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# *Plasmodium falciparum* Infection Does Not Affect Human Immunodeficiency Virus Viral Load in Coinfected Rwandan Adults

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**Background.** *Plasmodium falciparum* infection has been reported to increase human immunodeficiency virus (HIV) viral load (VL), which can facilitate HIV transmission. We prospectively studied the impact of mild *P falciparum* coinfection on HIV VL in Rwanda.

**Methods.** We measured plasma HIV VL at presentation with malaria infection and weekly for 4 weeks after artemether-lumefantrine treatment in Rwandan adults infected with HIV with *P falciparum* malaria. Regression analyses were used to examine associations between malaria infection and HIV VL changes. Samples with detectable virus underwent genotypic drug-resistance testing.

**Results.** We enrolled 28 HIV-malaria coinfecting patients and observed 27 of them for 5 weeks. Three patients (11%) were newly diagnosed with HIV. Acute *P falciparum* infection had no significant effect on HIV VL slope over 28 days of follow-up. Ten patients with VL <40 copies/mL at enrollment maintained viral suppression throughout. Seventeen patients had a detectable VL at enrollment including 9 (53%) who reported 100% adherence to ARVs; 3 of these had detectable genotypic drug resistance.

**Conclusions.** Unlike studies from highly malaria-endemic areas, we did not identify an effect of *P falciparum* infection on HIV VL; therefore, malaria is not likely to increase HIV-transmission risk in our setting. However, routine HIV testing should be offered to adults presenting with acute malaria in Rwanda. Most importantly, we identified a large percentage of patients with detectable HIV VL despite antiretroviral (ARV) therapy. Some of these patients had HIV genotypic drug resistance. Larger studies are needed to define the prevalence and factors associated with detectable HIV VL in patients prescribed ARVs in Rwanda.

**Keywords.** antiretroviral drug resistance; HIV; malaria; *Plasmodium falciparum*; Rwanda.

Measuring human immunodeficiency virus (HIV) viral load (VL) allows for the monitoring of antiretroviral (ARV) therapy efficacy, and the level of plasma HIV

viremia has been positively correlated with the risk of perinatal and sexual transmission [1, 2]. Because HIV and malaria are co-endemic and have high potential for morbidity, it is important to understand the interactions of these 2 infections. *Plasmodium falciparum* infection has previously been reported to increase transiently the VL of individuals infected with HIV [3–6]. For example, Hoffman et al [4] prospectively observed 27 patients in Malawi after successful treatment for malarial infection and found a significant decline in HIV VL over 4 weeks. Using a similar study design, at health centers in and around Kigali, Rwanda, we examined the effect of *P falciparum* infection on HIV VL kinetics in adults infected with HIV.

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## METHODS

The study protocol was approved by the institutional review boards of the Albert Einstein College of Medicine and the Rwanda National Ethics Committee.

### Study Population

The study was conducted from January 2011 to April 2011 (the local malaria transmission season). Adults with mild, symptomatic *P falciparum* infection seeking care at Nyacyonga, Kabuye, Kagugu, and Masaka Health Centers and the Women's Equity in Access to Care and Treatment outpatient healthcare clinics in Kigali, Rwanda were invited to participate. Mild malaria was defined as any level of symptomatic *P falciparum* parasitemia without evidence of vital organ dysfunction [7]. Individuals who had a positive malaria smear as well as a positive HIV antibody test were offered enrollment in the study. Written informed consent was obtained from all study participants.

### Study Procedures

A positive *P falciparum* blood smear was confirmed by 2 experienced microscopists, and a positive HIV antibody test was determined using the Abbott Determine Rapid Test Strips for HIV1/2 (Abbott Laboratories, Princeton, NJ) and the Uni-Gold HIV Rapid Test (Trinity Biotech, Ireland). All patients diagnosed with *P falciparum* infection were treated with artemether-lumefantrine for 3 days. Patients newly diagnosed with HIV were provided with linkage to care. For patients with previously diagnosed HIV infection who were already in care, medical records were reviewed to obtain history of opportunistic infections, ARV history, and recent CD4<sup>+</sup> T cell counts. Patients returned to clinic weekly for 4 additional study visits.

At enrollment and at each of the 4 weekly follow-up visits, blood pressure, heart rate, temperature, respiratory rate, malaria smear, and self-report of ARV adherence were obtained, and plasma was collected by venipuncture using K<sub>2</sub> EDTA blood collection tubes (BD Vacutainer, Franklin Lakes, NJ). Plasma was centrifuged and aliquoted within 2 hours of collection. Samples were stored in -80°C at the National Reference Laboratory (NRL) Division of the Rwanda Biomedical Center in Kigali before shipment to the Montefiore Medical Center (New York) in a dry shipper. A complete blood cell count was performed for each patient at the NRL, and percentage of parasitemia was calculated from 5 different fields using the following formula:

$$\left( \frac{\text{number of asexual parasites}}{\text{number of RBC}} \right) \times 100.$$

Plasma HIV VL assessments were performed at Montefiore Medical Center using the Abbott RealTime HIV Assay (Abbott Laboratories) with <40 copies/mL as the lower limit of detection. Patients with HIV VL <40 copies/mL at the time of diagnosis of malaria infection and at days 14 and 28 after

artemether-lumefantrine treatment were considered to have undetectable VL. Patients who had HIV VL >40 copies/mL at any of these 3 predetermined time points were considered to have detectable VL and had additional HIV VL measurements performed on samples drawn at days 7 and 21 to allow a more detailed analysis of their HIV VL trajectory.

### Human Immunodeficiency Virus Resistance Testing Analysis

Genotypes were performed on samples with detectable HIV VL to identify ARV drug-resistance mutations. Plasma samples were further ultracentrifuged at 17 000 rpm for 1 hour at 4°C before RNA extraction (QIAamp Viral RNA Minikit; Qiagen, Valencia, CA), and the region encompassing the HIV protease and reverse-transcriptase genes was amplified by nested primers. cDNA was synthesized (Superscript III One-step RT-PCR with Platinum Taq Kit; Invitrogen Corp., Carlsbad, CA) following the manufacturer's instructions, and PCR products were sequenced (3730XL DNA Analyzer; Applied Biosystems, Foster City, CA). Resistance mutations were defined according to the International AIDS Society-USA Panel and confirmed by published mutations in the Stanford HIV Drug Resistance database (<http://hivdb.stanford.edu/>). Standard phylogenetic analyses ruled out sequence contamination.

### Data Analysis

Bivariate analysis was conducted using the Mann-Whitney *U* or Wilcoxon rank-sign test for baseline continuous variables and  $\chi^2$  or Fisher's exact test for baseline dichotomous variables when appropriate. To identify predictors, such as participant characteristics that significantly correlated with HIV VL changes, we applied random slope regression models to test the interaction between time and predictor. These random slope models account not only for subject-by-subject variations in slopes but also for correlations of repeated measures. We used SAS version 9.3 (SAS Institute Inc., Cary, NC) and STATA version 12 (College Park, TX) to conduct these analyses. A 2-tailed *P* < .05 was considered statistically significant.

## RESULTS

Twenty-eight Rwandan adults infected with HIV and presenting with mild *P falciparum* infection were enrolled. Twenty-seven patients completed the 5-week study. One patient was lost to follow-up after the first visit and was excluded from analyses. For the 27 remaining patients, median age was 34 years (interquartile range [IQR], 29–40) and 52% were men. Follow-up blood smears were negative for malaria parasites at each time point.

### Human Immunodeficiency Virus Disease

At enrollment, 24 of the 27 patients were already known to be infected with HIV, and 3 patients (11%) were newly diagnosed. Eighteen patients reported being prescribed ARVs, and 24

**Table 1. Characteristics of the Cohort By Detectable and Undetectable (<40 copies/mL) HIV VL Status\***

Characteristic	Undetectable HIV VL (n = 10)	Detectable HIV VL (n = 17)	P Value
Age (years)	36.5 (31.3–40.8)	31 (29–40)	.48
Sex (% females)	60%	41%	.44 <sup>†</sup>
Days Ill	4.5 (2.8–7.0)	3.0 (2.3–7.0)	.53
Parasitemia (%)	0.41 (0.39–5.0)	0.35 (0.28–0.44)	.56
Temperature (°C)	37 (36.7–37.6)	37 (36.4–37.2)	.38
Hematocrit	42.2 (40.8–48.2)	41 (37.4–42.8)	.20
WBCs (cells/μL)	5250 (4175–8000)	3050 (2475–4825)	.01
Lymphocytes (cells/μL)	1640 (1418–1868)	1340 (940–1770)	.24
CD4 <sup>+</sup> T cells (cells/μL)	596 (455–758.5)	395.5 (240–519.5)	.01
% on ARV	80%	53%	.23 <sup>†</sup>
% on Cotrimoxazole	100%	82%	.27 <sup>†</sup>

Abbreviations: ARV, antiretroviral; HIV, human immunodeficiency virus; VL, viral load; WBC, white blood cell count.

\* Median and interquartile ranges are provided; geometric mean is reported for parasitemia. P Values are calculated using the Mann-Whitney U test.

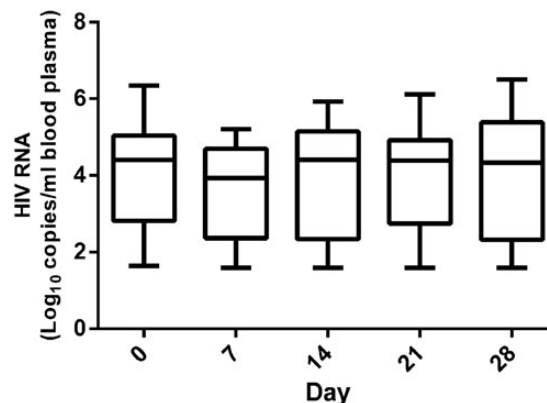
<sup>†</sup> Fisher's exact.

patients reported being prescribed co-trimoxazole; all patients reported 100% adherence to medications. All ARV regimens included a combination of nucleoside reverse-transcriptase inhibitors (NRTIs) and a non-NRTI (NNRTI) (efavirenz or nevirapine). Median CD4<sup>+</sup> T cell count was 484 cells/μL (IQR, 303–629), and for patients with known HIV infection at enrollment these values were recorded from within a median of 5 months (range, 3–19 months) before study entry; 1 newly diagnosed patient had no CD4<sup>+</sup> T cell count available. Presenting symptoms included fever, myalgias, headache, joint pain and gastrointestinal symptoms, and lack of clinical indicators of severe malaria. The geometric mean of percent parasitemia was 0.37%.

Ten of 27 patients were categorized as having an undetectable VL (<40 copies/mL) at enrollment and at days 14 and 28. Two of the patients with undetectable HIV VL did not report taking ARVs. The remaining 17 patients had detectable VL. There were no significant differences between the group with undetectable VL and detectable VL with regard to age, sex, number of days ill, temperature, hematocrit, and percentage of parasitemia. The group with undetectable VL had significantly higher CD4<sup>+</sup> T cell (596 cells/μL vs 395.5 cells/μL,  $P = .01$ ) and leukocyte counts (5250 cells/μL vs 3050,  $P$  value = .006) compared with the group with detectable VL, regardless of ARV prescription history (Table 1).

#### Effect of *Plasmodium falciparum* Infection on Human Immunodeficiency Virus Viral Load

Median VL among those with detectable virus was 25 641 copies/mL (IQR, 686–108 541) at enrollment and 23 713 copies/mL



**Figure 1.** Human immunodeficiency virus RNA concentrations at time of mild *P falciparum* infection (0), and weekly for 4 weeks. Regression analyses using both the random slope model and mixed effects linear model found no significant change over time. Box and whisker plots represent the median and interquartile ranges.

(IQR: 268–243 567) at day 28. Of the 17 patients who completed the study and had at least 1 detectable HIV VL measured at enrollment, day 14 or day 28, we obtained VL from days 7 and 21 in 16 of them. Human immunodeficiency virus VL measurements did not change significantly over the 5-week course of the study: there were no significant changes in the median log<sub>10</sub> HIV VL from the samples collected at enrollment to day 7 post-malaria treatment ( $P$  value = .09), day 14 ( $P = .50$ ), day 21 ( $P = .79$ ), or day 28 ( $P = .75$ ). To further investigate the effect of *P falciparum* infection on HIV VL kinetics, we performed regression analyses using both the random slope model and mixed effects linear model. These models also found no significant change in HIV VL over time (Figure 1).

To identify individuals who had a significant change of HIV VL after successful treatment for *P falciparum*, we used individual regression analysis and identified a single patient who had a significant decrease in HIV VL from enrollment to day 28 ( $P = .014$ ). At enrollment, this patient, who reported taking ARVs, had a 0.53% parasitemia, a CD4<sup>+</sup> T cell count of 327 cells/μL, and an HIV VL of 1089 copies/mL that decreased to 149 copies/mL at day 28. In contrast, 1 patient not on ARVs with an enrollment CD4<sup>+</sup> T cell count of 98 cells/μL had a significant increase in HIV VL over the study period, with 62 copies/mL at enrollment and  $3 \times 10^6$  copies/mL by day 28 ( $P = .04$ ). It should be noted that this patient was diagnosed with active tuberculosis 2 weeks after study enrollment.

#### Drug Resistance Mutations

To determine whether drug-resistant strains were present and associated with HIV viremia, we carried out genotyping for ARV drug-resistance mutations on the 17 samples with detectable HIV VL (Table 2). Two of these samples could not be successfully genotyped. Genotypic analyses revealed that 12 patients

**Table 2. HIV Drug-Resistance Genotypes in Samples With Detectable HIV Viral Loads**

Patient ID	NRTI	NNRTI	PI Major	PI Minor
No ARV				
52	None	None	None	L10I
18	None	None	None	None
25	None	None	None	None
28	None	None	None	None
41	None	None	None	None
65	None	None	None	None
81	None	None	None	None
Prescribed ARV				
40	D67G, K70R, M184V, K219Q	Y188L, K238N	None	None
68	M184V	Y181C, G190A	None	None
30	None	K103N	None	None
22	None	None	None	L10V
23	None	None	None	L33I
76	None	None	None	L10V
53	None	None	None	None
57	None	None	None	None

Abbreviations: ARV, antiretroviral; HIV, human immunodeficiency virus; ID, identification; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

(80%) did not have clinically relevant drug-resistance mutations, 1 sample (7%) had a single NNRTI mutation, and 2 samples (13%) demonstrated both NRTI and NNRTI mutations. All 3 patients in whom drug-resistant mutations were found reported current ARV use. Four samples were found to harbor minor protease inhibitor (PI) mutations that are considered naturally occurring polymorphisms (no patients had prior PI exposure).

## DISCUSSION

Prior studies have reported that *P falciparum* infection may transiently increase HIV VL [2–4, 6]. This had, until now, only been evaluated in highly endemic areas and before the availability of ARVs. To determine whether *P falciparum* infection impacts HIV VL trajectories in Rwanda, we carried out a prospective study and, in contrast to Hoffman et al [4], we did not detect a decline in HIV VL after successful treatment for *P falciparum*, suggesting that malaria does not increase HIV VL in our setting.

Our patients manifested very mild illness, with normal temperatures and hematocrit, and this may explain the lack of effect of malaria infection on HIV VL. Prior studies have shown that high parasitemia associated with fever are associated with the greatest change on HIV VL and that the inflammatory cytokine tumor necrosis factor drives HIV replication during malaria [3, 8]. In addition, a pre-malaria infection VL would provide

additional data to examine the effect of malaria on VL trajectory. Our study is one of the first to examine the HIV VL-malaria interaction in patients on ARV. Whether malaria infection can cause breakthrough VL during ARV suppression will have to be studied in regions where malaria infection increases HIV VL in ARV-naive patients. The 1 patient who demonstrated a significant increase in HIV VL in our study had active TB, and the association of TB with an increase in HIV VL is well documented [9, 10].

An unanticipated and important finding from our study was that all patients with previously diagnosed HIV infection who were prescribed ARVs reported 100% adherence, yet 8 of them had detectable HIV VL. The 3 samples that had NRTI or NNRTI drug-resistance mutations were all among the ARV-prescribed group, suggesting that in a fraction of cases, ARV resistance is leading to loss of HIV viral suppression. Among those with ARV resistance mutations, the genotyping data identified K65R, K70R, M184V, and K103N, which have been previously described in Rwanda [11]. Two patients (14%) had both NRTI and NNRTI mutations. Inferences regarding the epidemiology of HIV drug resistance in Rwanda require larger population-based studies [12]. The lack of HIV drug-resistance mutations among the majority of those reporting 100% ARV adherence and who had a detectable HIV VL suggests that lack of adherence may be a contributor to uncontrolled viremia [13]. Two patients in our study who had undetectable VL reported no current use of ARVs. Although elite control may be a possibility, recent studies suggest that self-report of no ARVs may be inaccurate [14, 15]. Larger studies in Rwanda are needed to define the prevalence of and factors associated with detectable HIV VL in patients prescribed ARVs to improve clinical outcomes.

We identified 3 new HIV infections in patients presenting with mild *P falciparum* infection. Prior studies have shown that persons infected with HIV have a higher rate of malaria infection [16–18]. It is conceivable that in some cases, patients' symptoms (such as fever and myalgias) were related to HIV retroviral syndrome rather than malaria, and their malaria infection was subclinical. A study from Uganda found that 11% of patients presenting with malaria-like illness had HIV [19]. Our study was not designed to determine whether adults infected with HIV have a higher rate of malaria infection in Rwanda, but offering routine HIV testing in adults presenting with malaria infection, or symptoms consistent with malaria, could be beneficial in linking patients to HIV care and improving outcomes.

## CONCLUSIONS

This prospective study of HIV-infected Rwandan adults coinfecting with *P falciparum* did not identify malaria-associated changes in HIV VL. Our findings are in contrast to previous reports from areas more highly malaria-endemic, where malaria

coinfection was found to be associated with a transient rise in HIV VL. The key observation in our cohort was that the majority of patients prescribed ARVs and reporting 100% adherence had detectable HIV VL. Human immunodeficiency virus drug-resistance mutations could account for lack of viral suppression in a subset of patients, but suboptimal adherence may also be a contributor to uncontrolled viremia. Improved adherence strategies, HIV VL monitoring, and drug resistance testing for patients receiving ARVs are needed to inform clinical practice and improve patient outcomes in Rwanda. In our cohort, 11% of coinfecting patients were newly diagnosed with HIV: additional studies are needed to determine whether malaria infection in Rwandan adults is more prevalent among individuals infected with HIV. If HIV infection is more prevalent in adults with malaria then HIV testing should be offered to all adults presenting with malaria in Rwanda.

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**Author contributions.** K. S. and J. P. D. designed the research. K. S., N. L., C. B., and K. K. performed the research. E. I., E. M., and K. A. provided assistance with study site selection and enrollment. K. S., N. L., A. G.-Y., and M. H. analyzed the data. K. S., R. M. P., M. J. K., and J. P. D. wrote the manuscript.

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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