MAJOR ARTICLE

Decreasing Efficacy of Antimalarial Combination Therapy in Uganda Is Explained by Decreasing Host Immunity Rather than Increasing Drug Resistance

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Background. Improved control efforts are reducing the burden of malaria in Africa but may result in decreased antimalarial immunity.

Methods. A cohort of 129 children aged 1–10 years in Kampala, Uganda, were treated with amodiaquine plus sulfadoxine-pyrimethamine for 396 episodes of uncomplicated malaria over a 29-month period as part of a longitudinal clinical trial.

Results. The risk of treatment failure increased over the course of the study from 5% to 21% (hazard ratio [HR], 2.4 per year [95% confidence interval {CI}, 1.3–4.3]). Parasite genetic polymorphisms were associated with an increased risk of failure, but their prevalence did not change over time. Three markers of antimalarial immunity were associated with a decreased risk of treatment failure: increased age (HR, 0.5 per 5-year increase [95% CI, 0.2–1.2]), living in an area of higher malaria incidence (HR, 0.26 [95% CI, 0.11–0.64]), and recent asymptomatic parasitemia (HR, 0.06 [95% CI, 0.01–0.36]). In multivariate analysis, adjustment for recent asymptomatic parasitemia, but not parasite polymorphisms, removed the association between calendar time and the risk of treatment failure (HR, 1.5 per year [95% CI, 0.7–3.4]), suggesting that worsening treatment efficacy was best explained by decreasing host immunity.

Conclusion. Declining immunity in our study population appeared to be the primary factor underlying decreased efficacy of amodiaquine plus sulfadoxine-pyrimethamine. With improved malaria-control efforts, decreasing immunity may unmask resistance to partially efficacious drugs.

Increased funding, implementation of effective antimalarial combination therapy, and improved disease-prevention efforts appear to be decreasing malarial morbidity and mortality in many areas of Africa [1, 2]. These changes are encouraging, but with partial control of malaria will come new challenges. In particular, malaria is characterized by the development of partial immunity after repeated exposure to parasites [3–5], and improved malaria-control efforts are likely to delay and diminish the acquisition of immunity [6]. Limited data on the consequences of loss of acquired immunity in African children exist.

Although disease prevention has become an increasingly important component of malaria-control efforts in Africa, prompt treatment with effective drugs will remain the cornerstone of control for the foreseeable future [7]. Response to antimalarial therapy depends on both drug-parasite and host-parasite interactions. Drug-parasite interactions can be altered by parasite mutations that enable the parasites to persist after drug treatment [8–10]. Host-parasite interactions are primarily determined by the acquisition of antimalarial immunity. With increasing immunity, the likelihood of successful response to partially efficacious antimalarials increases, with the immune system helping to clear parasites not killed by antimalarials. Indeed, prior studies have shown associations between increasing age or transmission intensity, both surrogates of acquired im-

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munity [3], and a lower risk of antimalarial treatment failure [11–14].

Treatment for uncomplicated malaria has changed dramatically in Africa in recent years in response to increasing drug resistance, moving recently from chloroquine or sulfadoxine-pyrimethamine (SP) monotherapy to broad advocacy for combination therapy [15]. Artemisinin-based combination therapies (ACTs) have shown the greatest efficacy [16], but an older combination regimen, amodiaquine plus SP (AQ+SP), has been highly efficacious in some areas [17–19] and is recommended by the World Health Organization (WHO) for treatment of uncomplicated malaria when ACTs are unavailable [15]. We recently observed a rapid decrease in the efficacy of AQ+SP for treating uncomplicated malaria in children in Kampala, Uganda. To determine whether the decreased efficacy of AQ+SP was due to increasing drug resistance, decreasing host immunity, or both, we analyzed the contributions of both parasite and host factors to treatment outcomes.

METHODS

Recruitment and follow-up of study participants. Between November 2004 and April 2005, children aged 1–10 years from households randomly selected from a neighborhood of Kampala, Uganda [20], were enrolled in a randomized trial of combination antimalarial therapies; an interim analysis of comparative results through June 2006 has been published [21]. Briefly, caretakers of study participants were asked to bring their children to a designated study clinic for all medical care. Malaria was diagnosed if a child had fever and parasitemia. On diagnosis of the first episode of uncomplicated malaria, study participants were randomly assigned to receive 1 of 3 antimalarial regimens (AQ+SP, artesunate plus AQ, or artemether-lumefantrine) for all episodes of uncomplicated malaria. After receiving treatment for malaria, study participants underwent active follow-up for 28 days and then passive follow-up for malaria after 28 days. Children underwent routine assessment and blood smear examination monthly (every 3 months after June 2006) to identify asymptomatic parasitemia. All participants were given insecticide-treated bed nets between May and June of 2006.

Treatment outcomes and laboratory techniques. Early treatment failures within 3 days of treatment were classified according to 2005 WHO guidelines [22]. Recurrent episodes of malaria occurring >63 days after a prior episode were considered new infections. For recurrent episodes of malaria occurring 4–63 days after a prior episode, parasites were genotyped with 6 markers to distinguish new infection from recrudescence [23]. *Plasmodium* species were evaluated using species-specific polymerase chain reaction (PCR) [24]. Parasite polymorphisms were assessed using PCR followed by sequence-specific restriction enzyme digestion [25, 26].

Statistical analysis. Statistical analysis was performed using Stata SE (version 10; StataCorp) and R software (version 2.5.1; R Foundation for Statistical Computing). Only data from the AQ+SP study arm were analyzed, because only this arm had adequate numbers of treatment failures to detect meaningful associations. AQ+SP treatments were given from November 2004 until March 2007; at that point, after a planned interim analysis and review by our data and safety monitoring board, this treatment arm was stopped because of an unacceptably high risk of treatment failure.

Our outcome measure was the 63-day risk of treatment failure, defined as recurrent malaria due to recrudescent parasites. We considered only treatments for new *Plasmodium falciparum* infections and excluded treatments for which no genotyping result was obtained. Treatments resulting in early treatment failures were also excluded, because these are commonly due to factors other than drug resistance [26, 27]. Risk of failure was estimated using the Kaplan-Meier product-limit formula. Data were censored for subjects who did not complete 63 days of follow-up and for new infections.

Predictor variables of interest included calendar time, parasite polymorphisms, age of subjects at enrollment, distance of residence from a swamp, recent asymptomatic parasitemia, and parasite density at treatment. Calendar time was evaluated as a continuous variable. Parasite polymorphisms with mixed alleles were categorized as containing the resistance-mediating polymorphism, as suggested by the similar risks of treatment failure in subjects with samples containing mixed alleles and those with samples containing only the polymorphism of interest. Age at enrollment was analyzed instead of age at treatment to enable independent evaluation of the effects of time and age, and age was evaluated as a continuous variable. Distance from a swamp, a surrogate marker of parasite exposure [28], was dichotomized at 50 m to best reflect the relationship with the risk of recrudescence. Asymptomatic parasitemia, a surrogate marker of host immunity [5], was defined as the presence of a positive blood smear in the absence of fever at least 28 days after and 5 days before treatment for malaria. Recent asymptomatic parasitemia was defined as the presence of at least 1 episode of asymptomatic parasitemia in the prior 180 days.

Associations between predictor variables of interest and the risk of treatment failure were estimated using Cox proportional hazards. Left censoring of recent asymptomatic parasitemia was accounted for using inverse probability of censoring weighting, with weights determined using logistic regression for covariates that significantly predicted censoring. Robust inference accounting for repeated measurements in the same subject was performed using the grouped-jackknife method. Possible confounding or interaction between duration of the interval of assessment for asymptomatic parasitemia and the presence of recent asymptomatic parasitemia was ruled out. Associations between time and the presence of parasite polymorphisms were

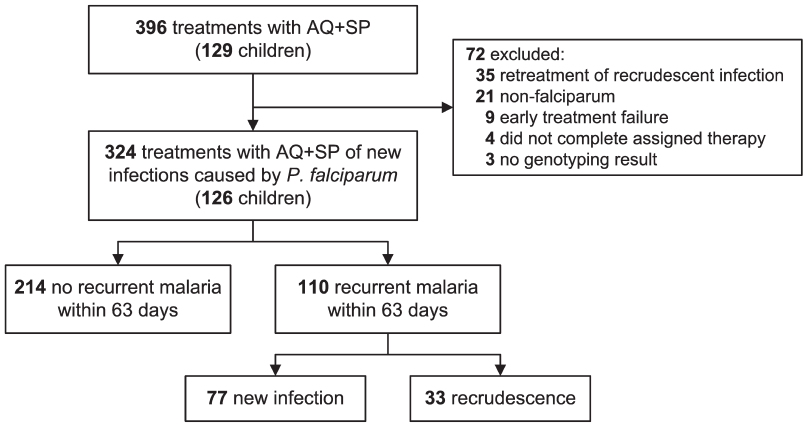

Figure 1. Malaria treatments and outcomes included in the present analysis. AQ+SP, amodiaquine plus sulfadoxine-pyrimethamine.

estimated using logistic regression analysis. Differences were considered significant at $P < .05$.

RESULTS

Characteristics of malaria episodes. A total of 396 AQ+SP treatments for malaria were given to 129 children over the course of the study (figure 1). Of these treatments, 324 given to 126 children were included in the analysis. Considering treatments included in the analysis, 47 children were treated once, 35 were treated twice, and the remaining 44 were treated up to 13 times during the study. The median age at the time of treatment was 7.2 years (interquartile range, 4.9–8.9 years), and the geometric mean parasite density was 14,515 parasites/μL (range, 16–464,000 parasites/μL).

Increasing risk of treatment failure over time. The 63-day risk of recurrent malaria after treatment with AQ+SP was 34%, and the 63-day risk of recrudescence (treatment failure), as determined by genotyping, was 11% (figure 1). The risk of recrudescence after treatment with AQ+SP increased significantly during the 29 months of the study, from 5% in the first quarter of the study to 21% in the last quarter (hazard ratio [HR], 2.4 per year [95% confidence interval {CI}, 1.3–4.3]; $P = .002$) (figure 2). In contrast, the risk of new infection after treatment was stable for the first 3 quarters and then decreased in the last quarter (HR, 0.49 [95% CI, 0.25–0.95]; $P = .04$). Thus, our results indicate decreasing antimalarial efficacy of AQ+SP, a drug combination that until recently showed excellent efficacy in Uganda [17, 18].

Parasite polymorphisms and changes over time. To investigate whether the observed increased risk of failure after treatment with AQ+SP was the result of increased parasite resistance to these drugs, we evaluated parasites collected at the time of each treatment for polymorphisms associated with resistance to SP or AQ. These included single-nucleotide polymorphisms (SNPs) in *dhfr* and *dhps* that are associated with SP treatment failure [10, 29] and SNPs in *pfcrt* and *pfmdr1* that appear to be associated with AQ treatment failure [30–33].

Considering markers of SP resistance, the SNPs *dhfr* 51I and *dhfr* 108N were present in nearly all of a random subset of 90 samples (99% and 100%, respectively). We therefore assumed in

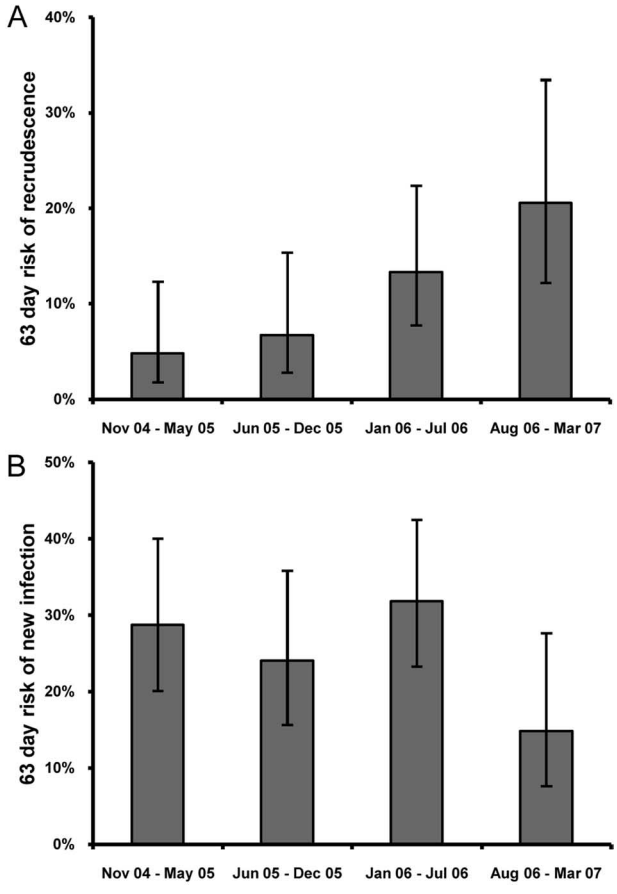

Figure 2. Risk of recurrent malaria over time after treatment with amodiaquine plus sulfadoxine-pyrimethamine. *A,* Risk of recrudescence (treatment failure). *B,* Risk of new infection. Whiskers represent 95% confidence intervals.

Table 1. Prevalence of parasite polymorphisms and association with failure of treatment with amodiaquine plus sulfadoxine-pyrimethamine.

Gene, SNP	Prevalence of SNP, proportion (%)	Risk of treatment failure with/without SNP, %	Association with treatment failure[a]	
			HR (95% CI)	*P*
dhfr, 59R	279/322 (87)	12.4/2.3	5.1 (0.7–38)	.12
dhps				
437G	310/323 (96)	11.5/0.0	∞ (0.4–∞)[b]	.38
540E	304/323 (94)	11.2/7.7	2.0 (0.4–11)	.42
pfmdr1				
86N	96/322 (30)	14.9/9.4	1.6 (0.8–3.2)	.15
184F	78/322 (24)	13.8/10.2	1.4 (0.7–3.0)	.39
1246Y	264/321 (82)	12.0/7.3	1.7 (0.6–4.8)	.35
dhfr/*dhps* quintuple[c]	261/321 (81)	12.7/4.0	3.7 (0.9–15)	.07
pfmdr1 double[d]	40/322 (12)	21.2/9.6	2.4 (1.1–5.0)	.03

NOTE. CI, confidence interval; HR, hazard ratio; SNP, single-nucleotide polymorphism.

[a] Associations were determined using Cox proportional hazards with inference accounting for repeated measures.

[b] The association for *dhps* 437G is reported as an odds ratio determined using Fisher's exact test, because there is no reliable method of estimating inference for this HR.

[c] *dhfr* 51I + 59R + 108N and *dhps* 437G + 540E.

[d] *pfmdr1* 86N + 184F.

data analysis that they were present in all samples. Of greater interest were the SNPs *dhfr* 59R, *dhps* 437G, and *dhps* 540E, which have demonstrated varied prevalence across Africa and are most clearly associated with SP treatment failure [27, 34]. These SNPs were assessed in all 324 samples. The prevalence of all 3 of these SNPs was high (table 1). Compared with infections with parasites without these polymorphisms, parasites with *dhfr* 59R, *dhps* 437G, and *dhps* 540E were all associated with a higher risk of failure. The presence of all 3 of these SNPs along with *dhfr* 51I and *dhfr* 108N (*dhfr*/*dhps* quintuple polymorphism) resulted in almost 4 times the hazard of treatment failure compared with infections with parasites that contained <5 of these polymorphisms. However, in the context of high baseline prevalence of the polymorphisms, none of these associations reached statistical significance. We found only 2 samples (0.6%) that contained the *dhfr* 164L allele, which has been associated with very poor response to SP [35].

Considering potential markers of AQ resistance, all 90 randomly selected samples contained *pfcrt* 76T, so this SNP was assumed to be present in all samples. Conversely, none of the 90 randomly selected samples contained *pfmdr1* 1034C or 1042D, so these SNPs were assumed to be absent from all samples. Other relevant *pfmdr1* SNPs were evaluated in all 324 samples (table 1). We found a higher risk of treatment failure with AQ+SP in subjects whose parasites contained *pfmdr1* 86N, 184F, or 1246Y. None of these individual associations approached statistical significance, but the combined presence of *pfmdr1* 86N and 184F (*pfmdr1* double polymorphism) significantly predicted treatment failure. Considering the combined effects of SNPs associated with SP and AQ resistance, subjects whose parasites did not contain the *dhfr*/*dhps* quintuple polymorphism had a low risk of failure regardless of the presence of the *pfmdr1* double polymorphism (table 2). The presence of the *dhfr*/*dhps* quintuple polymorphism increased the hazard of failure 3-fold, and the addi-

Table 2. Prevalence of single-nucleotide polymorphism (SNP) combinations and association with failure of treatment with amodiaquine plus sulfadoxine-pyrimethamine.

Combination of SNPs	Prevalence of combination, proportion (%)	Risk of treatment failure, %	Association with treatment failure[a]	
			HR (95% CI)	*P*
Without *dhfr*/*dhps* quintuple,[b] with or without *pfmdr1* double[c]	60/321 (19)	4.0	1.0 (reference)	. . .
With *dhfr*/*dhps* quintuple, without *pfmdr1* double	232/321 (72)	10.6	3.0 (0.7–12)	.12
With both *dhfr*/*dhps* quintuple and *pfmdr1* double	29/321 (9)	29.0	9.1 (2.0–41)	.004

NOTE. CI, confidence interval; HR, hazard ratio.

[a] Associations were determined using Cox proportional hazards with inference accounting for repeated measures.

[b] *dhfr* 51I + 59R + 108N and *dhps* 437G + 540E.

[c] *pfmdr1* 86N + 184F.

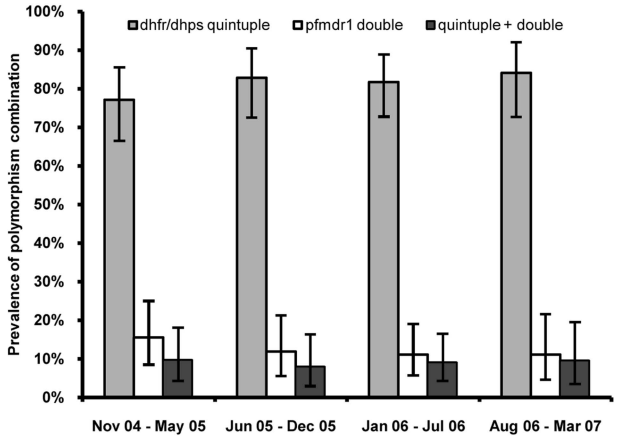

Figure 3. Prevalence of parasite polymorphisms over time. Whiskers represent 95% confidence intervals.

tion of the *pfmdr1* double polymorphism increased this hazard another 3-fold.

Because the clinical efficacy of AQ+SP decreased over the course of our study, it was of interest to determine whether the prevalence of key resistance-mediating polymorphisms increased over this time frame. In fact, the prevalence did not change significantly during the study for the *dhfr/dhps* quintuple polymorphism, the *pfmdr1* double polymorphism, or both together ($P = .4$, $P = .3$, and $P = .7$, respectively) (figure 3). In addition, adjustment for these polymorphism combinations did not change the association between time and the risk of treatment failure (HR excluding polymorphisms, 2.4 per year [$P = .004$]; HR including polymorphisms, 2.4 per year [$P = .004$]). These findings indicate that, although there were significant associations between certain parasite polymorphisms and the risk of treatment failure, the increasing risk of failure that we observed during our study was not due to an increase in parasite drug resistance mediated by the 12 SNPs that we evaluated.

Surrogate markers of host immunity and changes over time. Because the decreasing antimalarial efficacy of AQ+SP could not be explained by known markers of resistance to these agents, we considered the possibility that decreasing treatment response was due to waning host immunity. Although repeated infection is accompanied by the acquisition of clinically relevant antimalarial immunity, there is currently no straightforward marker for this immunity [36]. Therefore, to test the hypothesis that immunity influenced the risk of treatment failure in our study, we measured associations between 3 surrogate markers of immunity and failure: increasing age, exposure to parasites, and history of asymptomatic parasitemia.

In our cohort, we found an association between increased age at enrollment and the risk of treatment failure, but this association did not reach statistical significance (HR, 0.5 per 5-year increase [95% CI, 0.2–1.2]; $P = .1$). To further consider exposure to malarial parasites in our study population, we used spatial data as a surrogate for exposure. We recently showed that those living close to a swamp bordering the study site had a higher incidence of malaria than did those living farther away [28]. Considering AQ+SP treatment outcomes, those living within 50 m of the swamp (48% of subjects) had a significantly lower risk of treatment failure after therapy than did those living at least 50 m away (5% vs. 18%; HR, 0.26 [95% CI, 0.11–0.64]; $P = .003$), consistent with increased immunity in the group with highest exposure to parasites. Those living at least 50 m from the swamp showed a steady increase in the risk of treatment failure over the course of our study, consistent with a gradual decrease in host immunity in this relatively nonimmune group (HR, 2.0 per year [95% CI, 1.1–3.7]; $P = .02$) (figure 4). In contrast, those living within 50 m of the swamp did not show increased risk of treatment failure until late in our study (HR for the fourth quarter vs. the first 3 quarters, 3.5 [95% CI, 0.8–14.7]; $P = .09$). The prevalences of parasite polymorphisms were similar in the 2 groups (data not shown). When both surrogates of immunity were considered together, subjects expected to have the greatest immunity on the basis of age (>5 years at enrollment) and exposure (living within 50 m from the swamp) had a much lower risk of treatment failure than those expected to have the least immunity (age <5 years at enrolment and living at least 50 m from the swamp) (3% vs. 28%; HR, 0.12 [95% CI, 0.03–0.5]; $P = .005$). These findings suggest that antimalarial immunity strongly influenced the risk of treatment failure in our study.

Age at enrollment and distance from the swamp appear to be good surrogates for immunity, but they are not markers that could change during the study. To determine whether declining immunity was responsible for the increase in treatment failure over time, a surrogate marker of immunity that might vary over time was required. Another potential surrogate for malarial immunity is asymptomatic parasitemia, because immunity is required to control parasitemia and prevent the development of symptomatic malaria [3–5]. Recent asymptomatic parasitemia (within the prior 180 days, present for 34% of treatments) was

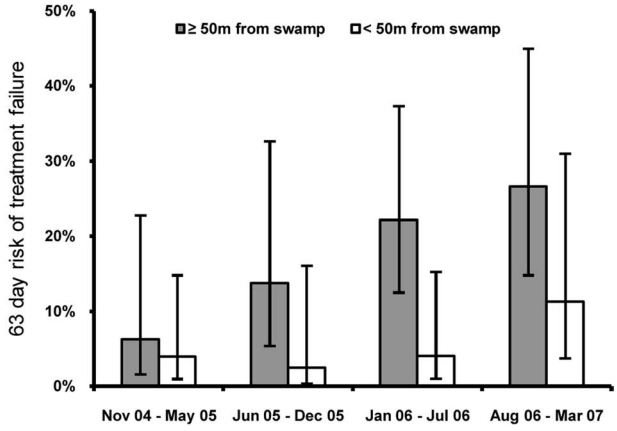

Figure 4. Risk of treatment failure in subjects over time, stratified by distance from the swamp. Whiskers represent 95% confidence intervals.

Table 3. Parasite and host factors associated with treatment failure.

	Model not including AP		Model including AP	
Risk factor	HR (95% CI)	*P*	HR (95% CI)	*P*
Calendar time (per year)	2.9 (1.5–5.4)	.001	1.5 (0.7–3.4)	.3
Parasite SNPs				
Without *dhfr*/*dhps* quintuple,[a] with or without *pfmdr1* double[b]	1.0 (reference)	. . .	1.0 (reference)	. . .
With *dhfr*/*dhps* quintuple, without *pfmdr1* double	2.8 (0.7–11.6)	.16	3.1 (0.7–12.5)	.12
With both *dhfr*/*dhps* quintuple and *pfmdr1* double	7.9 (1.8–35.3)	.007	7.7 (1.8–33.5)	.006
Parasite density >100,000 parasites/μL	2.2 (0.9–5.5)	.08	1.9 (0.7–4.8)	.18
Living ≤50 m from swamp	0.3 (0.1–0.8)	.01	0.3 (0.1–0.8)	.01
Age at enrollment (per 5-year increase)	0.6 (0.3–1.2)	.15	0.7 (0.4–1.4)	.3
AP observed in the last 180 days	. . .	. . .	0.06 (0.01–0.5)	.006

NOTE. Associations were determined using Cox proportional hazards with inference accounting for repeated measures. AP, asymptomatic parasitemia; CI, confidence interval; HR, hazard ratio.

[a] *dhfr* 51I + 59R + 108N and *dhps* 437G + 540E.

[b] *pfmdr1* 86N + 184F.

strongly associated with a lower risk of failure after therapy with AQ+SP (1% vs. 18%; HR, 0.06 [95% CI, 0.01–0.36]; $P = .002$). To test the hypothesis that a decrease in host immunity was responsible for the increasing risk of failure over the course of the study, we performed a multivariate analysis, first excluding and then including recent asymptomatic parasitemia as an explanatory variable. After adjustment for parasite resistance–mediating SNPs, parasite density, distance from the swamp, and age at enrollment, the association between time and risk of treatment failure remained strong (HR, 2.9 per year; $P = .001$) (table 3). However, the inclusion of recent asymptomatic parasitemia reduced the association between time and the risk of failure (HR, 1.5; $P = .3$). This finding suggests that a decrease in antimalarial immunity during our study largely drove the observed decrease in efficacy of AQ+SP.

DISCUSSION

Earlier analysis of data from our ongoing clinical trial showed that AQ+SP was inferior to 2 ACT regimens [21]. An additional 9 months of follow-up showed a further decrease in efficacy (figure 2), and the AQ+SP arm was subsequently discontinued in our study. Why did the efficacy of AQ+SP decrease so rapidly? An obvious explanation might be increasing resistance of malarial parasites in Kampala to the components of AQ+SP. However, during our study we did not find changes in the prevalence of key polymorphisms that mediate diminished responses to AQ or SP. An alternative explanation for the loss of drug efficacy is diminished host immunity, because antimalarial treatment responses to partially efficacious drugs are dependent on host immunity [11–14, 37], and study subjects benefited from a number of study-specific and national malaria-control measures that probably decreased their exposure to parasites. A straightforward measure of antimalarial immunity is not available [36], but a number of reasonable surrogates of immunity have been established. Two such surrogates, increasing age at enrollment and residence near a local area of high transmission, both showed an association with greater treatment efficacy, suggesting that baseline immunity played a major role in efficacy. A surrogate marker of immunity that could change over time—recent asymptomatic parasitemia—appeared to explain most of the decrease in AQ+SP efficacy during our study. Thus, our results suggest that, in the setting of preexisting diminished parasite susceptibility to AQ and SP, worsening drug efficacy was mediated not by increasing parasite resistance but by diminishing host immunity.

Antimalarial immunity usually increases with increasing age in individuals living in areas of endemicity, but it appeared to wane in our cohort. Factors that may have contributed to declining immunity in our study population included improved access to antimalarial combination therapy, community-wide changes in antimalarial treatment, and distribution of insecticide-treated bed nets. When our study began, chloroquine monotherapy was by far the most common antimalarial used in the community [38]. We know from prior data in Kampala that, although symptoms often improve after treatment with chloroquine, parasites fail to clear in almost 90% of patients, exposing them to continued parasitemia [39]. Access to prompt combination therapy improved the overall health of children in all 3 treatment arms of our trial and dramatically decreased the prevalence of asymptomatic parasitemia [21]. Asymptomatic parasitemia has been associated with protection from subsequent symptomatic malaria [40–44], and, in this study, we now show a strong association with protection from subsequent treatment failure with partially effective therapy. On a community level, the highly effective antimalarial artemether-lumefantrine, which began to be widely dispensed in Kampala in early 2006, may have decreased the transmission intensity of parasites in the area, because this therapy is highly effective in clearing parasites [21] and artemisi-

nins may provide additional transmission-blocking effects [45]. Finally, we distributed insecticide-treated bed nets to all study participants in May and June of 2006, cutting the incidence of malaria in half [28]. Although AQ+SP efficacy began to decline before distribution of the bed nets, the decrease in parasite exposure afforded by this control measure may have further contributed to declining immunity.

It should be emphasized that the effect of immunity on the efficacy of AQ+SP in our study was probably relevant only because local parasites were partially, but not completely, resistant to this therapy. With therapy to which there is a high level of parasite resistance, such as chloroquine or SP, drug efficacy may improve with increased immunity, although not to acceptable levels [46]. With therapy to which there is little or no resistance, efficacy will not vary significantly with immune status, because the drug will be able to clear parasites in almost all subjects. Despite our finding of a very high prevalence of parasite polymorphisms that are known to confer moderate resistance to SP, the presence of a polymorphism known to confer high-level resistance to SP (*dhfr* 164L) remains rare in Kampala. We also found polymorphisms in *pfcrt* and *pfmdr1* associated with resistance to amodiaquine; specific associations in prior studies have varied, but none of these polymorphisms have yet been associated with high-level resistance [30–33]. In contrast to these prior studies, in which *pfmdr1* 86Y was associated with treatment failure, we found an association between *pfmdr1* 86N and treatment failure. The reason for this discrepancy is unknown. Although we cannot exclude the possible presence of additional unmeasured parasite polymorphisms conferring high-level resistance to SP or AQ, it is unlikely that such polymorphisms would have increased enough in prevalence over the course of our study to explain the rapid decrease in efficacy that we observed. Rather, it is most likely that declining immunity in our cohort unmasked preexisting moderate resistance to AQ+SP in Kampala.

Given the treatment failure rate of 21% at the end of our study, AQ+SP is no longer an appropriate antimalarial therapy in Kampala. Fortunately, data from our ongoing trial have not revealed decreasing efficacy for the ACTs AQ plus artesunate and artemether-lumefantrine (data not shown), and it seems appropriate to recommend these drugs as first-line therapy across Africa. However, with limited availability of ACTs, non-ACT regimens are still widely used to treat uncomplicated malaria in Africa. In addition, the continued efficacy of antifolates in preventing malaria, including the important role of SP in intermittent preventive therapy of pregnant women, may require underlying antimalarial immunity [47]. Decreasing immunity may additionally increase the selective pressure for drug-resistant parasites, facilitating a further decline in efficacy [48].

With increased malaria-control efforts now starting to have a significant effect on malaria transmission in Africa [2, 7] and regional elimination under discussion [49, 50], declines in antimalarial immunity for many living in Africa are likely to be at least as dramatic as that seen for children in our cohort. Thus, as malaria-prevention efforts successfully decrease the burden of malaria in Africa, these efforts must be coupled with access to highly effective antimalarial therapy. In addition, careful monitoring of drug efficacy will be critical to identify emerging drug resistance that may be unmasked because of decreasing antimalarial immunity.

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