the most important factor determining the level and the slope of the curve. Using the nonlinear combination of regression coefficients ($\gamma\alpha + \beta$), we estimated that the hazard of developing AIDS or death for the individuals with baseline HIV-1 RNA >10,000 to be 2.3 [95% confidence interval (95% CI): 1.5–3.0] times of the hazard for those with baseline HIV-1 RNA <10,000 ($p < .0001$). To make the results more tangible, we plotted the predicted trajectory of CD4 (Fig. 3) and the predicted AIDS-free survival function (Fig. 4) in low (solid line) and high (dashed line) viral load categories. Individuals with higher viral load are predicted to have lower CD4 and the rate of disease progress is faster. Predictive margins show that CD4 depletion in individuals with baseline HIV-1 RNA >10,000 copies/ml is, on average, 17% (95% CI: 11–22)† faster than that in those with baseline HIV-1 RNA <10,000 copies/ml.

Among our study population, there were 13 confirmed cases of tuberculosis (TB) and three cases of hepatitis B virus (HBV). No cases of hepatitis C virus (HCV) were observed. We explored the effect of these coinfections on disease progression by including TB or HBV as covariates in our model and did not find any statistically significant effect of these coinfections on CD4 depletion or AIDS-free survival.

Discussion

We analyzed the incidence and probability estimates of the development of clinical AIDS or death among HIV-infected ART-naive individuals for 5 years in an urban clinic in Botswana. This cohort study of 436 individuals confirmed that patients with different levels of baseline HIV-1 RNA and CD4 follow different trajectories before ART initiation. Baseline viral load can be indicative of the patient's prognosis.

We found that CD4 depletion is much faster in patients with higher viral load. The Kaplan–Meier probability estimates showed that during each year of follow-up, the probability of the patients with baseline HIV-1 RNA >10,000 copies/ml having the composite outcome is much higher than that of those with HIV-1 RNA <10,000 copies/ml. The joint modeling showed that the hazard of developing the composite outcome for the individuals with baseline HIV-1 RNA >10,000 copies/ml to be 2.3 (95% CI: 1.5–3.0) times higher than that for those with baseline HIV-1 RNA <10,000 copies/ml ($p < .0001$). This is in line with the previous studies' finding that higher viral load was associated with shorter period between seroconversion and the onset of AIDS or eventual death.[2,8]

It has been shown that the virulence and the replicative capacity of the virus are highly correlated.[9] Therefore, viral set point during the asymptomatic stage of the disease has been used as a proxy for virulence of the HIV virus.[10] An individual with a high (low) viral load is most probably infected with a strain of HIV-1 that has a high (low) replicative capacity.[11] Because of the high level of virulence, the participants in this study with higher viral load developed AIDS, or die, faster. Individuals with high viral load may benefit from earlier ARV treatment initiation.

Comment

There are both clinical and public health benefits of using viral load as a yardstick to treatment initiation in resource-limited settings. From a clinical perspective, higher viral load is associated with faster CD4 decline and clinical disease progression. Therefore, treatment initiation based on viral load level may improve patient's clinical outcome. Moreover, from the public health angle, treatment initiation for individuals with high viral load may affect rates of HIV transmission.

*In this model, if $\gamma = 0$, then there is no association between CD4 and the event time, which implies the information from the longitudinal model does not improve the survival estimate.

†The results are expressed in percentage, because the outcome variable in the longitudinal model was in natural log.

Higher viral load also increases the likelihood of HIV transmission. Patients with high viral load are more likely to transmit the virus than an individual with lower viral load, either through vertical[12] or horizontal transmission. In a community-based study of 15,127 persons in a rural district of Uganda, viral load was found to be the chief predictor of the risk of heterosexual transmission of HIV-1.[13] A systematic review found that higher viral load consistently increased rates of HIV transmission.[14]

Treatment initiation for patients with high viral load can reduce the chance of virus transmission. A meta-analysis of the literature on sexual transmission of HIV showed that overall rate of transmission dropped from 5.65 per 100 PYs for heterosexual couples not on ART to 0.46 per 100 PYs for those on ART, a 92% reduction.[15] Another systematic review shows that risk of HIV sexual transmission among heterosexual discordant couples is minimal after the HIV-infected partner on ART is fully suppressed.[16]

Although the 2013 WHO guidelines recommend ART be initiated in all individuals with HIV with $CD4^+$ T-cell count ≤ 500 per mm^3 regardless of WHO clinical stage[5], Botswana ART guidelines, like those in many other resource-limited settings, as of October 2015, recommend ART initiation for adults with CD4 < 350 per mm^3. Therefore, there are a large number of HIV-infected individuals, particularly in sub-Saharan countries who may benefit if the guidelines are revised. A randomized clinical trial, the Botswana Combination Prevention Program, is evaluating the impact of treating individuals with high viral load on reducing population-level HIV incidence in rural communities. The results of this study may help to guide HIV prevention strategies in southern African countries.

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Author Disclosure Statement

No competing financial interests exist.

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