Interactions between HIV Infection and Malaria in Children Living in sub-Saharan Africa in the Era of Widening Access to Improved Interventions

By

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Over 65% of the world's HIV-infected population lives in sub-Saharan Africa where 350 million people are exposed to infection with malaria. Thus any biological interactions between HIV and malaria would have major public health implications for this region. There is growing evidence that HIV infection leads to negative malaria outcomes and these effects may differ across different populations and epidemiologic settings. In non-pregnant adults and pregnant women, HIV infection increases the risk of asymptomatic parasitemia and clinical malaria. Additionally, in non-pregnant adults, the response to antimalarial therapy may be compromised by HIV infection. There are scanty data describing the effects of HIV infection on the risk of malaria and on malaria outcomes in children, the population at highest risk of clinical malaria and malaria mortality.

Over the last two decades, treatment and control of both HIV infection and malaria in sub-Saharan Africa have undergone major changes. There has been rapid scale-up of programs providing antiretroviral therapy (ART) and trimethoprim-sulfamethoxazole (TS) prophylaxis for HIV care and in parallel, wide scale implementation of malaria control interventions, such as artemisinin-combination therapies (ACTs) for treatment of uncomplicated malaria, indoor residual spraying, and widespread distribution of insecticide treated bednets. It is not clear, how these interventions singly or in combination might affect the interactions between HIV infection and malaria in different risk groups

The goal of this dissertation was to contribute to knowledge on the effects of HIV infection on malaria in children in the era of increasing access to improved HIV and malaria interventions. Data from HIV-infected and HIV-uninfected children enrolled in cohort studies in two settings of differing malaria transmission intensity in Uganda were utilized. The dissertation focused on the risk of malaria in HIV-infected children and antimalarial treatment outcomes in those with uncomplicated malaria. The objectives of studies that comprised this dissertation were 1) to assess the effect of daily TS prophylaxis on the risk of malaria in HIV-infected children, 2) to determine the association between daily TS prophylaxis and infection with antifolate-resistant malaria parasites, 3) to determine the risk factors for malaria in young HIV-infected children living in an area of high malaria transmission, and 4) to determine the efficacies and safety of ACTs for the treatment of uncomplicated malaria in HIV-infected children.

HIV-infected children receiving daily TS had an 80% reduced risk of clinical malaria compared to HIV-uninfected children not receiving TS. However, TS use was associated with infection with parasites with a rare mutation, *dhfr* 164L, known to mediate high-level antifolate resistance. Among HIV-infected children less than 38 months of age; low CD4 cell percentage, interruption of daily TS prophylaxis, and ART use were independent risk factors for malaria. We hypothesize that non-nucleoside reverse transcriptase inhibitors given as part of ART alter the metabolism of certain ACTs or TS leading to reduced malaria prophylaxis in those receiving these drugs concomitantly. Artemether-lumefantrine (AL), dihydroartemisinin-piperaquine (DP) and artesunate-amodaiquine (AS/AQ) were all 100% efficacious for malaria treatment, however compared to AL, AS/AQ was associated with a higher risk of neutropenia 45% vs. 17% (p=0.001), malaise 27% vs. 5% (p=0.010) and anorexia 24% vs. 5% (p=0.014). Adverse events associated with AS/AQ treatment were highest among those who were concomitantly receiving ART. In the setting of high malaria transmission intensity, DP was superior to AL in preventing recurrent parasitemia (7.1% vs. 34%, p <0.001).

Findings from these studies support the use of daily TS prophylaxis in HIV-infected children living in sub-Saharan Africa. However, there is need for continuous surveillance to monitor the emergence and spread of rare mutations that may be associated with widespread TS use. Artemether-lumefantrine and dihydroartemisinin-piperaquine appear to be safe and efficacious for the treatment of malaria in HIV-infected individuals, even among those concomitantly receiving ART. However the potential interactions between NNRTIs and other drugs given as part of HIV care and ACTs are an urgent concern. Studies in more diverse HIV-infected populations living in different malaria transmission settings are needed to confirm these findings.

DEDICATION

I dedicate this dissertation to two special groups of people. First my family: my wonderful parents who taught me to reach for the sky and supported me every time I did, my four elder brothers who were always shining examples, my little sister who has always been there for me, and the kids: Kale, Tesi, Francine, Joni, Maya and Micah who are a tremendous source of inspiration. This work is also dedicated to those very special little children who live with HIV; watching their daily struggles made me want to do this.

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CHAPTER 1

HIV AND MALARIA: OVERVIEW

HIV infection

Human Immunodeficiency Virus (HIV) belongs to the retrovirus family of viruses and consists of two distinct viral types, HIV-1 and HIV-2 [1]. HIV-1 infection, which is the subject of this dissertation, causes more severe disease and is predominantly found in Central, East and Southern Africa, the United States and Europe [2, 3]. HIV is acquired through contact with body fluids or infected blood and the most common modes of transmission are through sexual intercourse, re-use of needles, blood transfusion and vertical transmission from mother to child during the perinatal and breasting feeding period [4]. The virus primarily infects CD4 T lymphocytes, but it also infects a variety of other cells, including monocytes and thymocytes [5]. HIV disease is characterized by progressive depletion of CD4 cells, resulting in increasing immunosuppression and escalating susceptibility to opportunistic infections and malignancies which define AIDS[5]. HIV pathogenesis also involves activation of a variety of immune cells and elevation of proinflammatory cytokines and chemokines in the plasma and lymph nodes [6]. Both immunosuppression and immune activation play a role in the effects of HIV on other morbidities.

Global burden of HIV

By the end of 2008, 33.4 million people worldwide were estimated to be living with HIV, of whom 2.1 million were children under 15 years of age. A total of 2.3 HIV million infections in adults and 430,000 HIV infections in children were acquired in 2008, and HIV accounted for 1.7 million deaths in adults and 280,000 deaths in children worldwide [7]. Recent data indicate that new HIV infections and deaths from HIV are on the decline. Successes in the control of the epidemic have been attributed to the expansion of access to effective prevention and control strategies, especially in sub-Saharan Africa and the rest of the developing world [8, 9]. However, HIV remains one of the most important infections worldwide.

HIV burden and control in sub-Saharan Africa

Sub-Saharan Africa bears the brunt of the HIV epidemic. By the end of 2008, an estimated 22.4 million persons living in sub-Saharan Africa were HIV-infected, representing 5.2% of the total adult population in this region, 67% of the world's HIV-infected population and over 90% of the world's pediatric HIV-infected population. AIDS caused 1.4 million deaths in sub-Saharan Africa in 2008. Although HIV infection is one of the most important public health problems in this region, its burden differs greatly across countries. Latest country reports provide prevalences ranging from 0.7% in Senegal and Niger to 26% in Swaziland. Southern Africa continues to be the worst affected sub-region[7].

HIV control strategies are aimed at reducing new infections and improving treatment and care of infected persons. By the end of 2008, The Global Fund to fight

HIV, TB and Malaria (GFTAM) and the United States Emergency Plan for AIDS relief (PEPFAR) were together supporting antiretroviral therapy (ART) treatment for 2.95 million people in low to medium income countries, the majority in sub-Saharan Africa[10]. This is a steep increase from 2% to 44% coverage in five years. Daily trimethoprim-sulfamethoxazole (TS) prophylaxis is recommended for all HIV-infected persons with symptomatic disease, adults with CD4 cell counts less than 350 cells/μl regardless of disease stage, and HIV-infected children with CD4 percentage less than 25% [11]. TS prophylaxis is rapidly becoming the standard of care for persons accessing HIV care in Africa. Increased availability of ART and TS prophylaxis in HIV-infected persons has been associated with longer survival, reduced risk of morbidity, including malaria, and improved overall quality of life [12-19].

Global burden of malaria

With over 40% of the world's population at risk and approximately 250 million clinical episodes and over 800,000 deaths annually, malaria is clearly one of the most important public health problems worldwide [20, 21]. 109 countries are malaria-endemic however, within these settings transmission varies from very low unstable transmission to very high perennial transmission [22]. As in the case of HIV control, efforts to decrease the global burden of malaria have recently escalated, with concentrated efforts in sub-Saharan Africa. In 2005, the Roll Back Malaria Partnership and World Health Assembly set a target to reduce the number of malaria cases by 50% between 2000 and the end of 2010 and by 75% by the end of 2015 [23]. In order to meet these targets there has been a rapid scale up of proven interventions, notably distribution of long lasting insecticide treated bed nets (ITNs), indoor residual spraying (IRS) with pesticides, intermittent preventive therapy in pregnant women (IPTp) and prompt and effective treatment with highly efficacious artemisinin combination therapies (ACT). Following successful deployment of these interventions, more than one-third of the endemic countries providing data reported a reduction in malaria cases of over 50% in 2008 [20].

Malaria in sub-Saharan Africa

Disease burden and control strategies

Nine out of ten malaria deaths and over 200 million cases of malaria occur in 45 endemic sub-Saharan African countries[20, 24]. It is estimated that 350 million people in this region live in high transmission settings [22]. The highest risk of malaria is in young children under five years of age and pregnant women. Eighty-five percent of malaria deaths in Africa occur in children under five years of age. However, expanding use of ITNs, IRS and uptake of ACTs for malaria treatment has led to recent progress in malaria control in Africa. Up to 50% reductions in malaria deaths have been reported in some highly endemic countries, including the Gambia, Rwanda and Zanzibar[20].

Malaria transmission and disease manifestation

Malaria is caused by a protozoan parasite of the genus *Plasmodium* and transmitted by the female Anopheles mosquito. There are five human *Plasmodium* species found in Africa: *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax* and *Plasmodium ovale* and of which *P. falciparum* is the most virulent. In an infected human host, *Plasmodium falciparum* stages include sporozoites present during the liver stage, asexual blood stages and gametocytes [25]. Clinical disease is mainly due to blood stage parasites. Malaria transmission intensity is markedly heterogeneous across Africa, ranging from one to over one thousand infective bites a year [26].

Clinical manifestations of malaria infection range from asymptomatic to severe life-threatening illness. While the immune responses to malaria are not fully understood, it is clear that host immunity is a major determinant of disease manifestation and response to therapy [25, 27]. Immunity to malaria is acquired through repeated exposure to infection and is characterized by the ability to control clinical disease and parasite density. In endemic settings, infants become susceptible to malaria at three to four months of age, after maternally-acquired immunity wanes. The risks of severe malaria and death increases in children two and four years old and decreases thereafter. Acquired immunity and subsequent protection for symptomatic disease is dominated by effectors that control parasite numbers and the development of antibodies to variant specific antigens (VSA) expressed by infected erythrocytes which are thought to be major targets of protective immunity against severe disease [28, 29]. By five years of age, children living in highly endemic areas have acquired partial immunity to malaria and subsequently the risk of severe and symptomatic malaria declines [29]. In contrast, persons living in low or unstable malaria transmission settings remain susceptible to clinical disease throughout their lives. During pregnancy there is an interruption of malaria immunity, leading to increased susceptibility to disease. In low transmission settings, pregnant women have a two to three-fold increased risk of severe malaria compared to non-pregnant adults, while in areas of stable transmission the major impact of malaria in pregnancy is asymptomatic parasitemia and chronic anemia in the mother and placental malaria and poor birth outcomes, such as low birth weight [24, 27, 30]. Pregnancy-specific malaria immunity develops with increasing parity and by the third pregnancy the malaria risk is substantially reduced [31, 32] . Primigravidae have a two- to four-fold increased risk of placental malaria compared to multigravidae [30].

Interactions between HIV infection and malaria in sub-Saharan Africa

Based on the progressive impairment of host immunity in advancing HIV disease and the reliance on host immunity to prevent malaria, it would be expected that HIV negatively affects the clinical course of malaria. Defense mechanisms against different stages of malaria parasites involve mechanisms of both cell mediated and humoral immunity that are impaired in HIV disease. For example, the destruction of Plasmodium sporozoites in the liver stage of malaria infection is reliant on cytotoxic T cells[33] and elimination of parasitized red blood stages is further dependent on antibodies production under CD 4 T

cell control [34]. Furthermore, immune activation of CD4 cells in HIV infection may increase the risk of severe malaria, as activated CD4 T cells are thought to play a role in the development of cerebral malaria [35]. These and other potential immunological interactions between HIV and malaria lead to the expectation of increased susceptibility to parasitemia and increased likelihood of clinical disease, severe disease, antimalarial treatment failure and possibly malaria mortality, with progressive HIV disease. However, studies conducted early in the HIV epidemic did not find consistent associations between the two infections, and a review of studies conducted between 1983 and 1996 concluded that there was no convincing evidence of an interaction between HIV infection and malaria with the exception of a possible increased risk of placental malaria among HIV-infected women[36]. Many of the studies reviewed at that time had methodological limitations and small sample sizes and they were not likely to be widely generalizable. Eight of the ten clinical studies were cross-sectional and only two were cohort studies. One of the cohort studies followed up recently infected HIV-seropositive subjects for only three months. None of the studies assessed the effects of level of immunosuppression among HIV-infected subjects and it was not possible determine possible modification of effects of HIV on malaria by age or malaria endemicity, both markers of malaria immunity. There is now literature from numerous studies conducted after 1996 in different populations that indicate that HIV infection leads to negative malaria outcomes and the effects of HIV infection may differ across different populations and epidemiologic settings.

Effect of HIV infection on malaria in pregnancy

Much of the evidence concerning HIV and malaria interactions comes from studies of pregnant women. Numerous such studies have demonstrated higher risks of parasitemia and of placental malaria in HIV-infected women compared to uninfected women during pregnancy and at the time of delivery [37-40]. As discussed above, in the general population, primigravidae are at highest risk of malaria in pregnancy and pregnancy-specific malaria immunity is acquired with subsequent pregnancies. However, the effect of HIV infection on the risk of malaria has been shown to be more marked among multigarvidae thus, the burden of malaria in pregnancy is not limited to primigravidae but present all gravidities in HIV-infected women [37, 39]. Immunological studies have described possible mechanisms by which HIV may impair immune mechanisms necessary for protection from malaria during pregnancy. It has been shown, for example that production of interferon-γ in response to malarial antigen stimulation, antibodies to certain VSAs and antibodies to merozoite proteins are reduced in HIV-infected women [41-44].

Compared to women with malaria or HIV infection alone, women co-infected with HIV and malaria have an increased risk of poor pregnancy outcomes, such as low birth weight, intra-uterine growth retardation and pre-term birth [45-47]. Studies evaluating the effect of malaria on risk of mother to child transmission of HIV (MTCT) have found inconsistent results. Some studies have reported higher risks of MTCT among mothers with placental malaria or peripheral parasitemia when compared to women

without malaria at delivery [48-50] while other studies have found no such relationship [38, 51].

There are few data on the effect of HIV infection on response to antimalarial therapy in women with malaria; however, there is evidence that intermittent preventive therapy (IPTp) with two doses of sulfadoxine-pyrimethamine (which is routinely given to pregnant women in Africa) is less effective in HIV-infected when compared to HIV-uninfected pregnant women [52]. This finding has led the World Health Organization (WHO) to recommend that in HIV-infected pregnant women, IPTp should consist of at least three doses of SP unless the woman is receiving TS prophylaxis as part of her HIV care [53]

Effect of HIV infection on malaria in non-pregnant adults

There is growing evidence of negative effects of HIV infection on malaria outcomes in non-pregnant adults living in sub-Saharan Africa. Four large cohort studies following HIV-infected and uninfected adults in areas of high malaria transmission in Uganda and Malawi have all shown that HIV infection is associated with a higher risk of parasitemia and clinical malaria and risks increase as CD4 counts decline[54-56]. Several other studies in settings with various levels malaria transmission intensities have also reported higher incidence rates of malaria in HIV-infected adults compared to HIV-uninfected individuals [57-61]. HIV infection has also been found to increase the risk of severe malaria and death in areas where malaria transmission is low or unstable [62-65]. A recent study that used existing data to model the impact of HIV infection on malaria in adults concluded that compared to HIV-uninfected individuals, those with HIV and CD4 cell counts of 200-499/ul have a three-fold higher malaria risk while those with CD4 cell counts less than 200/ul have a five-fold increased risk regardless of the level of malaria endemicity [66]. HIV-infected adults with CD 4 cell counts of 500/ul or more do not appear to be more vulnerable to malaria compared to the general population. HIV infection has been estimated to have increased the malaria parasite biomass in sub-Saharan Africa by 18% in 2005, with the highest impact in areas where both diseases are highly endemic [66]. Another study concluded that in five countries where the prevalence of HIV infection was greater than 20% and malaria transmission was unstable, the risks of clinical malaria was increased by up to 28% (95% CI 14-47%) and malaria deaths by 114% (95% CI 37-188%).

Studies of the response to malaria treatment in HIV-infected adults have been inconsistent. Efficacy against malaria infections for which antimalarial treatments are prescribed and post-treatment prophylaxis to prevent new infections are important properties for antimalarials used in malaria endemic countries. The need for prevention of new infections increases with increasing malaria transmission intensity. Some studies have found no impact of HIV infection on overall treatment outcomes [67, 68] while others found higher rates of recrudescent malaria among individuals with low CD 4 cell counts [69, 70]. Still other studies have reported higher rates of failures due to new infections and not recrudescent malaria [59]. Of note, the studies that have evaluated the effect of HIV infection on malaria treatment have predominantly evaluated non-artemisinin therapies which are now of limited use in sub-Saharan Africa. Two studies

have evaluated treatment outcomes after treatment of malaria with artemisinin monotherapy. Both reported poorer treatment outcomes in HIV-infected individuals [58, 71].

Studies evaluating the immune responses to malaria in HIV-infected adults have identified impairments in both T cell and B cell defense mechanisms that may play a role in the interactions observed in clinical studies adding to similar findings as those seen in the setting pregnancy. For example, a study in Burkina Faso assessing cell mediated immunity to malaria reported that HIV-infected adults with CD4 cell counts < 200 had reduced lymphocyte proliferation and cytokine (IL-2 and INF-gamma) production in response to a variety of *P. falciparum* antigens when compared to HIV-uninfected individuals [72]. Another study comparing humoral responses to five synthetic peptides of *P. falciparum* malaria found that adults with AIDS produced lower levels antibodies to three of these ring stage peptides [73]. However both studies concluded that only a subset of immune responses important in the defense against malaria are impacted by HIV infection.

Effect of HIV infection on malaria in children

There have been few studies of the interactions between HIV infection and malaria in children. This is probably because, although children are at highest risk of developing malaria, HIV infection is less common in this population. Furthermore, prior to extensive implementation of HIV treatment and care programs in Africa, HIV-infected children died very young so the numbers of such children available for study was limited. The relationship between HIV infection in children is unclear with respect to the risk and severity of malaria and response to antimalarial therapy. Early studies in infants and young children found equivalent risks of malaria in HIV-infected and HIV-uninfected children[74, 75], while another, more recent study found higher rates of malaria parasitemia in HIV-infected children[76]. There is evidence that HIV-infected children are at higher risks of severe malaria [77] and one study has reported a higher risk of malaria mortality among HIV-infected compared to HIV-uninfected children with severe malaria [78]. Studies of the effect of HIV infection on the response to antimalarial therapy are similarly few and inconsistent. Most studies have found no effect [68, 79, 80] while one study that assessed the response to chloroquine, which was widely ineffective in the study population and is no longer recommended for treatment of malaria, found higher rate of treatment failure in children less than five years of age [59].

Effect of improved HIV and malaria control interventions on interactions

Expanding access to TS prophylaxis and ART for HIV care, and to ACTs ITNs and IRS for malaria control, likely modifies some of the observed HIV-malaria interactions and introduce new linkages between the two infections such as interactions due to concomitant use of ARVs, TS and ACTs.

TS prophylaxis may play a dual role in malaria-HIV interactions. Its anti-parasitic activity may lead to a reduction in the risk of malaria while but it may also

accelerate the spread resistance to antifolate drugs, rendering sulfadoxine-pyrimethamine (SP), the only antimalarial drug currently recommended for prophylaxis in pregnancy, less effective and increasing the risk of infection with resistant parasites. A study conducted in rural Uganda reported that, compared to a malaria incidence of 50.8 episodes per 100 person years in HIV-infected adults, the malaria risk was reduced by 79% by TS prophylaxis [19]. Three other studies have also found that TS prophylaxis is associated with a reduction in the risk of malaria in HIV-infected adults and children [18, 61, 81]. Another study that evaluated treatment outcomes among HIV-infected children and adults in HIV care programs found that HIV-infected adults who were receiving daily TS prophylaxis had a lower risk of treatment failure compared to HIV-uninfected individuals following treatment with chloroquine and sulfadoxine-pyrimethamine. Last, a recent study conducted in an area of very high malaria transmission reported an equivalent risk of placental malaria in HIV-infected pregnant women receiving daily TS and in HIV-uninfected pregnant women [82].

ART use slows the rate of decline of immune function and results in immune reconstitution in HIV-infected individuals and may thus limit the negative effects of HIV infection on malaria outcomes. Furthermore, certain antiretroviral drugs such as protease inhibitors have been shown to have direct antimalarial properties. Thus in the ART era, HIV-infected individuals may no longer be at increased risk of malaria and may even have improved treatment outcomes compared to HIV-uninfected persons. Data on the effect of ART on the risk of malaria among HIV-infected individuals are scarce. One study of adults in Uganda found that compared to the baseline risk of malaria, the use of ART and TS was associated with a 92% reduction in the risk of malaria and a 95% reduction in risk with TS, ART and ITN use [19]. One cross sectional study in children reported that ART use was associated with an increased prevalence of asymptomatic malaria parasitemia[83].

ACTs are highly efficacious antimalarials thatt have been adopted as first-line therapy for the treatment of uncomplicated malaria in 43 of the 45 malaria endemic countries in sub-Saharan Africa[84]. There are scanty data evaluating the efficacy of these treatments in HIV-infected persons. The most recent WHO antimalarial treatment guidelines, which recommend ACT treatment for uncomplicated malaria, state that, with the exception of HIV-infected patients on zidovudine or effavirenz in whom AQ-containing regimens should be avoided, there is insufficient information to modify the general malaria treatment recommendations for patients with HIV/AIDS [85]. There is emerging evidence of adverse drug interactions between ARVs and ACTs and other antimalarials (specifically those that are metabolized by cytochrome P450 enzymes [86]. Non nucleoside reverse transcriptase inhibitors (NNRTIs), specifically nevirapine, may induce enzymes responsible for the metabolism of artesunate-amodiaquine (AS/AQ) and lumefantrine (AL), leading to poorer antimalarial treatment outcomes when these drugs are co-administered. NNRTIs are recommended as first-line ART regimens in Africa while all 43 countries in Africa that have adopted ACTs as first line antimalarial therapy use either AL or AS/AQ, making this potential interaction very important in Africa. Other antiretroviral drugs such as protease inhibitors and efavirenz are known to be potent inhibitors of P450 enzymes necessary for the metabolism of some antimalarials (e.g. lumefantrine and amodiaquine). Co-administration of these drugs would potentially lead to increased antimalarial drug toxicities. Two recent studies found that co-

administration of amodiaquine and efavirenz was associated with inhibition of CYP2C8 enzyme, decreased amodiaquine metabolism and subsequent hematological and liver toxicity [87, 88]. ART-antimalarial interactions may similarly lead to induction or inhibition of antiretroviral drug metabolism

Knowledge gaps in HIV-malaria interactions

As discussed above, research conducted over the last two decades has increased our understanding of the effects of HIV infection on the natural history of malaria. It is now clear that increasing immunosuppression in advancing HIV disease increases the risk of parasitemia and clinical malaria in pregnant women and in non-pregnant adults. In pregnant women, dual infection with HIV and malaria is associated with poor pregnancy outcomes, possibly including increased maternal to child transmission of HIV. In adults living in low malaria transmission settings, HIV infection increases the risk of severe malaria and possibly malaria mortality. Response to antimalarial therapy may be reduced in immunosuppressed adults receiving certain antimalarial drug regimens although the effect of HIV infection on ACT treatment outcomes is virtually unknown. There is emerging evidence to suggest that previously documented interactions may be modified by recently scaled-up HIV and malaria interventions. TS appears to be protective against malaria in some settings, but there are few data concerning either its protective effect against malaria or its effect on the spread of antifolate resistance in children. Few clinical studies have evaluated the effects of ART on the risk of malaria or the clinical implications of hypothesized ARV-antimalarial drug interactions. The biggest gap in knowledge is in children. This is the population at highest risk for clinical malaria, severe malaria and malaria mortality, and there are approximately two million HIV-infected children living in sub-Saharan Africa. However, little is known about the effects of HIV on the risk of malaria, malaria severity of and response to therapy.

DISSERTATION GOALS AND SPECIFIC AIMS

The overall goals of this dissertation are:

1. To contribute to knowledge on the effect of HIV infection on malaria in children living in sub-Saharan Africa
2. To assess the effects of widening access to TS prophylaxis, ART and ACTs on HIV effects on malaria in children living in sub-Saharan Africa.

This dissertation includes studies assessing the risk of malaria in HIV-infected children and evaluating the use of ACT regimens for malaria treatment in HIV-infected children.

Study 1: Effect of Trimethoprim-Sulfamethoxazole on the Risk of Malaria in HIV-Infected Ugandan Children Living in an Area of Widespread Antifolate Resistance

Specific aims

1. To compare the risk of malaria among HIV-infected children receiving TS prophylaxis and HIV-uninfected children not receiving TS prophylaxis in Kampala, Uganda
   Hypothesis 1: The risk of malaria among HIV-infected children receiving TS prophylaxis is lower than the risk of malaria among HIV-uninfected children not receiving TS prophylaxis
2. To determine the relationship between TS prophylaxis and prevalence of antifolate resistance-conferring mutations in *P. falciparum* infections.
   Study hypothesis: The prevalence of antifolate resistance-conferring mutations in *P. falciparum* infections is higher among HIV-infected children receiving TS prophylaxis than the prevalence of antifolate resistance-conferring mutations in *P. falciparum* infections among HIV-uninfected children not receiving TS prophylaxis

Study 2: Risk factors for malaria in a cohort of young HIV-infected children living in an area of high malaria transmission

Specific aim: To determine the independent effects of CD4 percentage level, ART use and interruption of TS prophylaxis on the risk of malaria in HIV-infected infants and children living in an area of very high malaria transmission intensity.
Study hypothesis 1: Decreasing CD4 percentage is associated with an increased risk of malaria among HIV-infected children
Study hypothesis 2: ART use is associated with a decreased risk of malaria among HIV-infected children
Study hypothesis 3: Interruption of TS prophylaxis is associated with an increase in the risk of malaria in HIV-infected children

Study 3: Efficacy and safety of artemisinin combination therapies for treatment of uncomplicated malaria in HIV-infected Ugandan children

Specific aims:

1. To determine the efficacies of artesunate-amodiaquine (AS/AQ), artemether-lumefantrine (AL), and dihydroartemisinin-piperaquine (DP) for the treatment of uncomplicated malaria in HIV-infected children living in two settings of differing malaria transmission intensity.
   Study Hypotheses:
   1) There is no difference in the efficacies of AS/AQ and AL for the treatment of uncomplicated malaria in HIV-infected children living in an area of moderate transmission intensity
   2) There is no difference in the efficacies of AL and DP for the treatment of uncomplicated malaria in HIV-infected infants and young children living in an area of very high transmission intensity

2. To determine the safety and tolerability of artesunate-amodiaquine, artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated malaria in HIV-infected children living in two settings of differing malaria transmission intensity.
   Study Hypotheses:
   1) There is no difference in the safety of AS/AQ and AL for the treatment of uncomplicated malaria in HIV-infected children
   2) There is no difference in the safety of AL and DP for the treatment of uncomplicated malaria in HIV-infected infants and young children

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CHAPTER 2

EFFECT OF TRIMETHOPRIM-SULFAMETHOXAZOLE ON THE RISK OF MALARIA IN HIV-INFECTED UGANDAN CHILDREN LIVING IN AN AREA OF WIDESPREAD ANTIFOLATE RESISTANCE

ABSTRACT

Background

Daily trimethoprim-sulfamethoxazole (TS) protects against malaria, but its efficacy may be diminished as antifolate resistance increases. This study assessed the incidence of *P. falciparum* malaria and the prevalence of resistance-conferring *P. falciparum* mutations in HIV-infected children receiving daily TS and HIV-uninfected children not taking TS.

Methods

Subjects were 292 HIV-infected and 517 HIV-uninfected children from two cohort studies in Kampala, Uganda observed from August 2006 to December 2008. Daily TS was given to HIV-infected, but not HIV-uninfected children and all participants were provided an insecticide treated bed net. Standardized protocols were used to measure the incidence of malaria and identify markers of antifolate resistance.

Results

Sixty-five episodes of *P. falciparum* malaria occurred in HIV-infected and 491 episodes in HIV-uninfected children during the observation period. TS was associated with a protective efficacy of 80% (0.10 vs. 0.45 episodes per person year, $p<0.001$). The prevalences of *dhfr* 51I, 108N, and 59R and *dhps* 437G and 540E mutations were each over 90% among parasites infecting both HIV-infected and HIV-uninfected children. The prevalence of the *dhfr* 164L mutation, which is associated with high-level anti-folate resistance, was significantly higher in parasites from HIV-infected compared to HIV-uninfected children (8% vs. 1%, $p=0.001$). Sequencing of the *dhfr* and *dhps* genes identified only one additional polymorphism, *dhps* 581G, in two of 30 samples from HIV-infected and 0 of 54 samples from HIV-uninfected children.

Conclusion

Despite a high prevalence of known antifolate resistance-mediating mutations, TS prophylaxis was highly effective against malaria, although its use was associated with presence of *dhfr* 164L mutation.

Introduction

Daily prophylaxis with the combination drug trimethoprim-sulfamethoxazole (TS) has been shown to reduce the risk of malaria in HIV-infected adults and children living in sub-Saharan Africa [1-4]. The WHO, UNAIDS and UNICEF recommend TS prophylaxis for all HIV-infected patients with symptomatic disease, HIV-infected asymptomatic adults with CD4 cell counts less than 350 cells/μl and HIV-infected asymptomatic children with CD4 cell percentages less than 25% [5]. Despite the evidence for significant benefits associated with TS use in HIV-infected patients, implementation of TS prophylaxis in sub-Saharan Africa remains low. A recent UNICEF report estimated that only 4% of the 4 million HIV-infected children in need of TS prophylaxis in sub-Saharan Africa are receiving this intervention [6]. There are concerns that expanded TS use may lead to the rapid selection and spread of *P. falciparum* parasites resistant to anti-folates, which may in turn diminish the efficacy of TS itself and of sulfadoxine-pyrimethamine (SP), which acts against the same two enzyme targets as TS. Cross-resistance between TS and SP is of particular concern as SP remains the only recommended drug for intermittent preventive therapy (IPT), a proven means of preventing malaria in pregnant women [7].

To characterize the benefits and risks of daily TS in HIV-infected children, this study evaluated the incidence of malaria and the prevalence of anti-folate resistance-conferring mutations in *P. falciparum* parasites in HIV-uninfected children not taking TS prophylaxis compared with HIV-infected children taking daily TS prophylaxis in an area of moderate malaria transmission.

Methods

The study was an observational cohort study conducted in Kampala, an urban setting in south-central Uganda where malaria is mesoendemic with stable transmission throughout the year. Study participants were from cohorts of HIV-infected and HIV-uninfected children followed up between 2004 and 2009. Details of both studies are described elsewhere [2, 8] and are summarized here briefly. The first study enrolled 599 children who tested HIV-negative or were presumed to be uninfected (432 children tested HIV negative, 167 children had no HIV test result available). Eligibility criteria included: 1) age 1-10 years, 2) agreement to come to the study clinic for any febrile episode or other illness, 3) agreement to remain in Kampala for the duration of the study, 4) agreement to avoid medications administered outside the study protocol, 5) lack of history of any known serious chronic disease requiring frequent medical attention, including HIV/AIDS, 6) weight $\geq$ 10 kg, and 7) provision of informed consent by the parent or guardian. All study participants were given an insecticide treated bednet (ITN) between May-June 2006. The second study enrolled 300 HIV-infected children from a pediatric HIV clinic in Kampala from October 2005 through August 2006 using convenience sampling. Eligibility criteria were the same as for the HIV-uninfected cohort, except for:

1) living within a 20 km radius of the study clinic, 2) no restriction based on a history of serious chronic disease, and 3) weight $\geq$ 5kg. All Participants were given an ITN at enrollment.

Similar protocols were used for follow-up of both cohorts. Subjects were followed for all of their medical problems in study clinics open seven days a week. Children who presented with new medical problems underwent standardized evaluation. Medications with antimalarial activity were avoided for the treatment of non-malarial illnesses whenever possible. HIV-infected subjects meeting standard WHO eligibility criteria were provided antiretroviral therapy according to local guidelines.

Exposure allocation and measurement: As shown in the directed acyclic graph, the exposure of interest was TS prophylaxis, which was prescribed to HIV-infected children only (figure 1). TS compliance was assessed during monthly study visits using self reported three-day recounts.

Outcome measurements: Malaria ascertainment was identical in both cohorts. Subjects who presented to the clinics with a documented fever (tympanic temperature $\geq$ 38.0˚C) or history of fever in the previous 24 hours had blood obtained by fingerprick for a thick blood smear. Thick smears were stained with 2% Giemsa for 30 minutes and parasite density estimated using a rigorous quality control system, as previously described [2]. If the thick blood smear was positive, the patient was diagnosed as having malaria. For repeat episodes of malaria in the same study participant, molecular genotyping was used to distinguish new from recrudescent infections, as previously described [9]. Infections were deemed to be recrudescent if at least one allele was shared at all six loci between consecutive episodes of malaria.

On the day malaria was diagnosed, samples were tested for the following polymorphisms associated with antifolate resistance: dihydrofolate reductase (*dhfr*) N51I, C59R, S108N, and I164L; and dihydropteroate synthetase (*dhps*) A437G and K540E using a nested polymerase chain reaction (PCR) followed by sequence-specific restriction enzyme digestion as previously described [10, 11]. For a subset of samples, the complete *dhfr* and *dhps* genes were amplified by nested PCR and sequenced. Amplicons were purified using Exosap (USB) and sequenced bi-directionally using nested primers at the Genomics Core Facility, UCSF. Sequence data were aligned and manually curated using Lasergene (DNASTAR). Laboratory investigators were blinded to clinical data at the time of molecular analysis.

Measurement of Covariates: Demographic data collected included date of birth and residence. ITN use was assessed at routine monthly visits.

Statistical analysis.

The period of observation began on August 1, 2006 (when enrollment for both cohorts was complete and all study participants had been given an ITN) for all participants and continued through December 15, 2008 (when follow-up for the HIV-uninfected cohort ended) or the date of premature study withdrawal. Data were entered and verified using Access (Microsoft Corporation, Redmond, WA). Analyses were performed using STATA version 10.0 (Stata Corp., College Station, TX). Baseline characteristics of malaria episodes – log-transformed parasite densities and temperature, were compared using two-sample t-tests. Categorical variables were compared using chi-square tests. Malaria

incidence was defined as the number of new episodes of malaria (recrudescences as determined by PCR were excluded). Negative binomial regression models were used to measure the association between use of TS prophylaxis and malaria incidence (expressed as an incidence rate ratio (IRR)), controlling for age as described by the following formula: $\lambda(X, T) = T + e^{(a + b_1 x_1 + b_2 x_2)}$ $IRR = e^{b}$ X=0 (unexposed) =HIV-uninfected, X=1 (exposed): HIV-infected. The protective efficacy of TS was defined as 1 – IRR. Logistic regression models were used to compare the risk of infection with antifolate resistant parasites adjusting for repeated measurements in the same individual assuming an exchangeable correlation structure as described by the formula $\text{Logit } [P(Yij=1 | X_{1ij}, X_{2ij})] = \beta 0 + \beta_1 X_{1ij} + \beta_2 X_{2ij}]$. A p-value < 0.05 was considered statistically significant.

Results

Study participants. Characteristics of study participants are shown in Table 1. A total of 517 HIV-uninfected children were in active follow-up by August 2006 and included in the study. Likewise, 292 of the 300 HIV-infected children initially enrolled were active and included in the present study. The median level of TS adherence in the HIV-infected population over the study period was 100%. Less than 100% TS adherence was reported in only 216/8257 (3%) of total assessments. Likewise ITN use was very high. Only 1% of monthly evaluations in both cohorts were associated with less than 100% compliance. The mean age at the beginning of the observation period was significantly higher for HIV-uninfected compared to HIV-infected participants (7.4 vs. 5.7 years, p<0.0001), primarily because the HIV-infected cohort was recruited about one year later than the uninfected cohort. A total of 452 (87%) HIV-uninfected children and 277 (94%) HIV-infected children were followed for the full observation period. The mean CD4 cell percentage in the HIV-infected cohort was 23% at baseline and 76 (26%) participants received antiretroviral therapy (ART) throughout the study period. Sixty-five (22%) HIV-infected children were initiated on ART during follow-up.

Characteristics of malaria episodes. The large majority of treatments for malaria were for new, rather than recrudescent infections (Table 2). Ninety-two percent of new malaria episodes were due to *P. falciparum* in both HIV-uninfected and HIV-infected children. The geometric mean parasite density of malaria infections was lower for HIV-infected (6,462 parasites/µl) compared to HIV-uninfected children (11,270 parasites/µl), however this difference was not statistically significant (p=0.397).

Malaria incidence. The protective efficacy of TS for the entire observation period was 80% (95% CI 72-85%) after adjusting for age, and efficacy did not decline over the three consecutive 9.5-months periods that constituted the observation period (IRRs: 0.19 (0.12-0.28), 0.26 (0.15-0.43) and 0.20 (0.10-0.38), respectively (Table 3)). The protective efficacy of TS was similar for HIV-infected participants receiving ART (76% (95% C.I. 63-84%)) and HIV-infected participants not receiving ART (83% (95% C.I 74-89%))

Prevalence of resistance-mediating polymorphisms. In new *P. falciparum* infections detected, the prevalences of five *dhfr* and *dhps* point mutations known to mediate

diminished response to therapy with SP and to be common in East Africa [12] (*dhfr* 51I, 108N, and C59R and *dhps* 437G and 540E) were all over 90%, independent of TS use (Table 4) and time. The simultaneous presence of all five of these mutations was also common for HIV-uninfected and HIV-infected participants (86% vs. 92%, p=0.222). In contrast, *dhfr* 164L, which has been associated with high-level SP resistance, was uncommon and significantly more common in HIV-infected compared to HIV-uninfected participants (8% vs. 1%, p=0.001). However, as with the other mutations, the prevalence of the *dhfr* 164L mutation did not change with time in parasites from HIV-infected (12%, 10%, 0% p=0.417) or HIV-uninfected participants (0%, 2%, 0%, p=0.375). To assess for the presence of novel mutations in the dhfr or dhps genes as a result of daily TS use, the complete genes in a subset of samples, including *dhfr* from 40 and *dhps* from 30 HIV-infected children receiving TS and *dhfr* from 73 and *dhps* from 54 HIV-uninfected children not receiving TS were sequenced. Sequencing confirmed the sequences at known polymorphic alleles in all samples but one, in which *dhfr* 108N/51I/164L mutations were seen in the initial analysis and 108N/51I/59R seen with sequencing; this discrepancy was presumably due to characterization of different strains in a mixed infection. Sequencing identified only one additional polymorphism, the previously reported *dhps* 581G mutation, which was seen in samples from 2 of 30 children receiving daily TS and none of the 54 HIV-uninfected children for whom sequence results were available (p=0.125).

Discussion

The presence of simultaneous cohorts including HIV-infected children receiving daily TS and HIV-uninfected children not receiving TS allowed for the assessment of the antimalarial preventive efficacy of TS. For ethical reasons it was not possible to randomize HIV-infected children to TS use and provision of this therapy in HIV-uninfected individuals is not recommended due to the risks of adverse events. The study could thus only compare HIV-infected children exposed to TS and HIV-uninfected children not exposed to TS.

Results from this study show that daily TS was highly efficacious in conferring protection against malaria in HIV-infected children, despite a high prevalence of resistance-mediating *P. falciparum* polymorphisms. TS use has recently been shown to protect against malaria in HIV-infected adults [1] and children [2] in Uganda. The study in adults assessed TS use in a very high malaria transmission setting and compared the incidence of malaria during a period before TS was used to the malaria incidence during a subsequent time period, after TS was introduced to the study cohort. Potential confounding by time varying factors, such as seasonality, was not clearly addressed and may have biased the findings of that study. The prior pediatric study assessed the combined effects of TS and ITN use on malaria risk. Therefore the present study adds to data showing that daily TS is protective against malaria.

It is not yet clear if continued TS protection will be seen with increasing prevalences of mutations in the target enzymes *dhfr* and *dhps* that limit antimalarial treatment efficacy of the related antifolate SP [13-16]. However, in the present study, despite some of the highest prevalences of five key *dhfr* and *dhps* mutations reported in

Africa, TS offered strong protective efficacy against malaria, and protective efficacy was maintained throughout the 29-month observation period.

Drugs that inhibit the folate pathway enzymes *dhfr* and *dhps* have important roles in the treatment of many infections. TS is active against many bacteria and opportunistic pathogens, leading to recommendations for its widespread use in HIV-infected individuals in Africa. SP, which inhibits the same two enzymes as TS, has been a standard antimalarial therapy in Africa for many years. Use of SP to treat malaria is now discouraged due to increasing resistance, especially in Southern and East Africa, with resistance mediated by step-wise selection and also selective sweeps of five key mutations [17]. Additional mutations in *dhfr* and *dhps*, notably *dhfr* 164L, mediate higher levels of resistance, but thus far these mutations are uncommon in Africa [18]. Despite decreasing malaria treatment efficacy, SP retains a key role in malaria control, as it is the only drug well-established to protect against malaria when used as intermittent preventive therapy in pregnant women [19] or infants [20]. The antifolates TS and SP remain critical elements of control efforts for HIV infection and malaria, but resistance-mediating mutations are common in *P. falciparum* in many areas, and key questions remain inadequately answered. This study considered the following three major concerns regarding daily use of TS in HIV-infected individuals.

First, did daily TS offer protection against malaria despite the presence of antifolate resistance-mediating mutations? Five key resistance-mediating mutations, which were already known to be common in much of East Africa [21], were remarkably common in parasites isolated from patients with malaria in our study. In this setting, daily TS nonetheless offered strong preventive efficacy against malaria. Thus, although the resistance-mediating mutations may have mitigated the protective efficacy of TS to some extent, the intervention nonetheless had a pronounced effect. This result bodes well for Africa, where the prevalence of these five key mutations is increasing, but other mutations that mediate higher-level resistance remain uncommon.

Second, did TS use select for parasites with additional antifolate resistance mediating mutations that might diminish any protective efficacy of antifolates? Blood samples were screened for the *dhfr* 164L mutation, which is common in parts of South America and Asia [17], but has not been prevalent in Africa. Recently, however, the mutation was seen in 4% of parasites isolated in Western Kenya [22], in 4.7% of parasites in Malawi from subjects receiving SP [23], and 4-14% of symptomatic subjects not receiving antifolates in two low transmission highland regions of Western Uganda [24]. In the present study *dhfr* 164L was uncommon in HIV-uninfected children who did not receive TS. However, compared to those who did not receive TS, the prevalence of the *dhfr* 164L mutation was significantly greater in HIV-infected children who received TS. These results indicate that, in certain settings, daily TS may select for *dhfr* 164L. The clinical significance of selection for this additional mutation is unknown, but biochemical studies have shown profound resistance in parasites harboring *dhfr* 164L [25]. However, it remains unclear whether the *dhfr* 164L mutation will spread in Africa. Indeed, as is the case with some other resistance-mediating polymorphisms that may engender a fitness cost (e.g. increased copy number of the putative drug transporter gene *pfmdr1*), the high level of antimalarial immunity of populations in areas of high malaria transmission in Africa may limit selection for *dhfr* 164L [18]. The complete *dhfr* and *dhps* genes were sequenced in a subset of samples, and only one additional polymorphism, the *dhps* 581G

mutation, was identified in two samples from children receiving daily TS. This mutation has been associated with high-level in vitro sulfadoxine resistance in *P. falciparum* [26], has been seen in Southeast Asia and South America [26, 27], and was present at a prevalence of 55% in a recent study in northern Tanzania [28]. In the present study, the prevalence of the 581G mutation was low and therefore there was insufficient power to determine if its presence was associated with TS use. Importantly, no novel *dhfr* or *dhps* mutations were identified.

Third, did the protective efficacy of TS decline over time? In this study, TS efficacy was maintained over the 29-month observation period. This is not surprising given the stability of the antifolate-mediating mutations assessed. Five of the six mutations evaluated were close to saturated throughout the study period, while the prevalence of the 164L mutation, which is not yet widespread, did not increase in those exposed or unexposed to TS. The evolution of antifolate resistance likely occurred over several years, limiting the ability of the study to detect changes over the relatively short observation period. Therefore, while the present study gives an indication that TS prophylactic efficacy may be sustained in areas where background antifolate resistance is extensive, more studies will be needed to assess its longer-term efficacy.

This study had some limitations. There was possible residual confounding due to unmeasured factors as a result of lack of randomization of the comparison groups. The data collected on residence and SES may have been insufficient to adjust adequately for this pathway of potential confounding. There is ongoing work to collect additional data on micro environmental (residence) factors. Data on ITN use was reported by subjects' mothers and may have been inaccurate. However it is unlikely that any misclassification of this variable was related to exposure status. Sixteen percent of presumed HIV-uninfected subjects were not tested for HIV, as they had either been excluded from the cohort prior to testing or declined to be tested. However, the lack of HIV infection among the 432 children who were tested suggests that very few of the cohort, if any, had this infection, and any bias resulting from misclassification of HIV status would be expected to underestimate differences seen between the HIV-infected and HIV-uninfected cohorts. The impact of TS on six well characterized polymorphisms in *dhfr* and *dhps* was considered in all samples, but due to logistical constraints, whole gene sequences were assessed in only a subset of samples chosen based on amplification and sequencing success; sequencing of only a portion of non-random samples might have introduced bias. However there is no reason to believe that success of gene amplification was related to TS use. It is however possible that the samples that were amplified were not sufficient to identify other rare polymorphisms whose distribution may have differed in exposed and unexposed subjects. Finally, the study was done in a relatively low malaria transmission setting, and findings may not be generalizable to other settings, particularly those with higher malaria transmission intensity. These limitations, however, do not alter the key conclusions of the study, as noted above.

Considering impacts on malaria, this study strongly supports the use of TS prophylaxis in HIV-infected children in Africa. There are concerns for use of TS in HIV-uninfected population due to potential spread of antifolate resistance and adverse events, however, toxicity was uncommon in our cohort of HIV-infected children. Given the excellent protective efficacy of TS, consideration might be given for use in use in populations at highest risk of adverse consequences of malaria in addition to other

transmission reduction measures such as ITNs. The study was not well-equipped to assess the impact of daily TS use on the selection of common antifolate resistance-mediating mutations, as these were already very common at our study site. However, the selection of an additional mutation, *dhfr* 164L, in children receiving daily TS is concerning, and suggests the need for continued surveillance to monitor the protective efficacy of TS and the prevalence of resistance-mediating polymorphisms.

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Figure 1: Directed Acyclic Graph

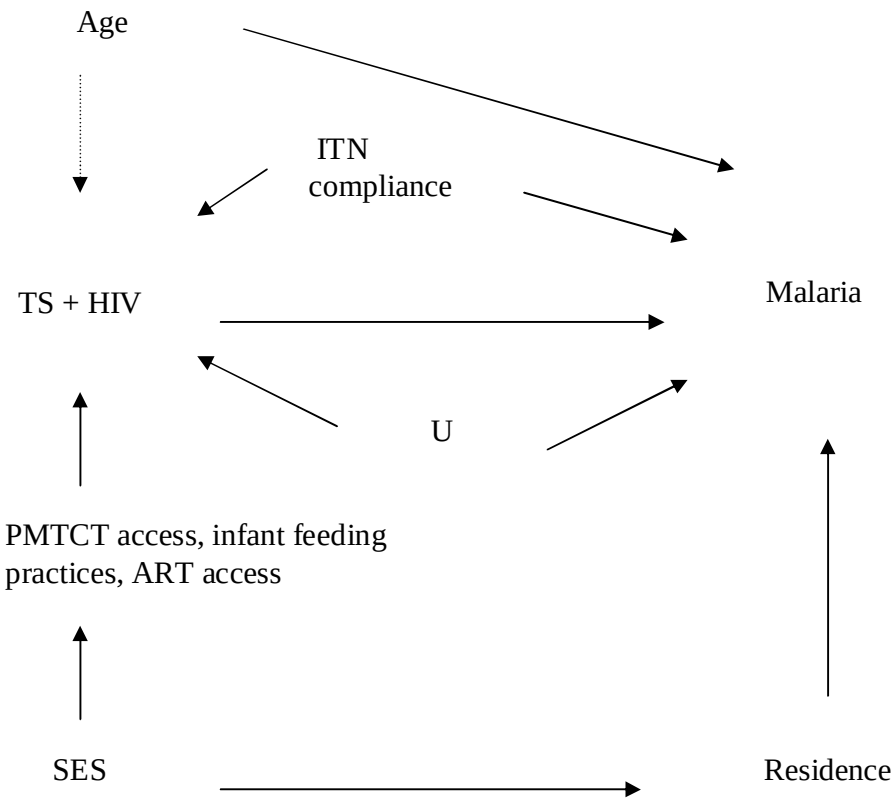

Table 1. Patient characteristics

Characteristic	HIV-uninfected*	HIV-infected*
Number of subjects	517	292
Total person years of observation	1095	665
Median duration of follow up in yrs (range)	2.1(0.2-2.4)	2.4 (0-2.4)
Mean age in yrs at the start of follow-up (SD)	7.4 (2.7)	6.0(2.6)
Mean CD4 percent at start of follow-up (SD)	N/A	23%(8.6)
Antiretroviral (ART) use during follow-up		
Total person years on ART	N/A	275
Number of participants on ART at start of follow-up	N/A	76 (26%)
Number of participants initiated on ART during follow-up	N/A	65 (22%)

*All HIV-uninfected children were not receiving TS while all HIV-infected children were receiving daily TS

Table 2. Characteristics of malaria episodes

Characteristic	HIV-uninfected* (n=517)	HIV-infected* (n=292)
Number of treatments for malaria	511	65
New episodes of malaria[†]	491	65
Species causing new episodes		
P. falciparum[‡]	451 (92%)	60 (92%)
P. malariae	23 (4%)	2 (3%)
P. ovale	14 (3%)	3 (5%)
P. vivax	3 (1%)	0
Geometric means parasite density per µL[§]	11230	6462

* All HIV-uninfected participants were not receiving TS and all HIV-infected participants were receiving daily TS

[†] After exclusion of episodes due to recrudescences identified by genotyping

[‡] Includes *P. falciparum* mono and mixed infections

[§] Only include new episodes of falciparum malaria

CHAPTER 3

RISK FACTORS FOR MALARIA IN A COHORT OF YOUNG HIV-INFECTED CHILDREN LIVING IN AN AREA OF HIGH MALARIA TRANSMISSION

Abstract

Introduction: HIV-infection and advancing immunosuppression have been shown to increase the risk of malaria in adults. However, there are conflicting data on whether HIV increases the risk of malaria in young children, and data on the effect of antiretroviral therapy (ART) on the risk of malaria are scarce. We evaluated the independent effects of CD4 cell percentage, ART, and the use of trimethoprim-sulfamethoxazole (TS) prophylaxis on the risk of malaria in a cohort of young HIV-infected children living in an area of intense transmission intensity in Tororo, Uganda.
Methods: Subjects were 55 HIV-infected children aged 3-19 months of age at enrollment and followed for a median of 23 months. All children received an insecticide treated bednet (ITN) and TS prophylaxis at enrollment. All children were ART-naïve at enrollment and received standard therapy (NNRTI + two NRTIs) if they became eligible according to standard WHO guidelines. Children were followed for all their health care needs, and malaria was diagnosed if they presented with fever and a positive thick blood smear. Malaria was treated with one of two artemisinin-based combination therapies (ACT). CD4 cell percentages were measured every three months. Independent risk factors for the monthly risk of malaria were identified using generalized estimating equations with adjustment for compliance with ITNs, age, location of residence and repeated measures in the same study participant.
Results: A total of 204 episodes of malaria were diagnosed over 1181 person months of follow-up. 50 subjects were initiated on ART and TS prophylaxis was interrupted in eight participants due to toxicity. Independent risk factors for malaria included a CD4 percentage < 20% (RR=2.26, 95% CI 1.40-3.66), ART use (RR=3.02, 95% CI 1.17-7.78) and TS interruption (RR=2.46, 95% CI 1.52-3.97).
Conclusions: In this cohort of young HIV-infected children living in an area of high malaria intensity, greater immunosuppression, interruption of TS prophylaxis, and ART use were independent risk factors for malaria. We hypothesize that certain drugs given as part of ART may induce metabolism of certain ACTs and possibly TS leading to reduced prophylaxis against malaria among those receiving these drugs.

Background

In sub-Saharan Africa, HIV-infection increases the risk of symptomatic malaria in pregnant women and non-pregnant adults [1-4]. In non-pregnant adults, this effect has been shown to be more pronounced as CD4 cell counts decline [3-5],with reported increases in risk as much as four to six times among those with CD4 cell counts less than 200 cells/µl compared to HIV-uninfected adults or those with CD4 cell counts more than 500 cells/µl [3, 4]. Although children are the population most vulnerable to malaria, the effects of HIV-infection on the risk of malaria in this population are unclear. While some studies have reported risks of anemia and mortality to be increased in children co-infected with HIV and malaria [6-9], there is no consensus on whether HIV-infection or declining CD4 cell levels are associated with an increased risk of symptomatic malaria in children. The majority of previous studies have found no effect [10, 11] however, one study reported an increased risk of symptomatic malaria and higher levels of parasitemia in HIV-infected children compared to HIV-uninfected children aged less than five years [12].

Interventions used for treatment and care of HIV may modify the risk of malaria. Daily Trimethoprim-sulfamethoxazole (TS) prophylaxis is recommended for symptomatic HIV-infected individuals, asymptomatic adults with CD4 cell counts less than 350 cells/µl and asymptomatic children with CD4 percentage less than 25% [13]. TS prophylaxis decreases the risk of malaria in HIV-infected adults and likely children [14-18]. However it is not clear if sub-optimal TS use, such as from interruptions as due to toxicity, diminishes its prophylactic efficacy against malaria. The coverage of antiretroviral therapy (ART) in sub-Saharan Africa is increasing [19, 20]. ART may, directly or indirectly, reduce the risk of malaria in HIV-infected individuals. The protease inhibitor class of antiretrovirals (PIs) have antimalarial activity and may thus directly prevent malaria [21, 22]. However PIs are not widely used in sub-Saharan Africa as they are generally reserved for second-line ART [23, 24]. Other ART regimens may reduce the risk of malaria through immune reconstitution. Clinical data on the effects of ART on the risk of malaria are scarce. A study in adults reported a lower risk of clinical malaria among HIV-infected adults treated with ART compared to those who were untreated, after adjusting for CD4 cell counts [15]. Contrary to this finding, a study that investigated the prevalence of *P. falciparum* parasitemia in HIV-infected children found a higher risk of asymptomatic parasitemia in children on ART compared to those not receiving ART [25]. In both these studies treated patients were receiving non-nucleoside reverse transcriptase (NNRTI)-based ART regimens.

Insecticide Treated bed nets (ITNs) are a proven intervention for the prevention of malaria [26-28]. Following findings that ITNs independently reduced the risk of malaria among HIV-infected adults living in a high transmission setting[15], the Centers for Diseases Control now includes this intervention into a basic care and prevention package distributed to HIV-infected individuals in sub-Saharan African countries [29, 30].

We have been following a cohort of young HIV-infected and uninfected children living in an area of very high malaria transmission intensity to assess the interactions of HIV and malaria in children. We used data from this cohort to evaluate the effects of ART use, TS use and CD4 percentage levels and ITN use on the risk of malaria in HIV-

infected children adjusting for other known factors associated with the risk of malaria. The structural relationships between exposures and the outcome are shown in figure one.

Methods

Study setting. The study is part of the Tororo Child Cohort (TCC) study, a prospective observational cohort study being conducted in Tororo District, Uganda. The entomological inoculation rate (EIR) in Tororo has been estimated to be 591 infective bites per person per year [31]. Details of the TCC study have been described elsewhere [32] and are summarized here. One-hundred HIV-unexposed (born to HIV-uninfected mothers), 203 HIV-exposed (born to HIV-infected mothers, and testing negative for HIV infection), and 48 HIV-infected infants were enrolled between August 2007 and April 2008 using convenience sampling. Eligibility criteria were: 1) age 1.5-12 months, 2) documented HIV status of mother and child, 3) agreement to come to the study clinic for any febrile episode or other illness, 4) agreement to avoid medications administered outside the study protocol, 5) living within a 30 km radius of the study clinic, 6) absence of active medical problem requiring in-patient evaluation at the time of screening, 7) currently breastfeeding if HIV-exposed, and 8) provision of informed consent from parent or guardian. Malaria outcomes including malaria incidence have been measured in the cohort for over two years. Children who met the following criteria were included in the present study: 1) subjects with confirmed HIV-infection at enrollment (n=48) or those who were HIV-exposed and sero-converted during breastfeeding (n=9) and 2) subjects who completed at least one full calendar month of follow-up after documentation of HIV-infection.

Study participant follow-up. At enrollment, daily TS was prescribed for prophylaxis and an ITN was provided to each participant. Subjects were followed for all of their health care needs at a dedicated study clinic open seven days a week. At monthly routine study visits, study participants' TS and ITN compliance, and breast feeding status were assessed through self-reporting by their mothers. TS prophylaxis was suspended in patients who developed severe adverse events and restarted after resolution of the adverse event. Study participants were encouraged to come to the clinic for all their health care needs. Subjects who met World Health Organization criteria [24] were initiated on ART (nevirapine (NVP) + lamivudine (3TC) + zidovudine (AZT) or stavudine(D4T)). Children with new medical problems underwent standardized medical evaluation and algorithms were developed to guide therapy for common illnesses. Children who presented with subjective or documented fever (tympanic temperature $\geq 38^0$C) in the previous 24 hours had blood obtained by finger prick for a thick blood smear. If the thick blood smear was positive, the patient was diagnosed with malaria. Children diagnosed with uncomplicated malaria were randomized to receive either artemether-lumefantrine or dihydroartemisinin-piperaquine for malaria treatment and those with complicated or severe malaria were treated with quinine.

Laboratory methods. Thick and thin blood smears were stained with 2% Giemsa for 30 minutes. A smear was judged to be negative if no parasites were seen after review of 100 high-powered fields. The diagnosis of malaria was based on initial readings of thick blood smears. Speciation of parasites was based on readings of thin smears. Final

microscopy results used a rigorous quality-control system that included re-reading of all blood smears by a second microscopist and resolution of any discrepancies between the first and second readings by a third microscopist. CD4 cell counts and percentages were measured every three months using standard methods.

Statistical analysis. Data were entered and verified in Epi-Info version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA). Analyses were performed using STATA version 10.0 (Stata Corp., College Station, TX). The study period was from 1st September 2007 to 31st December 2009 and the unit of analysis was a calendar month. The period of observation for each study participant began at the beginning of the first calendar month for which the participant had complete data and ended on December 31st 2009 or, for those who were withdrawn before 31st December 2009, at the end of the last calendar month for which they had complete follow-up. Participants were censored during any other months between the start and end of observation when follow-up was incomplete.

The outcome of interest was malaria incidence per month. All repeat episodes of malaria in the same individual which occurred within 14 days of diagnosis of a previous malaria diagnosis were considered to be recrudescent infections and not included in the analysis. Malaria was coded as a binary outcome and only six participants had more than one malaria diagnosis in the same month.

Age was categorized into quartiles (less than 12 months, over 12 to 18 months, over 18 to 24 months, over 24 to 38 months). ART use was categorized as: none (ART-naïve), partial (initiated ART during the month), or complete (received ART throughout the month). TS use was categorized as: complete (received TS throughout the month), partial (received TS for part of the month), or none (not on TS throughout the month). CD4 percentage levels were graded: as CD4 percentage $\geq$ 20%, or CD4 percentage < 20%. CD4 grades were imputed for the months when CD4 cell measurements were not done using the following rules: 1) when two consecutive CD4 percentage measurements did not change the CD4 grade, the months in between the two measurements were assigned the same CD4 grade, 2) when two consecutive CD4 percentage measurements changed CD4 grades either from low to high or high to low in patients in whom ART status did not change, the change in CD4 percentage was assumed to be linear in the direction of change and grades were assigned accordingly, 3) when two consecutive CD4 percentage measurements changed CD4 grades from low to high and ART was initiated between the two measurements, CD4 level was classified as low for the months prior to ART initiation and a linear increase in percentage was assumed after the first month of ART prescription. Other variables that were collected as potential risk factors for malaria were breastfeeding (not breastfed throughout the month, breastfed for part of the month, breastfed throughout the month), ITN use (consistent ITN use if participants were reported as having slept under an ITN the night prior to routine monthly assessment or inconsistent ITN use, otherwise) and residence (rural or urban).

Descriptive statistics were used to summarize baseline characteristics. Relationships between different exposures were explored using linear or logistic regression models adjusting for repeated measures in the same individual and assuming exchangeable correlation structure. Univariate and multivariable regression models, adjusting for repeated measures in the same individual, were used to determine the relative risks associated with exposures and the outcome. Variables found to be significantly associated with the outcome at univariate analysis were included in a

multivariable model together with interaction terms for ART use and CD4 grade and CD4 grade and age, A backward stepwise approach was used to select the final model considering significance levels at p values < 0.05 and p < 0.2 for interaction terms using two-sided tests. Stratified analyses were used to assess effect modification by age on the relationship between ART use and malaria. To further explore the potential confounding by unmeasured time-related confounders on the relationship between ART use and malaria, the 28-month study period was divided into three consecutive time periods: the initial ten calendar months of the study, the next nine months and the last nine months of the study and the effect of ART use on malaria in each of the three time periods was determined and compared to the overall effect unadjusted by time.
To confirm that imputation of CD4 percentage levels did not lead to differential misclassification of this exposure and subsequent confounding, we compared coefficients and inference for covariates from a model restricting the analysis to one month following CD4 measurements to those from a model using all eligible months and imputed CD4 level data. There were no differences in the directions of exposure-outcome associations. Inferences changed from non-significant trends to become statically significant for the relationships between residence and ART use and malaria incidence. The model with imputed data was used for the final analysis.

Results

Characteristics of the cohort. Fifty-five children aged between 2.7 months and 18.7 months were enrolled between September 2007 and October 2008. The median follow-up time per participant was 23 months and the total person-time was 1181 person-months (Table 1). By the end of the observation period, 50 participants (91%) were receiving ART. Of those receiving ART, 86% were receiving NVP, 3TC and AZT while 14% where receiving D4T, 3TC and NVP. None of the participants were switched to second-line therapy during the observation period. As expected, ART use was associated with increasing CD4 percentage levels (p-value<0.001) and age (p value <0.001). Regardless of ART use, CD4 percentage increased with age. The mean CD4 percentages were 26.5% (SD 8.2) in children less than 12 months of age, 31% (SD=9.3) in those aged 12-18 month old, 33.6% (SD=9.3) in those aged >18 – 24 months and 37.1% (SD=8.9) in children over 24 months (p<0.001). During the observation period, TS was stopped in eight participants, due to severe neutropenia, leading to a total of 15 person-months off TS (incidence 0.01/per person per month). In all cases, adverse events resolved spontaneously and TS was successfully restarted. All TS interruptions occurred in children receiving ART. TS adherence was high; 100% adherence was reported in 97.9% of the total monthly assessments. Only 1.3% of 1161 assessments for ITN compliance were associated with inconsistent ITN use. ITN compliance data were missing for 20 months. Missing data were not associated with age, ART use or TS use.

Risk factors of malaria. A total of 207 episodes of malaria were diagnosed during the observation period in 40 (73%) study participants (incidence 0.17 episodes per person per month). In the univariate analysis, increasing age, low CD4 percentage level, use of ART, absence of TS prophylaxis, inconsistent ITN use and rural residence were all significantly

associated with an increased risk of malaria (Table 2) and there was a trend towards a lower risk of malaria among those who were breastfeeding (RR: 0.65 [95%CI 0.39 – 1.07], p=0.089). After adjusting for other variables, breastfeeding was not associated with malaria risk (RR: 0.82 [95% CI 0.48 – 1.40], p=0.459) and not included in the final model.

ART use was the strongest risk factor for malaria. Children receiving ART throughout the month had a 3-fold increased risk of malaria compared to those who were not receiving ART (RR: 3.02, [95% CI 1.17 – 7.78], p=0.02). This effect was significant in children less than 18 months of age (RR: 2.45, [95% CI 1.21 – 4.93], p =0.012), but did not reach statistical significance in those aged 18 months or older (RR: 2.95 [95% CI 0.78 – 11.17], p=0.111). As shown in figure 2, the effect of ART was consistent among those with high and low CD4 percentage levels. The number of malaria episodes during the person time off ART and the person time on ART were compared. Seven episodes of malaria occurred prior to ART initiation (4%) compared to 189 (96%) episodes which occurred after ART initiation (p=0.027). Potential confounding by other unmeasured time-related factors, on the relationship between ART use and malaria was directly explored. Of the 204 episodes of malaria diagnosed in the observation period, 50 (25%) occurred in the first ten months of follow-up, 68 (33%) occurred during the middle nine months and 86 (42%) occurred during the last nine months (p=0.034). Although ART use was more common over time (p <0.001), its effect on malaria was maintained in all three consecutive periods of observation.

Interruption of TS prophylaxis was associated with 2.5 times the risk of malaria compared to uninterrupted TS use (RR: 2.46 [95% CI 1.52 – 3.97], p<0.001). CD4 percentage less than 20% was similarly associated with over twice the risk of malaria when compared to CD4 percentage of 20% or higher (RR: 2.26 [95% CI: 1.40 – 3.66], p=0.001). Age did not modify the effect of CD4 level of malaria (p=0.611) and as illustrated in figure 2, neither did ART use (p=0.359). To test for time dependent confounding between malaria and CD4 percentage level, the effect of previous malaria on CD4 percentage levels was assessed. Malaria was not associated with a decline in CD4 cell percentage (p=0.206) in the next calendar month.

Inconsistent ITN use, increasing age and rural residence remained independently predictive of malaria after adjusting for other variables (Table 2).

Discussion

Our study evaluated HIV-related risk factors for malaria in infants and young children receiving standard HIV care and living in an area of very high malaria transmission intensity. The overall incidence of malaria was high. Low CD4 percentage, interruption of TS prophylaxis and use of ART all independently increased the risk of malaria. Additionally, and as found in other studies of HIV-uninfected populations, inconsistent ITN use and rural residence were associated with an increased risk of malaria.

The finding of a higher risk of symptomatic malaria with advancing immunosuppression has been reported in studies of both untreated [3-5], and treated HIV-infected adults [15] but not in children. Indeed there are scanty data to suggest that HIV-infection increases the risk of symptomatic malaria in infants and young children

and leading to the suggestion that HIV-related immunosuppression may impair only well-developed antimalarial immunity [33]. Our study was not designed to assess the effect of HIV on the risk of malaria in young children; however the finding of an inverse relationship between the level of CD4 cell percentage and the risk of malaria suggests that advancing HIV-related immunosuppression increases the risk of malaria in this young age group. A previous study that followed infants living in an area of relatively low malaria transmission, from birth to 48 months of age, reported an equivalent risk of malaria in HIV-infected and uninfected children[10]. However that study did not account for level of immunosuppression. Another study of infants, enrolled at five to nine months of age, and followed for 13 months found the risk of malaria in children with clinical AIDS was not different from that of children with asymptomatic HIV-infection or those who were HIV-uninfected [11]. In both of these studies, the overall incidence of malaria was much lower than seen in our study population, suggesting that host immunity may play an increasingly critical role in the protection against malaria with increasing exposure to infection. Furthermore, the use of ITNs, daily TS and ACTs for malaria treatment may modify previously observed HIV-malaria interactions in young children. Our finding that the effect of CD4 percentage level on malaria was independent of ART use, suggests that immune reconstitution after ART initiation, adequately restores malaria-specific immune function. Studies in adults have reported that malaria is associated with a temporary decrease in CD4 cell counts [34, 35]. We did not find evidence of long-term reduction of CD4 cell levels after an episode of malaria.

TS prophylaxis is known to reduce the risk of malaria in HIV-infected persons. It is therefore not surprising that its interruption would increase the malaria risk. Serious adverse effects of daily TS prophylaxis, necessitating its interruption are rare [12, 36-38]. However our study findings show that among the few individuals in whom TS is withdrawn, its temporary absence has important negative effects for the risk of malaria.

It is not clear why ART use increased the risk of malaria in our population. PI antiretrovirals have been shown to have direct antimalarial activity in vitro and in vivo and it would be expected that NNRTIs and nucleoside reverse transciptase inhibitors would repair previously compromised host immunity leading to a similar reduction in the risk of malaria. In our study ART use was associated with an increase in CD4 levels, ruling out the possibility of a poor response to ART. We further evaluated confounding by age and other unmeasured time related factors that may have lead to an increase in malaria later in the study when most ART use occurred. We ruled out bias due to reverse causation (malaria leading to a reduction in CD4 percentage level and subsequent ART initiation) by ensuring time ordering in the analysis and assessing the effects of malaria on future CD4 percentage level. A recent prospective study in children reported that ART use was associated with a four-fold increased odds of asymptomatic *P. falciparum* parasitemia and three-fold higher parasite densities among those infected with *P. falciparum* [25] but that CD4 cell count levels were not associated with the risk of parasitemia. As in our study, participants in that study were predominantly receiving NVP, 3TC and AZT. It is possible that this effect is a result of pharmacokinetic interactions between certain antiretroviral drugs and TS or ACTs. Both trimethoprim and sulfamethoxazole are partially metabolized by cytochrome P450 (CYP) enzymes including CYP3A4 and CYP 2C9 [39-41] and NNRTIs, including nevirapine (NVP) which was predominantly used in our study population, are known to induce CYP3A4

[42, 43]. This would mean that persons on NNRTIs may be receiving a lower effective dose of TS than is sufficient for prevention of malaria. We were unable to test this hypothesis in our population as there was insufficient person-time off TS. Pharmacokinetic interactions between NNRTIs and artemisinin combination therapies (ACT) used for treatment of malaria may also have contributed to our observation of an increased risk of malaria with ART use. In our population, children with malaria were randomized to receive artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine for the treatment of malaria. There is potential for NVP to induce lumefantrine metabolism and thus attenuate any post-treatment prophylaxis provided by AL. But the limited person-time off ART in this HIV-infected cohort limited our ability to test this hypothesis. We plan to explore this hypothesis by comparing the risk of future malaria among HIV-infected children on ART receiving AL for malaria treatment and HIV-exposed children not on ART and receiving AL for malaria treatment.

Our study had some limitations. Its observational design may have led to confounding by unmeasured factors. Furthermore, because we were unable to randomize subjects to TS and ART use, we had insufficient power to fully assess interactions between these factors and other exposures. Our HIV-treated population was predominantly receiving one combination ART regimen and therefore we were unable to separate potential effects by different regimens and we are not able to generalize our findings to populations receiving other regimens. Regardless of these limitations however, we were still able to identify important risk factors for malaria in our population.

Our study provides the first data characterizing risk factors for malaria in infants and young children with perinatally acquired HIV-infection. In the setting of high malaria transmission, children with advancing immunosuppression are at increased risk of malaria. Our findings further demonstrate that in such settings, it is important to emphasize ITN compliance and to limit any interruptions of TS prophylaxis to the shortest time deemed necessary. The unexpected finding of a higher risk of malaria in children on NNRTI-based ART underscores the need to fully investigate interactions between ARVs and other therapies used in HIV-infected persons and ARVs and antimalarial drugs. More studies in diverse HIV-infected populations and malaria transmission settings are needed to confirm our findings.

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Figure 2: **Direct Acyclic Graph showing relationships between exposure and outcome variables**

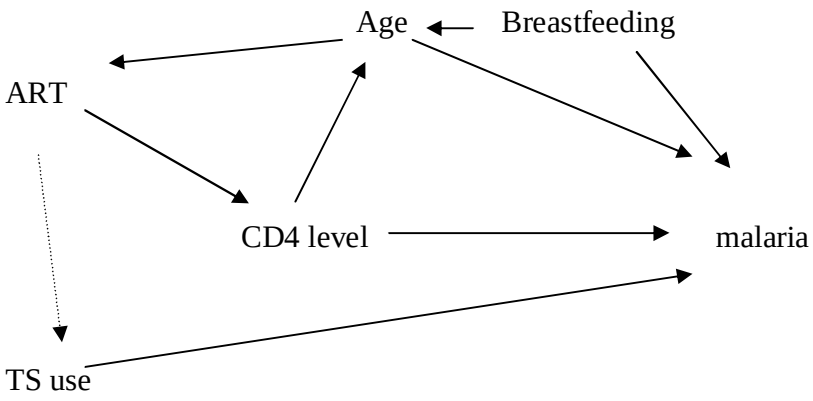

Figure 3: Relationship between ART use CD4 percentage and risk of malaria

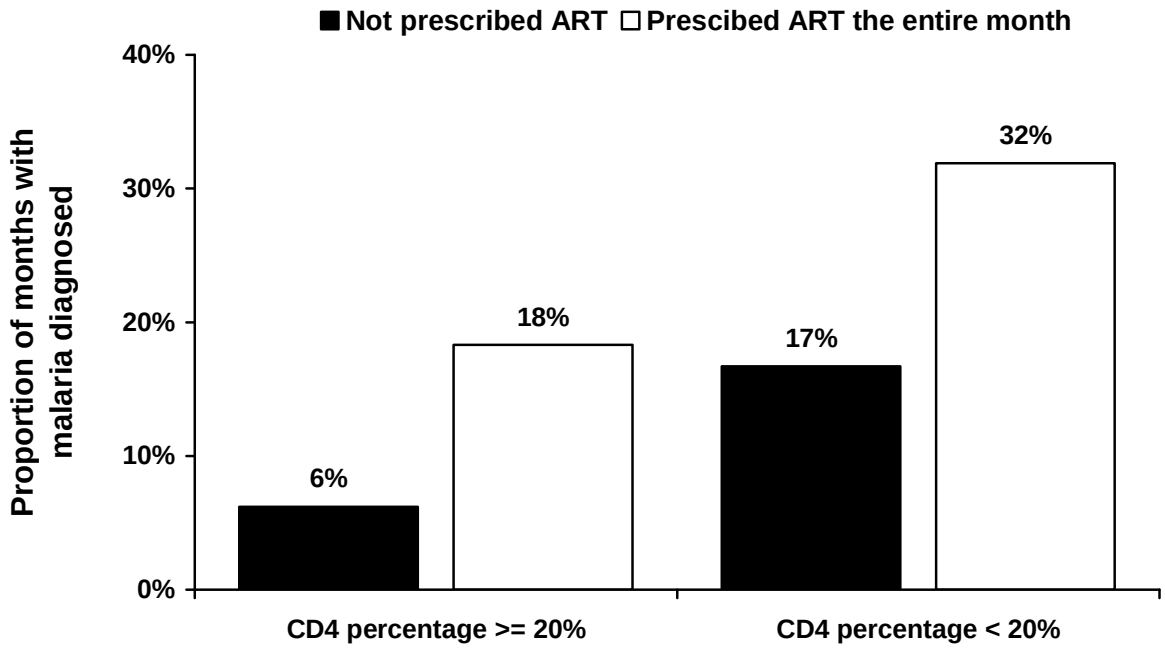

Table 3: Characteristics of study cohort

Characteristic	Result
Number of children	55
Total person months of follow up	1181
Median number of months of follow-up per child (range)	23 (1-28)
Mean age in months at start of observation period (SD)	7.5 (3.5)
Person months ART prescribed	
No ART prescribed	169 (14%)
ART prescribed part of the month	47 (4%)
ART prescribed the entire month	965 (82%)
Person months TS prescribed	
No TS prescribed	15 (1.3%)
TS prescribed part of the month	16 (1.4%)
TS prescribed the entire month	1150 (97%)
CD4 levels over study period	
Mean CD4 percent among participants not receiving ART (SD)	24% (7.3)
Mean CD4 percent among participants receiving ART (SD)	33% (8.5)
Did not sleep under ITN the previous night on routine monthly questionnaire *	15 (1.3%)
Person months with at least one episode of malaria diagnosed	204 (17%)

Table 4. Risk factors for being diagnosed with malaria for each month of observation

Risk factor	Categories	Proportion of person months with malaria diagnosed	Univariate analysis		Multivariate analysis	
			RR (95% CI)	p-value	RR (95% CI)	p-value
Age	2 - <12 months	29/275 (11%)	1.0 (reference)	-	1.0 (reference)	-
	12 - <18 months	49/287 (17%)	1.63 (1.02-2.62)	0.04	1.29 (0.82-2.04)	0.27
	18 - < 24 months	57/288 (20%)	1.90 (1.14-3.16)	0.01	1.51 (0.88-2.59)	0.13
	24 - < 38 months	69/331 (21%)	1.95 (1.12-3.40)	0.02	1.61 (0.92-2.82)	0.10
ART prescribed	None	13/169 (8%)	1.0 (reference)	-	1.0 (reference)	-
	Partial	5/47 (11%)	2.23 (0.88-5.60)	0.09	1.69 (0.69-4.14)	0.25
	Complete	186/965 (19%)	3.48 (1.37-8.81)	0.009	3.02 (1.17-7.78)	0.02
TS prescribed	Complete	197/1150 (17%)	1.0 (reference)	-	1.0 (reference)	-
	Partial	1/16 (6%)	0.88 (0.34-2.24)	0.79	0.78 (0.30-1.90)	0.55
	None	6/15 (40%)	2.93 (1.65-5.22)	<0.001	2.46 (1.52-3.97)	<0.001
CD4 percentage	CD4 % $\geq$ 20%	174/1068 (16%)	1.0 (reference)	-	1.0 (reference)	-
	CD4 % < 20%	30/113 (27%)	1.52 (0.93-2.48)	0.09	2.26 (1.40-3.66)	0.001
ITN use	Consistent ITN use	194/1146 (17%)	1.0 (reference)	-	1.0 (reference)	-
	Inconsistent ITN use	5/15 (33%)	2.07 (0.95-4.51)	0.07	1.77 (1.04-2.99)	0.03
Residence	Urban	34/354 (10%)	1.0 (reference)	-	1.0 (reference)	-
	Rural	170/827 (21%)	2.34 (1.22-4.47)	0.01	2.40 (1.27-4.53)	0.007

CHAPTER 4

EFFICACY AND SAFETY OF ARTEMISININ COMBINATION THERAPIES FOR TREATMENT OF UNCOMPLICATED MALARIA IN HIV-INFECTED UGANDAN CHILDREN

Abstract

Background: Artemisinin-based combination therapies (ACTs) are now widely recommended as first-line treatment for uncomplicated malaria. There are limited data on the efficacy and safety ACTs in HIV-infected children.
Methods: Two cohorts of HIV-infected Ugandan children were followed to measure the incidence of malaria and response to antimalarial therapy: 300 children aged 1-14 years followed for three years in urban Kampala, and 57 children aged 6 weeks - 3 years followed for two years in rural Tororo. All children were given insecticide treated bednets, trimethoprim-sulfamethoxazole prophylaxis and antiretroviral therapy if indicated. In Kampala, uncomplicated malaria was treated with AS/AQ during the first 23 months of the study and with AL during the last 30 months of the study. In Tororo subjects with malaria were randomized to receive AL or dihydroartemisinin-piperaquine (DP) for all uncomplicated malaria episodes. Adverse events and treatment outcomes were assessed after 28-days of follow-up.
Results: The incidence of malaria was 0.10 and 2.08 episodes per person year in Kampala and Tororo, respectively. A total of 41 AS/AQ and 37 AL treatments occurred in Kampala, and 125 AL and 87 DP treatments occurred in Tororo. All three ACTs were 100% efficacious after adjustment for genotyping. In Kampala, AS/AQ was associated with a higher risk of neutropenia ($p=0.001$), malaise ($p=0.010$) and anorexia ($p=0.014$) compared to AL. In Tororo, DP had the added benefit of lowering the risk of recurrent parasitemia compared to AL (7.1% vs. 34%, $p <0.001$).
Conclusions: Our findings support the use of AL and DP for the treatment of uncomplicated malaria in HIV-infected children. AS/AQ, though highly efficacious, was poorly tolerated and associated with a high risk of neutropenia.

Introduction

Widespread antimalarial resistance has led to a recent global shift in the treatment of malaria. The World Health Organization (WHO) now recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated falciparum malaria. By 2009, 43 of the 45 malaria endemic African countries had adopted an ACT, in all cases either artemether-lumefantrine (AL) or artesunate-amodiaquine (AS/AQ) as first line antimalarial therapy [1]. AL is the first-line antimalarial treatment in 22 sub-Saharan African countries and the most widely used ACT in the region [2]. Recent trials evaluating AL efficacy report 28-day cure rates for uncomplicated falciparum malaria of over 95% [3-5]. AS/AQ is the first-line therapy in 21 sub-Saharan African countries [2]. There is geographical variability in the efficacy of AS/AQ for treatment of uncomplicated malaria. Some studies, predominantly from West Africa, report 28-day efficacy rates of 95% or higher [3, 6, 7]; however, in other settings failure rates have exceeded 10% [7, 8]. In 2009, dihydroartemisinin-piperaquine (DP) was added to WHO's list of recommended ACTs for the treatment of uncomplicated malaria [1]. DP has consistently been shown to be as efficacious as other ACTs for the treatment of uncomplicated falciparum malaria [9-12] and superior in terms of preventing recurrent malaria after therapy [10, 13, 14].

ACTs have been shown to be very safe for use in the general population, with contraindications limited to women in their first trimester of pregnancy and young infants (< 5 kg) currently, due to insufficient safety data in these populations[15].

While there is compelling evidence to support the use of ACTs for malaria treatment in the general population, data evaluating their efficacy and safety in HIV-infected populations are limited. Data on the effect of HIV on antimalarial treatment efficacy are inconclusive. Although some studies have shown equivalent antimalarial efficacy in HIV-infected and HIV-uninfected persons, [16-18] others have reported lower efficacy in HIV-infected populations [19-21]. However, these studies were almost exclusively limited to adult populations and non-ACT treatment regimens that are no longer recommended. The increasing use of daily trimethoprim-sulfamethoxazole (TS) prophylaxis and antiretroviral therapy (ART) in HIV-infected Africans raises concerns about the safety of antimalarial therapy use in this population. In particular, there is emerging evidence of potential adverse pharmacokinetic interactions between different antiretrovirals and various ACTs [22, 23]; however, data evaluating the clinical relevance of these safety concerns are scarce.

In this study, data from 290 episodes of malaria treated with ACTs were used to evaluate the efficacy and safety of AS/AQ, AL and DP in HIV-infected children living in two regions of Uganda with different levels of malaria transmission intensity.

Methods

Study settings. Study subjects were HIV-infected children enrolled in two cohort studies in the Kampala and Tororo districts of Uganda. The study settings and parent studies are described in previous chapters. The entomological inoculation rate (EIR) in Kampala has

been estimated to be less than 10 infective bites per person year in 2005 (unpublished data) and 591 infective bites per person-year in Tororo [24].
The 300 and 57 HIV-infected subjects from Kampala and Tororo are described in the two previous chapters. Similar protocols were used for follow-up of both cohorts. Subjects were followed for all of their medical problems in study clinics open seven days a week with after hours care available at adjacent hospitals. Malaria ascertainment was identical in both studies and medications with antimalarial activity were avoided for the treatment of non-malarial illnesses whenever possible. Subjects meeting standard WHO eligibility criteria were provided antiretroviral therapy according to local guidelines. The preferred first-line ART regimen prescribed for infants and young children in Tororo was nevirapine (NVP), lamivudine (3TC) and zidovudine (AZT). In the Kampala-based cohort, where children were older, the preferred first line ART regimen was efavirenz (EFV), 3TC and AZT. In both studies, stavudine (D4T) was chosen in place of AZT when AZT was contraindicated and in the Kampala study, children receiving D4T-containing regimens at enrollment were continued on the these regimens.
Malaria treatment allocation. Children enrolled in the Kampala study diagnosed with uncomplicated malaria were treated with AS/AQ. In July 2007, AS/AQ was replaced with AL due to a high risk of neutropenia observed following treatment with AS/AQ [17]. Study subjects in Tororo aged 4 months or older and weighing at least 5 kg were randomized at the time of their first diagnosis with uncomplicated malaria to receive either AL or DP. All subsequent uncomplicated malaria episodes were treated with the same regimen. Treatments for uncomplicated malaria were given according to standard weight-based guidelines. For study participants treated with AS/AQ or DP, all treatment doses were directly observed at the study clinics. For AL, the first daily dose was directly observed, and caretakers were instructed to give the second daily dose at home. Patients who vomited within 30 minutes of taking study medication had their dose re-administered.
Outcome assessment: For both cohorts, patients diagnosed with uncomplicated malaria were asked to return to the study clinics on days 1, 2, 3, 7, 14 and 28 and whenever they felt unwell. Blood was obtained by finger prick for thick blood smears and storage on filter paper on all follow-up days, except day 1. Treatment outcomes were classified according to WHO guidelines as early treatment failure (ETF; complicated malaria or failure to adequately respond to therapy on days 0-3), late clinical failure (LCF; complicated malaria or fever and parasitemia on days 4-28), late parasitological failure (LPF; asymptomatic parasitemia on days 7-28), and adequate clinical and parasitological response (ACPR; absence of parasitemia on day 28, without previously meeting criteria for treatment failure) [25]. Patients were not assigned a treatment outcome in the event of: 1) use of antimalarials outside of the study protocol, 2) loss to follow-up, or 3) withdrawal of informed consent. Laboratory methods for malaria confirmation and to differentiate new from recrudescent infections were as described in chapter one.

During the 28-day follow up period participants were actively assessed for adverse events by comparing baseline to follow-up clinical symptoms and signs. An adverse event was defined as any untoward occurrence, irrespective of its suspected relationship to the study medications, as per International Conference of Harmonization guidelines. Adverse events were graded as mild, moderate, severe or life-threatening using the National Institutes of Health Division of AIDS toxicity tables for grading and

severity of adverse events [26]. Laboratory assessments in the Kampala-based study included a complete blood count and liver function tests (alanine aminotransferase) measured on days 0 and 14, while in the Tororo study, only hemoglobin measurements were done at baseline and on day 28.

Figures one and two are Directed acyclic graphs describing the relationship between ACT treatment and antimalarial response and ACT treatment and risk of adverse events applicable to the Kampala-based cohort where treatment was related to calendar time.

Statistical analysis. Data were entered and verified using Access (Microsoft Corporation, Redmond, WA) or Epi-Info version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA). Analyses were performed using STATA version 10.0 (Stata Corp., College Station, TX). Linear and logistic regression models, adjusting for repeated measures in the same subject were used to compare continuous and categorical baseline variables. Malaria incidence was calculated as the number of episodes of malaria per time of observation. For study participants living in Kampala, the period of observation began the day after enrolment and continued until each patient completed 3 years of follow up (between October 2008 and September 2009) or on the date of premature study withdrawal. For study participants living in Tororo the period of observation began the day after enrollment or the date children who sero-converted first tested HIV positive and continued until December 31, 2009 or the date of premature study withdrawal. Efficacy outcomes were 28-day risk of recurrent falciparum parasitemia (early treatment failure, late clinical failure or late parasitological failure) both unadjusted and adjusted by genotyping to distinguish recrudescence and new infections. Risks of recurrent parasitemia were estimated using the Kaplan-Meier product limit formula.

Data were censored for patients who did not complete follow-up. Pairwise comparisons of treatment efficacy for individual episodes of malaria were made using a Cox proportional hazards model with adjustment for repeated measures in the same patient. Logistic regression models, adjusting for repeated measures in the same individual, ART use, baseline CD4 percent and malaria episode as described by the formula, were used for pairwise comparisons for risks of adverse events of AS/AQ and AL. The parameter of interest is represented as β1 in the formula: $[P(Yij=1| X_1ij, X_{2ij}, X_{3ij})] = \beta0 + \beta_1X_{1ij} + \beta_2X_{2ij} + \beta_3X_{3ij} + \beta_4X_{4ij}$ (X1= ACT treatment, X2=ART use X3=malaria episode, X4=CD4 level at the start of the treatment period) Exchangeable correlation structure was assumed for repeated measures in the same individual. A p-value < 0.05 was considered statistically significant.

Results

Study subjects. 300 HIV-infected children living in Kampala and 57 HIV-infected children living in Tororo were included in this study (Figure 3). Characteristics of study participants are shown in Table 1. Kampala participants were older than those in Tororo. Immunological status, as measured by CD4 percentage, was comparable in the two cohorts however; over 90% of Tororo participants were receiving ART by the end of the observation period, compared to 50% of Kampala participants. The total person time of follow-up for Kampala-based participants was 805 person-years and for Tororo-based participants, 102 person-years. The incidence of malaria was over 20-fold higher in

Tororo than in Kampala (2.08 episodes per person year vs. 0.10 episodes per person year, respectively). Of the 300 subjects enrolled in Kampala, 50 (17%) were treated for malaria at least once (median = 1 treatment, range 1-4), resulting in 41 AS/AQ treatments and 37 AL treatments. In contrast, 41 of the 57 (72 %) subjects enrolled in Tororo were treated for malaria at least once (median = 4 treatments, range 1-24), resulting in 125 AL treatments, 87 DP treatments, and one quinine treatment.

Baseline characteristics of malaria episodes. All malaria episodes diagnosed in both cohorts were uncomplicated with the exception of one episode treated with quinine in Tororo, due to a danger signs (single convulsion). Characteristics of malaria episodes on the day of diagnosis are shown in Table 2. Over 93% of episodes of malaria were due to *P. falciparum* in both cohorts. Parasite densities were marginally higher in Tororo compared to Kampala (geometric mean 11032 vs. 6646 parasites/µl, p=0.12). The prevalence of anemia (Hb <10 g/dl) was higher in Tororo compared to Kampala (53% vs. 13%, p <0.001). Concomitant ART use was higher in Tororo compared to Kampala (92% vs. 41%, p<0.001). All patients were taking TS as the time they were diagnosed with malaria, with the exception of 5 (6%) episodes in Kampala and 8 (4%) episodes in Tororo where TS was being held due to toxicity.

Antimalarial treatment outcome. Treatment outcomes for all episodes of falciparum malaria treated with ACTs are presented in Table 3. In Kampala, there were only two episodes of recurrent asymptomatic parasitemia after 28 days both of which were due to new infections. The risk of recurrent parasitemia unadjusted by genotyping were similar for AS/AQ and AL treated patients in Kampala (2.4% vs. 2.7% respectively, HR=0.97, 95%C.I. 0.07-14.19, p=0.98). In Tororo, the risk of recurrent parasitemia after 28 days was significantly higher for patients treated with AL compared to those treated with DP (34% vs. 7.1%, HR=5.69, 95% C.I. 2.68-12.08, p <0.001). However, as in Kampala, both treatments were 100% efficacious in terms of preventing recurrent parasitemia due to recrudescent parasites.

Safety and tolerability. Cough, gastrointestinal symptoms and malaise were the most commonly identified adverse events after antimalarial treatment in both cohorts (Table 4). Most identified adverse events were either mild or moderate in intensity and difficult to distinguish from malaria symptoms. Three serious adverse events occurred in Kampala, all due to life- threatening neutropenia that resolved spontaneously. In Kampala, there was a significantly higher risk of adverse events in patients treated with AS/AQ compared to those treated with AL (78% vs. 49%, RR=1.52, 95% CI 1.09-2.11, p=0.014). After adjusting for ART use and malaria episode, AS/AQ was associated with a significantly higher risk of neutropenia (p=0.008), malaise (p=0.01) and anorexia (p=0.01), compared to AL. Subjects treated with AS/AQ and ART concomitantly had a higher risk of neutropenia than those treated with AS/AQ and not receiving ART (62% vs. 21%, p=0.001). AZT was responsible for the higher risk of neutropenia in children receiving ART. Children on AZT-containing regimens were 2.7 times more likely to develop neutropenia compared to those on D4T-containing regimens (67% vs. 25%, p=0.004). All five patients who developed neutropenia after AL treatment were receiving AZT-containing ART regimens. In Kampala, ART use was an independent risk factor for anorexia (p=0.003) and neutropenia (<0.001). Among subjects treated in Tororo, there was no significant difference in the risk of an adverse event after treatment with AL or DP (50% vs. 55%, RR=1.07, 95% CI 0.83-1.37, p=0.60). The risks of individual adverse

events were similar in both treatment arms, with the exception of diarrhea, which occurred more commonly in DP compared to AL treated subjects (p=0.04). ART was a risk factor for diarrhea (p=0.02).

Discussion

This study evaluated the incidence of malaria and response to therapy in HIV-infected children living in settings of contrasting malaria transmission intensity and receiving standard HIV care. No episodes of severe malaria occurred. In the context of routine TS prophylaxis and ITNs use, the incidence of malaria was relatively low in our urban setting. However, the burden of malaria remained high among children living in high transmission settings despite these interventions. All three of the ACTs evaluated were 100% efficacious. At the high transmission site, DP had the added benefit of superior post-treatment prophylactic effect compared to AL. AL and DP were safe and well tolerated, however, AS/AQ was less well tolerated compared to AL and was associated with a high risk of neutropenia, especially in the setting of concomitant ART use.

This study adds to the scanty data evaluating antimalarial treatment among HIV-infected children. Host immunity has been shown to be an important determinant of antimalarial treatment outcomes [27, 28] and it has been hypothesized that HIV-related immunosuppression interferes with malaria-specific immunity, leading to poor antimalarial treatment outcomes. Studies comparing immune responses to *P. falciparum* antigens in HIV-infected and uninfected individuals have reported lower responses in HIV-infected pregnant and non-pregnant adults [29-31]. In this study, the observations of 100% efficacy of AS/AQ, AL and DP, after exclusion of new infections, suggests that any immunosupression due to HIV may not significantly impair response to therapy when high efficacious ACTs are used. In addition, concomitant use of TS prophylaxis in HIV-infected individuals may improve treatment outcomes, as TS has been shown to be protective against malaria in HIV-infected adults and children [32, 33].

Co-administration of drugs metabolized through the cytochrome P450 pathways, such as NNRTI or protease inhibitor antiretroviral drug classes, and antimalarials such as AS/AQ or AL, may induce metabolism and reduce bioavailability of antimalarials [34]. Specifically, there is potential for NNRTIs to induce lumefantrine metabolism when the two are administered together [35]. In this study, AL efficacy was not compromised in individuals receiving ART, the vast majority of who were receiving nevirapine or efavirenz.

Increased antimalarial toxicity in HIV-infected persons may result from synergistic effects of HIV disease or concomitantly administered therapies. Toxicity may be further worsened by pharmacokinetic drug interactions. Most adverse events reported in our study population were similar in category and severity to those reported from previous studies in the general population; however, the overall risks of adverse events were higher in this HIV-infected population, particularly for those treated with AS/AQ. Previous studies have reported risks of adverse events following AS/AQ treatment ranging from 4% to 29% in the general population [36, 37]. In contrast 78% of participants treated with AS/AQ in this study experienced at least one adverse event. The 45% risk of neutropenia is of particular concern and is substantially higher than has been

reported in the general population [38, 39]. The neutropenia observed in this study was likely a result of multiple etiologies. Amodiaquine is known to have cytotoxic effects on mononuclear leukocytes and to inhibit granulocyte-monocyte colony formation [40-42]. Similarly, HIV-infection causes bone marrow abnormalities and is associated with peripheral cytopenias, including neutropenia [43, 44]. TS and antiretroviral therapies, specifically zidovudine, are also known risk factors for neutropenia [45-50]. In addition to these synergistic effects, pharmacokinetic interactions between ART, TS and AQ may have further increased the risk of AS/AQ toxicity. There are data to suggest that co-administration of amodiaquine and efavirenz, through inhibition of CYP2C8 enzyme, decreases amodiaquine metabolism and increases the risk of hematological and liver toxicity [51, 52]. Over half of the subjects in this study who were concomitantly treated with AS/AQ and ART were receiving efavirenz. Of note, AS/AQ was not associated with increased levels of liver transaminase enzymes. Malaise, anorexia, and vomiting were more common among AS/AQ treated than AL treated subjects. These symptoms have been reported in previous studies in the general population [38, 53]; however, overall risks were higher in the present study. Overlapping effects of ART likely contributed to the high risks of these adverse events.

In Tororo, DP and AL were well tolerated and the risk of the most common adverse events was similar in both treatment groups. However, the risk of diarrhea was higher among those treated with DP. This finding is consistent with previous studies, which have reported diarrhea to be more common in DP-treated participants compared to those receiving other therapies [9, 54, 55]. In Tororo, ART was not found to be associated with a higher risk of adverse events. However, over 90% of Tororo participants were receiving ART, thus limiting the statistical power of our study to measure the independent effects of concomitant ART use. Furthermore, our ability to identify adverse events was partially reduced by failure to elicit subjective symptoms in this cohort of very young children.

This study had other limitations. In Kampala, antimalarial treatment was not randomized and this may have led to confounding, specifically from factors related to calendar time such as HIV disease progression, and ART use. Advancing HIV disease over time may have led to more frequent HIV-related toxicities in the latter part of the study, coinciding with AL treatments. This effect would have led to overestimation of adverse events in the period when AL was used and thus biased the study results towards the null. On the other hand, better control of HIV disease with longer ART use over time would have lowered the risks of HIV-related toxicities over time, leading to fewer adverse events during the time AL was used for malaria treatment and biasing the study results away from the null. Adjusting for CD4 percentage at the start of the treatment periods may have limited the effects of such potential biases. However it is unlikely that this adjustment ensures that our results represent causal relationships between individual antimalarials and adverse events in Kampala. Comparison of the antimalarial efficacy and safety between Kampala and Tororo did not offer a straightforward comparison. The study population was much younger in Tororo and the adverse event assessment was not identical, including limited laboratory assessment in Tororo. Lastly, the results of this study may not be widely generalizable to other HIV-infected child populations.

AL and AS/AQ are by far the most commonly used ACTs for malaria treatment in sub-Saharan Africa and DP, a newer ACT, is gaining prominence as a promising

antimalarial for widespread deployment. As malaria endemic countries worldwide move towards universal use of ACTs for uncomplicated malaria treatment, our study provides reassuring evidence that AL and DP are highly efficacious and safe for the treatment of uncomplicated malaria in HIV-infected children, including those receiving TS prophylaxis and ART. While AS/AQ was found to be highly efficacious, the poor tolerability and higher neutropenia risk compared to AL that we found reiterates previous concerns of its use in HIV-infected individuals, particularly those receiving antiretroviral therapy. More clinical and pharmacokinetic studies in diverse HIV-infected populations are needed to fully evaluate antimalarial use in HIV-infected populations in the setting of increasing HIV control interventions.

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Figure 5: Directed acyclic graph showing relationship between ACT and antimalarial outcomes

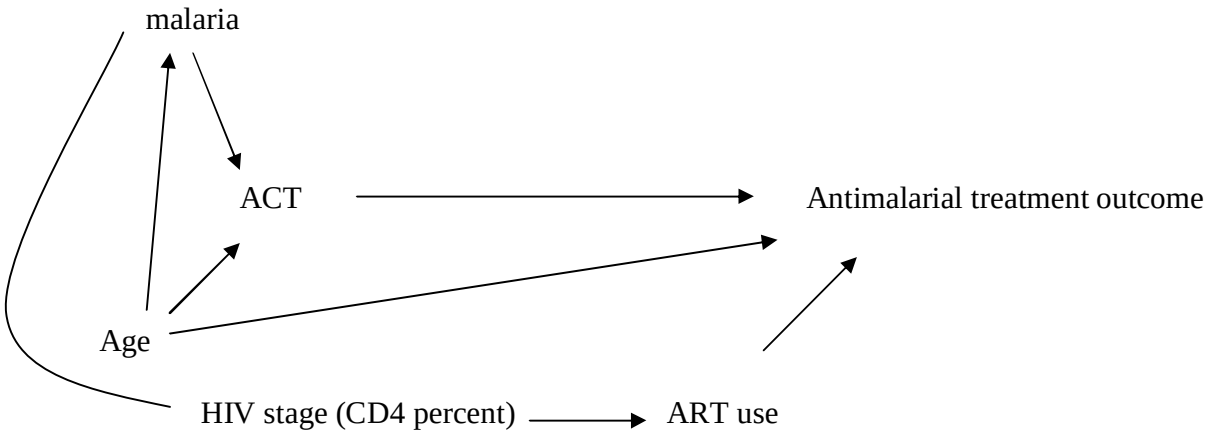

Figure 6: Directed acyclic graph showing relationship between ACT and adverse events (applies to Kampala study where ACT treatment was not randomized)

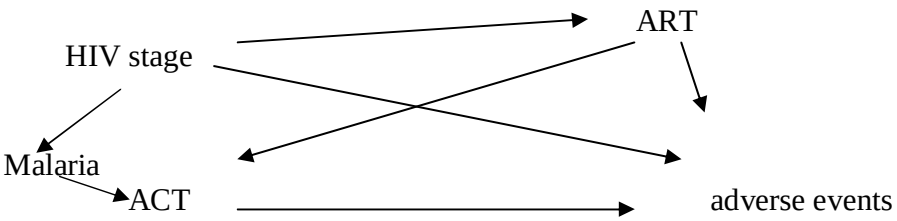

Figure 7. Study participant flow

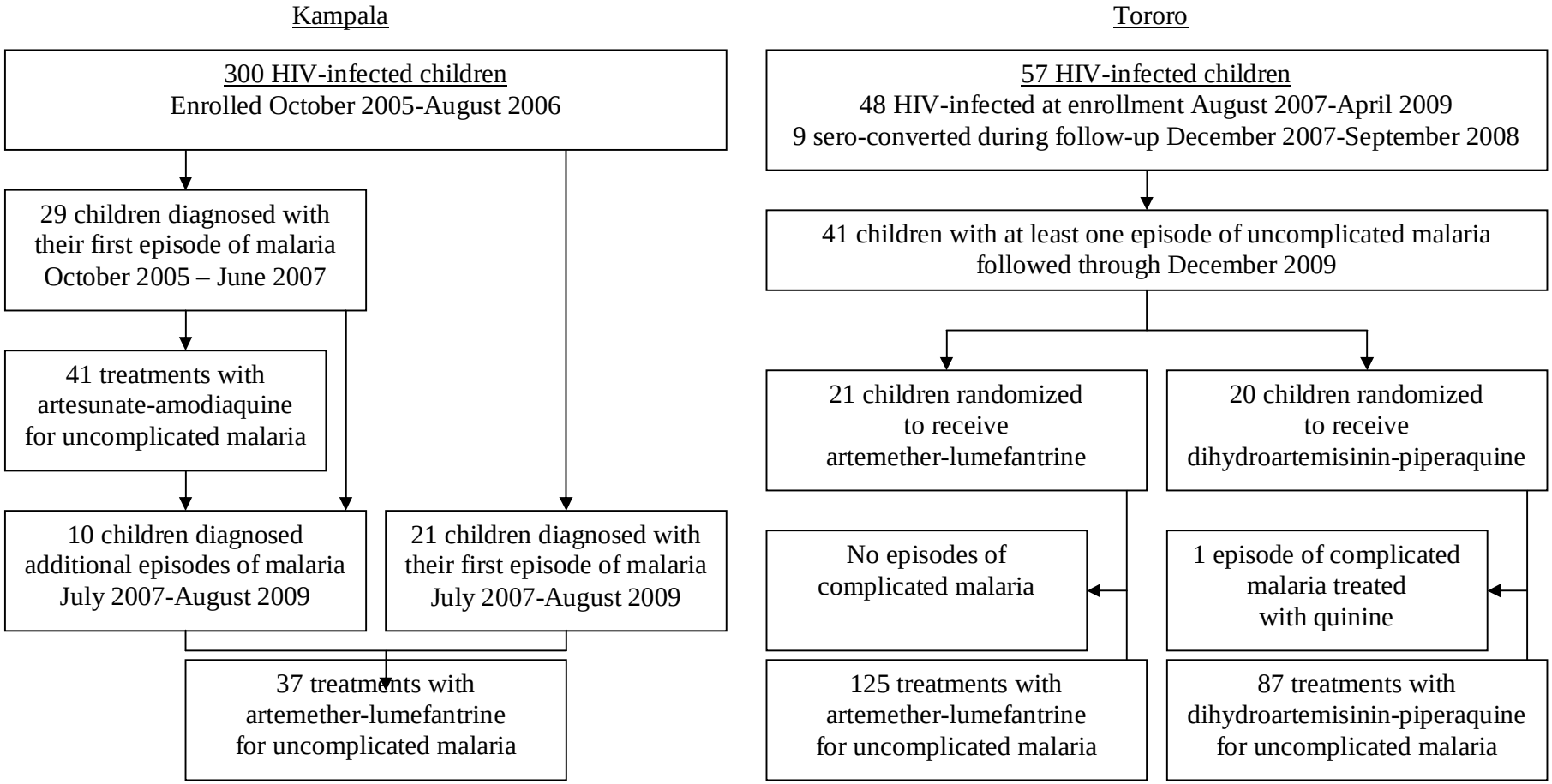

Table 4: Characteristics of study participants

Characteristic	Site	
	Kampala	**Tororo**
Number of study participants	300	57*
Mean age at start of observation period in years (SD)	5.7 (2.6)	0.6 (0.3)
Mean CD4 percent at start of observation period (SD)	21.3 (9.6)	21.2 (10.6)
Median duration of follow-up in months (IQR)	34 (32-36)	24 (21-26)
Number of participants on ART before enrollment (%)	35 (12%)	0
Number of participants initiated on ART during follow-up (%)	113 (38%)	52 (91%)
Number of participants treated for malaria (%)	50 (17%)	41 (72%)
Total number of treatments for malaria	78	213
Incidence of malaria per person year (95% CI)	0.10 (0.08-0.12)	2.08 (1.81-2.38)

*****Includes 48 HIV-infected children at enrolment and 9 HIV-exposed children who sero-converted during follow-up

Table 5. Baseline characteristics of individual malaria episodes treated with ACTS

Characteristic	Kampala		Tororo	
	AS/AQ (n=41)	**AL (n=37)**	**AL (n=125)**	**DP (n=87)**
Mean age in years (SD)	7.4 (2.9)	7.4 (3.0)	1.7 (0.6)	1.6 (0.6)
Plasmodium species				
P. falciparum	37 (90%)	36 (97%)	121	84 (96.5%)
P. malariae	2 (5%)	1 (3%)	(96.8%)	2 (2.3%)
P. ovale	2 (5%)	0	2 (1.6%)	1 (1.2%)
Mean Temperature, ^{0}C (SD)	38.2 (1.1)	38.1 (1.1)	2 (1.6%)	38.2 (1.1)
Geometric parasite density/ul	5183	8714	38.3 (1.1)	7419
Gametocytes present, n (%)	2 (4.9%)	3 (8.1%)	14542	6 (6.9)
Mean hemoglobin, g/dl (SD)	11.9 (1.5)	10.9 (1.7)	8 (6.4%)	9.4 (1.5)
Mean neutrophil count per mm^3	3358	2924	10.1 (1.4)	-
(SD)	(2478)	(1518)	-	79 (91%)
Concomitant ART use, n (%)	13 (32%)	19 (51%)	117 (94%)	0
AZT+3TC+EFV	6 (15%)	11 (30%)	0	79 (91%)
AZT+3TC+NVP	2 (5%)	3 (8%)	110 (88%)	0
D4T+3TC+NVP	1 (2%)	3 (8%)	0	0
D4T+3TC+EFV	3 (7%)	1 (3%)	7 (6%)	0
AZT+3TC+ABC	1 (2%)	1 (3%)	0	85 (98%)
Concomitant TS use, n (%)	40 (98%)	33 (89%)	119 (95%)	

Table 6. WHO 28-day treatment outcomes for episodes of falciparum malaria treated with ACTs

Treatment outcome	Kampala		Tororo	
	AS/AQ (n=37)	**AL (n=36)**	**AL (n=121)**	**DP (n=84)**
Lost to follow-up	0	1 (2.8%)	1 (0.8%)	1 (1.2%)
Early treatment failure	0	0	0	0
Late clinical failure	0	0	13 (11%)	1 (1.2%)
Late parasitological failure	1 (2.7%)	1 (2.8%)	30 (23%)	5 (6.0%)
Recurrent parasitemia due to new infection	*1 (2.7%)*	*1 (2.8%)*	*42 (34%)*	*6 (7.1%)*
Recurrent parasitemia due to recrudescence	*0*	*0*	*0*	*0*
Genotyping unsuccessful	*0*	*0*	*1 (0.8%)*	*0*
Adequate clinical and parasitological response	36 (97.3%)	34 (94.4%)	77 (64%)	77 (92%)

Table 7. Common adverse events identified within 28 days of malaria diagnosis

Adverse event	Kampala		Tororo	
	AS/AQ (n=41)	AL (n=37)	AL (n=125)	DP (n=87)
Malaise	11 (27%)	2 (5%)	1 (1%)	0
Anorexia	10 (24%)	2 (5%)	1 (1%)	2 (2%)
Vomiting	6 (15%)	1 (3%)	12 (10%)	7 (8%)
Diarrhea	3 (7%)	2 (5%)	12 (10%)	18 (21%)
Nausea*	6 (16%)	1 (3%)	-	-
Cough	24 (59%)	13 (35%)	47 (38%)	36 (41%)
Pruritus*	4 (10%)	4 (11%)	-	-
Neutropenia†	14/31 (45%)	5/30 (17%)	-	-
Serious Adverse Events	2 (7%)	1 (3%)	0	0

*Tororo-based participants too young for assessment of adverse event
† Adverse event not evaluated in Tororo-based study

CHAPTER 5

CONCLUSION
SUMMARY OF STUDY FINDINGS AND IMPLICATIONS FOR PUBLIC HEALTH AND FUTURE RESEARCH

Summary

Over the last two decades, evidence from numerous studies conducted in sub-Saharan Africa has increased our understanding of the interactions between HIV-infection and malaria. However, in the same time period, treatment and control of both HIV and malaria have undergone major changes. There has been rapid scale-up of HIV treatment programs providing antiretroviral therapy (ART) and trimethoprim-sulfamethoxazole (TS) prophylaxis and in parallel, wide scale implementation of malaria control strategies, such as artemisinin-combination therapies (ACTs) for treatment of uncomplicated malaria, indoor residual spraying, and widespread distribution of insecticide treated bednets (ITNs). It is not clear, how these interventions singly or in combination might affect the interactions between HIV-infection and malaria in different risk groups. There is a clear need for more data on HIV-malaria interactions in children, as there is a growing number of HIV-infected children in Africa, now estimated at two million [1], and this is the population at highest risk of malaria. This dissertation aimed at contributing to knowledge on the effects of HIV-infection on malaria in children in the setting of widening access to improved interventions. The studies conducted utilized data from two cohorts of HIV-infected children living in two malaria transmission settings in Uganda. The dissertation addressed aspects of prevention of and risk factors for malaria, and treatment of uncomplicated malaria in HIV-infected children.

Study summaries

The studies in this dissertation addressed the following research questions

1. What is the effect of daily TS prophylaxis on the risk of malaria in HIV-infected children living in an area of high antifolate resistance?

The study addressing this question was conducted in Kampala, Uganda where malaria transmission intensity is moderate. Despite a very high prevalence of antifolate resistance, HIV-infected children receiving TS had an 80% lower risk of malaria compared to HIV-uninfected children not receiving TS. However, TS use was associated with an increased prevalence of malaria infections with parasites with the uncommon mutation, *dhfr* 164L, known to mediate very high-level antifolate resistance. Daily TS use is becoming part of standard care for HIV-infected individuals and its protective effects appear to be larger than any increased risk of malaria due to HIV-infection. Therefore, from a public health stand point, HIV-infection may no longer be considered a risk factor for malaria among those accessing care for HIV infection as such individuals are now, paradoxically, protected from malaria.

2. What are the risk factors for symptomatic malaria in HIV-infected infants and young children living in an area of very high malaria transmission intensity?

In HIV-infected adults, the risk of malaria is inversely associated with CD4 cell count implying that HIV-related immunosuppression progressively impairs malaria-specific immunity [2-4]. In adults, ART use has been found to reduce the risk of malaria [5] . In HIV-infected children, protective and risk factors for malaria have not been characterized. The second study identified HIV-related risk factors for malaria in a cohort

of children aged 2.6 – 38 months living in Tororo, Uganda, an area of very high malaria transmission. Interruption of daily TS prophylaxis, low CD4 percentage and use of ART were found to increase the risk of malaria. The finding of an increase in the risk of malaria when TS prophylaxis is interrupted is consistent with findings from the first study and thus strengthens the evidence of a causal relationship between TS use and malaria protection. This study provides the first data showing that as in the case of adults, the level of immunosuppression in HIV-infected children increases the risk of malaria. This further suggests that CD4 T cells, possibly through activation of cytokine-dependent mechanisms [6], play an important role in non-specific immune mechanisms necessary for protection against malaria in individuals who have not developed malaria-specific immunity.

ART use was independently associated with a higher risk of malaria. Treated children in our study were receiving nevirapine, lamivudine and zidovudine. This finding is puzzling however one other study has reported an increased risk of asymptomatic malaria in children treated with the same ART regimen as was used in our cohort [7]. We hypothesize that the increase in the risk of malaria in children on ART is due to pharmacokinetic drug interactions between certain antiretroviral drugs and TS and antiretorival drugs and ACTs. Non-nucleoside reverse transcriptase inhibitor (NNRTI) antiretrovirals, such as nevirapine, are known to induce P450 enzymes that play a role in metabolizing both trimethoprim and sulfamethoxazole. Thus, children receiving NNRTI-based ART and TS could have been experiencing lower TS levels than those not on ART. Nevirapine may also induce metabolism of the artemether-lumefantrine in those treated for malaria with this ACT. This may reduce the post-treatment prophylaxis provided by AL and predispose to more new infections compared to those treated with AL but not receiving nevirapine-based ART.

3. Are ACTs efficacious and safe for the treatment of uncomplicated malaria in HIV-infected children?

Artemether-lumefantrine (AL), artesunate-amodiaquine (AS/AQ) and dihydroartemisinin-piperaquine (DP) are the most important ACTs used for the treatment of uncomplicated malaria in sub-Saharan Africa. They are known to be highly efficacious and safe for use in the general population but little is known about their use in HIV-infected populations. Overlapping toxicity profiles and drug interactions between different antiretroviral drugs, ACTs and TS may increase the risks of adverse events in patients receiving treatment for malaria and HIV. Pharmacokinetic interactions between ART and ACTs may also induce metabolism of individual drugs in ACT combinations resulting in reduced antimalarial efficacy. The study compared the efficacies and safety of three ACTS for the treatment of uncomplicated malaria in HIV-infected children. We compared AL and AS/AQ in the cohort of children aged 1-14 years old in Kampala, and DP and AL in the cohort of children aged 6 weeks – 3 yrs living in Tororo. All three ACTs were remarkably efficacious in both cohorts. However, AS/AQ was relatively poorly tolerated and associated with a high risk of neutropenia, with was highest risk seen in those receiving zidovudine-based ART.

Public Health implications

The findings from these studies have important public health implications. Our data support the wide use of daily TS prophylaxis in HIV-infected children living in sub-Saharan Africa, regardless of malaria transmission intensity or background antifolate resistance. However, the finding of rare mutations known to mediate high-level antifolate resistance indicates the need for ongoing surveillance for these mutations and any others that may emerge with continued TS use. Daily TS appears to be well tolerated in HIV-infected children, but clinicians should be aware that its interruption in the small proportion of patients who develop evidence of toxicity is associated with a substantially increased risk of malaria, specifically in young children living in high transmission settings. Our findings further support the prioritization of HIV-infected populations for distribution of ITNs and further indicate the need to emphasize consistent use of these ITNs in such populations.

Recently published antimalarial treatment guidelines indicate that there are limited data for use in formulating treatment recommendations in HIV-infected populations [8]. Our data suggest that AL and DP are highly efficacious and safe for the treatment of uncomplicated malaria in HIV-infected individuals regardless of ART use. We reiterate previous concerns regarding the use of AS/AQ for the treatment of malaria in HIV-infected individuals and receiving ART.

Implications for future research

This dissertation identified important areas for future study. While it appears that the benefits of TS prophylaxis to malaria control outweigh any risks at this time, we identified the potential risk of the selection of rare mutations such as *dhps* 164L and possibly *dhps* 581G, with TS use. In addition to ongoing surveillance to monitor the spread of these mutations, more studies are needed to identify the clinical implications of the spread of these mutations on the efficacy of TS and sulfadoxine-pyrimethamine, an antimalarial used for intermittent preventive treatment in pregnant women.

Studies that have evaluated immune responses to malaria in HIV-infected individuals have been largely restricted to adults and pregnant women [9-14]. Given the finding of a higher risk of malaria among infants and young children with low CD4 percentage level, studies assessing the role of CD4 T cells in malaria immunity among non-immune populations are warranted.

The need for studies to elucidate potentially important pharmacokinetic interactions between commonly used antimalarials, antiretrovirals and TS and their clinical implications is underscored by findings from this dissertation. There is growing interest in this area of research with the expectation of drug interactions based on knowledge of linkages in metabolic pathways of these therapies [15, 16]; and our findings suggest potentially important clinical implications of these interactions for both prevention and treatment of malaria in HIV-infected persons. Specifically, it is critical for future studies to determine if nevirapine (and possibly efavirenz) induces TS metabolism, leading to an increase in the risk of malaria. One such study could involve comparing TS

blood levels and the risks of malaria in three groups of HIV-infected populations: those receiving NVP-based ART and TS, those receiving PI-based ART and TS, and those receiving TS and no ART. Such a study would not only study NVP-TS interactions but also provide a potential alternative ART regimen. It would additionally test the hypothesis that PI-based regimens, directly reduce the risk of malaria. It is also important to determine if NNRTI antiretrovirals such as NVP induce the metabolism of lumefantrine, an incredibly important antimalarial used as first-line therapy in the majority of malaria endemic African countries. Although one of the studies in this dissertation did not find evidence that the 28-day efficacy of artemether-lumefantrine was reduced for malaria treatment, the finding of a higher risk of malaria among children on ART may partially be due to increased AL clearance leading to reduced post-treatment prophylaxis against malaria. Finally, our findings of a very high risk of neutropenia among children treated with AS/AQ and efavirenz-based ART supports the hypothesis that zidovudine impairs amodiaquine metabolism. This has been shown in a pharmacokinetic study in health volunteers [17] but has not been confirmed in HIV-infected individuals.

The research in this dissertation was confined to two distinct study populations. Our study population in Kampala included children aged 1 – 14 years living in an area of relatively low but stable malaria transmission intensity. Our study population in Tororo included recently infected children with HIV aged 2 months – 38 months living in an area of very high malaria transmission intensity. While these contrasting populations provided the opportunity to compare interactions in two distinct populations, they may not represent the entire scope of HIV-infected pediatric populations with different levels of malaria exposure. There is therefore a need for more studies to confirm our findings in a wider scope of HIV-infected populations living in different malaria transmission settings.

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