

Effect of HIV-1 Infection on Antimalarial Treatment Outcomes in Uganda: A Population-Based Study

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(See the editorial commentary by Kublin and Steketee, on pages 1–3.)

Background. Human immunodeficiency virus (HIV) infection may increase the burden of malaria by increasing susceptibility to infection or by decreasing the response to antimalarial treatment. We investigated the seroprevalence rate of HIV-1 infection and its effect on antimalarial treatment outcomes in adults and children with uncomplicated falciparum malaria in Uganda.

Methods. This retrospective study included 1965 patients ≥ 18 months old who were randomized to receive 1 of 3 antimalarial regimens at 7 sites in Uganda. HIV-1 testing was performed using 2 enzyme-linked immunosorbent assays and Western blot analysis of stored blood spots. The primary study outcome was clinical treatment failure at 28 days after antimalarial treatment. Molecular genotyping was used to distinguish clinical treatment failures due to new infections from those due to recrudescences.

Results. The HIV-1 seroprevalence rate was 2.5% in 1802 patients < 18 years old and 31% in 163 patients ≥ 18 years old presenting with malaria. HIV-1 infection was associated with a > 3 -fold (hazard ratio [HR], 3.28 [95% confidence interval {CI}, 1.25–8.59]) increased risk of clinical treatment failure for adults, but there was no increased risk for HIV-1-infected children. Molecular genotyping revealed that clinical treatment failures were due to new infections (HR, 6.35 [95% CI, 1.64–24.5]) rather than to recrudescences (HR, 1.51 [95% CI, 0.27–8.58]).

Conclusions. The HIV-1 seroprevalence rate was surprisingly high in adults presenting with malaria. This finding supports the implementation of routine HIV counseling and testing for adults with uncomplicated falciparum malaria. HIV-1 infection increased the susceptibility to new malarial infections but did not increase the risk of recrudescences in adults.

Malaria and HIV infection are each responsible for staggeringly high morbidity and mortality in sub-Saharan Africa. In regions where both diseases are endemic, HIV infection may increase the burden of malaria by increasing the susceptibility to infection. In vitro, antibody and interferon- γ responses to *Plasmodium falciparum* are impaired by HIV infection, although some cellular effector responses appear to be unaffected [1–3]. Recent clinical studies from Uganda have shown

that HIV infection is associated with an increased risk of clinical malaria and parasitemia in nonpregnant adults [4–6] and that the association strengthens with decreasing immune competence [4, 6].

Few studies have examined the effect that HIV infection has on the response to antimalarial treatment [7–11]. In areas where malaria is highly endemic, immunity plays an important role in determining the response to antimalarial treatment, as is evidenced by the finding that increasing age is associated with a decreasing risk of clinical treatment failure (CTF) [12]. Impaired immunity associated with HIV infection could diminish the effectiveness of antimalarial treatment by diminishing the role that the immune system plays in the clearance of parasites. In addition, HIV-infected patients may be at increased risk of malarial disease due to a newly acquired infection. A greater number of episodes of parasitemia at presentation, which is associated with a less favorable response to treatment [12] and is observed in HIV-infected populations [4], could

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also adversely influence the response to antimalarial treatment.

It is critical to the success of both control programs for malaria and HIV care efforts to evaluate outcomes in the HIV-infected population after treatment with standard antimalarial regimens. Because of the profound influence that acquired immunity has on susceptibility to malaria, the greatest burden of malaria in sub-Saharan Africa is in children, and treatment response rates improve with advancing age [13]. Thus, it is essential to examine the effect that HIV infection has on antimalarial treatment outcomes over a broad age range. In the present study, we evaluated the clinical response to antimalarial treatment in HIV-1-infected and HIV-1-uninfected children and adults presenting with uncomplicated falciparum malaria to 7 district health centers in Uganda.

PATIENTS, MATERIALS, AND METHODS

Study Patients

The study patients were participants of randomized clinical trials comparing the efficacy and safety of combination antimalarial drug regimens at 7 district health centers in Uganda. These centers are Uganda Ministry of Health sentinel malaria surveillance sites that were selected to represent the diversity of malaria transmission rates in Uganda. These studies have been described in detail elsewhere [14, 15] and are summarized here.

The trials, conducted between 2002 and 2004, included the following treatment arms: sulfadoxine-pyrimethamine (S-P) plus chloroquine, S-P plus amodiaquine (AQ), and (for 4 of the 7 sites) AQ plus artesunate. For all trials, patients presenting to the outpatient departments of the sentinel health centers with symptoms suggestive of uncomplicated malaria and a positive thick-blood smear at screening were referred to study physicians for further evaluation.

Patients were recruited for the trials if they met the following inclusion criteria: (1) ≥ 6 months old; (2) history of fever during the preceding 24 h or axillary temperature $\geq 37.5^\circ\text{C}$; (3) no history of serious side effects to study medications; (4) no evidence of concomitant febrile illness; (5) provision of informed consent; (6) ability to participate in 28-day follow-up; (7) no history of treatment with an antifolate or AQ during the preceding week; (8) not pregnant; (9) no danger signs (prostration, inability to drink, recent convulsion, or persistent vomiting) or evidence of severe malaria [16]; (10) *P. falciparum* mono-infection; (11) parasite density of 2000–200,000 parasites/ μL ; and (12) hemoglobin level ≥ 5.0 g/dL. Consent was obtained to use blood samples in future biological studies.

Enrolled patients were randomly assigned to receive study medications, dosed in accordance with standardized weight-based World Health Organization (WHO) guidelines [17]. All patients were provided with a 3-day dose of acetaminophen for treatment of their febrile symptoms. After enrollment, patients were asked to return to the study clinics for follow-up

visits on days 1, 2, 3, 7, 14, and 28. Blood was obtained by finger prick for thick-blood smears and for storage on filter paper on all follow-up days except day 1. Filter-paper blood spots were stored at room temperature in sealed sample bags containing silica gel. Patients who were classified as having CTFs were re-treated with quinine (10 mg/kg 3 times daily for 7 days).

Study Design and Procedures

The present study was a secondary analysis using retrospective HIV-1 antibody testing of patients who participated in the trials. Although samples from all 3138 patients who completed follow-up were available for HIV-1 antibody testing, testing was performed only on samples from a subset of patients ≥ 18 months old, to avoid potential misclassification of younger children. The study was approved by the Uganda National Council of Science and Technology and the institutional review boards of Makerere University; the University of California, San Francisco; and the University of California, Berkeley.

HIV-1 testing. HIV-1 serostatus was determined using filter-paper blood spots that were collected during the conduct of the clinical trials and delinked from patient identifiers. Filter-paper blood spots collected on the day that malaria was diagnosed were cut and labeled with a new unique identifier. Samples were screened for HIV-1 antibodies by 2 ELISAs (Vironostika HIV-1 Plus O Microelisa System [bioMérieux] and Genetic Systems rLAV EIA [Bio-Rad Laboratories]). Patients were classified as HIV-1 positive if the results of both ELISAs were positive, and patients were classified as HIV-1 negative if the results of both ELISAs were negative. Western blot analysis (Genetic Systems HIV-1 Western Blot; Bio-Rad Laboratories) was performed on samples with discordant ELISA results, and the results were classified as positive, negative, or indeterminate. Western blot analyses with indeterminate results were repeated, and the results were subsequently classified as positive or negative.

Antimalarial treatment outcomes and genotyping. The primary outcome of the study was clinical response at 28 days after antimalarial treatment. CTF was classified as early or late in accordance with 2003 WHO guidelines [16]. Patients were classified as having an early treatment failure (ETF) if they developed danger signs or complicated malaria or did not adequately respond to antimalarial treatment after 3 days. Patients were classified as having a late clinical failure (LCF) if they had danger signs, complicated malaria, or fever and parasitemia on days 4–28 and had not previously met the criteria for ETF. CTFs were further classified as due to either recrudescences or new infections, on the basis of molecular genotyping results. All patients with ETFs were considered to have recrudescences. In patients with LCFs, filter-paper blood spots obtained at enrollment and on the day the LCF was determined were analyzed for polymorphisms in merozoite surface protein-2 (*MSP-2*), according to methods published elsewhere [18]. Outcomes were

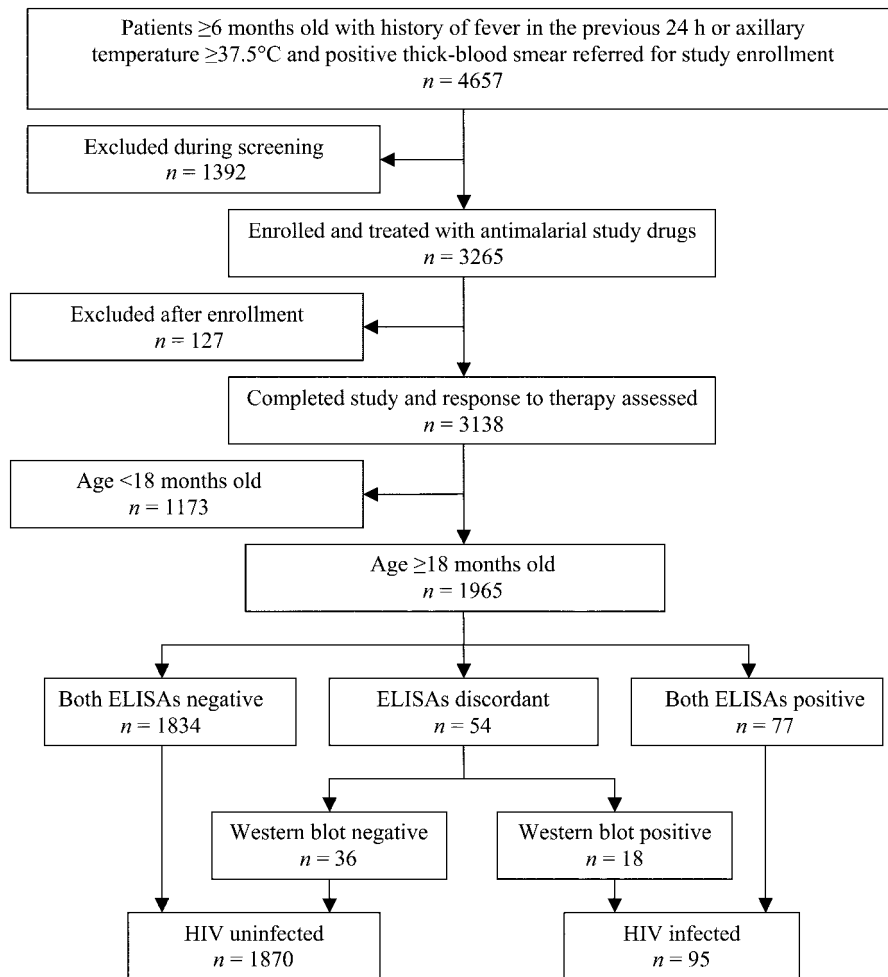


Figure 1. Trial profile

classified as recrudescences if all *MSP-2* alleles present on the day of recurrence were present at enrollment. Outcomes were classified as new infections if any *MSP-2* alleles differed from those present at enrollment.

Statistical Analysis

The study was designed to test the hypothesis that HIV-1 infection increases the risk of CTF in patients with uncomplicated falciparum malaria. The primary outcome variable was the risk of CTF, either unadjusted or adjusted by genotyping to distinguish recrudescences from new infections. Patients ≥ 18 years old were classified as adults, and patients < 18 years old were classified as children. Data were entered in SPSS software (version 10; SPSS) and analyzed using Stata statistical software (version 7.0; StataCorp). Risks of CTF were estimated using the Kaplan-Meier product limit formula, and censoring was included in the computation of risk when results adjusted by genotyping were used. Associations between HIV-1 infection and response to antimalarial treatment were estimated using a

Cox proportional hazards model controlling for age and treatment group. Stratified analyses based on age groups were pre-planned because of the known associations between age and both the HIV-1 seroprevalence rate and the response to antimalarial treatment. Categorical variables were compared using χ^2 or Fisher's exact tests, and continuous variables were compared using Student's *t* test. All reported *P* values were 2-sided, and $P < .05$ was considered to be statistically significant.

RESULTS

Study patients. Of 4657 patients who presented to the district health centers with fever and positive thick-blood smears, 3138 were enrolled in the antimalarial treatment trials and had a recorded treatment outcome (figure 1). Patients were excluded from the trials for the following most common reasons: unable to participate in 28-day follow-up (28%), previous antimalarial treatment within 1 week of presentation (22%), evidence of severe malaria or danger signs (18%), concomitant febrile illness (15%), and nonfalciparum malaria (5%). Because appro-

appropriate diagnostic tests were not available for HIV testing of infants, enrolled patients <18 months old were excluded from the present study. The final study population was 1965 patients, with 1802 patients <18 years old and 163 patients ≥18 years old (figure 2).

HIV-1 seroprevalence rate. HIV-1 infection was present in 95 (4.8%) of 1965 patients. The HIV-1 seroprevalence rate varied according to age: it was 30.7% in patients ≥18 years old and 2.5% in patients <18 years old ($P < .001$) (figure 2).

The clinical characteristics of the study population, according to HIV-1 serostatus, are shown in table 1. The ages of HIV-1-infected and HIV-1-uninfected patients were similar after stratification of patients into those <18 years old and those ≥18 years old. Pretreatment parasite densities were slightly higher in HIV-1-infected patients than in HIV-1-uninfected patients for both children (28,186 vs. 20,706 parasites/ μL ; $P = .09$) and adults (20,537 vs. 15,174 parasites/ μL ; $P = .11$), but these differences did not reach statistical significance. In adults, the mean temperature on presentation was higher in HIV-1-infected patients than in HIV-1-uninfected patients. There were no differences in antimalarial treatment regimens between HIV-1-infected patients and HIV-1-uninfected patients.

Antimalarial treatment outcomes and HIV-1 serostatus. In the entire study population, the risk of CTF due to either recrudescences or new infections was 36%. The risk of CTF was significantly higher for children than for adults (39% vs. 11%; $P < .001$), which is consistent with the findings in many other African studies demonstrating an increasing response to antimalarial treatment with increasing age. After stratification of CTFs according to genotyping results, 37% of the CTFs were due to recrudescences and 63% were due to new infections.

For adults, the risk of CTF was >3-fold higher for HIV-1-infected patients than for HIV-1-uninfected patients (hazard ratio [HR], 3.28 [95% confidence interval {CI}, 1.25–8.59]; $P = .02$) (table 2). In contrast, for children, the risk of CTF

was not significantly higher for HIV-1-infected patients than for HIV-1-uninfected patients (HR, 1.21 [95% CI, 0.75–1.96]; $P = .44$). The higher risk of CTF for HIV-1-infected adults was attributable to new infections, not recrudescences (table 2). The risk of new infections was >6-fold higher for HIV-1-infected adults than for HIV-1-uninfected adults (HR, 6.35 [95% CI, 1.64–24.5]; $P = .007$). For children, there was a trend toward a higher risk of recrudescences for HIV-1-infected patients (HR, 1.67 [95% CI, 0.86–3.26]; $P = .13$), but there was no association between HIV-1 infection and risk of new infections (HR, 0.94 [95% CI, 0.47–1.89]; $P = .86$). The relationship between HIV-1 infection and CTF was not confounded by treatment group for either children or adults.

The cumulative risk of CTF for adults is shown in figure 3. At <21 days after treatment, the risk of CTF was <5% for both HIV-1-infected and HIV-1-uninfected adults. However, by day 28 after treatment, this risk increased to 20% for HIV-1-infected adults and remained low (7%) for HIV-1-uninfected adults. For both HIV-1-infected and HIV-1-uninfected adults, the risk of recrudescences ≤28 days after treatment was <5%. In contrast, the risk of CTF due to new infections was >16% for HIV-1-infected adults and <3% for HIV-1-uninfected adults.

DISCUSSION

Both susceptibility to malaria and response to antimalarial treatment are dependent on the immunologic response of the host [19]. In areas where malaria is endemic, antimalarial immunity develops with repeated exposure to parasites and is usually well developed by the age of 5 years [20]. Therefore, in areas of high endemicity, both the incidence of malaria and the risk of CTF are highest in children [13]. The immunosuppression caused by HIV infection might be associated with the failure to protect against malarial infection and the development of clinical disease [21]. In the present study, we found

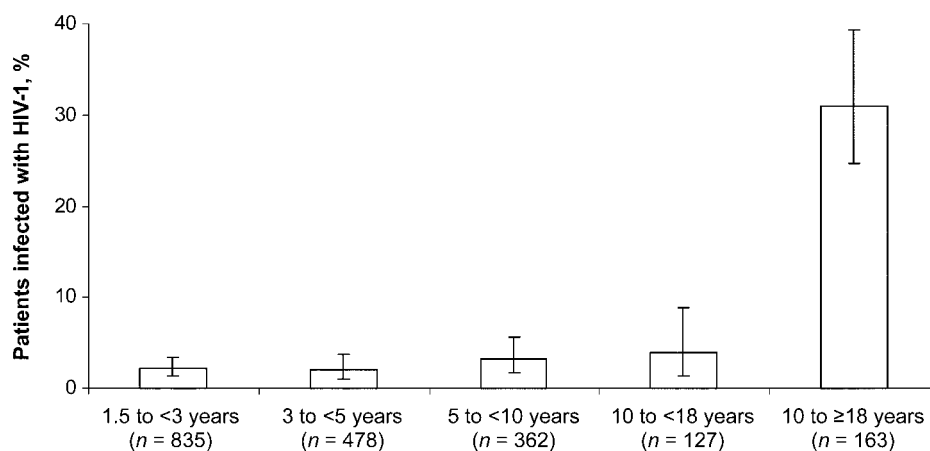


Figure 2. Proportion of patients with malaria and infected with HIV-1, by age group. Error bars represent 95% confidence intervals.

Table 1. Baseline characteristics of HIV-1-infected and HIV-1-uninfected children and adults.

Parameter	Age group					
	1.5–17 years old			≥18 years old		
	HIV-1 infected (n = 45)	HIV-1 uninfected (n = 1757)	P	HIV-1 infected (n = 50)	HIV-1 uninfected (n = 113)	P
Age, median (range), years	3.5 (1.5–15)	3.0 (1.5–17)	.11	28.5 (18–54)	28 (18–65)	.43
Female sex, %	62	51	.14	62	75	.09
Geometric mean parasite density, parasites/μL	28,186	20,706	.09	20,537	15,174	.11
Temperature, mean ± SD, °C	37.6 ± 1.1	37.5 ± 1.1	.48	37.5 ± 1.4	37.1 ± 0.9	.05
Treatment group, no. (%) of patients			.61			.50
CQ and S-P	19 (42)	687 (39)		27 (54)	51 (45)	
AQ and S-P	19 (42)	688 (39)		16 (32)	47 (42)	
AQ and AS	7 (16)	382 (22)		7 (14)	15 (13)	

NOTE. AQ, amodiaquine; AS, artesunate; CQ, chloroquine; S-P, sulfadoxine-pyrimethamine.

that 30.7% of adults presenting to district health centers in Uganda with uncomplicated falciparum malaria were coinfecting with HIV-1. This seroprevalence rate is much higher than that seen in the general population of Ugandan adults. Thus, in adults, presentation with malaria predicted HIV-1 infection. The risk of CTF after antimalarial treatment was >3-fold higher for HIV-1-infected adults than for HIV-1-uninfected adults, and these apparent CTFs were principally due to new infections. The HIV-1 seroprevalence rate was much lower in children, and antimalarial treatment outcomes in children were much less affected by underlying HIV-1 infection. Our results suggest that HIV-1 infection reverses acquired antimalarial immunity, thereby increasing the susceptibility of previously protected adults.

Accumulating evidence from sub-Saharan Africa indicates that malaria occurs with increasing frequency [4–6] and severity [22] in HIV-infected adults, compared with that in HIV-uninfected adults. The Uganda Ministry of Health reported that, in 2003, 4.1% of adults in the country were infected with HIV [23]. In our study population, adults who presented with malaria had a high risk of HIV-1 infection (30.7%), suggesting that this group should be offered voluntary counseling and treatment (VCT) for HIV infection. Moreover, our estimate of the HIV-1 seroprevalence rate in patients presenting with un-

complicated falciparum malaria may be conservative, because patients with concomitant febrile illnesses, severe malaria, or nonfalciparum malaria were excluded from the study.

Few studies have examined the effect that HIV infection has on the response to antimalarial treatment. Two small studies using quinine therapy and outcome measures at 7 days after treatment found that HIV infection had no effect on the response to antimalarial treatment [7, 8]. In a study in Kampala, Uganda, that had a limited sample size and used an inadequate treatment regimen, CTF after treatment with chloroquine was more common in HIV-infected children than in HIV-uninfected children (100% vs. 65%; $P = .10$), but the difference was not statistically significant, and no association between HIV serostatus and clinical outcome was observed in older patients ($P = .22$) [9]. In a study in Ethiopia, delayed clearance of malarial parasites after treatment was associated with coinfection with HIV [10].

The present study is the first, to our knowledge, to evaluate the effect that HIV-1 infection has on outcomes in adults and children receiving combination therapy for uncomplicated falciparum malaria. The parasite density was slightly higher in HIV-1-infected patients than in HIV-1-uninfected patients, but this difference did not reach statistical significance and is unlikely to be of clinical importance. This finding is in contrast to those of

Table 2. Association between HIV-1 infection and clinical treatment failure in children and adults with malaria.

Parameter	Age group				
	1.5–17 years old		≥18 years old		
	HR (95% CI)	P	HR (95% CI)	P	
All treatment failures	1.21 (0.75–1.96)	.44	3.28 (1.25–8.59)	.02	
Treatment failures due to recrudescences	1.67 (0.86–3.26)	.13	1.51 (0.27–8.58)	.64	
Treatment failures due to new infections	0.94 (0.47–1.89)	.86	6.35 (1.64–24.5)	.007	

NOTE. The hazard ratio (HR) was calculated for HIV-1 infection in patients with malaria, controlling for age and treatment group. CI, confidence interval.

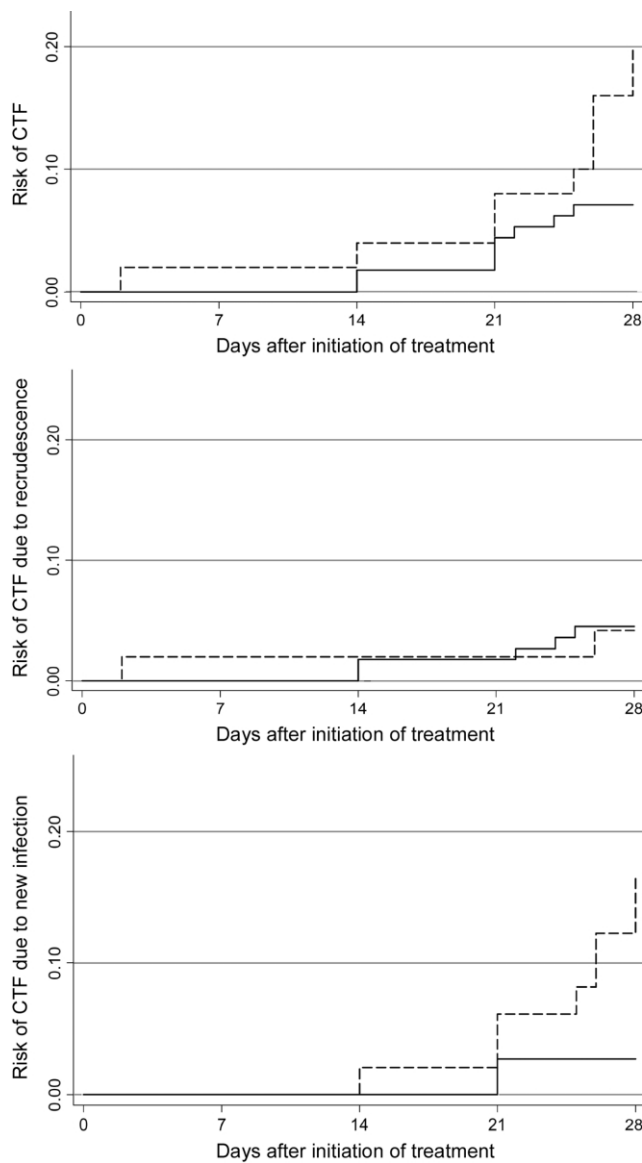


Figure 3. The cumulative risk of clinical treatment failure (CTF) in adults with malaria. Patients were divided into outcome categories and by HIV-1 serostatus. A dotted line represents HIV-1-infected patients, and a solid line represents HIV-1-uninfected patients.

studies clearly documenting higher parasite densities with increasing immunosuppression due to HIV infection [4, 24].

For children, the risk of CTF was the same for HIV-1-infected and HIV-1-uninfected patients. For adults, HIV-1 infection was not associated with an increased risk of CTF due to recrudescences. However, in contrast to the finding in children, HIV-1 infection was associated with a >6-fold increased risk of new malarial infections. Therefore, HIV-1-infected adults were much more likely to develop symptomatic malaria after antimalarial treatment than were HIV-1-uninfected adults. Our finding of an increased risk of CTF in HIV-1-infected adults is consistent with evidence in the literature, although our risk estimates appear to

be higher than those reported previously, possibly because the small samples in our study population resulted in low precision in the estimates. In addition, because mixed populations of parasites may exist during infections, misclassification of new infections and recrudescences could occur. However, considering all outcomes without adjustment by genotyping, HIV-1-infected adults still had a significantly higher risk of CTF than did HIV-1-uninfected adults.

Why were HIV-1-infected adults, but not HIV-1-infected children, more likely to develop malaria? We propose that the effect of HIV-1 on antimalarial immune responses is relatively unimportant for children, in whom these responses are not yet well developed. Therefore, in these children, although HIV-1 infection may have altered the antimalarial immune responses somewhat, its effect was not strong enough to markedly alter the incidence of disease. In contrast, in adults, in whom antimalarial immunity is generally strong enough to prevent most episodes of symptomatic disease, HIV-1 infection altered the antimalarial immune responses enough to allow a marked increase in the incidence of disease.

Our study has several important limitations. It was restricted to patients with uncomplicated falciparum malaria and those >18 months old. Therefore, our results may not be applicable to patients with other febrile illnesses, complicated falciparum malaria, or other forms of malaria or to infants. This is an important caveat, because the strongest effect that HIV infection has on malaria may occur in infants and in those with severe malaria [22, 25]. We also did not measure CD4 cell counts in our study. Previous studies have demonstrated that HIV infection is associated with an increased risk of clinical malaria and parasitemia and that the association strengthens with decreasing immune competence. In addition, our assertion that the HIV-1 seroprevalence rate was higher in patients presenting with malaria than in the general population was made on the basis of national statistics and not on the HIV-1 seroprevalence rate in control subjects in our study communities. Last, because of the relatively low HIV-1 seroprevalence rate in children and the relatively small number of adults presenting with malaria, the precision of our estimates of the association between HIV-1 infection and CTF was low, as is shown by the wide CIs. Although we did not find a statistical association between HIV-1 infection and antimalarial treatment outcome in children, we found a trend toward a higher rate of recrudescences in this group that may be clinically important but will require a larger study for elucidation. The low HIV-1 seroprevalence rate further limited our ability to fully explore whether the treatment group or the study site modified the relationship between HIV-1 and antimalarial treatment outcome.

In summary, we found that Ugandan adults presenting with malaria had an HIV-1 seroprevalence rate that was much higher than that reported in the general population. HIV testing is

not routinely performed in patients with uncomplicated malaria. Our study suggests that, in Ugandan adults, presentation with malaria may offer an unrecognized opportunity to identify HIV disease before the development of more serious complications. With the availability of rapid HIV diagnostic tests and sufficient allocation of resources, routine VCT for HIV could be initiated when adults present with malaria [26, 27]. We also found that HIV-1-infected adults had a higher risk of CTF than did HIV-1-uninfected adults, but this increased risk was due to new infections rather than to recrudescences. Therefore, we found no evidence to support differential antimalarial treatment policies for HIV-1-infected and HIV-1-uninfected adults with uncomplicated falciparum malaria. However, because of the increased risk of CTF in HIV-1-infected adults, emphasis on preventive measures for malaria, such as the use of insecticide-treated bed nets, should be an essential component of treatment programs for malaria and HIV in areas where malaria is endemic. The interactions between HIV infection and malaria merit continued surveillance as antiretroviral therapy and trimethoprim-sulfamethoxazole prophylaxis are implemented in sub-Saharan Africa and as new antimalarial treatments, such as artemisinin-based combination therapies, are introduced.

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