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Increased Efficacy of Sulfadoxine-Pyrimethamine in the Treatment of Uncomplicated *Falciparum* Malaria among Children with Sick Cell Trait in Western Kenya

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The role of the sickle cell hemoglobin type as a determinant of treatment outcome with sulfadoxine-pyrimethamine was retrospectively studied in young children with uncomplicated *falciparum* malaria who lived in an area with intense perennial malaria transmission. Between 1993 and 1997, 2795 treatments involving 813 children were monitored. Sick cell trait (HbAS) was present in 17.7% of the children. Two-and-a-half percent of the children experienced early clinical treatment failure by day 2–3, and 17.3% of the children were parasitemic on day 7. Treatments in HbAS children were less likely than those in HbAA children to result in persistence of parasitemia by day 3 (relative risk [RR], 0.66; 95% confidence interval [CI], 0.47–0.93; $P = .02$) or in parasitologic treatment failure on day 7 (RR, 0.51; 95% CI, 0.36–0.71; $P < .0001$). These results suggest that the HbAS phenotype should be included among factors that determine sulfadoxine-pyrimethamine treatment outcome.

Sickle cell hemoglobin (HbS) is a mutant allele of β -globin and is one of the well-known balanced polymorphisms providing heterozygotes (HbAS) with 60%–90% protection against high-density *Plasmodium falciparum* parasitemia, acute uncomplicated

malaria, severe malaria, and malaria mortality [1–3]. This advantage has provided a strong selection pressure in large parts of sub-Saharan Africa with moderate-to-intense malaria transmission, resulting in high population frequencies of this mutation.

Over the last decade, several countries in sub-Saharan Africa, including Kenya, have replaced or are in the process of replacing chloroquine with sulfadoxine-pyrimethamine for the first-line treatment of uncomplicated *P. falciparum* malaria. With a general concern that the effective life of sulfadoxine-pyrimethamine may be limited in Africa [4], close monitoring of the efficacy of this antimalarial treatment is essential for evaluating geographic variation in the development of resistance to it and assessing its durability in specific areas. In vivo antimalarial treatment failures may be caused by many factors other than the intrinsic susceptibility of *P. falciparum* to the antimalarial drug being tested [5]. An understanding of the different factors that influence the clinical and parasitologic response is important for the correct interpretation of estimates of drug efficacy.

The presence of the sickle cell trait (HbAS) or other host-genetic factors that confer protection against malaria is seldom taken into account in the assessment of antimalarial efficacy. There are several studies suggesting that host-genetic factors play a role in determining antimalarial treatment outcome, including evidence for a reduced effect of chloroquine and artemisinin derivatives against *P. falciparum* in α -thalassemia [6–8]. Little is known, however, of the role of sickle cell hemoglobin. *P. falciparum* parasites grown in HbSS erythrocytes in vitro have been found to be less susceptible to chloroquine than those infecting HbAA erythrocytes, whereas no difference was seen

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between HbAS and HbAA erythrocytes [9]. Only one study has compared the treatment response with antimalarials *in vivo* by hemoglobin S phenotype [10]: the study, conducted in the 1980s in western Kenya, showed an enhanced response to chloroquine in HbAS children who were <10 years of age with uncomplicated malaria. To our knowledge, studies involving the potential role of the HbAS phenotype in determining treatment outcome with other antimalarials have not been published. We retrospectively studied the role of the sickle cell trait as determinant of the clinical and parasitological response to sulfadoxine-pyrimethamine in children <5 years of age with acute uncomplicated *P. falciparum* malaria.

Methods

Study area and population. This study was conducted between April 1993 and March 1997 in 15 villages on the shores of Lake Victoria in western Kenya, as part of a larger ongoing community-based study of the acquisition of natural immunity to malaria in children <5 years of age (Asembo Bay Cohort project [ABCP]). The study site and design of the ABCP have been described elsewhere in detail [11, 12]. In brief, the population was ethnically homogeneous, with >95% belonging to the Luo tribe. This area has intense perennial malaria transmission, with a mean of 60–300 infected bites per year [13]. Between 60% and 90% of the children <5 years of age have detectable *P. falciparum* parasitemia at any time. High-grade resistance to chloroquine was widespread [14].

Each child enrolled in the ABCP was visited every 2 weeks by a village monitor. At each visit, a morbidity questionnaire was completed, which included questions on the history of antimalarial use in the previous 2 weeks, and the body temperature taken. During every other visit (i.e., every 4 weeks), the child's weight and height were measured, and a blood sample (250–500 μ L) was obtained by finger or heel prick for the determination of hemoglobin concentrations and the presence of malaria parasites. Mothers were told to bring their children to the village monitor at any time that the child was ill in between scheduled visits. In case of a documented fever (axillary temperature $\geq 37.5^{\circ}\text{C}$), an additional malaria smear was obtained. During the study period, sulfadoxine-pyrimethamine was not widely available in the area outside of the Centers for Disease Control and Prevention/Kenya Medical Research Institute research setting. Previous analysis indicated that HbAS and HbSS occur in 17.4% and 3.3%, respectively, of infants born in the ABCP [2]. The corresponding frequencies observed in pregnant women in this study area are 23% and 0%.

Design of treatment study. ABCP birth-cohort children were enrolled in this treatment study if they fulfilled the following criteria: infection with microscopically detected *P. falciparum* (pure infections or mixed infections with either *P. malariae* or *P. ovale*); age <60 months; axillary temperature $\geq 37.5^{\circ}\text{C}$; no signs of severe malaria (hemoglobin ≥ 5.0 g/dL, parasitemia $\leq 100,000/\mu\text{L}$, and normal mental status) [15]; and no history of sulfadoxine-pyrimethamine treatment in the previous 14 days [16]. The treatment dose with sulfadoxine-pyrimethamine was one-fourth of a tablet for infants, one-half of a tablet for 1–3-year-old children, and a

full tablet for 4-year-old children [17]. Each tablet contained 25 mg pyrimethamine and 500 mg sulfadoxine. The brands of sulfadoxine-pyrimethamine used during the 4-year study period were Fansidar (Hoffman–La Roche), Falcidin (Cosmos), and Orodar (Elys Chemical Industries). The quality of the local brands was confirmed by high-performance liquid chromatography. The tablets were crushed and given with water under supervision of the village monitor. Every child was observed for 30 min to see if vomiting occurred, and, if so, the full dose was repeated.

Follow-up schedule. Each child was visited at home on days 2 and 7, at which time a morbidity questionnaire was answered and the axillary temperature and a blood sample for a malaria blood smear were obtained. If the study participants could not be found on the scheduled days, the village monitor revisited the homes on days 3 or 4 and 8, 9, or 10.

Laboratory methods. Thick and thin blood smears were stained with Giemsa and examined for parasites. Parasite densities were counted against 300 leukocytes and expressed per cubic millimeters of blood, using an assumed leukocyte count of 8000 cells/mm³. Hemoglobin concentrations were measured using the HemoCue Hb Test System (HemoCue). Plasma and blood cell pellets were frozen at -20°C . DNA was extracted from the frozen blood cell pellets by use of the Puregene and Capture column DNA extraction kits (Gentra Systems), as directed by the manufacturer. A polymerase chain reaction method, as described by Wu et al. [18], was used to determine the presence of hemoglobin A and S.

Definitions. Treatment failures and successes were defined using a modified version of the World Health Organization (WHO) classification system [16]. Early clinical failure was defined as having either clinical deterioration requiring alternative treatment or persistence of fever with an increased *P. falciparum* parasitemia on day 2, compared with the pretreatment density, or persistence of any *P. falciparum* parasites with fever by day 3 or 4. Parasitologic treatment failure was defined as the presence of *P. falciparum* parasites on day 7, regardless of the presence of symptoms. Cumulative treatment failure was defined as having either an early clinical failure by day 2–4 or a parasitologic treatment failure by day 7. Halofantrine (SmithKline Beecham) at a dose of 3×8 mg/kg was used for retreatment of children experiencing clinical failures. Children were classified as stunted, underweight, or wasted if the height-for-age, weight-for-age, and weight-for-height *z* scores, respectively, were less than -2 . Moderate anemia was defined as a hemoglobin concentration <8.0 g/dL.

Data management and statistical analysis. The software packages SUDAAN (release 8; SAS callable version) and SAS (version 8.0) were used for the bivariate and multivariate analyses, respectively. Confidence intervals and *P* values were corrected for multiple observations per child. We estimated the association between the different phenotypes and sulfadoxine-pyrimethamine efficacy, using a binomial regression analysis in PROC GENMOD (SAS) with a log link function to obtain estimates of the adjusted risk ratios and using generalized estimating equation methods to account for multiple treatments per child [19, 20].

To verify whether associations between Hb phenotype and sulfadoxine-pyrimethamine efficacy could be attributed to confounding, factors associated with either Hb phenotype or treatment failure in bivariate analysis (*P* < .10) were added to the multivariate

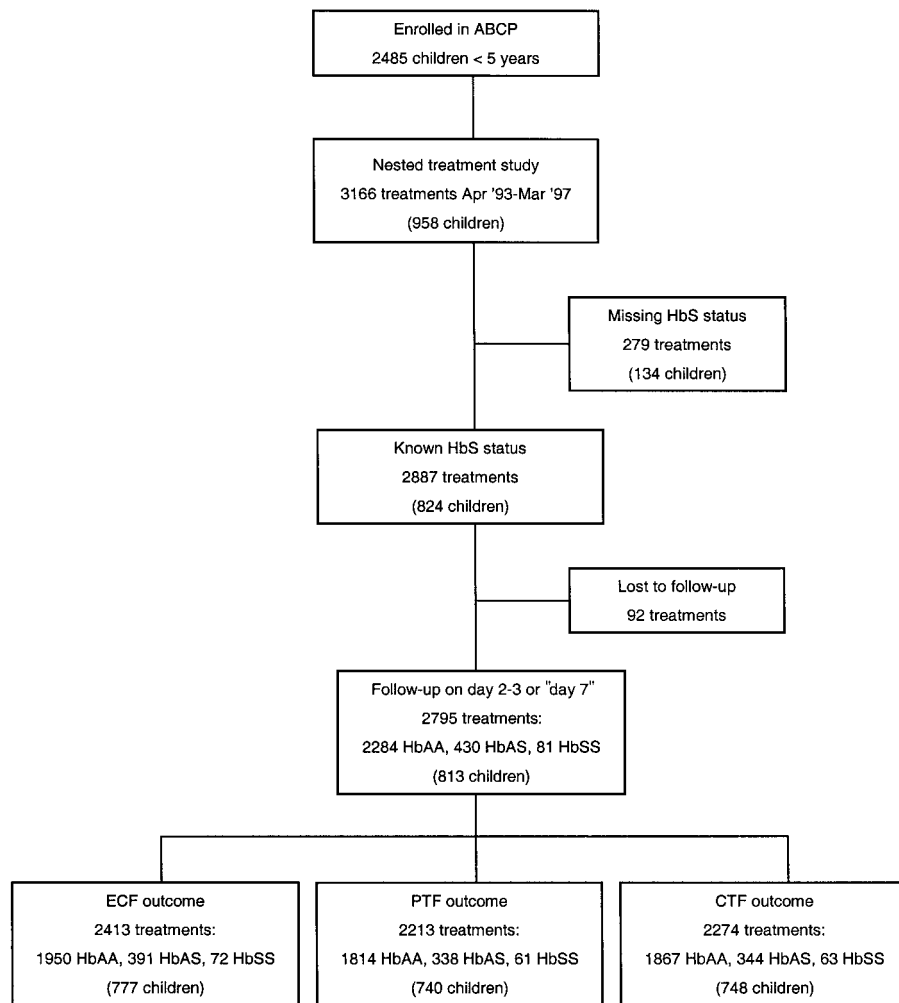


Figure 1. Profile of a study of hemoglobin phenotype and sulfadoxine-pyrimethamine efficacy in young children in western Kenya, 1993–1997. ABCP, Asembo Bay Cohort Project; CTF, cumulative treatment failure; ECF, early clinical failure; PTF, parasitologic treatment failure.

models as covariates. The following factors were considered to be potential confounders: year of study, treatment dose (in milligrams per kilogram) of sulfadoxine-pyrimethamine on the basis of body weight, history of recent chloroquine intake, and presence of pretreatment moderate anemia [21]. The following factors were considered to be potential intermediate factors: age, history of recent sulfadoxine-pyrimethamine intake, pretreatment parasite density, and nutritional z scores [21]. The differentiation between confounding and intermediate factors (which are covariates that may be affected by the exposure and represent a step in the causal path between exposure and outcome [22]) was made a priori. Intermediate factors were added as covariates because they can also be confounders [22]. None of the individual intermediate factors influenced the point estimate of the main association between phenotype and treatment failure, and all modeling could therefore be done using the GENMOD procedure [23]. Age was evaluated as an interaction term in the model (age × HbS) to assess the association between the HbAA and HbAS phenotypes and sulfadoxine-

pyrimethamine efficacy in the different age groups. For all statistical tests, 2-sided $P < .05$ was considered to be significant.

Results

Between April 1993 and April 1997, 3166 treatments were given to 958 children who were part of the ABCP birth cohort. Overall, 3063 treatment episodes were followed-up, 83.5% could be followed-up successfully within 4 days, on day 2 (64.9%), day 3 (14.0%), or day 4 (4.6%), and 76.7% could be followed-up successfully on “day 7” (52.8% on day 7 and 24.0% on days 8–10). This resulted in an overall success rate of follow-up by day 7 of 78.8% or 2495 treatments. The Hb phenotype was available for 86.0% (824/958) of the children and 91.3% (2795/3063) of the treatments that contributed follow-up data on days 2–4 or 7 (figure 1).

Of the 824 children with a known HbS status, 79% had the HbAA phenotype, 17.7% had the HbAS phenotype, and 3.3% had the HbSS phenotype. There were no differences between the characteristics of the children receiving treatments that could be successfully followed until day 7 and those that were either lost to follow-up before that day or had an unknown HbS status, except that missing HbS status occurred more frequently in children born in the second half of the study (1995–1997; $P < .0001$).

The pretreatment characteristics of the 2795 treatments are described in table 1. Children with HbAS contributed fewer treatments (median, 2 vs. 3; $P = .04$), were older at the time of their first treatment, had lower-density parasitemias and less moderate anemia, and were less likely to have been treated with sulfadoxine-pyrimethamine in the previous 35 days than HbAA children. Treatments in HbAS children were associated with higher mean height-for-age Z (HAZ) scores than those in HbAA children, but this difference was not statistically significant. However, HbAS children were less likely to be stunted (HAZ score of less than -2 ; 27.9% vs. 36.0%; $P = .03$). Compared with HbAA children, HbSS children contributed fewer treatments (median, 1 vs. 3; $P = .05$), had significantly lower median parasite densities, were less moderately anemic, were older at their first treatment, and had lower HAZ and weight-for-age z scores than HbAA children (none of these were statistically significant).

Children with HbAS were equally as likely to be parasitemic as HbAA children on day 2, but they were more likely to have cleared their parasitemia by day 3. HbAS was not associated with faster fever clearance. Overall, 2.5% (61/2413) of the treatments resulted in an early clinical failure, and 17.3% (383/2213) resulted in parasitologic treatment failure. The cumulative treatment failure risk by day 7 was 19.5% (444/2274). In both bivariate and multivariate analyses, HbAS was associated with a significantly lower parasitologic and cumulative treatment

failure risk by day 7 (table 2). Although HbAS was associated with a reduced risk of cumulative treatment failure among all age groups, this association was particularly apparent in the first 6 months of life (3.1% [2/61]) versus in children 6–59 months of age (18.7% [63/337]) (figure 2). Several variables (study year, sulfadoxine-pyrimethamine dose based on body weight, moderate anemia, and recent chloroquine intake) were omitted from the final model, because they were not found to be effect modifiers or confounders or to affect the precision of the estimated effect of the HbAS phenotype on treatment outcome. Thus, to evaluate the role of the intermediate factors on the association between the hemoglobin phenotypes and treatment outcome, we compared a model with all the potential intermediate factors to the crude model. The efficacy estimates did not differ much between these 2 models for any of the outcomes. The prevalence of pure *P. malariae* or *P. ovale* infections on day 7 was similar between treatments in HbAS and HbAA children (4.9% vs. 3.4%; relative risk, 1.41; 95% confidence interval, 0.32–6.13).

Eighty-one treatments were contributed by 27 children with HbSS. Although the risks of parasitologic and cumulative treatment failure were lower in HbSS children than in children with HbAA, none of these differences was statistically significant (table 2). The presence of pure *P. malariae* or *P. ovale* infections on day 7 was more likely in children with HbSS than HbAA (16.7% [2/12] vs. 3.4% [14/409]; $P = .02$).

Discussion

We found that in this area of high malaria transmission pressure, among young children who required treatment for uncomplicated malaria, sulfadoxine-pyrimethamine was half as likely to fail for those with the sickle cell trait (HbAS) than those with normal hemoglobin (HbAA). Apart from age, this is the strongest determinant of treatment outcome in young

Table 1. Characteristics of 813 children (644 with the HbAA phenotype, 142 with the HbAS phenotype, and 27 with the HbSS phenotype) <5 years of age receiving 2795 treatments for uncomplicated falciparum malaria in western Kenya, 1993–1997.

Patient characteristic	Phenotype (no. of treatments)			P	
	HbAA (n = 2284)	HbAS (n = 430)	HbSS (n = 81)	HbAS vs. HbAA	HbSS vs. HbAA
Male, no. (%)	1110 (48.6)	205 (47.7)	26 (32.1)	.88	.26
Age in months, median (IQR)	12.4 (7.1–20.5)	12.0 (7.9–20.3)	13.2 (8.3–20.0)	.65	.43
Age in months at first treatment, median (IQR)	5.8 (3.6–8.7)	6.3 (4.4–9.5)	7.1 (4.7–10.8)	.02	.07
Axillary temperature, mean (SE)	38.3 (0.02)	38.3 (0.05)	38.2 (0.13)	.43	.28
Parasite density, median (IQR) ^a	9773 (3240–22227)	7307 (2240–17067)	4853 (1573–14693)	<.0001	.0004
Mixed species vs. pure <i>P. falciparum</i> , no. (%)	143 (6.3)	35 (8.1)	8 (9.9)	.21	.29
Gametocytes, no. (%)	138 (6.1)	31 (7.3)	5 (6.2)	.33	.98
Receipt of sulfadoxine-pyrimethamine in preceding 15–35 days, no. (%)	301 (13.2)	40 (9.3)	11 (13.6)	.03	.94
Hemoglobin level <8.0 g/dL, no. (%)	580 (31.7)	84 (24.6)	12 (18.2)	.03	.05
Height-for-age z score, mean (SE)	−1.52 (0.05)	−1.31 (0.10)	−1.84 (0.36)	.07	.38
Weight-for-age z score, mean (SE)	−0.63 (0.05)	−0.54 (0.12)	−0.85 (0.40)	.49	.59
Weight-for-height z score, mean (SE)	0.49 (0.05)	0.40 (0.12)	0.50 (0.27)	.48	.99

NOTE. IQR, interquartile range.

^a Determined for those with malaria parasitemia only.

Table 2. Results of the univariate multivariate analysis, showing Hb phenotype as a determinant of treatment failure among young children with uncomplicated falciparum malaria in western Kenya, 1993–1997.

Patient characteristic, phenotype	No. of patients	Patients with characteristic, %	Crude RR (95% CI)	P	Adjusted RR (95% CI) ^a	Adjusted P
Parasitemic on day 2						
HbAS	308	52.6	0.97 (0.87–1.08)	.56	0.98 (0.88–1.09)	.72
HbSS	64	59.4	1.09 (0.89–1.34)	.41	1.12 (0.91–1.37)	.28
HbAA	1512	54.4	Reference group	—	Reference group	—
Febrile on day 2						
HbAS	308	8.8	0.95 (0.62–1.45)	.80	1.01 (0.67–1.52)	.97
HbSS	64	12.5	1.34 (0.73–2.45)	.35	1.30 (0.70–2.39)	.41
HbAA	1512	9.3	Reference group	—	Reference group	—
Parasitemic on day 3 ^b						
HbAS	64	26.6	0.66 (0.47–0.93)	.02	0.74 (0.52–1.04)	.08
HbAA	335	40.3	Reference group	—	Reference group	—
Febrile on day 3 ^b						
HbAS	64	9.4	1.05 (0.42–2.58)	.92	1.02 (0.42–2.47)	.97
HbAA	335	9.0	Reference group	—	Reference group	—
Early clinical failure ^c						
HbAS	391	1.5	0.57 (0.22–1.50)	.26	0.62 (0.24–1.60)	.32
HbSS	72	2.8	0.99 (0.33–3.00)	.99	1.00 (0.33–3.03)	.99
HbAA	1950	2.7	Reference group	—	Reference group	—
Parasitological treatment failure						
HbAS	338	9.8	0.51 (0.36–0.71)	<.0001	0.54 (0.39–0.75)	.0002
HbSS	61	13.1	0.70 (0.44–1.12)	.13	0.74 (0.48–1.15)	.19
HbAA	1814	18.9	Reference group	—	Reference group	—
Cumulative treatment failure						
HbAS	344	11.3	0.53 (0.39–0.72)	<.0001	0.56 (0.41–0.76)	.0002
HbSS	63	15.9	0.74 (0.50–1.10)	.14	0.79 (0.55–1.14)	.21
HbAA	1867	21.2	Reference group	—	Reference group	—
Gametocytes on day 7 ^d						
HbAS	338	28.7	1.06 (0.87–1.28)	.58	1.12 (0.92–1.37)	.25
HbSS	61	39.3	1.46 (0.98–2.17)	.06	1.73 (1.32–2.26)	<.0001
HbAA	1813	26.5	Reference group	—	Reference group	—

NOTE. CI, confidence interval; RR, relative risk.

^a Adjusted for age, pretreatment parasite density, sulfadoxine-pyrimethamine intake in previous 15–35 days, and height-for-age z score.

^b Only 5 observations in HbSS children (data not shown).

^c Includes 125 observations contributed on day 4 (103 HbAA, 19 HbAS, and 3 HbSS).

^d Both “crude” and adjusted RR are controlled for pretreatment gametocytemia.

children identified in our antimalarial treatment studies (unpublished observations). An enhanced efficacy, although less pronounced, was also seen with sickle cell anemia (HbSS), but numbers of HbSS children were small (27 children and 81 treatments), and the difference was not statistically significant.

Children who carried the sickle cell trait were older at the time of their first treatment, were less likely to have received SP in the previous month, and had lower pretreatment parasite densities than children with HbAA. They were also less likely to be stunted, possibly reflecting the beneficial long-term cumulative impact on health and nutritional status because of the lower risk of clinical malaria and severe anemia [24, 25]. Age, pretreatment parasite densities (independent of age), recent sulfadoxine-pyrimethamine intake, and malnutrition are all known determinants of antimalarial efficacy [26–30] and are also affected by the sickle cell trait. Thus, the trait could determine antimalarial treatment outcome through these intermediate factors. However, the multivariate analysis showed that only a small proportion of the lower risk of treatment failure in HbAS children could be attributed to these factors. Although the exact mechanisms of

protection of the sickle cell trait against malaria morbidity are only partly understood, there are several other possible mechanisms by which HbAS might enhance the effect of sulfadoxine-pyrimethamine.

There are several examples of pharmacodynamic and kinetic interactions of other antimalarials with malaria resistance genes, including with the artemisinin derivatives and α -thalassemia [6, 31, 32], chloroquine and α -thalassemia [6, 8, 31], and chloroquine and HbS [33]. It is unknown, however, if such drug-specific modulation exists for sulfadoxine or pyrimethamine and the HbAS phenotype. It is possible that the uptake and or retention of either of the 2 components of sulfadoxine-pyrimethamine may be higher in HbS containing red cells infected with *P. falciparum*. Erythrocytes with HbS can have excess α -globin chains, which can promote lipid peroxidation by binding to red cell membranes, especially under low oxygen tension experienced by red cells infected with mature parasite stages that are cytoadhered in the microcapillary bed [34]. As a result, the membrane permeability may increase and favor the increased uptake of a drug. The increased efficacy of sulfadoxine-pyrimethamine in HbAS indi-

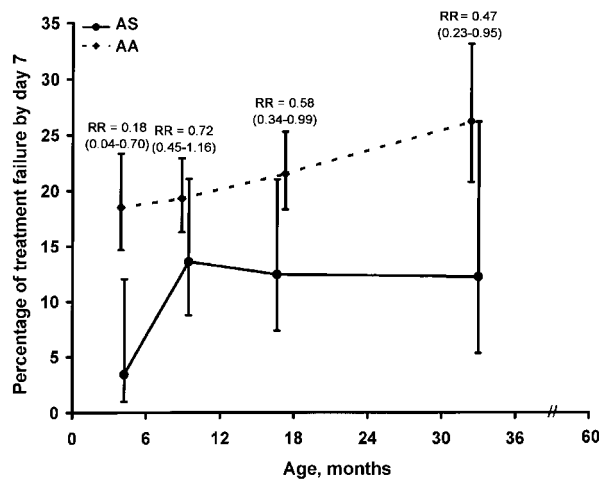


Figure 2. Cumulative treatment failure among young children in western Kenya by day 7 after treatment of uncomplicated falciparum malaria with sulfadoxine-pyrimethamine, by hemoglobin phenotype and age. Error bars represent the 95% confidence intervals (CIs) for the failure risk estimate. Ninety-five percent CIs for the relative risk (RR) are shown in parentheses.

viduals may also be explained by physiologic and immunologic mechanisms resulting in enhanced clearance of parasites, irrespective of an impact on the uptake and/or retention of the antimalarial drug. For example, under conditions of low oxygen tension, a retardation of parasite growth and parasite invasion has been observed *in vitro* in nonsickled AS cells [9, 35–37], possibly due to the loss of K^+ ions caused by altered membrane permeability [34]. The natural exposure of all *P. falciparum*-infected erythrocytes to low oxygen tension occurs while sequestered in the deep tissues during the last half of the parasite's asexual life-cycle, where the parasite matures to a schizont and develops merozoites. Since sulfadoxine-pyrimethamine has a narrow time-window of drug action, which targets the parasite during this schizont stage, both mechanisms would coincide and may interact to effectively kill parasites, thus preventing subsequent parasite multiplication and enhancing clearance. In addition, preferential phagocytosis of parasitized erythrocytes expressing neoantigens has been suggested as a common and powerful mechanism of protection in several red cell disorders [38, 39]. In sickle cell trait, a binding of larger amounts of antibodies to neoantigens of infected cells [40], as well as an altered immune reactivity [41], has been observed.

The present treatment study is based on a retrospective analysis of data from a larger cohort study, which was not designed to look at the impact of the hemoglobin phenotype on treatment outcome with sulfadoxine-pyrimethamine. Thus, hemoglobin type was not available for 14% of the children enrolled in this treatment–follow-up study. A fifth of the treated children were lost to follow-up by day 7. The larger cohort study included routine monthly finger-prick blood samples, and, although most

caregivers consented to treatment follow-up, a relatively high proportion subsequently refused to have their child pricked for the additional day-2 and day-7 treatment–follow-up visits. Treatments in children with an unknown phenotype or without follow-up on day 2 or 7 did not differ in age, severity of illness, or other predictors of treatment outcome from those with a complete follow-up or known phenotype. Thus, this is unlikely to have been a source of substantial bias. Because low pretreatment parasite density was not an exclusion criteria in this study and because we aimed to represent early clinical failure, as opposed to early treatment failure, a modified version of the WHO criteria to define treatment outcome was used, which excluded the 75% decline in parasitemia by day 2 or 3 as a criterion for early treatment or parasitologic failure. Use of the old WHO definition for parasitologic failure by day 7, commonly used at the time this study was conducted [42], or the current WHO classification [16], which combines clinical and parasitologic criteria, did not change our results (data not shown).

Results of this study, together with those of the previous study of chloroquine efficacy in this same area [10], suggest that the sickle cell trait may enhance the effect of antimalarial drugs and that this protection extends beyond the known time interval (~2–16 months of age) during which HbAS was previously found to contribute to reduced mortality in western Kenya [2]. Whether a similar beneficial effect of sickle cell trait on treatment outcome can be found among young children in areas of lower or more seasonal transmission pressure, as well as among semi-immune adolescents and adults, deserves further investigation.

We conclude that sickle cell trait enhances the efficacy of sulfadoxine-pyrimethamine to clear *P. falciparum* parasites in young children with uncomplicated malaria. This is also the group usually targeted for *in vivo* studies of antimalarial drug efficacy in malaria-endemic areas of Africa. Thus, in areas with a high prevalence of sickle cell trait among the study population, overall estimates of drug efficacy may be somewhat higher than those seen in areas where sickle cell trait is less common. Furthermore, the improved treatment efficacy conferred by HbAS may become more apparent with the emergence of higher levels of antimalarial drug resistance, which will result in more parasites escaping the static or killing effects of the drug, requiring that host factors provide a greater contribution to the radical clearance of parasites [43]. An unequal distribution of HbAS by chance between treatment groups or over time in longitudinal monitoring studies might be an important source of bias, which is a particular problem in studies of small sample sizes. Thus, one should question whether determination of HbS should be included in the routine monitoring of antimalarial treatment efficacy. This would not be feasible in most settings in malaria-endemic countries, nor would it be indicated, as long as there is no reason to suspect that the study sample has a biased distribution of HbAS relative to the underlying population. Nevertheless, these results do suggest that the presence of hemoglobin S should be taken into account

in studies that aim to determine factors that affect treatment outcome with sulfadoxine-pyrimethamine and possibly other antimalarials.

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