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THE IMPACT OF AGE, TEMPERATURE, AND PARASITE DENSITY ON TREATMENT OUTCOMES FROM ANTIMALARIAL CLINICAL TRIALS IN KAMPALA, UGANDA

GRANT DORSEY, ANNE F. GASASIRA, RHODERICK MACHEKANO, MOSES R. KAMYA, SARAH G. STAEDKE, AND ALAN HUBBARD

Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California; Department of Medicine, Makerere University Medical School, Kampala, Uganda; Department of Biostatistics, School of Public Health, University of California, Berkeley, California

Abstract. Antimalarial drug treatment policy in sub-Saharan Africa is generally guided by the results of clinical drug efficacy studies in patients with uncomplicated *Plasmodium falciparum* malaria. The selection criteria used to enroll these patients often vary and may have a significant impact on treatment outcomes. In Kampala, Uganda, we investigated the impact of age, baseline temperature, and pre-treatment parasite density on estimates of treatment efficacy using a statistical modeling approach in 2,138 patients enrolled in six clinical trials involving seven different treatment regimens. Decreasing age, increasing temperature, and increasing parasite density were all independent predictors of an increased risk of treatment failure across all treatment groups. Compared with an unrestrictive approach to subject selection, enrolling only patients fulfilling selection criteria recommended by the World Health Organization (age < 5 years old, documented fever, and parasite density < 200,000/μL) increased the risk of treatment failure by 25–60% for the different treatment regimens. Caution should be taken when comparing results from drug efficacy studies with different subject selection criteria.

INTRODUCTION

The emergence of antimalarial drug resistance has forced many countries in sub-Saharan Africa to review their national treatment policies. Accurate data on the degree and distribution of drug resistance are essential for the formulation of rational drug policies. Typically, drug efficacy data are generated from *in vivo* studies assessing the therapeutic response to antimalarial therapy in patients with symptomatic uncomplicated malaria. The World Health Organization (WHO) has provided standardized guidelines for the assessment and monitoring of antimalarial drug efficacy with the aim of supporting programmatic decision-making. These protocols have been designed to collect minimal essential information among populations at greatest risk and have been widely adopted.[1] In areas of high transmission, the WHO guidelines recommend studying patients less than five years of age with a documented fever and a parasite density between 2000 and 200,000/μL at enrollment.

Response to antimalarial therapy may be influenced by host and parasite factors and can vary with the population studied. The criteria used to select subjects for an antimalarial drug efficacy trial defines the study population and can impact on treatment outcome and study results. For example, in endemic areas, increasing age is associated with greater antimalarial immunity and improved therapeutic response,[2,3] and a higher parasite burden at the time of treatment may be associated with less favorable outcomes.[4] The recommended WHO patient selection criteria are restrictive, and do not cover the full range of patients with uncomplicated malaria. In practice, the criteria used to enroll patients into antimalarial drug efficacy studies often vary with respect to age, parasite density, and baseline temperature.[5]

Since 1998, our group has conducted six antimalarial drug efficacy studies involving seven different treatment regimens in Kampala, Uganda. These studies have used similar protocols in a common target population with a relatively unrestricted approach to patient enrollment. To investigate the impact of subject selection criteria on treatment outcome, we

evaluated independent predictors of treatment failure and estimated the effect that common variations in selection criteria had on the results obtained in our studies.

MATERIALS AND METHODS

Study site. All studies were conducted between August 1998 and June 2003 at the outpatient department of Mulago Hospital in Kampala, Uganda. Mulago Hospital is a tertiary referral hospital that principally serves a poor urban population. Malaria is mesoendemic in Kampala, with transmission peaking after each of two rainy seasons (Ugandan Ministry of Health, unpublished data).

Patients included in this analysis came from six previously published clinical trials. The treatment arms and study dates are as follows: study 1: chloroquine (CQ), August 1998 to March 1999;[3] study 2: CQ versus sulfadoxine-pyrimethamine (SP), March 1999 to August 1999;[6] study 3: SP versus amodiaquine (AQ) versus SP plus AQ (SP + AQ), September 1999 to July 2000;[7] study 4: SP versus SP + AQ versus SP plus artesunate (SP + AS), July 2000 to August 2001;[8] study 5: SP versus SP + AQ versus SP plus CQ (SP + CQ), March 2001 to January 2002;[9] and study 6: SP + CQ versus SP + AQ versus AQ plus AS (AQ + AS), October 2002 to June 2003.[10]

Informed consent was obtained from all adult participants and from parents or legal guardians of minors. All protocols were reviewed and approved by the Institutional Review Boards of Makerere University, Kampala and the University of California, San Francisco. In addition, studies 4–6 were approved by the Ugandan National Council for Science and Technology, and study 6 was approved by the International Clinical Studies Review Committee at the National Institutes of Health.

Subject selection. The protocols used for all of the studies were similar, and detailed methods have been published previously as referenced earlier. Briefly, in all studies with the exception of study 4, subjects were recruited from the population of patients presenting to the Mulago Hospital outpatient department with symptoms suggestive of malaria and a

positive screening blood smear (10% Leishman's stain for 10 minutes). In study 4, a cohort of healthy children was recruited from the surrounding community and followed for one year; each time a participant presented with a new episode of fever a screening blood smear was performed. All subjects included in the analysis of our six studies fulfilled the following selection criteria: 1) age $\geq$ 6 months, 2) tympanic temperature $\geq$ 38°C (equivalent to axillary temperature of 37.5°C) or a history of fever in the previous 48 hours, 3) absence of severe malaria[11] or danger signs (inability to stand or drink, lethargy, recent convulsions, persistent vomiting), 4) willingness to provide informed consent, 5) residence within Kampala 6) *Plasmodium falciparum* monoinfection, and 7) parasite density $\geq$ 2,000 asexual parasites/μL. Additional restrictions to subject selection were used as follows. In study 4, the upper limit for age was 6 years. In study 6, the upper limit of age was 10 years, and a provision to exclude patients with a parasite density $\geq$ 200,000 asexual parasites/μL was added after 14% of the patients were recruited into the trial.

Baseline evaluation and treatment. In all studies, baseline temperatures were measured using an electronic tympanic thermometer and blood was removed either by venipuncture or finger prick for thin and thick smear preparation. Thick and thin blood smears were stained using 2% Giemsa for 30 minutes. Parasite density was calculated by counting the number of asexual parasites per 200 white blood cells from the thick blood smear with the assumption of a white blood cell count of 8,000/μL.

All study drugs were obtained from reputable pharmaceutical companies and medications were dosed according to modified weight-based guidelines from the WHO for administration of fractions of tablets.[12] All therapy was directly observed. Patients were observed for 30 minutes after administration of medication, and the dose was repeated if vomiting occurred.

Patient follow-up and outcome classification. In all studies clinical outcome was assessed after 14 days using the WHO clinical classification system (adequate clinical response [ACR], early treatment failure [ETF], or late treatment failure [LTF])[12] with the modification that after day 3, patients with parasitemia and a history of recent undocumented fever were considered LTF. For the SP arm of study 2 and all patients in studies 4 and 6, outcomes were assessed after 28 days with genotyping used to distinguish recrudescence from new infections. Genotyping was successful in 97% of the samples analyzed. The few samples (n = 10) with unsuccessful genotyping were classified as due to recrudescence. With 28-day follow-up, outcomes were reclassified as failures if repeat therapy was due to recrudescent parasites and not a failure if repeat therapy was due to new infections, as previously described.[13] Progression to complicated malaria was defined as treatment failure accompanied by criteria for severe malaria[14] or danger signs[12] and referral to the hospital for intravenous quinine therapy.

In all studies, outcomes were not classified if any of the following occurred: 1) development of an alternative febrile illness that would interfere with outcome classification, 2) loss to follow-up, 3) self-medication with antimalarial drugs, 4) development of severe malaria or danger signs on day 0 after leaving the clinic, and 5) withdrawal of informed consent.

Statistical analysis. Data from each study had previously been entered and verified with Epi-Info version 6.04 (Centers for Disease Control and Prevention, Atlanta GA). We created a pooled database using SPSS version 10 (SPSS Inc., Chicago IL). Information extracted from the original patient data included age, treatment group, baseline temperature, pre-treatment parasite density, the day repeat therapy was given, whether repeat therapy was due to recrudescence or new infection, and whether the patient progressed to complicated malaria (severe malaria or danger signs) after Day 0. Analysis was done on STATA statistical software version 8.0 (STATA Corporation, College Station, TX) and R statistical software (R Foundation, Free Software Foundation, Boston, MA).

Comparisons of baseline characteristics among the different treatment groups and identification of independent predictors of progression to complicated malaria were made using generalized estimating equations with exchangeable correlation and robust standard errors.[15] In our analysis of predictors of treatment failure, failure was defined as all early treatment failures and any late treatment failure in studies with 14-day follow-up and any late treatment failure due to recrudescence in studies with 28-day follow-up. Predictor variables included age, baseline temperature, and baseline parasite density normalized by natural log transformation. We used an exploratory form of discrete survival analysis to model the time until treatment failure for these data. Specifically, we categorized time to failure into five blocks (0–3, 4–7, 8–14, 15–21, and 22–28 days). We then defined the outcome for each of these blocks to be either 1 (failure) or 0 (not failed yet). Patients were right-censored at the last block if they had not failed therapy. Finally, we fit an exploratory (logistic) regression model using POLYCLASS,[16] which chooses the best model among a large class of potential models (including main effects, splines, and multiplicative interactions). This logistic regression model (which actually models the hazard of treatment failure at each block) can be used to generate the cumulative probability of failure for any combination of treatment group and baseline covariates. In this way, we can use parametric extrapolation to estimate the relative efficacy of different treatments among different potential populations.

In a related set of analyses, we estimated the relative efficacy of treatment among progressively more restricted populations, defined first by age, then age and temperature, and finally by age, temperature, and parasite density. To do so, we took the resulting POLYCLASS model fit described above and used it to determine the predicted probability of treatment failure (by the last time block). We then computed the average of these predicted probabilities among the progressively more restrictive populations, which is the estimate of the marginal probability of treatment failure for each treatment within the specified population.

RESULTS

Baseline characteristics and treatment outcomes. Baseline characteristics and treatment outcomes for patients from each of the seven treatment groups are presented in Table 1. Among studies with no upper limit for age, approximately half (51%) of the patients were less than five years old. Geometric mean parasite densities and mean baseline temperatures were equivalent for all the treatment groups with the exception that the SP + AS group had a higher average parasite density ($P < 0.001$) and baseline temperature ($P < 0.001$).

TABLE 1
Baseline patient characteristics and treatment outcomes*

Treatment group	No.	Age (years)			Temperature °C†		Parasite density (asexual parasites/µL)				14-Day clinical failure‡	28-Day clinical failure§	Complicated malaria¶ no. (%)
		Median	< 5	Range	Mean	> 38.0°C	Geometric mean	> 100,000	> 200,000	> 500,000			
CQ	352	4.0	55%	0.5–60	38.0	48%	35,520	26%	11%	2%	49%	NA	9 (3)
SP	549	3.7	63%	0.5–50	37.9	50%	38,940	26%	12%	2%	14%	32%	6 (1)
AQ	131	4.0	53%	0.5–50	38.0	53%	29,970	21%	9%	2%	7%	NA	0
SP + CQ	265	5.0	49%	0.5–44	37.8	46%	32,500	23%	5%	1%	8%	36%	2 (1)
SP + AQ	541	4.0	63%	0.5–50	38.0	49%	36,400	26%	9%	1%	2%	8%	5 (1)
SP + AS	181	3.3	91%	0.6–5.7	38.3	66%	61,080	41%	15%	2%	0	17%	0
AQ + AS	119	4.0	56%	0.5–10	38.0	45%	29,890	22%	2%	1%	0	3%	0
Total	2,138	4.0	61%	0.5–60	38.0	50%	37,150	26%	9%	1%	–	–	–

* CQ = chloroquine; NA = not available; SP = sulfadoxine-pyrimethamine; AQ = amodiaquine; AS = artesunate.
† Tympanic temperature.
‡ Proportion of patients with early or late treatment failure according to the World Health Organization classification system.
§ Proportion of patients with early or late treatment failure adjusted for genotyping. Including only those patients enrolled in studies with 28-day follow-up and with successful genotyping.
¶ Progression to severe malaria or danger signs after day 0.

An elevated temperature at enrollment ($\geq$ 38.0°C tympanic) was documented in 50% of the patients; the remaining patients had a history of recent fever. Among studies with no upper limit for parasite density, 27% of the patients had parasite densities > 100,000 asexual parasites/µL and 11% of patients had parasite densities > 200,000 asexual parasites/µL. The risk of clinical failure increased considerably when follow-up was extended from 14 to 28 days (adjusted by genotyping), as previously reported.[8]

Model for treatment failure. Our final POLYCLASS model included treatment group effects, baseline variables (age, temperature, parasite density), and variations by time block. Decreasing age, increasing temperature, and increasing parasite density were all independently associated with an increasing risk of clinical treatment failure across all treatment groups. Figure 1 provides a visual presentation of the effect of changing the level of each individual predictor variable on the risk of treatment failure relative to a low risk patient population (age = 10 years, baseline temperature = 37.5°C, and pre-treatment parasite density = 10,000 asexual parasites/µL).

The modeled risks of treatment failure based on variations in patient selection criteria across all treatment groups are presented in Table 2. Restricting enrollment to only children less than five years of age resulted in an appreciable increase in estimates of treatment failure risk compared with the enrollment of patients with no restrictions on age, temperature, or parasite density. Further restricting enrollment to only patients with a documented fever led to even greater estimates of treatment failure risk. Restricting enrollment to pre-treatment parasite densities < 200,000/µL led to a slight decrease in estimates of treatment failure risk. Comparing results based on patients fulfilling the current WHO protocol (age less than five years, documented fever, and parasite density < 200,000/µL) to an unrestricted patient population, the relative risk of treatment failure was 1.25 for the CQ treatment group and ranged from 1.44 to 1.60 in the remaining treatment groups.

Risk for progression to complicated malaria. A total of 22 of 2,138 patients (1%) treated for uncomplicated malaria progressed to complicated malaria requiring intravenous quinine therapy. The risk of progression to complicated malaria was limited to children less than five years of age (22 of 1,304 versus 0 of 834; $P = 0.0002$) and always occurred within the first three days of follow-up. Restricting our analysis to children less than five years of age, there were no patients who progressed to complicated malaria receiving artesunate-containing combination therapy (0 of 232 versus 22 of 1,072; $P = 0.03$). In a multivariate analysis restricted to children less than five years of age not receiving artesunate, the only significant independent predictor of progression to complicated malaria was increasing parasite density at enrollment (odds ratio = 3.2 for each log increase in parasite density, 95% confidence interval = 2.1–4.7, $P < 0.0001$). The risk of progression to complicated malaria stratified by parasite density in children less than five years of age not receiving artesunate is presented in Figure 2. The risk was low (< 1%) for parasite densities less than 100,000/µL, with the greatest interval increase in risk occurring with parasite densities > 500,000/µL compared with parasite densities of 200,000–500,000/µL (23.8% versus 5.9%).

DISCUSSION

Clinical response to antimalarial therapy involves a complex interaction between the parasites, the drugs, and the host response. *In vivo* tests are considered the gold standard method of measuring antimalarial drug resistance.[12] These studies traditionally focus on the interaction between the parasite and drug; however, host factors may have important effects on the results of *in vivo* studies.[17,18] For example, a semi-immune patient might be cured despite being infected with parasites genetically resistant to the drug given.[2] In this study, we show that age, temperature, and parasite density are all independent predictors of antimalarial drug efficacy across a wide range of commonly used treatment regimens. Identification of such predictor variables can help improve our understanding of the biologic nature of treatment response. In addition, our results show that the selection criteria used to enroll patients can have a significant impact on estimates of antimalarial drug efficacy.

Host immunity plays a critical role in the clearance of malarial parasites. For example, the ability of monocytes to kill intracellular parasites is a function of antibody-dependent mechanisms[19] and the production of specific antibodies has been associated with protection against clinical attacks of malaria.[20] Acquired antimalarial immunity develops following
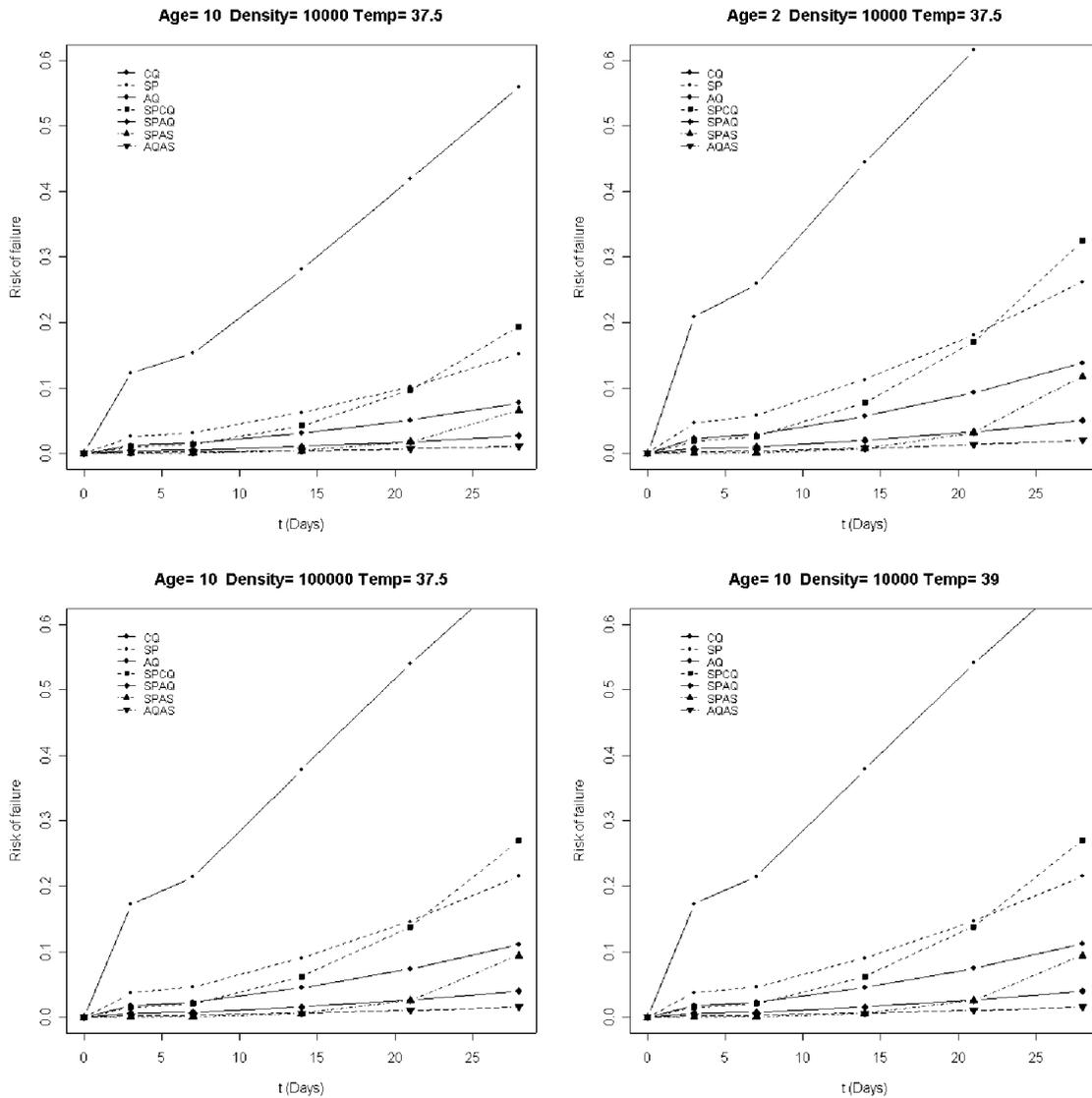
FIGURE 1. Cumulative probability of failure for each treatment group based on combinations of baseline covariates estimated from a POLYCLASS regression model. Upper left graph displays results of a baseline low risk patient population (age = 10 years, baseline temperature = 37.5°C, and pre-treatment parasite density = 10,000 asexual parasites/μL). Upper right graph demonstrates the independent effects of decreasing age. Lower left graph demonstrates the independent effects of increasing parasite density. Lower right graph demonstrates the independent effects of increasing temperature. CQ = chloroquine; SP = sulfadoxine-pyrimethamine; AQ = amodiaquine; AS = artesunate.

repeated exposure to infecting parasites. The association between increasing age and a decreasing risk of treatment failure has been previously reported in studies of different antimalarial treatments conducted in a variety of epidemiologic settings,[2,21–25] and it has been proposed that age is the best

surrogate marker of acquired immunity in endemic areas.[2] The impact of acquired immunity on the response to antimalarial therapy should be independent of the drug-parasite interaction, supported by the consistent association between age and treatment response across a wide range of antima-

TABLE 2
Estimated risk of 28-day treatment failure based on selection criteria and treatment*

Patient selection criteria	Treatment group						
	CQ	SP	AQ	SP + CQ	SP + AQ	SP + AS	AQ + AS
All patients	71%	27%	15%	34%	5.8%	13%	2.4%
Age < 5 years	84%	37%	22%	43%	8.1%	17%	3.2%
Age < 5 years, temperature > 38°C	90%	44%	25%	51%	9.6%	21%	4.1%
Age < 5 years, temperature > 38°C, parasite density < 200,000/μL†	89%	41%	24%	49%	9.0%	20%	3.8%

* For definitions of abbreviations, see Table 1.
† Patient selection criteria based on the World Health Organization protocol for high transmission areas.
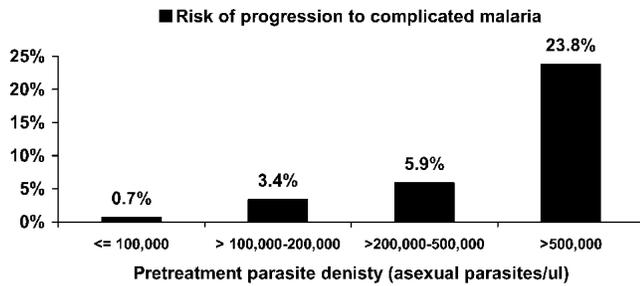
FIGURE 2. Complicated malaria defined as fulfilling criteria for severe malaria or danger signs. The analysis was restricted to patients less than five years of age not receiving artesunate (n = 1,072).

larial treatment regimens observed in this study. The relationship between age and treatment response is likely to vary with the level of malaria transmission intensity and the rate at which antimalarial immunity is acquired. In areas of higher transmission, protective immunity is acquired earlier in life.[26] Thus, the age associated with the sharpest decrease in the rates of antimalarial treatment failure should be inversely proportional to the level of transmission intensity.

In this study, higher parasitemia at presentation was associated with an increased risk of treatment failure across a wide range of antimalarial treatment regimens. Treatment with antimalarial drugs will optimally reduce the number of parasites between 100- and 10,000-fold per asexual cycle.[27] Parasite reduction appears to be a first-order process, such that a fixed fraction of the parasite population is removed with each successive cycle provided that the minimum parasiticidal concentration of the drug is exceeded.[28] A higher parasite biomass at the time of treatment initiation has previously been proposed to increase in the risk of treatment failure.[27] Although precise quantification of parasite biomass in patients infected with *P. falciparum* malaria is not possible due to fluctuations during the parasite life cycle and sequestration, peripheral parasitemia has been used as a relative estimate of the burden of infection. Higher parasitemia at presentation has been associated with an increased risk of early and late treatment failure in patients treated with chloroquine[24,25,29] and for late treatment failure in patients treated with artesunate,[4] quinine,[30] and mefloquine.[21] In endemic areas, patients with *P. falciparum* malaria are frequently infected with multiple genetically distinct strains.[13] Patients with higher pre-treatment parasitemias may be more likely to harbor a resistant strain, or to experience the spontaneous selection of resistant mutants. Additionally, a higher pretreatment parasite burden may predispose to recrudescence by simply increasing the probability that a parasite will survive the effect of short-acting artemisinins, for which parasite drug resistance has not been reported.[4]

We observed that increasing baseline temperature was an independent predictor of treatment failure across all treatment groups. The underlying biologic mechanism for this association is unclear. A higher temperature at presentation may represent a surrogate marker of a less effective host immune response to infection. We are unaware of previous studies reporting an independent association between baseline temperature and treatment failure, which may be due to a true lack of association, a failure to consider temperature as a potential predictor variable, or a failure to enroll patients

with a wide range of baseline temperatures as done in this study. Further studies of the association between temperature and response to antimalarial therapy are warranted.

Ideally, patients enrolled in studies of treatment of uncomplicated malaria should be at low risk for progression to complicated disease requiring hospitalization. In this study, we found that none of the 834 patients more than five years of age progressed to complicated disease. Interestingly, none of the 328 patients treated with an artesunate-containing regimen (all ≤ 10 years old) developed complicated disease, suggesting that this highly active, rapidly acting drug may protect against early disease progression. The only predictor of progression to complicated disease in this study was a higher pre-treatment parasite density. Among children less than five years old who did not receive artesunate, the risk was of progression to complicated disease was only 1% for those with parasite densities $< 200,000/\mu L$ and 9% for those $\geq 200,000/\mu L$, supporting the WHO recommendations of excluding patients above this threshold.

Attempts have been made to standardize selection criteria for antimalarial trials from similar epidemiologic settings. The WHO recommends that in areas of high transmission study subjects be limited to children less than five years old with a documented fever and parasite density $< 200,000$ asexual parasites/μL. However, the selection criteria used in antimalarial drug efficacy studies from high transmission areas frequently vary. In an extensive review of 108 studies from highly endemic areas of Africa between 1996 and 2002, 28% of the studies enrolled patients more than five years of age.[5] Similarly, considerable variability existed in criteria for temperature and parasite density. Few of these studies controlled for these differences in their analyses, making it difficult to directly compare results and monitor trends in drug resistance. Our analysis shows the practical implications that subject selection criteria may have on the results of antimalarial drug efficacy trials. Compared with an unrestrictive approach (enrolling all patients with uncomplicated malaria), limiting enrollment to only patients fulfilling selection criteria recommended by the WHO would have led to a 25% increase in the reported risk failure to CQ and a 44–60% increase in the reported risk of failure to the other treatment regimens evaluated. An awareness of the effects of age, temperature, and parasite density is important when comparing studies and monitoring trends in drug resistance. A standardized approach to subject selection criteria should be used in studies from similar epidemiologic settings when possible, or results should be stratified to allow for direct comparison of similar patient populations.

Authors' addresses: Grant Dorsey and Sarah G. Staedke, Department of Medicine, San Francisco General Hospital, University of

California, Parnassus Avenue, Box 0811, San Francisco, CA, 94143, Telephone: 415-206-4680, Fax: 415-648-8425, E-mails: grantd@itsa.ucsf.edu and staedke@itsa.ucsf.edu. Anne F. Gasasira and Moses R. Kamya, Department of Medicine, Makerere University Medical School, Anatomy Building, Room C-443, Kampala, Uganda, Telephone: 256-41-251-387, Fax: 256-41-540-524, E-mails: a_gasasira@hotmail.com and mkamya@info.com.ug. Rhoderick Machekano and Alan Hubbard, Department of Biostatistics, School of Public Health, University of California, 735 U-Hall 7360, Berkeley, CA, 94172, Telephone: 510-642-8365, Fax: 510-642-5815, E-mails: rodmach@uclink.berkeley.edu and hubbard@stat.berkeley.edu.

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