Plasma Folate Level and High-Dose Folate Supplementation Predict Sulfadoxine-Pyrimethamine Treatment Failure in Pregnant Women in Western Kenya Who Have Uncomplicated Malaria

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Sulfadoxine-pyrimethamine (SP) inhibits folate metabolism by the malaria parasite. We investigated the association between folate levels and SP failure in pregnant women. Data from a trial to assess the effect that folate supplementation has on SP failure in 467 pregnant women were analyzed. Plasma folate levels were determined at enrollment and at day 7. High baseline folate levels, high parasite densities, and age <20 years were risk factors for SP failure. High-dose (5 mg daily) folate supplementation or high folate levels at day 7 were independent risk factors. Therefore, pregnant women receiving SP should receive low-/moderate-dose folate supplementation.

Trial registration. http://www.clinicaltrials.gov identifier: NCT00130065.

Insecticide-treated nets (ITNs) and intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) are recommended to reduce the effects of malaria during pregnancy [1]. When used as IPT, repeated doses (at least 2) are administered, at least 1 month apart, after the first trimester; IPT is practiced in most African countries where malaria is endemic [1]. SP inhibits folate metabolism by the malaria parasite; high-dose folate supplementation has been documented to increase the risk of SP treatment failure [2]. High innate folate levels have been associated with treatment failure in children [3]. We studied the effect that folate supplementation has on the efficacy of SP treatment in pregnant women [2], and here we report the results of a secondary analysis of the effect that plasma folate levels have on SP treatment efficacy in parasitemic pregnant women.

Participants and methods. Enrollment, data collection, and laboratory methods have been described elsewhere [2]. During 2002–2005, pregnant women of all gravidities who had both uncomplicated malaria during the second or third trimester and a hemoglobin level of >7 g/dL were studied. At the time, SP was used as the first-line treatment for malaria and for IPT [4], with an efficacy of 60%–80% in children who were 6–59 months old [5].

The study was approved by the Kenya Medical Research Institute (Nairobi) and by the Centers for Disease Control and Prevention (Atlanta, Georgia). All participants gave informed consent.

Treatment consisted of either 5 mg of folate, 0.4 mg of folate, or placebo for 14 days, followed by 5 mg of folate, according to national policy. The first dose was given with SP (Lab and Allied), under supervision. From August 2004 onward, all participants received ITNs. Follow-up visits were at days 2, 3, 7, 14, 21, and 28 or whenever a woman felt that such a visit was necessary. At follow-up visits, signs and symptoms of malaria and side effects were noted. Axillary temperature was measured, and a blood smear was made. At days 0 and 7, nonfasting plasma was collected and was stored at -20°C, for folate analysis. After SP treatment failure, quinine treatment was administered. Outcome measures were the prevalence of SP treatment failure at days 3, 7, 14, and 28.

Plasma folate levels were measured by use of an Abbott AxSYM System (Abbott Laboratories). This method is based on ion-capture technology, whereby negatively charged complexes are formed during a reaction between folate and a reagent composed of folate-binding protein and monoclonal antibodies. These complexes are captured through electrostatic interaction with a positively charged glass-fiber matrix. Folate is then quantified by measuring the population of unoccupied folate binding–protein sites.

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We defined malaria as the presence of asexual parasites (any species) in thick blood smears, independent of clinical signs. A high parasite density was a density within the highest tercile of the study population. Women who were <20 years old were considered to be young. Low and high folate levels were defined as <3 and >15.4 ng/mL, respectively [6]. A low SP dose was defined as <25 mg of sulfadoxine per kilogram of body weight and was based on maternal weight at enrollment.

For continuous variables, Student's *t* test was used; for nonnormally distributed variables, the Kruskal-Wallis test was used; for proportions, either the χ^2 test or Fisher's exact test was used. The folate level at day 0 was log-transformed, and linear regression was used to determine factors affecting it. Factors examined included maternal age, trimester of pregnancy, sickle-cell trait, indicators of socioeconomic status, season (rainy or dry), study site, high parasite density, anemia, and HIV-infection status.

We used Kaplan-Meier curves and Cox proportional-hazards regression analysis to examine factors affecting the time to treatment failure, for days 3, 7, 14, and 28. Factors included young age, high parasite density, high body temperature, treatment dose (in milligrams per kilogram of body weight), and sickle-cell trait [7–9]. Because low folate levels did not seem to affect the time to treatment failure, we condensed folate levels into high versus normal/low. Because the folate level at day 7 can be affected by folate-treatment group and, in turn, can affect treatment failure (it is on the causal pathway between folate-treatment group and treatment failure), we developed 2 models for day 14 and day 28, one that used folate level at day 7 and one that used treatment groups. Factors were removed from models when their significance was $P > .05$; to allow for comparison, factors significant in a multivariate model at one time point were kept in the models for the other time points, even if they were not significant at the other time point. In addition, study site and receipt of an ITN were retained in all models, to account for differences in exposure, as were HIV-infection status and treatment group. The SAS system for Windows (version 8) was used for all analyses. All tests were 2-sided; $P < .05$ was considered to be significant.

Results. Of the 488 women who were enrolled, 467 were included in the analysis of the time to treatment failure [2]; *Plasmodium falciparum* was found in 458 (98.1%), *P. falciparum*/*P. malariae* in 8 (1.7%), and *P. malariae* in 1 (0.2%). Although 275 (58.9%) of the women reported having had fever during the preceding week, only 16 (3.4%) had fever at the time of enrollment. As we have reported elsewhere [2], at days 14 and 28, the overall treatment-failure rates were 18.6% and 42.9%, respectively.

Only 15 (3.3%) of the 458 women with a folate result at enrollment had low folate levels. In multiple linear regression analyses, factors associated with folate level at enrollment included hemoglobin level (0.02 ng/mL increase per g/dL hemoglobin increase [95% confidence interval {CI}, 0.01–0.03]), residence in a house with a mud wall (0.04 ng/mL [95% CI, 0.01–0.08 ng/mL]), and rainy season (0.04 ng/mL [95% CI, 0.01–0.08 ng/mL]). At enrollment, folate levels were comparable in all groups; at day 7, the group that had received 5 mg of folate had a significant median increase of 9.2 ng/mL (interquartile range, 2.4–16.3 ng/mL) in folate level, whereas the group that had received 0.4 mg of folate had a median increase of 2.7 ng/mL (interquartile range, 0–7.5 ng/mL). Of the 12 women with a high folate level at enrollment, 10 had a high parasite count ($P < .001$); these 12 women were significantly more likely to experience treatment failure (figure 1) ($P < .001$). There was no association between folate level at day 0 or 7 and sickle-cell carrier status (data not shown). At day 7, 100 women (2 in the placebo group, 23 in the 0.4-mg group, and 75 in the 5-mg group) had folate levels >15.4 ng/mL. Of these 100 women with high folate levels, 91 completed the study and 48 (38 in the 5-mg group and 10 in the 0.4-mg group) experienced treatment failure; the treatment-failure percentage did not significantly increase when the folate level was >15.4 ng/mL.

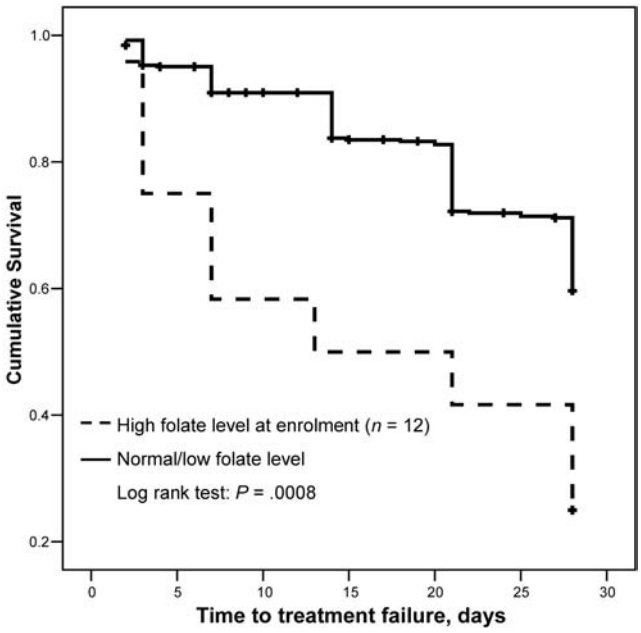

Figure 1. Cumulative treatment survival rates, by folate level at enrollment, in parasitemic pregnant women in western Kenya who were treated with SP during 2002–2005.

Of 466 women with known HIV status, 159 (34.1%) were HIV infected. Compared with HIV-uninfected women, HIV-infected women were significantly more likely to be older, to be multigravid, to have higher parasite densities, to be enrolled during the dry season, and to be anemic. No association with folate level was seen. The prevalence of treatment failure at day 28 was 42.9% in HIV-infected women and 43.1% in HIV-negative women. In univariate analysis, HIV infection was not associated with risk of treatment failure; in multivariate analysis, however, such an association was present at day 7 and was nearly significant at days 14 and 28 ($P = .07$) (table 1).

Table 1. Factors associated with time to sulfadoxine-pyrimethamine treatment failure in pregnant women in western Kenya during 2002–2005, by follow-up time point: multivariate survival analysis.

Characteristic	Hazard ratio (95% confidence interval)					
	Day 3	Day 7	Day 14 (model 1)	Day 14 (model 2)	Day 28 (model 1)	Day 28 (model 2)
Age <20 years	2.80 (0.87–9.05)[a]	**3.36** (1.48–7.63)	2.02 (0.94–4.34)[a]	**2.23** (1.19–4.19)	1.14 (0.73–1.78)	1.28 (0.84–1.93)
Primigravidae	1.62 (0.52–5.09)	0.90 (0.42–1.91)	1.31 (0.64–2.69)	1.32 (0.72–2.40)	**2.50** (1.60–3.91)	**2.24** (1.48–3.39)
Residence						
Kisumu	Reference	Reference	Reference	Reference	Reference	Reference
Bondo	0.80 (0.24–2.66)	0.97 (0.43–2.20)	1.81 (0.84–3.89)	1.41 (0.76–2.62)	**2.32** (1.47- 3.68)	**1.96** (1.29–2.99)
Siaya	1.56 (0.56–4.35)	1.17 (0.55–2.47)	1.32 (0.62–2.78)	1.45 (0.80–2.62)	**1.87** (1.21–2.89)	**1.92** (1.29–2.86)
Received an insecticide-treated net	0.70 (0.29–1.69)	0.93 (0.49–1.78)	0.85 (0.45–1.62)	0.82 (0.50–1.36)	**0.51** (0.35–0.74)	**0.56** (0.40–0.78)
HIV infected	1.97 (0.77–5.07)	**1.97** (1.01–3.85)	1.48 (0.77–2.85)	1.63 (0.97–2.76)[a]	1.36 (0.92–1.99)	1.38 (0.97–1.96)[a]
High parasite density	1.05 (0.44–2.51)	**2.10** (1.12–3.93)	**2.74** (1.52–4.93)	**1.82** (1.12–2.94)	**1.82** (1.28–2.57)	**1.48** (1.07–2.04)
Intervention						
Placebo	Reference	Reference	NI	Reference	NI	Reference
Folate						
0.4-mg dose	0.94 (0.27–3.28)	1.02 (0.46–2.27)		1.09 (0.58–2.04)		0.90 (0.60–1.34)
5-mg dose	2.63 (0.93–7.46)[a]	1.72 (0.84–3.54)		**2.01** (1.15–3.51)		**1.66** (1.16–2.38)
Folate level >15.4 ng/mL						
At day 0	**6.35** (1.66–24.28)	**5.30** (1.92–14.59)	2.59 (0.74–9.04)	**4.08** (1.67–10.00)	1.84 (0.77–4.44)	**2.73** (1.33–5.62)
At day 7	NI	NI	**2.00** (1.11–3.58)	NI	**1.86** (1.31–2.65)	NI

NOTE. Hazard ratios that are significant are in boldface type. Anemia and rainy season are not included in the table because they were not significant in any multivariate model. Factors that were examined but that were not found to be associated with treatment failure were trimester of enrollment, Luo ethnicity, education level, indicators of socioeconomic status, axillary temperature at enrollment, documented fever, fever during the week preceding enrollment, low body mass index, and reported use of an insecticide-treated net before enrollment (data not shown). Model 1 used the folate level at day 7; model 2 used the treatment group. NI, not included.

[a] $P = .05–.10$ (by Cox proportional hazards regression analysis).

There was no association between folate level and SP dose (in milligrams per kilogram of body weight) received, presence of sickle-cell trait, and season at enrollment. Maternal anemia was associated with treatment failure in the univariate but not in the multivariate analysis.

The most important factor(s) for treatment failure were as follows: day 3—high folate level at enrollment ($P = .007$); day 7—young age ($P = .004$), high parasite density ($P = .02$), and high folate level at enrollment ($P = .013$); day 14—either high parasite density ($P < .001$) and high folate level at day 7 ($P = .02$), in model 1, or young age ($P = .01$), high parasite density ($P = .02$), high-dose folate supplementation ($P = .01$), and high folate level at enrollment ($P = .002$), in model 2; day 28—either primigravidae ($P < .001$), enrollment at a rural site (Bondo, $P < .001$; Siaya, $P = .005$), receipt of an ITN ($P < .001$), high parasite density ($P < .001$), and high folate level at day 7 ($P < .001$), in model 1, or primigravidae ($P < .001$), enrollment at a rural site (Bondo, $P = .002$, Siaya, $P = .001$), receipt of an ITN ($P < .001$), high parasite density ($P = .02$), high-dose folate supplementation ($P = .006$), and high folate level at enrollment ($P = .006$), in model 2.

Discussion. We observed high treatment-failure rates for SP administered to pregnant women. Young age and high initial parasite densities were risk factors for treatment failure, as has been reported elsewhere [7]. High physiological folate levels at enrollment, high-dose (5 mg/day) folate supplementation, and high folate levels at day 7 were the most consistent risk factors across all time points, and these results were independent of age and parasite density at enrollment. The most likely explanation is that high folate levels counteract the effect that SP has on the malaria parasite [2].

Low folate levels (<3 ng/mL) were uncommon (3.3%), but the presence of malaria parasites may mask true folate deficiency [10]. Nutritional folate deficiency may be uncommon in this area, as it has been reported to be in other regions [11, 12]. However, folate levels are affected by the type of test used, which makes direct interstudy comparisons difficult [13]. We used reference folate levels from healthy American adults [6] because no reference folate values for pregnant or nonpregnant African populations are available.

The effect that high-dose folate supplementation has on SP treatment failure has been documented [2], whereas lower doses

of 0.4–1.5 mg daily have been reported not to increase the risk of treatment failure [2, 14]. Thus, there may be a narrow therapeutic window for folate supplementation in women who also are being treated with SP. Folate is important for both the development of the fetus and maternal health, but a high-folate diet or excessive folate supplementation may put the mother at risk for SP treatment failure. It is noteworthy that a very high folate level did not always result in treatment failure, indicating that other factors are also important.

We did not determine CD4 counts, and thus we cannot determine the effect that HIV immunosuppression might have on treatment responses. The hazard ratio of HIV infection was significant at day 7 and was almost significant at days 14 and 28. It is possible that our sample size was too small to allow a stronger conclusion.

We did not use genotyping to discriminate between reinfection and recrudescence. This may explain why both receipt of an ITN and location of residence are associated with treatment outcome at day 28; nevertheless, the correlation with folate level and folate supplementation was also observed at day 14, which is not as affected by new infections. We did not obtain pharmacokinetic data and did not identify molecular markers for SP-resistant genes. In a pharmacokinetic study, for sulfadoxine, the area under the curve was 43% lower in pregnant women than in nonpregnant women, whereas it was 17% lower for pyrimethamine [15]; this finding may have consequences for the period of synergy between sulfadoxine and pyrimethamine.

In the present study, only parasitemic women were included; however, the described interaction between folate and SP efficacy also has important implications for the use of SP as IPT, which targets women regardless of whether they are parasitemic. The high folate levels achieved with the 5-mg/day dose not only reduce the ability of SP to clear existing infections (the treatment effect) but, with sustained use of high-dose folate supplementation, also will reduce the efficacy of SP in preventing new infections during the posttreatment prophylactic period.

The present study's finding that folate levels markedly affect SP efficacy provides further support for our previous observation that high-dose (5 mg/day) folate supplementation should clearly be avoided in pregnant women who are receiving SP for the treatment or prevention of malaria. The present study's findings may also be of relevance to the use of other common folic-acid antagonists in the treatment of pregnant women, such as the use of sulfamethoxazole-trimethoprim (cotrimoxazole) to prevent opportunistic infections and malaria in HIV-infected women. Both the present study and a previous study in The Gambia [2, 14] suggest that the commonly recommended folate-supplementation dose of 0.4 mg/day or even doses up to 1.5 mg/day do not affect SP efficacy and can be used safely. The other factors found to be associated with SP treatment failure—age, parasite density, and physiological folate levels—are not easily modified, highlighting the importance of ITN use in this vulnerable group.

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