MAJOR ARTICLE

High Risk of Neutropenia in HIV-Infected Children following Treatment with Artesunate plus Amodiaquine for Uncomplicated Malaria in Uganda

Anne F. Gasasira,[1] Moses R. Kamya,[1] Jane Achan,[1] Tsedal Mebrahtu,[3] Joan N. Kalyango,[1] Theodore Ruel,[3] Edwin Charlebois,[3] Sarah G. Staedke,[4] Adeodata Kekitiinwa,[2] Philip J. Rosenthal,[3] Diane Havlir,[3] and Grant Dorsey[3]

[1]Department of Internal Medicine, Makerere University, and [2]Pediatric Infectious Diseases Clinic, Mulago Hospital, Kampala, Uganda; [3]Department of Medicine, San Francisco General Hospital, University of California, San Francisco; and [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

(See the editorial commentary by Olliaro on pages 992–3)

Background. Artemisinin-based combination therapies are rapidly being adopted for the treatment of malaria in Africa; however, there are limited data on their safety and efficacy among human immunodeficiency virus (HIV)–infected populations.

Methods. We compared malaria treatment outcomes between cohorts of HIV-infected and HIV-uninfected children in Uganda who were observed for 18 and 29 months, respectively. Malaria was treated with artesunate plus amodiaquine, and outcomes were assessed using standardized guidelines. HIV-infected children received trimethoprim-sulfamethoxazole prophylaxis and antiretroviral therapy in accordance with current guidelines.

Results. Twenty-six HIV-infected participants experiencing 35 episodes of malaria and 134 HIV-uninfected children experiencing 258 episodes of malaria were included in the study. Twelve HIV-infected children were receiving antiretroviral therapy, 11 of whom were receiving zidovudine. Malaria treatment was highly efficacious in both the HIV-infected and HIV-uninfected cohorts (28-day risk of recrudescence, 0% and 3.6%, respectively); however, there was a trend towards increased risk of recurrent malaria among the HIV-uninfected children (2.9% vs. 13.2%; $P = .08$). Importantly, the risk of neutropenia 14 days after initiation of treatment with artesunate plus amodiaquine was higher among HIV-infected children than among HIV-uninfected children (45% vs. 6%; $P < .001$). The severity of all episodes of neutropenia in HIV-uninfected children was mild to moderate, and 16% of episodes of neutropenia in the HIV-infected cohort were severe or life-threatening (neutrophil count, <750 cells/mm^3). In the HIV-infected cohort, the risk of neutropenia was significantly higher among children who received antiretroviral therapy than among those who did not receive antiretroviral therapy (75% vs. 26%; $P = .001$).

Conclusions. Artesunate plus amodiaquine was highly efficacious for malaria treatment in HIV-infected children but was associated with a high risk of neutropenia, especially in the context of concurrent antiretroviral use. Our findings highlight an urgent need for evaluation of alternative antimalarial therapies for HIV-infected individuals.

Artemisinin-based combination therapy is now recommended for treatment of uncomplicated malaria in sub-Saharan Africa. Most African countries have chosen 1 of 2 regimens as first-line therapy: artesunate plus amodiaquine (AS-AQ) or artemether-lumefantrine [1]. AS-AQ is now available as a coformulated single tablet, and rapidly increased uptake of this treatment is anticipated. However, safety assessment of AS-AQ has been limited.

The safety of AS and other artemisinins has been extensively studied, and these drugs appear to be very safe at the dosages used to treat malaria [2, 3]. AQ has been used to treat malaria for decades; however, concerns about safety of this drug remain. When used for malaria chemoprophylaxis, AQ was associated with rare but serious adverse events, including agranulocytosis, aplastic anemia, and hepatotoxicity [4, 5]. These problems led to removal of AQ from the World Health Organization essential drugs list in 1990. Subsequent

Received 23 September 2007; accepted 13 November 2007; electronically published 22 February 2008.

Reprints or correspondence: Dr. Anne Gasasira, MU-UCSF Research Collaboration, P.O. Box 7475, Kampala, Uganda (agasasira@berkeley.edu).

Clinical Infectious Diseases 2008; 46:985–91

1058-4838/2008/4607-0004$15.00
DOI: 10.1086/529192

reevaluation led to appreciation that the risk of toxicity associated with short-term AQ treatment appeared to be lower than that associated with AQ chemoprophylaxis [6], and AQ is now recommended by the World Health Organization for use in combination regimens to treat malaria [7].

The safety of AS-AQ in HIV-infected populations has not been studied. Overlapping toxicities of AQ and drugs commonly used to manage HIV infection, such as trimethoprim-sulfamethoxazole (TMP-SMX), and potential drug interactions with antiretroviral therapy merit concern. Therefore, we compared the efficacy and safety of AS-AQ for the treatment of uncomplicated malaria in a cohort of HIV-infected children receiving standard HIV care with the efficacy and safety of such treatment in a cohort of HIV-uninfected children, in Kampala, Uganda.

METHODS

Study participants. This study included children who had received a diagnosis of uncomplicated malaria, were treated with AS-AQ, and participated in 2 parallel cohort studies in Kampala, Uganda. Details of these 2 cohorts have been published previously and are described here briefly [8, 9]. A total of 601 HIV-uninfected children were recruited into a community-based cohort from a geographically defined census population, using probability sampling from November 2004 through April 2005. 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 (e.g., AIDS, sickle cell disease, and malignancy); and (6) weight, >10 kg. Children in the HIV-uninfected cohort were randomized to receive AS-AQ, artemether-lumefantrine, or AQ plus sulfadoxine-pyrimethamine at the time of their first episode of uncomplicated malaria. Only children receiving AS-AQ were included in this analysis. Insecticide-treated bednets (ITNs) were provided to all participants in this cohort during the period May–June 2006. Beginning in February 2007, HIV testing was offered to all participants remaining in the cohort, using a serial EIA testing algorithm according to national guidelines. We included up to the first 3 treatments with AS-AQ given to participants in the community-based cohort to match the maximum number of treatments given to participants in the HIV-infected cohort.

A total of 300 HIV-infected children were enrolled from a pediatric HIV clinic from October 2005 through August 2006. Eligibility criteria were the same as those for the HIV-uninfected cohort, except for (1) living within a 20-km radius of the clinic, (2) no restriction based on a history of serious chronic disease, and (3) weight, >5 kg. All HIV-infected cohort participants were prescribed TMP-SMX prophylaxis and were provided with ITNs. Those children meeting World Health Organization eligibility criteria were provided antiretroviral therapy.

Study participant follow-up. Children from both cohorts were observed in separate study clinics (open 7 days per week) for all of their medical problems using similar protocols. Children who presented with new medical problems underwent standardized medical evaluation. Standardized treatment algorithms were developed to guide therapy for malaria and nonmalarial illnesses. Medications with antimalarial activity were avoided for the treatment of nonmalarial illnesses. Malaria was diagnosed if a child had fever (documented tympanic temperature, >38.0°C, and/or history of fever in the previous 24 h) and any level of parasitemia. Study participants were withdrawn from the study cohorts if they moved out of the study area, could not be located for any consecutive 60-day period, withdrew consent, or died.

Treatment of malaria. AS-AQ treatment was directly observed and administered as follows: AQ (10 mg/kg on the first 2 days, followed by 5 mg/kg on the third day) and AS (4 mg/kg for 3 days). Patients treated for malaria were asked to return on days 1, 2, 3, 7, 14, and 28 after treatment initiation or any other day that they felt ill. Follow-up evaluation consisted of a standardized history and physical examination. Blood specimens were obtained by finger prick for thick blood smears on all follow-up days, except day 1. Complete blood cell counts and alanine aminotransferase (ALT) levels were assessed on the day that malaria was diagnosed and on day 14 of follow-up.

Malaria outcomes and assessment of efficacy and safety of antimalarial therapy. Treatment outcomes were classified, according to 2005 World Health Organization guidelines, as early treatment failure, late clinical failure, late parasitological failure, and adequate clinical and parasitological response [10]. Clinical treatment failures within the first 14 days were treated with standard doses of quinine. Cases of symptomatic malaria diagnosed >14 days after a previous episode of malaria were treated with the same assigned treatment regimens to which the study participants were initially randomized. Late parasitological treatment failures were not treated unless the patient developed symptomatic malaria. An adverse event was defined as any untoward medical occurrence, irrespective of its suspected relationship to the study medications, in accordance with International Conference of Harmonization guidelines. Adverse events were graded as mild, moderate, severe, or potentially life-threatening using the National Institutes of Health Division of AIDS tables for grading the severity of adverse events.

Laboratory methods. Microscopic examination was used to diagnose malaria. Parasite densities were estimated by counting the number of asexual parasites per 200 WBCs and calculating parasite densities, assuming a WBC count of 8000 cells/

μL. Molecular genotyping was used to distinguish recrudescent from new infections, as described elsewhere [11]. Complete blood cell counts (Coulter AcT5diff instrument; Beckman Coulter) and ALT levels (Cobus Integra instrument; Roche) were measured at College of American Pathologists–certified laboratories in Kampala, Uganda.

Statistical analysis. Data were double-entered in Access (Microsoft), and statistical analysis was performed using Stata, version 8 (Stata). Pairwise comparisons of categorical variables were made using generalized estimating equations with adjustment for repeated measures in the same patient, using exchangeable correlation and robust standard errors. Efficacy and safety data were evaluated using an intention–to-treat analysis. Efficacy outcomes included 28-day risk for recurrent parasitemia, both unadjusted and adjusted by genotyping to distinguish recrudescence and new infection. Risks of treatment failure were estimated using the Kaplan-Meier product limit formula. Data were censored for patients who did not complete follow-up and for new infections (based on outcomes adjusted by genotyping). Pairwise comparisons of treatment efficacy for individual episodes of malaria were made using a Cox proportional hazards model, with adjustment for repeated measurements in the same patient. To identify risk factors for neutropenia in HIV-infected children treated with AS-AQ, we used a logistic model, adjusted for repeated measurements in the same patient. In this model, neutropenia of any severity was the dependent variable, and previous AS-AQ treatment and antiretroviral therapy were included as independent variables.

To evaluate the clinical consequences of neutropenia after AS-AQ treatment among HIV-infected study participants, we conducted a nested case-control study. We randomly selected 3 control subjects for each patient with a neutropenic case, matched for antiretroviral treatment, age, and CD4 cell count or CD4 percentage (by age). We compared the prevalence of significant clinical events during the period of neutropenia among the patients with the prevalence of significant clinical events during the same period among matched control subjects with use of the χ^2 test or Fisher's exact test. $P < .05$ was considered to be statistically significant. Significant clinical events were defined as any febrile illness, bacterial skin infection, septicemia, and pneumonia. Duration of neutropenia was defined as the period from the date that AS-AQ therapy was started to the date that the next normal neutrophil value (>1300 neutrophils/mm^3) was measured. Routine laboratory evaluations (performed every 3 months) were used to monitor resolution of laboratory abnormalities.

Role of the funding source and ethical approval. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the article. The first author had full access to all study data and had final responsibility for the decision to submit for publication. Ethics approval for both cohort studies was obtained from the Uganda National Council of Science and Technology, Makerere University Research and Ethics Committee, and University of California, San Francisco, Committee on Human Research.

RESULTS

Study participants and baseline characteristics. In the HIV-infected cohort, 26 children received a diagnosis of malaria at least once over an 18-month period, resulting in 35 treatments with AS-AQ. In the HIV-uninfected cohort, 134 children were randomized to receive AS-AQ, and a total of 258 treatments over a 29-month period were included in this study. Among participants treated with AS-AQ for a second or third time, the median duration between treatments was 133 days (range, 34–327 days) for the HIV-infected patients and 114 days (range, 18–649 days) for the HIV-uninfected patients. Of the 134 community-based cohort participants who were treated with AS-AQ, 104 were tested for HIV infection, and all test results were negative. Among patients who were not tested for HIV infection, 21 were excluded from the study (12 moved from the study area, 6 could not be located for >60 consecutive days, and 3 withdrew consent) before testing was offered, and 9 refused testing. A comparison of the baseline characteristics of patients with episodes of malaria treated with AS-AQ in the 2 cohorts is presented in table 1. All HIV-infected children were receiving TMP-SMX therapy at the time that they were treated with AS-AQ, and none of the HIV-uninfected cohort participants were prescribed TMP-SMX. Members of the HIV-infected cohort were slightly older at the time that they were treated with AS-AQ, more frequently required use of ITNs, and had a higher mean ALT level at baseline, although the risk of an abnormally elevated ALT level at baseline was similar in the 2 groups (8.5% in the HIV-infected cohort vs. 3.1% in the HIV-uninfected cohort; $P = .13$). Participants in the HIV-uninfected cohort were more likely to have received repeated regimens of AS-AQ. Members of the community-based cohort who were tested for HIV infection and those who were not tested did not differ with regard to the baseline characteristics shown in table 1 (data not shown).

Treatment efficacy of AS-AQ. Members of both cohorts responded well to treatment of uncomplicated malaria with AS-AQ (table 2). There was a trend towards an increased risk of recurrent malaria within 28 days after treatment initiation in the HIV-uninfected cohort, compared with the HIV-infected cohort (13.2% vs. 2.9%; $P = .08$). However, treatment failures because of recrudescence were uncommon, with a risk of 3.6% in the HIV-uninfected cohort and 0% in the HIV-infected cohort ($P = .25$). Times to fever and parasite clearance were also similar for the 2 groups (table 2).

Safety and tolerability of AS-AQ. New or worsening cough was more common in the HIV-infected cohort during the 14

Table 1. Baseline characteristics of patients on the day that malaria was diagnosed.

Variable	HIV-uninfected cohort (n = 258)	HIV-infected cohort (n = 35)	P
AS-AQ treatment episode			
First	134 (52)	26 (74)	
Second	76 (29)	7 (20)	.03
Third	48 (19)	2 (6)	
Age, mean years ± SD	6.3 ± 2.5	7.2 ± 3.2	.05
Weight, mean kg ± SD	19.8 ± 6.3	20.2 ± 6.8	.69
ITN use	42 (16)	31 (89)	<.001
Tympanic temperature, mean °C ± SD	37.9 ± 1.2	38.2 ± 1.1	.22
Geometric parasite density, mean parasites/μL (range)	10152 (32–735,560)	6810 (16–428,440)	.37
Parasite species			
Plasmodium falciparum alone	245 (95)	30 (86)	
P. falciparum mixed infection	2 (1)	1 (3)	.08
Non–*P. falciparum* infection	6 (2)	1 (3)	
Unable to determine species	5 (2)	3 (9)	
WBC count, mean cells/mm^3 ± SD	7383 ± 3469	6445 ± 3371	.13
Neutrophil count, mean neutrophils/mm^3 ± SD	4302 ± 2806	3487 ± 2428	.11
Platelet count, mean platelets/mm^3 ± SD	218,516 ± 92,692	197,514 ± 121,301	.23
Hemoglobin level, mean g/dL ± SD	11.7 ± 1.4	11.8 ± 1.3	.52
ALT (SGPT) level, mean U/L ± SD	21.2 ± 17.0	30.3 ± 27.1	.006

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; AS-AQ, artesunate plus amodiaquine; ITN, insecticide-treated bednets; SGPT, serum glutamic–pyruvic transaminase.

days following therapy with AS-AQ, but other clinical events were similar in the 2 cohorts (table 3). Neutropenia occurring 14 days after therapy initiation was significantly more common in the HIV-infected cohort than in the HIV-uninfected cohort (45% vs. 6%; $P < .001$). Neutrophil counts were normal (>1300 neutrophils/mm^3) prior to initiation of therapy in all subjects who developed neutropenia. The risk of anemia, thrombocytopenia, and elevated ALT level did not differ between the cohorts. All episodes of neutropenia in the HIV-uninfected cohort were mildly or moderately severe (median neutrophil count, 1080 neutrophils/mm^3; range, 760–1300 neutrophils/mm^3), and 16% of HIV-infected participants developed severe or life-threatening neutropenia (median neutrophil count, 560 neutrophils/mm^3; range, 400–680 neutrophils/mm^3). Compared with the HIV-uninfected cohort, HIV-infected children treated with AS-AQ had >7 times the odds of neutropenia of mild severity or greater and >24 times the odds of neutropenia of moderate severity or greater (tables 4 and 5).

Among the 31 HIV-infected children who received AS-AQ and for whom neutrophil values were available, 12 (39%) were concurrently receiving antiretroviral drugs. The risk of neutropenia following therapy with AS-AQ was higher among those receiving antiretroviral therapy than among those not receiving antiretroviral therapy (75% vs. 26%; $P = .001$, after controlling for prior AS-AQ therapy). All but 1 of the study participants receiving antiretroviral therapy were receiving zidovudine ($n = 11$), the antiretroviral agent most closely associated with bone marrow suppression. There was also a trend towards a higher risk of neutropenia among HIV-infected children treated with AS-AQ for the second or third time, compared with those receiving their first treatment (75% vs. 35%; $P = .11$, after controlling for antiretroviral use) (table 5).

To assess the clinical significance of neutropenia, we compared the risk of significant clinical events among HIV-infected children during the period of neutropenia with that among HIV-infected control subjects not treated with AS-AQ over the same period; there was a trend towards a higher risk of any significant clinical event among patients treated with AS-AQ (57% vs. 35%; $P = .16$). The risk of pneumonia was higher among neutropenic patients treated with AS-AQ than among control subjects (43% vs. 19%; $P = .008$). None of the episodes of pneumonia diagnosed during this risk period required hospitalization.

DISCUSSION

Our results show that, in Uganda, AS-AQ was efficacious for the treatment of uncomplicated malaria in both HIV-infected children receiving TMP-SMX prophylaxis and in HIV-uninfected children. However, treatment with AS-AQ was associated with a remarkably higher risk of neutropenia in HIV-infected children, compared with HIV-uninfected children. HIV-

Table 2. Response to malaria therapy with artesunate plus amodiaquine, according to HIV infection status.

Outcome	HIV-uninfected cohort (n = 258)	HIV-infected cohort (n = 35)	P
28-Day WHO classification			
Adequate response	217/258 (84)	33/35 (94)	
Early treatment failure	2/258 (1)	0/35 (0)	
Late clinical failure	19/258 (7)	0/35 (0)	
Late parasitological failure	12/258 (5)	1/35 (3)	
Outcome not assessed	8/258 (3)	1/35 (3)	
Risk of early failure or recurrent infection,[a] % (95% CI)	13.2 (9.6–18)	2.9 (0.4–18.6)	.08
Risk of early failure or recrudescence,[a,b] % (95% CI)	3.6 (1.9–6.9)	0 (0–0)	.25
Fever clearance			
Documented fever on day 1	10/255 (4)	4/35 (11)	.05
Documented fever on day 2	2/253 (1)	1/34 (3)	.28
Documented fever on day 3	3/254 (1)	0/34 (0)	
Parasite clearance			
Positive smear result on day 2	9/252 (4)	2/34 (6)	.48
Positive smear result on day 3	0/254 (0)	0/34 (0)	

NOTE. Data are proportion (%) of patients, unless otherwise indicated. WHO, World Health Organization.

[a] Data were calculated using the Kaplan-Meier product limit formula.

[b] Data were PCR-corrected.

infected study participants who concurrently received antiretroviral therapy or had a history of repeated treatments with AS-AQ had the highest risk of neutropenia. This neutropenia appeared to have clinical consequences, because HIV-infected study participants had an increased risk of pneumonia during neutropenic episodes, compared with matched control subjects.

Neutropenia associated with AS-AQ treatment was most likely caused by AQ. Artemisinins have excellent safety profiles [3], and the addition of AS to other drugs does not appear to adversely affect safety and tolerability [2, 12]. In contrast, neutropenia is a well documented, albeit uncommon, adverse effect associated with AQ [6]. AQ and its metabolites exhibit cytotoxic effects on mononuclear leukocytes and inhibit granulocyte-monocyte colony formation [13]. Rate ratios of serious toxicity associated with AQ when used as chemoprophylaxis were reported as 1:2100 for blood dyscrasias and 1:31,000 for deaths due to blood dyscrasias [14]. However, short-term AQ treatment for malaria is generally considered to be safe [6]. The 6%

Table 3. Risk of new or worsening adverse events within 14 days after initiation of therapy with artesunate plus amodiaquine.

Adverse event	Proportion of patients (%)		P
	HIV-uninfected cohort	HIV-infected cohort	
Common symptom			
Cough	64/257 (25)	19/35 (54)	<.001
Anorexia	65/257 (25)	7/35 (20)	.53
Weakness and/or malaise	44/257 (17)	9/35 (26)	.24
Abdominal pain	45/237 (19)	6/35 (17)	.77
Vomiting	37/257 (14)	4/35 (11)	.66
Pruritis	26/257 (10)	3/35 (9)	.95
Diarrhea	19/257 (7)	3/35 (9)	.91
Laboratory finding			
Neutropenia	15/253 (6)	14/31 (45)	<.001
Thrombocytopenia	1/250 (0.4)	0/34 (0)	
Anemia	10/256 (4)	3/34 (9)	.13
Elevated alanine aminotransferase level	2/255 (0.8)	0/34 (0)	

Table 4. Severity of adverse events during the period of neutropenia.

Adverse event severity	No. (%) of patients	
	HIV-uninfected cohort	HIV-infected cohort
No adverse event	238 (94)	17 (55)
Mild	12 (4.7)	5 (16)
Moderate	3 (1.2)	4 (13)
Severe	0 (0)	3 (9.7)
Life-threatening	0 (0)	2 (6.5)

NOTE. Severity of adverse events during the period of neutropenia was stratified as follows: mild, 1000–1300 neutrophils/mm^3; moderate, 750–999 neutrophils/mm^3; severe, 500–749 neutrophils/mm^3; and life-threatening, <500 neutrophils/mm^3.

risk of neutropenia that was observed in our HIV-uninfected cohort is consistent with prior studies of AQ safety [6, 12]. However, in our HIV-infected cohort, the 45% risk of neutropenia, including a 16% risk of severe neutropenia, is cause for concern.

In addition to AQ use, several other factors may have contributed to the high risk of neutropenia seen in our HIV-infected children. HIV infection is associated with hematological disturbances, possibly attributable to viral inhibition of hematopoetic precursor cells or infections due to other pathogens [15]. Neutropenia has been reported in 10%–50% of HIV-infected individuals [16], with the risk increasing with increasing level of immunosuppression [15]. In addition, use of concomitant medications likely contributed to neutropenia. Neutropenia is one of the most commonly reported adverse reactions associated with TMP-SMX prophylaxis [17, 18]. Different antiretroviral drug classes are independently associated with blood disorders [19]. Nucleoside reverse-transcriptase inhibitors have been associated with hematological events. Zidovudine, in particular, is associated with increased risk of neutropenia in children and adults [20]. Finally, pharmacological interactions between antiretroviral drugs, TMP-SMX, and AQ may have potentiated AQ toxicity. Recent studies have revealed that efavirenz is an inhibitor of CYP2C8, a hepatic enzyme necessary for AQ metabolism [21], and that concomitant administration of efavirenz and AS-AQ is associated with increased AQ exposure [22]. TMP has also been shown to inhibit CYP2C8 [23].

In adults, HIV infection and increasing level of immunosuppression are associated with diminished antimalarial treatment response and an increased risk of recurrent infection after antimalarial therapy [24–26]. However, little is known about the effect of HIV infection on antimalarial efficacy in children, the population most vulnerable to malaria. We compared the efficacy of AS-AQ in HIV-infected children with that in HIV-uninfected children. No recrudescent malaria occurred in HIV-infected children treated with AS-AQ. There was a trend toward more episodes of recurrent malaria because of reinfection within 1 month after AS-AQ therapy among HIV-uninfected children, presumably because these children did not receive TMP-SMX prophylaxis and widespread ITN administration. Thus, there was no evidence of diminished efficacy of AS-AQ in HIV-infected children and, as recently described, administration of TMP-SMX and ITNs appeared to offer protection against malaria [9].

Our study had some limitations. The low incidence of malaria among our HIV-infected cohort limited the number of AS-AQ treatments and the precision of our estimates. However, because of the strong association between HIV infection and neutropenia after AS-AQ use, these results were highly statistically significant. We did not test 22% of community-based control subjects for HIV infection, because they had either been excluded from our cohort prior to testing or declined testing. However, the lack of HIV infection among any of the 104 children who were tested suggests that very few of the community-based cohort—if any—had this infection. Any bias resulting from misclassification of HIV status would be expected to underestimate differences seen between the HIV-infected and community-based cohorts.

The findings of this study could have major implications regarding malaria treatment recommendations in Africa. AS-AQ has been chosen as first-line antimalarial therapy in 15 countries in Africa, and in several other countries, including Uganda, AS-AQ is the alternative first-line antimalarial therapy

Table 5. Risk factors for new or worsening adverse events during the period of neutropenia.

Variable	Outcome	
	OR (95% CI)[a]	*P*
Association between HIV infection and severity of adverse events during the period of neutropenia		
Adverse event of mild severity or greater	7.6 (3.9–15.0)	<.001
Adverse event of moderate severity or greater	24.6 (6.8–89.1)	<.001
Risk factor for adverse events during the period of neutropenia in HIV-infected patients		
Prior treatment with artesunate-amodiaquine	1.6 (0.9–2.9)	.11
Antiretroviral therapy	2.3 (1.4–3.6)	.001

[a] Data were calculated using generalized estimating equations with adjustment for repeated measures in the same patient.

after artemether-lumefantrine [1]. TMP-SMX prophylaxis is now recommended for all HIV-infected Africans by many authorities, and it is increasingly being implemented. Similarly, antiretroviral therapy is increasingly available to HIV-infected Africans. Our findings suggest that, for HIV-infected individuals, particularly those receiving zidovudine-containing antiretroviral therapy, the treatment of malaria with AS-AQ should be avoided if possible. As alternatives, artemether-lumefantrine has proven efficacy without known serious toxicity risks [8, 27], and dihydroartemisinin-piperaquine has recently shown excellent efficacy [28–30]. However, other antimalarial drug combinations may also lead to toxicity because of HIV-specific factors or drug interactions, and research on the safety of malaria therapies for HIV-infected Africans is an urgent priority.

Acknowledgments

We thank all of the parents and guardians, for giving their consent; the study participants, for their cooperation; and our study teams, including the clinic team (Nestor Mbabazi, Caroline Akello, Fred Kateera, Sabrina Bakeera-Kitaka, Israel Kalyesubula, Rosemary Mbabazi, Egibwa Catherine, Sam Balikowa, George Kizito, Tamara Clark, Denise Njama, Catherine Maiteki, Bridget Nzarubara, Julia Mwesigwa, Joanita Nankabirwa, Basaliza M. Karakire, Norah Asaba, and Naome Kilama) and the data laboratory quality-control, field, and administrative teams.

Financial support. National Institutes of Health (AI062677 to D.H. and AI052142 to P.J.R.).

Potential conflicts of interest. All authors: no conflicts.

References

1. World Health Organization (WHO). Global antimalarial drug policies database—AFRO. Antimalarial treatment policies for *P. falciparum* and *P. vivax* by country in WHO Africa region. Available at: http://www.who.int/malaria/amdp_afro.html. Accessed 10 September 2007.
2. Price R, van Vugt M, Phaipun L, et al. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. Am J Trop Med Hyg **1999**; 60:547–55.
3. Ribeiro IR, Olliaro P. Safety of artemisinin and its derivatives: a review of published and unpublished clinical trials. Med Trop (Mars) **1998**; 58(Suppl 3):50–3.
4. Markham LN, Giostra E, Hadengue A, Rossier M, Rebsamen M, Desmeules J. Emergency liver transplantation in amodiaquine-induced fulminant hepatitis. Am J Trop Med Hyg **2007**; 77:14–5.
5. Neftel KA, Woodtly W, Schmid M, Frick PG, Fehr J. Amodiaquine induced agranulocytosis and liver damage. BMJ **1986**; 292:721–3.
6. Olliaro P, Nevill C, LeBras J, et al. Systematic review of amodiaquine treatment in uncomplicated malaria. Lancet **1996**; 348:1196–201.
7. World Health Organization (WHO). WHO guidelines for the treatment of malaria 2006. Geneva: WHO/HTM/MAL/2006.1108, **2006**.
8. Dorsey G, Staedke S, Clark TD, et al. Combination therapy for uncomplicated falciparum malaria in Ugandan children: a randomized trial. JAMA **2007**; 297:2210–9.
9. Kamya MR, Gasasira A, Achan J, et al. Effects of trimethoprim-sulfamethoxazole and insecticide treated bednets on malaria among HIV infected Ugandan children. AIDS **2007**; 21:2059–66.
10. World Health Organization (WHO). Susceptibility of *Plasmodium falciparum* to antimalarial drugs: report on global monitoring: 1996–2004. Geneva: WHO/HTM/MAL/2005.1103, **2005**.
11. Greenhouse B, Myrick A, Dokomajilar C, et al. Validation of microsatellite markers for use in genotyping polyclonal *Plasmodium falciparum* infections. Am J Trop Med Hyg **2006**; 75:836–42.
12. Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N. Artesunate combinations for treatment of malaria: meta-analysis. Lancet **2004**; 363:9–17.
13. Winstanley PA, Coleman JW, Maggs JL, Breckenridge AM, Park BK. The toxicity of amodiaquine and its principal metabolites towards mononuclear leucocytes and granulocyte/monocyte colony forming units. Br J Clin Pharmacol **1990**; 29:479–85.
14. Phillips-Howard PA, West LJ. Serious adverse drug reactions to pyrimethamine-sulphadoxine, pyrimethamine-dapsone and to amodiaquine in Britain. J R Soc Med **1990**; 83:82–5.
15. Adetifa IM, Temiye EO, Akinsulie AO, Ezeaka VC, Iroha EO. Haematological abnormalities associated with paediatric HIV/AIDS in Lagos. Ann Trop Paediatr **2006**; 26:121–5.
16. Levine AM, Karim R, Mack W, et al. Neutropenia in human immunodeficiency virus infection: data from the women's interagency HIV study. Arch Intern Med **2006**; 166:405–10.
17. Anglaret X, Chene G, Attia A, et al. Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1–infected adults in Abidjan, Cote d'Ivoire: a randomised trial. Cotrimo-CI Study Group. Lancet **1999**; 353:1463–8.
18. Toure S, Gabillard D, Inwoley A, Seyler C, Gourvellec G, Anglaret X. Incidence of neutropenia in HIV-infected African adults receiving cotrimoxazole prophylaxis: a 6-year cohort study in Abidjan, Cote d'Ivoire. Trans R Soc Trop Med Hyg **2006**; 100:785–90.
19. Koka PS, Reddy ST. Cytopenias in HIV infection: mechanisms and alleviation of hematopoietic inhibition. Curr HIV Res **2004**; 2:275–82.
20. Kline MW, Van Dyke RB, Lindsey JC, et al. Combination therapy with stavudine (d4T) plus didanosine (ddI) in children with human immunodeficiency virus infection. The Pediatric AIDS Clinical Trials Group 327 Team. Pediatrics **1999**; 103:62.
21. Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL. Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. Clin Pharmacol Ther **2007**; 82:197–203.
22. German P, Greenhouse B, Coates C, et al. Hepatotoxicity due to a drug interaction between amodiaquine plus artesunate and efavirenz. Clin Infect Dis **2007**; 44:889–91.
23. Wen X, Wang JS, Backman JT, Laitila J, Neuvonen PJ. Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. Drug Metab Dispos **2002**; 30:631–5.
24. Kamya MR, Gasasira AF, Yeka A, et al. Effect of HIV-1 infection on antimalarial treatment outcomes in Uganda: a population-based study. J Infect Dis **2006**; 193:9–15.
25. Shah SN, Smith EE, Obonyo CO, et al. HIV immunosuppression and antimalarial efficacy: sulfadoxine-pyrimethamine for the treatment of uncomplicated malaria in HIV-infected adults in Siaya, Kenya. J Infect Dis **2006**; 194:1519–28.
26. Van Geertruyden JP, Mulenga M, Mwananyanda L, et al. HIV-1 immune suppression and antimalarial treatment outcome in Zambian adults with uncomplicated malaria. J Infect Dis **2006**; 194:917–25.
27. Omari AA, Gamble C, Garner P. Artemether-lumefantrine for uncomplicated malaria: a systematic review. Trop Med Int Health **2004**; 9: 192–9.
28. Ashley EA, McGready R, Hutagalung R, et al. A randomized, controlled study of a simple, once-daily regimen of dihydroartemisinin-piperaquine for the treatment of uncomplicated, multidrug-resistant falciparum malaria. Clin Infect Dis **2005**; 41:425–32.
29. Kamya MR, Yeka A, Bukirwa H, et al. Artemether-lumefantrine versus dihydroartemisinin-piperaquine for treatment of malaria: a randomized trial. PLoS Clin Trials **2007**; 2:20.
30. Karema C, Fanello CI, Van Overmeir C, et al. Safety and efficacy of dihydroartemisinin/piperaquine (Artekin((R))) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwandan children. Trans R Soc Trop Med Hyg **2006**; 100:1105–11.