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Short Report: Prevalence of Asymptomatic Parasitemia and Gametocytemia among HIV-Infected Ugandan Children Randomized to Receive Different Antiretroviral Therapies

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Abstract. In a recent randomized controlled trial, the use of protease inhibitor (PI)-based antiretroviral therapy (ART) was associated with a significantly lower incidence of malaria compared with non-nucleoside reverse transcriptase inhibitor-based ART in a cohort of human immunodeficiency virus-infected Ugandan children living in an area of high malaria transmission intensity. In this report, we compared the prevalence of asymptomatic parasitemia and gametocytemia using data from the same cohort. The prevalence of asymptomatic parasitemia did not differ between the two ART treatment arms. The PI-based arm was associated with a lower risk of gametocytemia at the time of diagnosis of malaria (6.6% versus 14.5%, $P = 0.03$) and during the 28 days after malaria diagnosis (3.4% versus 6.5%, $P = 0.04$). Thus, in addition to decreasing the incidence of malaria, the use of PI-based ART may lower transmission, as a result of a decrease in gametocytemia, in areas of high malaria transmission intensity.

The effects of human immunodeficiency virus (HIV) on malaria have been well documented and include increased malaria incidence, increased parasitemia, and worse clinical outcomes.[1–3] Antiretroviral treatment (ART) for HIV that is also effective in preventing malaria and reducing transmission would have important public health implications for HIV-infected patients living in areas where malaria is highly endemic, such as sub-Saharan Africa. The HIV protease inhibitors (PIs) have been shown to inhibit the growth of *Plasmodium falciparum*, the most common cause of malaria in Africa, in culture and in animal models.[4–7] In addition to activity against the asexual stage of the parasite, PIs have shown *in vitro* activity against gametoctyes, the sexual stage of the parasite responsible for transmission to mosquitoes.[8] However, there are limited clinical data on the effects of PIs on asymptomatic parasitemia and gametocytemia.

We recently published the results of an open-label randomized trial comparing PI-based versus non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART for the prevention of malaria among HIV-infected children living in Tororo, a highly malaria-endemic area of Eastern Uganda.[9] In this study the use of PI-based ART was associated with a 41% reduction in the incidence of malaria, largely attributable to ART-antimalarial drug interactions resulting in a significant reduction in the risk of recurrent malaria after treatment with artemether-lumefantrine (AL). In this report, we compare the prevalence of asymptomatic parasitemia and gametocytemia between ART treatment arms in this trial. Briefly, study participants were HIV-infected children 2 months to 5 years of age who were either eligible for ART initiation or already receiving first-line ART with virologic suppression. Participants were randomized to receive either an NNRTI-(nevirapine or efavirenz)-based or PI-(ritonavir-boosted lopinavir)-based regimen. Participants also received an insecticide-treated bed net at enrollment and daily trimethoprim-sulfamethoxazole prophylaxis. Routine assessments every 4 weeks included a thick blood smear. Parents/guardians were encouraged to bring their children to a dedicated study clinic anytime they were ill. Children were diagnosed with malaria if they presented with a documented fever (tympanic temperature ≥ 38.0°C) or history of fever in the previous 24 hours and had a positive blood smear. Uncomplicated malaria was treated with AL, and participants were followed for 28 days after therapy according to standard guidelines. The study observation period began on the day after initiation of ART study drugs and ended with premature withdrawal from study or at the end of the study period (April 30, 2012). Asymptomatic parasitemia was defined as the presence of parasites in the absence of reported or documented fever, excluding a 28-day follow-up period after each episode of malaria. Participants with asymptomatic parasitemia were not given antimalarial therapy, in accordance with local guidelines. Gametocytemia was defined as the presence of gametocytes on thick smear. Blood smears were stained with Giemsa and the prevalence of asexual and sexual parasites were assessed as previously described.[10] Microscopists were blinded to the study participants' assigned ART regimen. Statistical analyses were performed with Stata version 11 (Stata, College Station, TX) using an intention-to-treat approach. The prevalences of asymptomatic parasitemia and gametocytemia stratified by the type of clinic visit were compared between the two ART treatment arms using generalized estimating equations, with adjustment for repeated measures in the same patients using exchangeable correlation and robust standard errors. A two-sided P value of ≤ 0.05 was considered statistically significant.

A total of 418 children were screened, 186 enrolled, and 184 initiated on ART study drugs (93 in the NNRTI arm and 91 in the PI arm). The primary reasons children were not enrolled included not meeting eligibility criteria for initiating ART ($N = 137$), being on ART with a detectable viral load ($N = 34$), and not being HIV infected ($N = 32$). Of those initiated on study drugs, 174 participants (94.6%) completed the study. A total of 3,216 routine blood smears were performed, of which 396 (12.3%) were positive for asymptomatic parasitemia. There was no difference in the prevalence of asymptomatic parasitemia between the PI-based and NNRTI-based treatment arms (11.8% versus 13.7%, $P = 0.36$). The prevalence of asymptomatic parasitemia was also similar between the two treatment arms when results were stratified

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TABLE 1
Associations between antiretroviral therapy (ART) group and asymptomatic parasitemia*

Duration since last episode of malaria	Prevalence of asymptomatic parasitemia		RR (95% CI)	*P* value
	PI-based ART	NNRTI-based ART		
All routine visits	193/1640 (11.8%)	203/1486 (13.7%)	1.18 (0.83–1.70)	0.36
30–60 days	22/120 (18.3%)	21/132 (15.9%)	0.85 (0.43–1.65)	0.62
> 60–120 days	33/164 (20.1%)	38/174 (21.8%)	1.10 (0.66–1.83)	0.71
> 120 days or no prior malaria	138/1356 (10.2%)	144/1180 (12.2%)	1.22 (0.84–1.79)	0.29

*PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; RR = rate ratio; CI = confidence interval.

by time since last episode of symptomatic malaria (Table 1). In addition, among those episodes of asymptomatic parasitemia, geometric mean parasite densities were similar between the PI-based and NNRTI-based treatment arms (1,137 versus 828 asexual parasites/μL, $P = 0.48$). The prevalence of gametocytemia at the time of monthly routine visits was also similar between the PI-based and NNRTI-based treatment arms (2.5% versus 3.2%, $P = 0.31$). A total of 437 episodes of malaria were diagnosed (*P. falciparum* = 414, *P. malariae* = 12, *P. ovale* = 10, and *P. vivax* = 1). All episodes of malaria with gametocytes detected were caused by *P. falciparum*. The prevalence of gametocytemia was significantly higher on the days malaria was diagnosed compared with monthly routine visits (11.2% versus 2.8%, $P < 0.001$). On the day malaria was diagnosed the prevalence of gametocytemia was significantly higher in the NNRTI-based arm compared with the PI-based arm (14.5% versus 6.6%, $P = 0.03$). The prevalence of gametocytemia was also significantly higher in the NNRTI-based arm compared with the PI-based arm during the 28-day malaria follow-up period (6.5% versus 3.4%, $P = 0.04$) (Table 2).

Previously, we reported that the risk of recurrent malaria after antimalarial therapy was significantly lower in the PI-based arm of our trial due most likely to drug interactions leading to a prolonged post-treatment prophylactic effect.[9] Specifically, the ritonavir component of the PI regimen inhibited the metabolism of lumefantrine, leading to prolongation of exposure to lumefantrine, and thus prolonged protection against recurrent malaria after treatment. To explore whether prolonged lumefantrine exposure after treatment also contributed to the lower risk of gametocytemia on the day malaria was diagnosed in the PI-based arm, results were stratified by duration since the last episode of malaria (Figure 1). The prevalence of gametocytemia was lower in the PI-based arm at each time point after prior therapy. Even considering episodes with no prior malaria or over 120 days since the last episode of malaria, the risk of gametocytemia remained significantly higher in the NNRTI-based arm compared with the PI-based arm (21.4% versus 7.5%, $P = 0.009$). Thus, the impact of the PI on gametocytemia is not explained by the pharmacokinetic effects of the PI on lumefantrine.

In summary, in this study of HIV-infected Ugandan children a PI-based ART regimen was associated with a significantly lower prevalence of gametocytemia on the day malaria was diagnosed and during 28 days after antimalarial therapy compared with an NNRTI-based regimen. However, there was no significant difference between the two ART treatment arms in the prevalence of asymptomatic parasitemia or gametocytemia at the time of monthly routine visits. These data provide further evidence to suggest that the use of a PI-based ART regimen may reduce the transmission potential for malaria among populations of HIV-infected children living in areas of high malaria endemicity, caution should be taken when generalizing these results to other patient populations.

Direct antimalarial effects of HIV PIs at levels achievable in humans have been supported by several laboratory-based studies.[4–6,11,12] Considering clinically relevant effects of PIs, recent data are available from two randomized trials in humans and the results have been mixed. In secondary analyses of a multicenter trial of HIV-infected women randomized to either PI-based or NNRTI-based ART, there were no differences in the incidence of malaria, diagnosed clinically or with laboratory confirmation.[13,14] However, this study was done with adults experiencing a relatively low incidence of malaria. In contrast, the HIV-infected children we studied in an area where malaria is highly endemic, a PI-based ART regimen was associated with a 41% reduction, compared with an NNRTI-based regimen, in the incidence of malaria.[9] Differences in the results of these studies can be explained by differences in the level of endemicity and the primary mechanism through which PIs prevented malaria. In Ugandan children, as in the multicenter study, the incidence of first episode of malaria after study enrollment was not significantly different between the two ART treatment arms. However, in Ugandan children the risk of recurrent malaria within 63 days of a prior episode was 54% in the NNRTI-arm compared with 28% in the PI-arm. This finding appears to be best explained by increased lumefantrine exposure after antimalarial therapy with AL among patients receiving PIs, leading to prolonged post-treatment prophylaxis against malaria. In this report, we add to these findings with the observation that PIs are also associated with a lower risk of gametocytemia. This finding might be explained by PIs leading to decreased asexual parasitemia with subsequent impact on gametocytemia, by PIs decreasing gametocytogenesis, or by direct gametocytocidal effect of PIs. The findings in this study argue against the first possibility, as we found no significant

TABLE 2
Associations between antiretroviral therapy (ART) group and gametocytemia*

Type of clinic visit	Prevalence of gametocytes		RR (95% CI)	*P* value
	PI-based ART	NNRTI-based ART		
All routine visits	41/1641 (2.5%)	48/1492 (3.2%)	1.38 (0.74–2.56)	0.31
Day malaria diagnosed	12/181 (6.6%)	37/256 (14.5%)	2.15 (1.07–4.31)	0.03
Days 1–28 malaria follow-up	39/1150 (3.4%)	102/1581 (6.5%)	1.98 (1.02–3.86)	0.04

*PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; RR = rate ratio; CI = confidence interval.

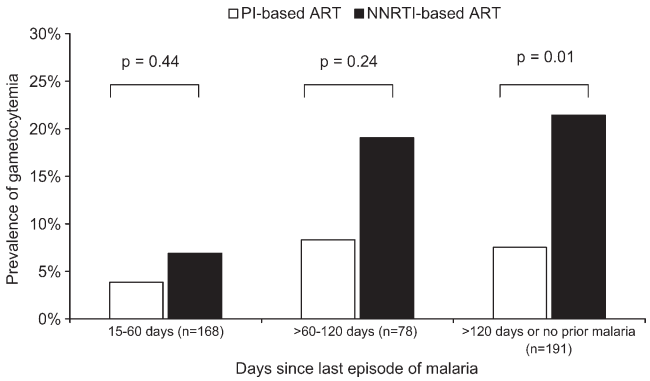

FIGURE 1. Association between the antiretroviral therapy (ART) group and the prevalence of gametocytemia stratified by duration since the last episode of malaria. PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor.

difference in the risk of asymptomatic parasitemia in the PI- and NNRTI-based ART arms. Because PIs were associated with a decreased risk of gametocytemia in the setting of malaria, suggests they impacted on gametocytemia by inhibiting gametocytogenesis or by directly exerting gametocytocidal activity. Indeed, *in vitro* studies have reported inhibition of gametocytogenesis by a range of PIs, including lopinavir, the active PI in our study, although only tipranavir exhibited significant gametocytocidal activity against *P. falciparum*.[8]

Results from our group and others suggest that the use of a PI-based ART regimen may provide several advantages over an NNRTI-based regimen in Africa. First, in children living in areas of high malaria endemicity, a PI-based regimen reduced the incidence of malaria, potentially offering major improvements in morbidity and mortality. Second, a PI-based regimen reduced the risk of gametocytemia, which likely will lead to reduced malaria transmission. Third, compared with NNRTI-based therapy, PI-based ART has been associated with higher rates of virologic suppression among HIV-infected African children with[15] and without[16] prior exposure to single-dose nevirapine. Thus, consideration of broader implementation of PI-based ART in malaria-endemic regions of Africa is warranted.

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