Effects of *Plasmodium falciparum* Parasite Population Size and Patient Age on Early and Late Parasitological Outcomes of Antimalarial Treatment in Children[▽]

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The design and interpretation of trials assessing the chemotherapeutic effects of antimalarial drugs depend on our understanding of how different selection criteria affect treatment outcomes. In this study, we analyzed the effects of baseline parameters on the initial parasite elimination rate and the risk of subsequent recrudescence as a marker for incompletely eliminated asexual blood-stage parasites in pediatric patients with uncomplicated *Plasmodium falciparum* infection treated with amodiaquine in a high-transmission area. We found that (i) parasite population size and patient age independently determine early and late parasitological treatment outcome measurements; (ii) the rate of recrudescence is higher in patients 1 to 3 years of age than in patients aged <1 or >3 years; (iii) patients aged >5 years with parasite densities between 2,000 and 10,000/μl have a lower recrudescence rate (13%; 95% confidence interval [CI], 8% to 21%) than patients aged <5 years with parasite densities of >10,000/μl (40%; 95% CI, 30% to 50%); and (iv) the sensitivity of detecting recrudescences outside this high-risk group, i.e., in patients of >5 years of age or with parasite densities of <10,000/μl, is as low as 27% or 22%, respectively. In conclusion, these findings highlight the need to use adequate selection criteria and to report parasitological outcome results adjusted for the readily available determinants of chemotherapeutic failure, i.e., patient age and baseline parasitemia. The thresholds may vary by transmission intensity and drug regimen. A better understanding of the limitations of antimalarial regimens in high-risk subgroups of patients has important implications for setting policy recommendations.

Antimalarial treatment trials provide the empirical evidence base for antimalarial treatment policies in countries where malaria is endemic (44). These trials use variable endpoints to address a range of public health questions. For instance, trials conducted to monitor the emergence or the spread of in vivo resistance or to determine the chemotherapeutic efficacy of a new regimen are designed to measure the ability of the treatment to completely eliminate asexual blood-stage parasites and, thus, to prevent recrudescence infections. On the other hand, the delay of reinfection caused by the effects of slowly eliminated drugs on the erythrocytic and, in some cases, hepatic stages of *Plasmodium falciparum* infection (14) can be an important additional clinical benefit of antimalarial treatment. This has been investigated in studies that use a combined measurement of the chemotherapeutic and the “posttreatment prophylactic” effects (“recurrence rate”) or that simply determine the requirement for retreatment (12). Regardless of the objectives in these trials, the characterization of the limitations of an antimalarial regimen in important subgroups of patients at high risk of failure, who may also be disproportionately susceptible to adverse clinical outcomes, will provide critical information for setting policy recommendations. In areas of

high malaria transmission, the value of an antimalarial drug depends largely on its performance in nonimmune populations at highest risk of *P. falciparum* malaria, i.e., young children and pregnant women in areas of high endemicity.

The differential effects of parasite population size and host age on the risk of recrudescence have long been known (8, 22, 31, 41). These associations were subsequently, though not universally (33), shown to operate in diverse transmission settings (11, 18, 32, 35, 39). Fewer studies have addressed the interaction of these factors on the response to antimalarial drugs (10, 17, 32, 37, 42).

The mechanisms of the underlying biological processes are poorly understood; thus far, antibody responses to selected parasite antigens (ring-infected erythrocyte antigen, merozoite surface protein 1) have been implicated in age-dependent, antiparasitic immunity (23, 24). It appears plausible that humoral and/or cellular immune responses play a role both in the initial, rapid parasite elimination phase during treatment when drug concentrations are highest and in the suppression of slowly replicating asexual parasites, preventing patent recrudescence of infections. Similarly, the pretreatment size of a given parasite population is likely linked to the treatment outcome by affecting the probability of survival of a subpopulation of asexual blood-stage parasites. In cases of multiclonal infections, this subpopulation will likely consist of drug-resistant parasites (28), but the mechanisms for differential parasite survival in sensitive infections remain elusive (22).

Using data from previously published controlled trials of amodiaquine, we studied in detail the relationships between (i) pre-

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treatment asexual-parasite density, host age, and other baseline parameters and (ii) early and late response rates to antimalarial treatment. We used graphical analyses for visualizing relationships and survival analysis to reflect specific biological processes (the “time to event,” i.e., the time to recrudescence, is thought to be a function of the antiparasitic potency of a regimen [43]). Based on these results, we aimed to describe the effects of the different sets of selection criteria on outcome estimates and how our ability to detect recrudescences in comparison to those in the subgroup at highest risk of parasitological failure was affected.

MATERIALS AND METHODS

Clinical study. For the purpose of this analysis, we pooled previously analyzed data sets from patients treated with amodiaquine for uncomplicated *P. falciparum* malaria in two independent trials conducted from 1999 to 2000 at the Albert Schweitzer Hospital, Lambaréné, Gabon (“study A” [4] and “study B” [1]). The study protocols were approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital. The study region is characterized by the perennial transmission of malaria (40). Chloroquine resistance is prevalent (3). Patients aged 0.3 to 10.8 years (0.3 to 3.6 years in study A [4] and 1.4 to 10.8 years in study B [1]) attending the outpatient pediatric department of the hospital were eligible for enrollment if the following inclusion criteria were met: an asexual *P. falciparum* parasitemia of 1,000 to 200,000 parasites/ μ l, a tympanic temperature of $>37.5^{\circ}\text{C}$ or a history of fever in the preceding 24 h, and written informed consent given by a parent or guardian of the patients. Exclusion criteria were signs of severe disease, adequate antimalarial treatment in the preceding 72 h, and a hemoglobin level of <5 g/dl. The patients were actively monitored daily until day 3 or until the second consecutive negative blood slide and, thereafter, on days 7, 14 (study B only), 21 (study B only), and 28. The parents were encouraged to return to the clinic in case of a change in the condition of their child (passive follow-up). Asexual-parasite density was determined using the previously described Lambaréné method (5). Amodiaquine was formulated as a 1% suspension of an amodiaquine base in study A (Flavoquine; Hoechst Marion Roussel, France) (4) or as tablets of an amodiaquine base in study B (200 mg, Camoquin; Parke-Davis, France) (1). Supervised treatment was administered in target doses of 10 mg/kg of body weight once daily for three consecutive days. The doses of tablets were rounded to the nearest quarter tablet, and syrup was administered in spoons of 5 ml. Full doses of drugs were readministered if patients vomited or spit out the first dose within 1 hour after administration. Patients who vomited the study drug more than once were withdrawn from the study and treated with rescue medication. The day 28 per protocol cure rate was the primary endpoint of the two study protocols. The parasitological cure was defined as initial asexual-parasite clearance before day 7 and no subsequent recrudescence between day 7 and day 28 (44). Early treatment failures were defined, based on standard clinical and parasitological criteria, as microscopically detectable recrudescence infections occurring before day 7 (1, 3), and late treatment failures were defined parasitologically as those occurring between day 7 and day 28 (1, 3). The PCR-based genotyping of pairs of parasite isolates from baseline and the day of asexual-parasite recurrence (after day 14) was used to classify recurrent infections as either recrudescences or new infections (23).

Statistical analyses. We used the nonparametric rank sum test for univariate comparisons of continuous baseline variables with skewed distributions. The risk of recrudescence and the corresponding confidence interval (CI) were estimated with the nonparametric Kaplan-Meier survivor function. Observations of patients who did not complete follow-up because of protocol violations, withdrawals, and reinfections before day 28 were right censored in the survival analysis; i.e., the data from the last evaluable visit were used in the survival analysis. Whenever appropriate, we also calculated incidence point estimates (“cure rate” or “failure rate”) and the respective differences in risk from the per protocol population as well as the corresponding CIs. The per protocol population consisted of patients with a known efficacy outcome (patients with new infections between days 21 and 28 were classified as “cured”). Proportions were compared with the Fisher exact test, and CIs were calculated using the accurate Wilson method for small sample sizes (6). We constructed, via maximum likelihood analysis, Cox proportional-hazard and logistic regression models for analyzing the effect of several risk factors on survival and binary outcomes, respectively, using a forward selection approach to include baseline parameters with *P* values of <0.1 . The proportional-hazard assumption was tested using weighted Schoenfeld residuals (16) and reported if violated. The degrees of correlation of independent variables included in the models were examined using the Spearman

TABLE 1. Outcomes of pediatric patients treated with amodiaquine for uncomplicated *Plasmodium falciparum* malaria

Outcome classification ^a	No. (%) of patients in indicated age range (yr)		Total no. of patients
	0.3 to <5	5 to <11	
Cured	63 (58) ^b	45 (79) ^b	108
Early treatment failure	4 (4) ^b	1 (2) ^b	5
Late treatment failure	42 (39) ^b	11 (19) ^b	53
Per protocol population	109	57	166
Lost to follow-up	15 (11) ^c	7 (10) ^c	22
Protocol violations, SAE	14 (10) ^c	8 (11) ^c	22
Total	138	72	210

^a Patients who were lost to follow-up and had protocol violations or severe adverse events (SAE) were included in the survival analysis of the risk of recrudescence when dropout occurred after day 7.

^b The denominator corresponds to the per protocol population.

^c The denominator corresponds to total patient population.

rank correlation coefficient. The reported unadjusted and adjusted hazard ratios indicate 1-unit changes of the corresponding variables. To objectively define a combination of thresholds of baseline parameters with the best discriminatory performance in correctly identifying patients with recrudescence infections, we compared the areas under the receiver operating characteristic (ROC) curve for different combinations of incrementally changed thresholds (1-unit increments). We used fractional polynomial regression as a flexible parametric method for fitting the smooth curves of unknown relationships between Y and X variables (34). Log₁₀-transformed parasite reduction ratios (PRR) were calculated as the fractional reductions of microscopically determined asexual-parasite densities per asexual-parasite life cycle (an average of 48 h [PRR_{48h}]) (43). The initial asexual-parasite clearance time (PCT) was computed as the time from the start of treatment until the first of two consecutive negative malaria blood slides taken on two subsequent days. In addition, we calculated stratified recrudescence ratios of the number of negative slides to the number of all slides (negativity) at 24 and 48 h after the start of treatment and tested their significance using Mantel-Haenszel statistics. All statistical analyses were performed, graphs were constructed, and the curves were fitted using Stata/MP version 10.0 for Mac OS X.

RESULTS

Study cohort and baseline parameters. A total of 210 patients were studied (Table 1); 166 patients (79%) reached a defined efficacy endpoint. Fifty-three recrudescence infections were detected until day 28 (corresponding to a failure rate of 32%; 95% CI, 25% to 40%). The Kaplan-Meier survival estimate was 67% (95% CI, 60% to 74%) for the pooled data set, but there was a striking difference between studies A and B, with survival estimates of 53% (95% CI, 41% to 63%) and 80% (95% CI, 70% to 87%), respectively. Early treatment failures occurred in five patients, four of these in children of <5 years of age. Patients aged <5 years had a 19% higher rate of recrudescence infections than older children (95% CI, 6% to 33%; *P* = 0.01), corresponding to a hazard ratio of 2.4 (95% CI, 1.2 to 4.8; *P* <0.01). Patients with recrudescence infections were significantly younger (median of 2.3 years versus 3.6 years) and harbored higher numbers of circulating ring-stage parasites than patients who did not experience recrudescence infections until day 28 (40,000 versus 18,000 parasites/ μ l [medians]) (Table 2). None of the other baseline parameters, including tympanic temperature and plasma creatinine concentration, were differentially distributed in unadjusted univariate comparisons among patients with or without recrudescence infections (Table 2). We found no evidence for underdosing or differences in daily amodiaquine doses among patients who did

TABLE 2. Univariate analysis of the relationships between the baseline parameters and the risk of subsequent recrudescence

Category	Patient characteristic	Value for patients with ^a :		P value
		No recrudescence ^b	Recrudescence ^c	
Patient	Age (yr)	3.6 (2.7–4.6)	2.3 (1.7–3.1)	<0.01
	Tympanic temp (°C)	38.0 (37.6–38.3)	38.0 (37.7–38.9)	0.08
Parasitology	Asexual parasite density/ μ l	18,000 (14,000–27,000)	40,000 (26,000–73,000)	<0.001
Hematology and chemistry	Hemoglobin concn (g/dl)	9.5 (9.2–9.9)	9.8 (9.1–10.1)	0.9
	Creatinine concn (mmol/liter)	54 (49–57)	55 (50–64)	0.15
	Alanine aminotransferase (U/liter)	26 (23–29)	24 (21–33)	0.9
Pharmacology	Amodiaquine (dose [mg/kg]/day)	11.4 (11.1–11.5)	11.1 (10.7–11.6)	0.6
	Frequency of vomiting on days 0–2 (%)	10 (6–17)	6 (2–16)	0.4
	Frequency of diarrhea on days 0–2 (%)	23 (17–32)	22 (13–35)	1.0

^a Values outside parentheses are medians or percentages. Values in parentheses represent the 95% CIs.

^b Twelve out of 116 patients vomited on days 0 to 2, and 27 out of 116 experienced diarrhea on days 0 to 2.

^c Three out of 50 patients vomited on days 0 to 2, and 11 out of 50 experienced diarrhea on days 0 to 2.

or did not experience recrudescence (Table 2). There was also no difference in the combined rates of vomiting or the numbers of times drugs were readministered (Table 2).

Relationships between host age, parasite population size, and risk of recrudescence. The rate of recrudescence was highest in children 1 to 3 years of age (37%, 26/71; 95% CI, 26% to 48%). Children who were <1 year old (16%, 3/19; 95% CI, 6% to 37%) had a rate similar to that of children of >3 years of age (15%, 13/87; 95% CI, 9% to 24%) (Fig. 1a). A one-sided comparison between the group of children of <1 year of age and the group of children who were 1 to 3 years of age was borderline significant ($P = 0.07$). The two-way graph of the $\log_{10}$ -transformed parasite density data against the rate of recrudescence infections plotted separately by age group (<5 years versus >5 years) suggested that the reduced rate of recrudescence in older children may be mediated mainly by their increased ability to deal with moderate to high parasite population sizes, ranging from 10,000 to 100,000/ μ l (Fig. 1b). Of note, parasite densities of >100,000/ μ l were associated with a high rate of recrudescence regardless of the age of the patient; however, the numbers were small (41% [7/17] versus 64% [3/9]), and the CIs were proportionally large (22% to 64% versus 12% to 65%) (Fig. 1b). Two-thirds of the patients with recrudescence infections had an asexual-parasite density of >25,000/ μ l at baseline.

The Cox proportional-hazard model on the time to recrudescence corroborated the univariate associations of the host and parasite density with the rate of recrudescence described in Table 2. Age (in years) (adjusted hazard ratio [AHR] of 0.9; 95% CI, 0.8 to 1.0; $P = 0.03$) and $\log_{10}$ -transformed asexual-parasite density (AHR of 2.1; 95% CI, 1.2 to 3.6; $P < 0.01$), but not tympanic temperature (in degrees Celsius) (AHR of 1.1; 95% CI, 0.9 to 1.4; $P = 0.3$), resulted in lower and higher hazards, respectively (likelihood ratio chi-square result = 18; P value for the whole model < 0.001; Nagelkerke's coefficient of determination = 0.2). In our data set, host age and parasite density were not correlated (Spearman's rank correlation coefficient = -0.1 ; $P = 0.2$) (see Fig. 3). However, the baseline tympanic body temperature was significantly correlated with patient age and pretreatment parasite density (Spearman's rank correlation coefficients of -0.3 ; $P < 0.001$ and 0.2 ; $P <$

FIG. 1. (a) Two-way graph of the proportions of recrudescence infections with increasing age. Error bars indicate upper and lower limits of the binomial exact 95% CIs. (b) Two-way graph of the proportions of recrudescence infections with increasing pretreatment asexual-parasite density/ μ l stratified by age of patients. Error bars indicate upper and lower limits of the 95% CIs.

FIG. 2. (a) Kaplan-Meier plot of the survivor function of recrudescence for patients with pretreatment asexual-parasite densities of $<10,000/\mu\text{L}$ or $\geq 10,000/\mu\text{L}$. Log rank test for the equality of survivor functions ($P < 0.01$). (b) Kaplan-Meier plot of the survivor function of recrudescence for patients aged ≥ 5 years or 1 to 4 years. Log rank test for equality of survivor functions ($P < 0.01$). The inclusion of patients aged <1 year in the <5 -year age group changed neither the shape of curves nor the significance level.

0.01, respectively). The addition of “study” (A or B) as a categorical variable did not improve the model (P was >0.1 for “study” and age) because “study” (A or B) and age were highly correlated (Spearman’s rank correlation coefficient = 0.8; $P < 0.001$). A separate proportional-hazard model where age was replaced by the “study” variable (A or B) gave results comparable to those of the first model: “study” and baseline parasitemia, but not baseline body temperature, resulted in significantly lower and higher hazards, respectively (data not shown). This supports the conclusion that age is a key determinant of the hazard of experiencing a recrudescence infection. Since age and the rate of recrudescence are nonlinearly related (Fig. 1a), we also evaluated a proportional-hazard model by including patient age and baseline parasite density as dichotomous variables (ages below or above 5 years and parasitemia below or above $10,000/\mu\text{L}$). Ages of <5 years and baseline parasite density of $>10,000/\mu\text{L}$ gave significantly higher hazards (AHR of 2.3, 95% CI, 1.2 to 4.5; $P = 0.01$; and AHR of 1.9, 95% CI, 1.1 to 3.5; $P = 0.02$, respectively). The Kaplan-Meier

FIG. 3. Scatter plot of pretreatment asexual-parasite density over age. Failures cluster within panel 1 (age of <5 years and $>10,000$ parasites/ μL).

plots in Fig. 2a and b illustrate the impact of an age of <5 years and a baseline parasitemia of $>10,000$ parasites/ μL on survival estimates.

A logistical regression analysis of the risk of recrudescence in patients classified according to age and parasite density (Fig. 3) revealed that the adjusted odds for recrudescence were four times higher (95% CI, 2 to 8; $P < 0.001$) for patients who were <5 years old and had parasite densities of $>10,000$ parasites/ μL than for patients who did not fall into this category. Moreover, ROC curve analysis of the risk of recrudescence showed that the combination of an age of <5 years and parasitemia of $>10^4$ parasites/ μL resulted in the highest area under the ROC curve (0.67).

Relationships between host age, parasite population size, and early treatment response rates. The time to asexual-parasite clearance below the microscopic-detection threshold (PCT) was positively correlated with the baseline asexual-parasite density (Spearman’s rank correlation coefficient = 0.3; $P < 0.01$). Patient age was negatively correlated with the PCT (Spearman’s rank correlation coefficient = -0.2 ; $P < 0.01$) (Fig. 4). In contrast to the differential recrudescence rates between infants and children who were 1 to 3 years of age, the PCTs in the age groups of <1 year and 1 to 3 years were equivalent (geometric means of 77 h and 73 h, respectively). Of note, three out of five parasitologically defined early treatment failures occurred in infants, all of whom were 6 months old. A Cox proportional-hazard model on the time to asexual-parasite clearance demonstrated that age (in years) (AHR of 0.6; 95% CI, 0.5 to 0.9; $P < 0.01$) and $\log_{10}$ -transformed asexual-parasite density (AHR of 1.1; 95% CI, 1.0 to 1.1; $P = 0.01$), but not tympanic temperature (in degrees Celsius) (AHR of 0.9; 95% CI, 0.8 to 1.1; $P = 0.3$), resulted in lower and higher hazards, respectively, than in analyses without these variables. The PRR_{48} was highly correlated with baseline parasite density (Spearman’s rank correlation coefficient = 0.3; $P < 0.001$), though this is likely an artifact since supposedly high PRR_{48} s in patients with low baseline density are invariably associated with subpatent parasite densities by 48 h and, hence, “missing

FIG. 4. Two-way graph of geometric mean times to asexual-parasite clearance with increasing age. Parasite clearance signifies the time point when asexual-parasite densities become undetectable via light microscopy, i.e., “subpatent.” Error bars indicate upper and lower limits of the 95% CIs.

data” (74 out of 195 patients with an evaluable 48-hour slide result).

Association between in vivo pharmacodynamic parameters and the risk of recrudescence. It has been suggested that the PCT and especially the PRR_{48} are related to differential parasite drug sensitivities (43). We, therefore, explored the relationship between these parameters and the risk of recrudescence. A Cox proportional-hazard model on the time to recrudescence demonstrated that PCT (in hours) (AHR of 1.1; 95% CI, 1.0 to 1.2; $P = 0.001$), but not the PRR_{48} (AHR of 1.2; 95% CI, 0.9 to 1.7; $P = 0.2$), resulted in a significantly higher hazard for recrudescence (likelihood ratio chi-square result = 8; P value for whole model = 0.2; Nagelkerke’s coefficient of determination = 0.1). In an alternative approach, we used the slide negativity rates at 24 h (6% [9/142] versus 0% [0/53] in patients without and with subsequent recrudescence, respectively) and 48 h (39% [53/137] versus 40% [21/53] in patients without and with subsequent recrudescence, respectively) after the start of treatment for calculating clinically more useful ratios of the risk of recrudescence, but the ratios were low (<0.1) and nonsignificant ($P = 0.1$ and $P = 0.9$, respectively).

Sensitivity to detect treatment failures in subpopulations of patients. The patients were categorized into groups below and above 5 years of age (by neglecting the heterogeneous response rates to amodiaquine of children of <5 years of age). The patients were further categorized by baseline parasitemia using a threshold of 10,000 parasites/ μ l based on earlier results (Fig. 3). The sensitivity to detect failure in patients of different categories was calculated by defining the subpopulation of patients of <5 years of age with >10,000 parasites/ μ l as the internal standard. Table 3 shows that the sensitivities for detecting failure in the comparison groups were $\leq 27\%$.

The impact of the selection criteria on failure estimates. The use of selection criteria (<5 years of age and >10,000 parasites/ μ l) that were more stringent than the current WHO recommendations (<5 years of age and >2,000 parasites/ μ l) produced a significantly higher estimate for the risk of recrudescence

TABLE 3. Sensitivity and specificity to detect recrudescence in subgroups of patients classified by using threshold criteria for host age and parasite population size

Patient subgroup	Age (yr)	Parasite density (parasites/ μ l)	Sensitivity (%)	Specificity (%)
1	≥ 5	<10,000	21	85
2	Any	<10,000	22	63
3	≥ 5	Any	27	75
4	<5	$\geq 10,000$	100	62

than an unrestricted approach using the total patient population in this study (estimated difference in the risk of recrudescence = 14%; 95% CI, 3% to 26%; $P = 0.01$) (Table 4).

DISCUSSION

The interest in amodiaquine, especially in combination with sulfadoxine-pyrimethamine or artesunate, as a replacement drug in areas of chloroquine resistance continues to grow (7, 13, 38) despite the important limitation of geographically heterogeneous response rates (1) and the potential for high degrees of amodiaquine cross-resistance with chloroquine (2). An overall parasitological failure rate of 35% clearly neither warrants the use of amodiaquine as monotherapy nor encourages its use in combination with other drugs.

In this study, we have attempted to systematically dissect the relationships between host age as the best surrogate marker for blood stage-specific immunity (21), the pretreatment parasite population size, and early as well as late outcome measurements of antimalarial treatment trials in children. Our results confirmed previously, mainly separately, reported associations: (i) parasite population size and host age independently determine the risk of recrudescence, and (ii) patient age is nonlinearly related to the risk of recrudescence, with the highest rates of recrudescence observed in patients who were 1 to 3 years old, compared to those observed in patients aged <1 or >3 years, after treatment with amodiaquine in the high-transmission study area. Moreover, we demonstrated that these associations equally applied to early and late outcome measurements. We could not confirm recently reported associations between body temperature and the risk of recrudescence (10, 11), which may be due to the smaller sample size of our study and, hence, its limited power or, alternatively, to the lack of a

TABLE 4. Impact of the selection criteria on the estimated risk of recrudescence by day 28

Set of selection criteria	Criterion		% of patients (95% CI) showing recrudescence rate by day 28	% Difference in risk estimates (96% CI) ^b	P value
	Age (yr)	Parasite density (parasites/ μ l)			
1	<11	>2,000–200,000	25 (20–32)	5 (–4–15)	0.3
2 ^a	<5	>2,000–200,000	30 (23–39)	14 (3–26)	0.01
3	<5	$\geq 10,000$ –200,000	40 (30–50)	9 (–3–22)	0.1

^a Selection criterion set 2 corresponds to WHO protocol recommendations for high-transmission settings (1).

^b The estimates of the differences in risks of recrudescence for selection criterion sets 1 and 2 are in comparison to set 1, and that for set 3 is in comparison to set 2.

causal relationship. In contrast to rapidly acting, artemisinin-containing combination therapies, the comparatively low per-cycle $\log_{10}$ parasite reduction ratio of amodiaquine (median $\text{PRR}_{48} = 2.5$) unmasked the significant impact of host age on the time to parasite clearance.

The observed age-dependent distribution of the risk of failure suggests an apparent rapid decline and a subsequent slow acquisition of the ability of the host to assist the effect of the drug during the first and after the third year of life, respectively. This pattern is reminiscent of the phenomenon of age-dependent negative conversion rates (25) and resembles the dynamics of drug-resistant parasite clearance (9). The lower risk of recrudescence in infants corresponds to a short period of postnatal protection (15, 36), possibly mediated by maternal antibodies (23, 24) and fetal hemoglobin (30) among other factors. The lower risk for recrudescence breakthrough infections in infants may also provide a credible alternative explanation for the recently reported, age-dependent protective effects of intermittent preventive treatment regimens (19).

Intriguingly, the time to parasite clearance was prolonged in infants, and three out of four parasitologically defined early treatment failures occurred in this age group. If this finding is not due to chance, it may indicate that the immune mechanisms related to the initial parasite clearance under drug treatment (e.g., related to splenic clearance) (27) differ from those that govern the control of slowly replicating subpatent infections and cause differential survival rates of asexual parasites (20, 26). In favor of this hypothesis, the body temperature—a crude measure of unspecific immune responsiveness—was also elevated in infants. Clearly, more data from infants are needed to elucidate the dynamic nature of these relationships.

The proportional-hazard model on the risk of recrudescence used in this study could account for only 20% of the data variation. This may indicate a lack of consideration of alternative determinants—our model did not include other important independent variables, e.g., ex vivo chemosensitivity of isolates and pharmacokinetic parameters (33)—or simply a large stochastic element in the distribution of the risk. We attempted to use calculated parasite reduction ratios to estimate in vivo pharmacodynamic effects but failed to detect any meaningful relationship between these ratios and the treatment outcome.

Recrudescences clustered in a subpopulation of patients characterized by an age of <5 years and a baseline parasite density of >10,000 parasites/ μl . The sensitivity to detect treatment failures in older children and children with <10,000 asexual parasites/ μl was as low as 21%. Against the backdrop of this figure, we endorse earlier recommendations (10) for using adequate protocols and to report study results stratified or adjusted by age and, ideally, pretreatment parasite density. This is especially important for large meta-analyses of antimalarial trials, which have mainly reported averaged treatment outcomes (29). For proof-of-principle studies, e.g., in phase II, trials with semi-immune populations (older children, adults) may be desirable due to safety and ethical considerations—with the important limitation that the results may tell only about 25% of the “truth” of the performance of a drug in particularly susceptible high-risk populations. Obviously, this figure may represent a worst-case scenario, since our findings cannot easily be extrapolated to other transmission settings and different drugs (32). In summary, a better understanding

of the limitations of an antimalarial regimen in high-risk subgroups of patients can provide important information for setting policy recommendations.

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