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Impact of Sulfadoxine-Pyrimethamine Resistance on Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and Preventing Low Birth Weight

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Abstract

Background—Owing to increasing sulfadoxine-pyrimethamine (SP) resistance in sub-Saharan Africa, monitoring the effectiveness of intermittent preventive therapy in pregnancy (IPTp) with SP is crucial.

Methods—Between 2009 and 2013, both the efficacy of IPTp-SP at clearing existing peripheral malaria infections and the effectiveness of IPTp-SP at reducing low birth weight (LBW) were

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assessed among human immunodeficiency virus–uninfected participants in 8 sites in 6 countries. Sites were classified as high, medium, or low resistance after measuring parasite mutations conferring SP resistance. An individual-level prospective pooled analysis was conducted.

Results—Among 1222 parasitemic pregnant women, overall polymerase chain reaction–uncorrected and –corrected failure rates by day 42 were 21.3% and 10.0%, respectively (39.7% and 21.1% in high-resistance areas; 4.9% and 1.1% in low-resistance areas). Median time to recurrence decreased with increasing prevalence of *Pfdhps*-K540E. Among 6099 women at delivery, IPTp-SP was associated with a 22% reduction in the risk of LBW (prevalence ratio [PR], 0.78; 95% confidence interval [CI], .69–.88; $P < .001$). This association was not modified by insecticide-treated net use or gravidity, and remained significant in areas with high SP resistance (PR, 0.81; 95% CI, .67–.97; $P = .02$).

Conclusions—The efficacy of SP to clear peripheral parasites and prevent new infections during pregnancy is compromised in areas with >90% prevalence of *Pfdhps*-K540E. Nevertheless, in these high-resistance areas, IPTp-SP use remains associated with increases in birth weight and maternal hemoglobin. The effectiveness of IPTp in eastern and southern Africa is threatened by further increases in SP resistance and reinforces the need to evaluate alternative drugs and strategies for the control of malaria in pregnancy.

Keywords

malaria in pregnancy; sulfadoxine-pyrimethamine resistance; intermittent preventive treatment; effectiveness

Each year, approximately 32 million pregnancies in Africa are at risk of *Plasmodium falciparum* (*Pf*) infection [1]. Malaria in pregnancy (MiP) is associated with severe anemia, low birth weight (LBW), and perinatal mortality, primarily during the first and second pregnancies [2].

A cornerstone of MiP prevention is intermittent preventive therapy in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) [3]. Administered as IPTp, SP both clears existing infections that consist of drug-sensitive parasites (treatment effect) and prevents incident infections (prophylactic effect) [4]. IPTp-SP reduces the risk of maternal anemia, LBW, and neonatal mortality [4,5], and the World Health Organization now recommends a dose of SP at each scheduled antenatal care (ANC) visit starting as early as possible in the second trimester [6, 7]. Currently, 33 malaria-endemic countries in Africa employ IPTp for MiP control [8,9], and SP remains the only antimalarial recommended for IPTp [6].

The effectiveness of IPTp in eastern and southern Africa is threatened by parasite resistance to SP, conferred by the successive acquisition of polymorphisms in the parasite genes encoding the targets of sulphadoxine and pyrimethamine: dihydropteroate synthase (*Pfdhps*) and dihydrofolate reductase (*Pfdhfr*), respectively. To varying degrees, these mutations are prevalent throughout Africa, and their association with the failure of SP treatment in children has rendered SP unsuitable for therapy. Nevertheless, even in areas where parasites routinely harbor up to 5 *Pfdhfr* and *Pfdhps* resistance-conferring mutations (the *Pfdhfr*–*Pfdhps* “quintuple mutant” haplotype), SP has remained effective as IPTp, presumably owing to pregnant women's acquired, partial immunity [4, 7]. However, the recent

emergence of a more highly resistant “sextuple mutant” parasite in Tanzania was associated with a loss of IPTp-SP efficacy [10, 11]. Because prior parasite mutations have disseminated widely in Africa and undermined antimalarial policies [12, 13], further studies are needed to quantify the impact of molecular markers of SP resistance on the efficacy of IPTp-SP across Africa.

We conducted multicountry studies [14–19] in sub-Saharan Africa to investigate 2 effects of IPTp-SP: (1) its efficacy at clearing existing peripheral infections in asymptomatic pregnant women receiving their first dose of IPTp-SP; and (2) the effectiveness of IPTp-SP at increasing mean birth weight, reducing LBW, or improving other birth outcomes. Here we present individual-level pooled analyses of peripheral parasite clearance and birth outcomes to better quantify the impact of SP resistance on IPTp-SP effectiveness.

Methods

Study Sites and Study Period

The studies were conducted between 2009 and 2013 at 8 sites in 6 countries: 2 countries in West Africa (Mali [2 sites] and Burkina Faso [16]) and 4 in eastern and southern Africa (Zambia [19, 20], Malawi [2 sites] [15, 17, 21], Uganda [14], and Kenya [18]).

Study Design

In Vivo Module: 42-Day In Vivo Follow-up—To determine the efficacy of IPTp-SP in clearing existing peripheral *Pf* malaria infections or preventing new infections, prospective single-arm 42-day in vivo treatment efficacy studies of single-dose SP were conducted in all sites except Uganda. Eligible women were human immunodeficiency virus (HIV)–uninfected, asymptomatic women of any gravidity who were due to receive their first dose of IPTp-SP according to national guidelines, with parasitemia confirmed by microscopy. The study design was identical, except in Zambia where the study duration was limited to 35 days because national treatment guidelines recommend monthly IPTp-SP. The study was designed to detect a parasitological failure risk of 10% in primigravidae and secundigravidae (G1-G2 [paucigravidae]) and 5% in multigravidae (G3+) with 10% and 5% precision, respectively, allowing for 15% loss to follow-up. This required 162 paucigravidae and 86 multigravidae per site; however, not all sites met these requirements. Following informed consent, a brief clinical examination; axillary temperature measurement; and collection of blood by fingerprick for malaria smears, rapid diagnostic tests, and filter-paper dried blood spots (DBSs) for polymerase chain reaction (PCR) were performed at enrollment and then weekly at the antenatal clinic. IPTp-SP was administered at enrollment and at the end of follow-up. Women who were smear positive on or after day 4 were classified as failing treatment, and received rescue treatment according to national guidelines. A sample of SP from each batch in each site was assessed and met the criteria for quality assurance (content analysis and dissolution) according to the United States Pharmacopeia monograph for SP tablets [22].

Molecular Module—In the in vivo study, microscopically detected peripheral parasite recurrence within 42 days was classified as recrudescence or reinfection by comparing

alleles of parasite genes encoding merozoite surface proteins 1 and 2 and glutamate-rich protein, using standard PCR methods and genomic DNA extracted from DBSs [23].The frequency of polymorphisms conferring parasite SP resistance at each study site was estimated using a pooled second-generation sequencing approach on parasites collected prior to SP receipt [24]. For each site, genomic DNA was pooled from women at enrollment in the in vivo studies (except Uganda, for which samples from children aged 6–59 months with acute uncomplicated malaria were used), amplified across *Pfdhfr* and *Pfdhps* loci conferring SP resistance (*Pfdhfr* codons 51, 59, 108, and 164, and *Pfdhps* codons 436, 437, 540, 581, and 613), and sequenced on a Roche GS Junior instrument (454 Life Sciences, Branford, Connecticut). The returned reads were quality-filtered, aligned to reference sequences, and manually scored for polymorphisms in *Pfdhfr* and *Pfdhps* that confer SP resistance [24].

Delivery Module—To determine the impact of IPTp-SP on the prevalence of adverse maternal and newborn outcomes, facility-based crosssectional studies were conducted at the time of delivery among HIV-uninfected women of any gravidity at all sites. Following informed consent obtained at delivery, participant ANC cards and clinic records were examined for information on doses of IPTp-SP, and peripheral and placental samples (DBSs, impression smear, and/or histology) collected. Maternal hemoglobin (Hb) was measured with HemoCue (HemoCue Inc, Cypress, California); anemia was defined as Hb < 11 g/dL and moderate-to-severe anemia as Hb < 9 g/dL. Newborns were weighed using digital scales (±10 g) and gestational age was assessed by Ballard score within 12 hours of birth, except in Uganda where only the date of the last menstrual period was available. LBW was defined as birth weight <2500 g and preterm delivery as gestational age <37 weeks. Small for gestational age (SGA) was defined as birth weight for gestational age <10th percentile using an ultrasound-derived fetal size nomogram [25]. Women enrolled in the in vivo studies were excluded from the delivery surveys. Individual studies were designed to detect a 2-fold difference in the prevalence of LBW or placental malaria in women who had received ≥2 doses of IPTp-SP vs none; sample sizes varied between 507 and 1103 depending on the prevailing prevalence of the endpoint and SP use.

Ethics Statement

The protocols were approved locally in each country and by the Liverpool School of Tropical Medicine (Liverpool, United Kingdom) and the Centers for Disease Control and Prevention (Atlanta, Georgia). Written informed consent was obtained from each woman.

Data Analyses

For the in vivo module, modified intention-to-treat analysis was used. We assessed therapeutic response to treatment by the Kaplan–Meier product limit formula to calculate the cumulative risks of recurrence of asexual parasites (unadjusted and PCR adjusted for reinfections) [26] and compared failure risk across sites by gravidity group using proportional hazards regression [16].

In the delivery module, we investigated the relationship between the number of SP doses and delivery outcomes and employed propensity scores to minimize the potential for

confounding of these relationships (see Supplementary Data for further details) [27, 28]. For binary outcomes, a Poisson distribution was assumed, allowing the results to be reported as prevalence ratios (PRs) and corresponding 95% confidence intervals (CIs) for each incremental dose of IPTp-SP. For continuous variables, results were expressed as the mean difference (MD) and 95% CI for each incremental dose of IPTp-SP. Forest plots report the sample sizes, summary statistics, and PRs or MDs and 95% CIs for the raw and weighted analyses. The primary outcomes were LBW, mean birth weight, and placental parasitemia.

Finally, we used meta-regression to determine the potential for SP resistance to modify the effectiveness of IPTp, where SP resistance was expressed either as the in vivo failure rate or as the frequency of the A437G, K540E, or A581G mutations in the parasite *Pfdhps* gene, which predict the frequency of quadruple, quintuple, and sextuple *Pfdhfr–Pfdhps* mutant haplotypes, respectively.

Results

In Vivo Module

Between July 2009 and May 2012, a total of 1222 asymptomatic parasitemic pregnant women were enrolled in 6 in vivo studies (7 sites) in 5 countries (Supplementary Figure 1); 1064 (87.1% [range, 66.3%–95.1%]) were followed successfully until the day of recurrence or completion of follow-up. The majority of women were paucigravidae (66.8%), with a mean gestational age of 23.5 weeks (Table 1). Use of insecticide-treated nets (ITNs) the night prior to enrollment varied from 21.7% (Zambia) to 77.1% (Mali).

Parasites from all sites except Mali and Burkina Faso harbored high frequencies (>90%) of mutations in *Pfdhfr*, except at locus 164, at which mutations were absent; in contrast, mutations in *Pfdhps* were more variable across sites, ranging from 27.5% to 100%, 0% to 100%, and 0% to 5.7% at codons 437, 540, and 581, respectively (Table 1; Supplementary Table 2). For further analyses of resistance, we classified sites based on the frequencies of *Pfdhps*-K540E mutation: high resistance (Kenya, Uganda, and both sites in Malawi) (>90% *Pfdhps*-K540E); moderate resistance (Zambia) (50%–90% *Pfdhps*-K540E); or low resistance (<50% *Pfdhps*-K540E) (Mali and Burkina Faso). By days 28 and 42, the pooled PCR-uncorrected risks of recurrence were 15.2% (95% CI, 13.3%–17.4%) and 21.3% (95% CI, 19.0%–23.8%), respectively; corresponding PCR-corrected failure risks (recrudescence) were 7.6% (95% CI, 6.2%–9.3%) and 10.0% (95% CI, 8.3%–11.9%). Recrudescence was more common among paucigravidae than multigravidae (adjusted hazard ratio, 2.7 [95% CI, 1.7–4.5]).

There was a trend toward increasing risk of treatment failure by the degree of SP resistance (Figure 1): the PCR-uncorrected risk of recurrence by day 42 was 4.9% in low-resistance sites, 21.0% in the single moderate-resistance site, and 39.7% in the high-resistance sites. PCR-corrected 42-day recrudescence rates were 1.1%, 10.7%, and 21.1% for the 3 categories, respectively (Supplementary Table 3). Therefore, relative to low resistance sites, the risks of treatment failure were 9.6- and 23.0-fold higher in moderate- and high-resistance sites, respectively (Supplementary Table 3). The median time to recurrence decreased with increasing prevalence of *Pfdhps*-K540E and ranged from 42 days in Mali to 18 days in

Machinga, Malawi, reflecting differences in reinfection rather than time to recrudescence (Figure 2).

Delivery Module

Between July 2009 and April 2013, 6099 delivering women were enrolled between 7 studies at 8 sites in 6 countries; 45.1% of them were paucigravidae (Table 1). Approximately half (49.4%) had received exactly 2 doses of antenatal SP; 9.4%, 23.8%, and 17.4% had received zero, 1, or ≥3 doses, respectively. Most women who received zero doses were from Mali (46.3%) or Burkina Faso (31.1%); most women who received ≥3 doses were from Kenya (47.3%) or Blantyre, Malawi (35.5%). ITN use the night prior to enrollment varied from 35.6% (Machinga, Malawi) to 95.6% (Kenya).

Overall, 9.6% of infants were LBW (Table 2). Overall, each dose of antenatal SP was associated with a 22% reduction in the risk of LBW (PR, 0.78 [95% CI, .69–.88]; $P < .001$; Figure 3). After stratification into 3 resistance strata, the effect of each dose of SP was similar in low-resistance (PR, 0.75 [95% CI, .64–.88]; $P = .001$), moderate-resistance (PR, 0.73 [95% CI, .51–1.03]; $P = .07$), and high-resistance areas (PR, 0.81 [95% CI, .67–.97]; $P = .02$) (Figure 3). Furthermore, meta-regression indicated that the effect of SP on the risk of LBW was not modified by resistance (Figure 4; Supplementary Figure 2). The effect of IPTp-SP did not differ significantly by ITN use (P value interaction term = .93) or gravidity (P value interaction term = .38) (Figure 3; Supplementary Table 4).

Overall, each incremental dose of IPTp-SP was associated with a mean increase of 49 g (95% CI, 22–76 g; $P < .001$) in birth weight (Table 2; Figure 3). This association varied between SP resistance strata: Each dose of SP was associated with 111-g (95% CI, 71–151 g; $P < .001$) and 56-g (95% CI, 18–94 g; $P = .004$) increases in the moderate- and high-resistance strata, respectively (Figure 3), but not in the low-resistance strata (mean increase, 20 g [95% CI, −20 to 59 g]; $P = .33$). Overall, there was no evidence for a linear relationship between the effect on mean birth weight and resistance (Figure 3; Supplementary Table 5). Additionally, there was no evidence of effect modification by ITN use ($P = .38$) or gravidity ($P = .22$) (Supplementary Table 4).

Each dose of IPTp-SP was associated with an increase in maternal Hb (MD, 0.26 g/dL [95% CI, .16–.36 g/dL]; $P < .001$) and less moderate-to-severe anemia (Hb < 9 g/dL; PR, 0.85 [95% CI, .79–.92]; $P = .001$); these associations were similar across the gravidity groups, among ITN users and nonusers (Supplementary Table 4), and across resistance strata (Supplementary Figures 9, 11, 21, and 23). IPTp-SP was also associated with a 23% lower risk of placental infection detected by impression smear (PR, 0.77 [95% CI, .63–.94]; $P = .01$), and a nonsignificant reduction in peripheral malaria (PR, 0.84 [95% CI, .69–1.02]; $P = .07$; Figure 5), and this was not modified by resistance levels (Table 2; Supplementary Table 5).

Discussion

Our analyses show that SP resistance mutations compromise the clearance of parasites in pregnant women. Compared with the Malian sites with the lowest prevalence of resistance

markers, the risk of SP failure was 23- to 40-fold higher in high-resistance sites in eastern Africa. In addition, high-resistance sites had a greater number of reinfections and a shorter time to reinfection during the 42-day follow-up, suggesting that SP resistance attenuates the effectiveness of SP to prevent new infections. Despite this, our findings indicate that, even in areas with high SP resistance, IPTp-SP is associated with beneficial impacts on birth weight and maternal Hb.

In areas with high SP resistance, which include much of eastern and southern Africa, SP was associated with a 19% reduction in LBW and a 15% reduction in maternal anemia at delivery. The association with reduced LBW was present in multigravidae as well as paucigravidae, consistent with a previous meta-analysis of clinical trials that compared 2-dose IPTp-SP with more frequent doses [7]. It is surprising that parasite SP resistance, as expressed both by parasite molecular markers and by in vivo efficacy of parasite clearance, did not affect the associations between IPTp-SP and birth outcomes: Irrespective of resistance, IPTp-SP is associated with a similar reduction in LBW and increase in maternal Hb. This finding may be explained by several factors. There may be a difference in the degree to which resistance affects the ability of IPTp-SP to treat or prevent infections vs the ability to prevent malaria-associated morbidity if partial suppression of parasitemia may be sufficient to reduce some of the adverse effect on fetal growth and maternal anemia. SP may also have secondary, off-target effects on bacterial or fungal infections that promote fetal growth and maternal health [31]. The impact of SP may also be affected by attributable fraction of malaria to LBW and thus malaria transmission intensity and the degree of protective immunity among pregnant women. Last, malaria is only one of many causes of LBW, and the relationship between population levels of parasite resistance and effectiveness of SP on LBW is likely to be affected by the prevalence of other risk factors, thus requiring larger sample sizes to show a trend.

It has been suggested that further increases in the level of resistance may result in a more definitive loss of the effectiveness of IPTp-SP. Two recent studies from northeastern Tanzania suggested that SP failed to inhibit growth of parasites with the sextuple haplotype [10, 11]; that is, parasites that harbor the quintuple mutant *Pfdhfr–Pfdhps* haplotype along with the additional *Pfdhps*-A581G mutation. These parasites are known to be associated with increased treatment failure in patients with acute malaria [32]. Women harboring these highly resistant parasites had higher parasite densities [33], more placental inflammation [10], and newborns with lower birth weights than women infected with parasites with the quintuple mutation who received IPTp-SP [11]. Taken together, these data suggest that the additional *Pfdhps*-A581G mutation, which confers a greater level of resistance than the quintuple mutant, should be included in the molecular surveillance of SP resistance to guide IPTp-SP implementation.

Several limitations must be noted. Among women enrolled at delivery, those who received fewer doses of IPTp-SP may have also received less antenatal care in general, and frequency of ANC visits is associated with birth weight [34]. However, the number of ANC visits was not collected as part of our surveys, precluding adjustment. Also, although all sites used the documented number of SP courses as the primary source of exposure data, not all sites verified with the participant whether she had actually taken these courses. In Zambia and

Uganda, the policy to use high-dose folic acid (5 mg) during ANC may have resulted in an overestimation of the SP treatment failure rates [35]. Furthermore, loss to follow-up in the Zambia and Machinga, Malawi sites was 33.7% and 38.8%, respectively, but baseline characteristics were similar among completers and noncompleters. The prevalence of LBW and preterm birth was lower and the mean gestational age at delivery higher (41.6 weeks) at the Machinga, Malawi site compared with the other sites. Sensitivity analysis to assess the effect of removing the Machinga study showed no change in overall conclusion (data not shown). The bimodal distribution of SP resistance in our studies limited our ability for trend analysis: 7 of the 8 sites clustered at the 2 extremes of the resistance spectrum, with only a single study representative of moderate resistance, consistent with known partitioning of mutant *Pfdhps* haplotypes across sub-Saharan Africa. Furthermore, none of the studies were conducted in areas such as northern Tanzania, which has a much higher prevalence (>50%) of parasites harboring the sextuple mutant haplotype. Finally, we were unable to assess the correlation between malaria transmission intensity and IPTp-SP effectiveness in this limited sample of studies.

This is the first prospective multicountry effort to link population-level parasite SP resistance to the efficacy and effectiveness of IPTp-SP. Our pooled analysis suggests that, with increasing resistance, there is a progressively diminished efficacy of IPTp-SP in clearing existing infections and a shortening of the posttreatment prophylaxis period. Although concerning, our analysis also suggests that IPTp-SP remains associated with improvements in birth weight and maternal hemoglobin level in areas where the prevalence of the *Pfdhps*-K540E mutation was >90%, but where the additional *Pfdhps*-A581G mutation was still rare. Although parasites harboring the *Pfdhfr–Pfdhps* quintuple mutant are now highly prevalent in areas across eastern and southern Africa, the sextuple mutant parasites are still uncommon, but are spreading [36–38]. Our data underline the need for studying alternative drugs for IPTp and alternative strategies to IPTp, such as intermittent screening and treatment during pregnancy. Our data also suggest that molecular surveillance of SP resistance could be a useful tool to guide IPTp-SP implementation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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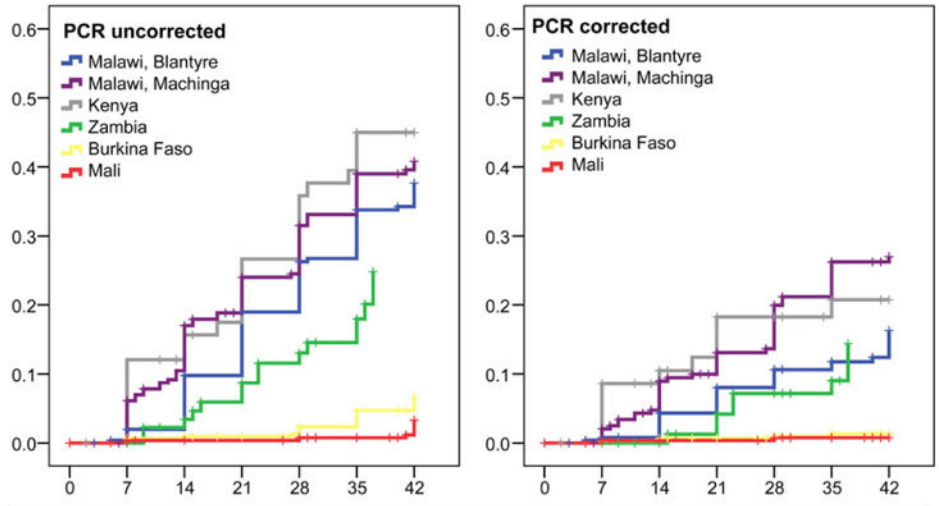

Country	Failure rate (95% CI)	HR (95% CI)	Number of women at risk					
			7	14	21	28	35	42
PCR uncorrected								
Malawi, Blantyre	37.6% (31.6%, 44.3%)	16.6 (8.0; 34.5)	253	226	206	178	156	139
Malawi, Machinga	40.4% (34.1%, 47.4%)	16.1 (7.7; 33.4)	246	211	178	153	125	107
Kenya	44.9% (33.0%, 58.9%)	20.4 (9.2; 45.4)	58	51	46	40	34	29
Zambia	21.0% (13.1%, 32.7%)	7.1 (3.0; 17.0)	92	85	68	60	50	n/a
Burkina Faso	6.5% (4.2%, 9.9%)	2.0 (.9; 4.5)	307	304	298	292	286	276
Mali	3.1% (1.6%, 6.2%)	ref	266	259	258	258	252	251
PCR corrected								
Malawi, Blantyre	16.2% (11.7%, 22.3%)	23.5 (5.4; 104.7)	251	225	206	178	156	139
Malawi, Machinga	26.5% (20.7%, 33.6%)	40.0 (9.1; 176.1)	246	211	178	153	125	107
Kenya	20.6% (11.9%, 34.3%)	33.2 (6.9; 159.2)	58	51	46	40	34	29
Zambia	10.7% (5.2%, 21.4%)	13.7 (2.7; 70.3)	92	85	68	60	50	n/a
Burkina Faso	1.3% (.5%, 3.5%)	1.7 (.3; 10.1)	307	304	298	292	286	276
Mali	0.8% (.2%, 3.0%)	ref	266	259	258	258	252	251

Figure 1.

Parasitological failure rates among asymptomatic, parasitemic pregnant women receiving their first dose of intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine (SP). The protocol in Zambia administered SP at 1 month and thus only contributes data through day 35 inclusive in this analysis. The therapeutic response was estimated using the Kaplan–Meier product limit formula [26]. In the polymerase chain reaction (PCR)–uncorrected analysis, recurrences were treated as treatment failures, and all other events (eg, withdrawal or protocol deviations) resulted in censoring at the time of that event, or at the time of their last follow-up visit in case of loss to follow-up. A similar strategy was used for the PCR-corrected analysis, except that patients with new *Plasmodium falciparum* infections (reinfections) were censored at the time of parasite reappearance [26]. To compare the failure risk across sites, hazard ratios (HRs) were estimated using Cox regression adjusting for gravidity and net use with a shared frailty component to account for the individual heterogeneity of the sites. Abbreviations: CI, confidence interval; ref, reference.

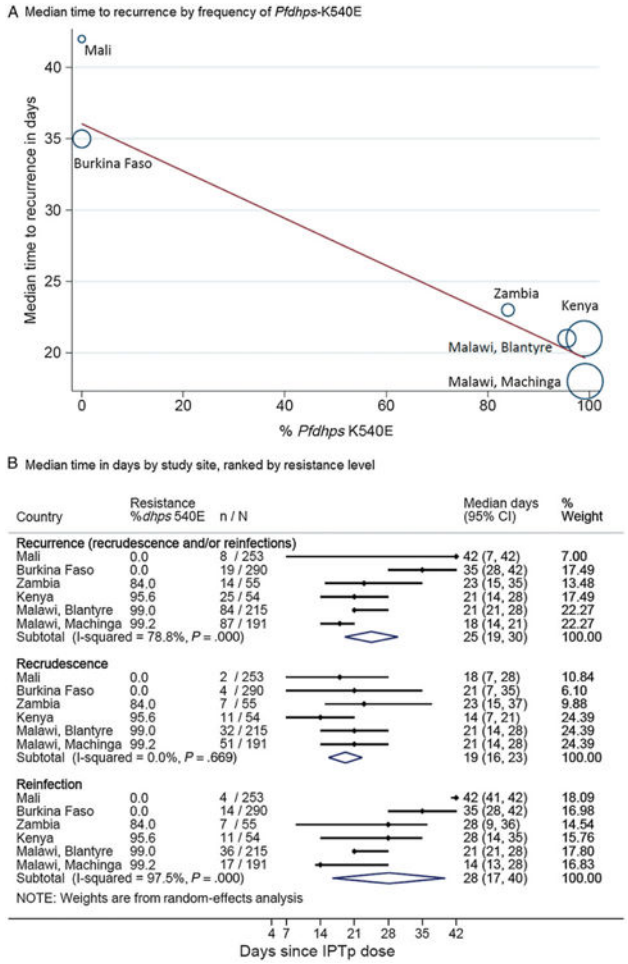

Figure 2.

Median time to recurrence, recrudescence or reinfection of *Plasmodium falciparum* among women participating in the intermittent preventive therapy in pregnancy in vivo module. A, Median time to recurrence by frequency of pyrimethamine: dihydropteroate synthase (*Pfdhps*)-K540E. The circles represent the estimated median time to recurrence of *P. falciparum* infection at each site, sized according to the precision of each estimate (the inverse of its within-study variance, σ^2). The line represents the linear prediction of the relationship between median time to recurrence and prevalence of *Pfdhps*-K540E. *B*, Median time in days by study site, ranked by resistance level. Abbreviations: CI, confidence interval; IPTp, intermittent preventive therapy in pregnancy.

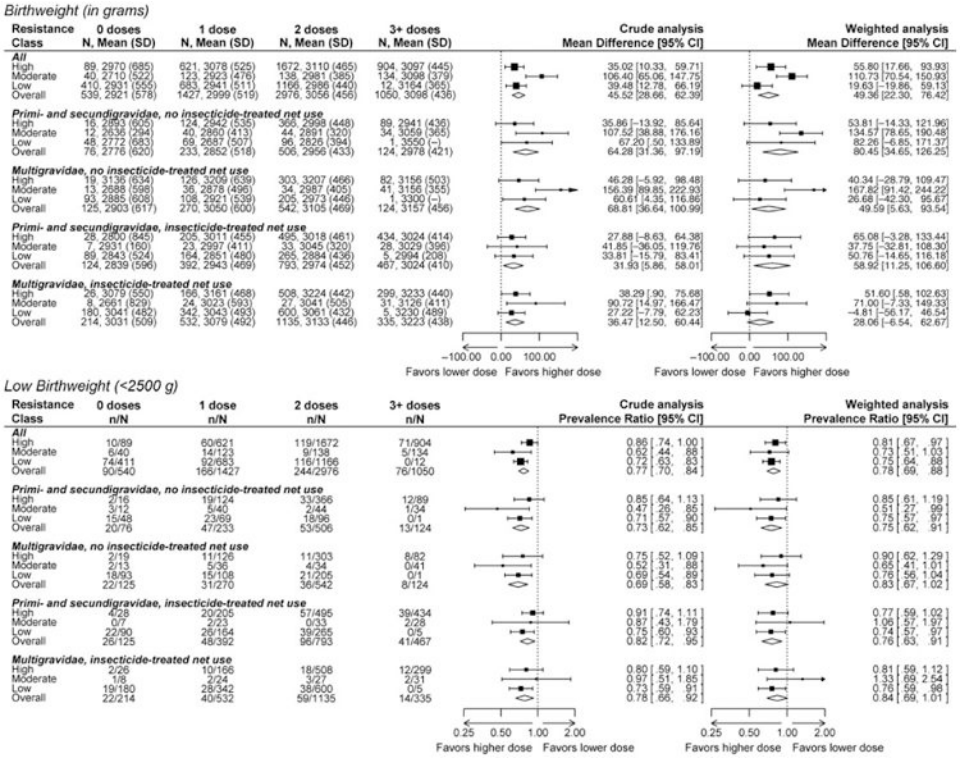

Figure 3.

Associations between each incremental dose of intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine (SP) and mean birth weight and the prevalence of low birth weight, stratified by SP resistance, gravidity, and insecticide-treated net use. Abbreviations: CI, confidence interval; SD, standard deviation.

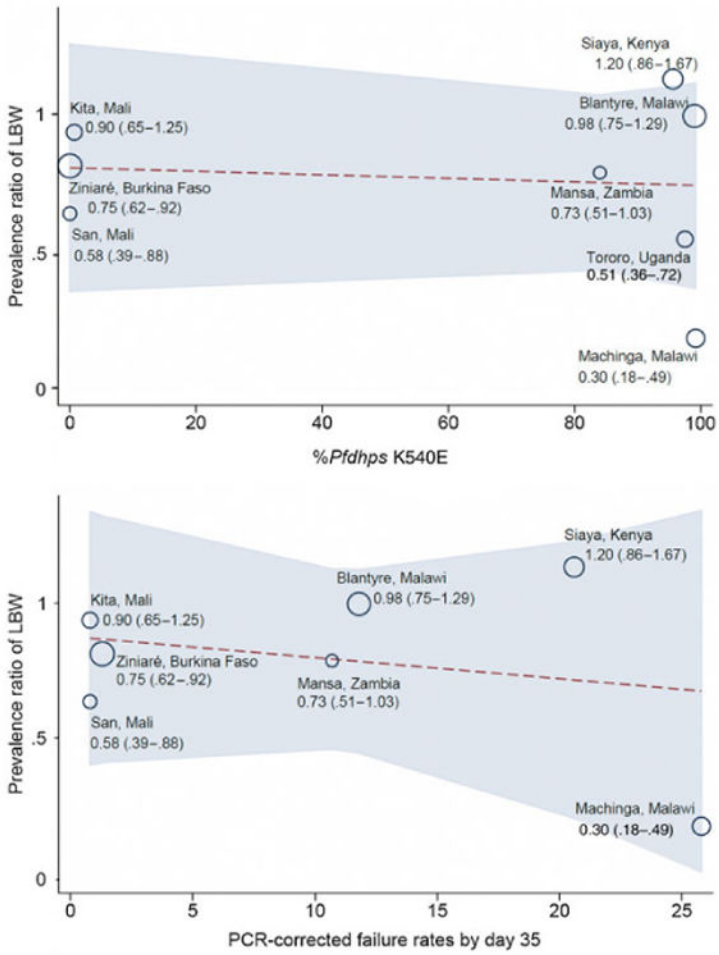

Figure 4.

Meta-regression bubble plot of the prevalence of low birth weight (LBW) by the frequency of the pyrimethamine: dihydropteroate synthase (*Pfdhps*)-K540E mutation (8 sites, top panel) and in vivo failure rates in asymptomatic pregnant women (7 sites, bottom panel). The Tororo site in Uganda did not conduct an in vivo study and is missing from the bottom panel. The circles represent the estimated prevalence ratio of LBW, sized according to the sample size from each site. The gray area represents the 95% confidence interval, and the dotted line is the linear prediction. In the bottom panel, the prevalence ratio of LBW was regressed against the polymerase chain reaction (PCR)–corrected failure rate by day 35, as this was the latest standardized day available from all countries (in Zambia, the study was ended on day 35 rather than on day 42). The numbers under each site represent the prevalence ratio and its confidence limit.

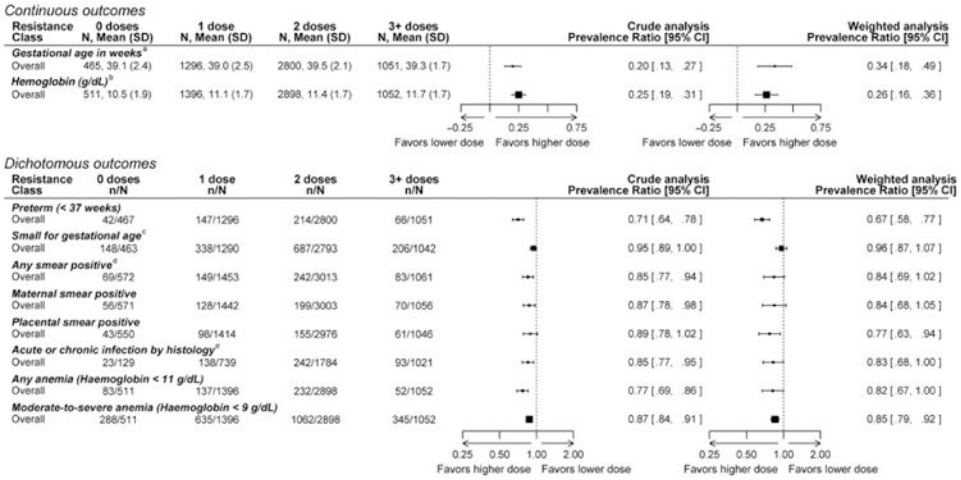

Figure 5.

Associations between each incremental dose of intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine and all secondary outcomes. [a]Gestational age, determined by Ballard score or where unavailable by last menstrual period, was missing from a variable number of individuals at each site; data completeness varied from 76% to 100%. [b]Hemoglobin level (g/dL), assessed by HemoCue at delivery. [c]Small for gestational age defined as birth weight for gestational age <10th percentile using an ultrasound-derived fetal size nomogram for a sub-Saharan African population [25]. [d]Malaria infection defined as either a positive peripheral smear (maternal malaria) or a positive placental impression smear (composite endpoint). [e]Active placental infection (acute or chronic) by placental histology, classified on a 5-point scale as described by Rogerson et al [30]. Placental histology was not done in the 3 sites in West Africa (low resistance strata). Abbreviations: CI, confidence interval; SD, standard deviation.

Table 1

Baseline Characteristics of Pregnant Women Enrolled Into the In Vivo and Delivery Modules

Module	San and Kita, Mali [16]	Zinairé, Burkina Faso [16]	Mansa, Zambia [19]	Blantyre, Malawi [17]	Tororo, Uganda [14]	Machinga, Malawi [15]	Siaya, Kenya [18]	Total
In vivo	(n = 266)	(n = 307)	(n = 92)	(n = 253)	*a*	(n = 246)	(n = 58)	(N =1222)
Primi-/secundigravidae	183 (68.8)	199 (64.8)	67 (72.8)	172 (68.0)		150 (61.0)	45 (77.6)	816 (66.8)
Gravidity missing	0 (0.0)	0 (0.0)	11 (12.0)	0 (0.0)		1 (0.4)	0 (0.0)	12 (1.0)
Age, y, mean (SD)	21.2 (5.1)	23.7 (5.5)	20.7 (5.5)	22.1 (5.0)		21.7 (5.0)	19.8 (4.8)	22.1 (5.3)
Gestational age, wk, mean (SD)	25.3 (3.2)	25.2 (3.1)	21.4 (2.9)	22.3 (3.3)		21.0 (3.9)	23.7 (3.7)	23.5 (3.8)
Hemoglobin, g/dL, mean (SD)	9.7 (1.5)	10.1 (1.4)	10.1 (1.8)	10.5 (1.5)		9.8 (1.3)	10.3 (1.9)	10.0 (1.5)
Knows date of LMP	44 (16.5)	59 (19.2)	80 (87.0)	162 (64.0)		206 (83.7)	56 (96.6)	607 (49.7)
Uses a bed net	205 (77.1)	183 (59.6)	20 (21.7)	62 (24.5)		88 (35.8)	44 (75.9)	602 (49.3)
Parasite density, geometric mean (95% CI)	750 (626–897)	671 (577–780)	768 (557–1059)	19371 (16599–22606)		269 (235–309)	1101 (809–1498)	1460 (1271–1678)
Pfdhps-K540E, %	0.7	0	84.0	100	97.5	99.2	95.6	
Pfdhps-A581G, %	0	0	0	1.6	0.2	1.5	5.7	
Delivery	(n = 1047)	(n = 1311)	(n = 435)	(n = 1141)	(n = 565)	(n = 710)	(n = 890)	(n = 6099)
Primi-/secundigravidae	436 (41.6)	334 (25.5)	218 (50.1)	612 (53.6)	298 (52.7)	303 (42.7)	550 (61.8)	2751 (45.1)
Gravidity missing	1 (0.1)	0 (0.0)	3 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.3)	7 (0.1)
Age, y, mean (SD)	25.2 (7.0)	25.4 (6.0)	24.3 (6.2)	24.2 (5.5)	24.6 (6.1)	24.1 (6.1)	22.7 (5.6)	24.4 (6.1)
Slept under ITN previous night	899 (85.9)	820 (62.5)	181 (41.6)	702 (61.5)	374 (66.2)	253 (35.6)	851 (95.6)	4080 (66.9)
House sprayed with insecticide (IRS)	0 (0.0)	0 (0.0)	149 (34.3)	6 (0.5)	15 (2.7)	0 (0.0)	24 (2.7)	194 (3.2)
IPTp doses								
0	265 (25.3)	178 (13.6)	40 (9.2)	23 (2.0)	32 (5.7)	14 (2.0)	20 (2.2)	572 (9.4)
1	302 (28.8)	401 (30.6)	123 (28.3)	159 (13.9)	203 (35.9)	142 (20.0)	123 (13.8)	1453 (23.8)
2	470 (44.9)	729 (55.6)	138 (31.7)	582 (51.0)	320 (56.6)	529 (74.5)	245 (27.5)	3013 (49.4)
3+	10 (1.0)	3 (0.2)	134 (30.8)	377 (33.0)	10 (1.8)	25 (3.5)	502 (56.4)	1061 (17.4)

Module	San and Kita, Mali [16]	Zinairé, Burkina Faso [16]	Mansa, Zambia [19]	Blantyre, Malawi [17]	Tororo, Uganda [14]	Machinga, Malawi [15]	Siaya, Kenya [18]	Total
Dose of folate administered, mg	0.40	0.40		0.25		0.40	0.40	
Plasmodium falciparum parasite rate, year 2010[b]	0.530	0.638	0.206	0.394	0.382	0.482	0.565	

Data are presented as No. (%) unless otherwise specified.

Abbreviations: CI, confidence interval; IPTp, intermittent preventive treatment in pregnancy; IRS, indoor residual spraying; ITN, insecticide-treated net; LMP, last menstrual period; *Pfdhps*, pyrimethamine: dihydropteroate synthase; SD, standard deviation.

[a] Not conducted.

[b] Malaria Atlas project [29].

Table 2

Delivery Module Outcomes

Outcome	San and Kita, Mali	Ziniaré, Burkina Faso	Mansa, Zambia	Blantyre, Malawi	Tororo, Uganda	Machinga, Malawi	Siaya, Kenya	Total
Birthweight, g								
no./No.[a]	983/1047	1288/1311	435/435	1141/1141	565/565	710/710	870/890	5992/6099
Mean (SD)	3030 (489)	2913 (475)	2976 (437)	2982 (450)	3082 (535)	3240 (444)	3140 (470)	3037 (483)
LBW								
no./No.[a] (%)	99/983 (10.1)	182/1288 (14.1)	34/435 (7.8)	112/1141 (9.8)	56/565 (9.9)	23/710 (3.2)	69/870 (7.9)	576/5993 (9.6)
Mean gestational age, wk[b]								
Mean (SD)	39.8 (1.5)	38.6 (1.5)	38.2 (2.0)	38.7 (1.7)	38.5 (3.6)	41.6 (1.4)	39.5 (1.8)	39.3 (2.1)
Preterm								
no./No.[a] (%)	13/971 (1.3)	70/995 (7.0)	61/429 (14.2)	146/1128 (12.9)	120/496 (24.2)	9/710 (1.3)	50/885 (5.6)	469/5614 (8.4)
Small for gestational age								
no./No.[a] (%)	331/968 (34.2)	293/992 (29.5)	76/428 (17.8)	249/1128 (22.1)	82/496 (16.5)	165/710 (23.2)	183/866 (24.7)	1379/5588 (24.7)
Any smear positive[c]								
no./No.[a] (%)	118/1043 (11.3)	61/1309 (4.7)	28/435 (6.4)	56/1138 (4.9)	114/565 (20.2)	55/710 (7.8)	90/883 (10.2)	522/6083 (8.6)
Maternal smear positive								
no./No.[a] (%)	100/1041 (9.6)	56/1301 (4.3)	21/435 (4.8)	52/1137 (4.6)	108/565 (19.1)	37/710 (5.2)	79/883 (9.0)	453/6072 (7.5)
Placental smear positive								
no./No.[a] (%)	80/1029 (7.8)	27/1246 (2.2)	19/435 (4.4)	12/1138 (2.2)	99/565 (17.5)	39/710 (5.5)	81/863 (9.4)	357/5986 (6.0)
Acute or chronic infection by histology[d]								
no./No.[a] (%)	N/A	N/A	50/435 (11.5)	153/1110 (13.8)	166/565 (29.4)	62/709 (8.7)	65/854 (7.6)	496/3673 (13.5)
Hb, g/dL[e]								
Mean (SD)	10.6 (1.9)	11.4 (1.6)	11.2 (1.5)	11.9 (1.6)	11.1 (1.8)	11.0 (1.8)	11.5 (1.9)	11.3 (1.8)
Anemia								
no./No.[a] (%)	463/854 (54.2)	487/1278 (38.1)	170/434 (39.2)	325/1129 (28.8)	247/565 (43.7)	321/710 (45.2)	317/887 (35.7)	2330/5857 (39.8)

Outcome	San and Kita, Mali	Ziniaré, Burkina Faso	Mansa, Zambia	Blantyre, Malawi	Tororo, Uganda	Machinga, Malawi	Siaya, Kenya	Total
Moderate-to-severe anemia								
no./No.[a] (%)	141/854 (16.5)	86/1278 (6.7)	22/434 (5.1)	32/1129 (2.8)	57/565 (10.1)	91/710 (12.8)	75/887 (8.5)	504/5857 (8.6)

Low birth weight defined as <2500 g; preterm as <37 weeks; anemia as Hb < 11 g/dL; moderate-to-severe anemia as Hb <9 g/dL; small for gestational age as birth weight for gestational age <10th percentile using an ultrasound-derived fetal size nomogram for a sub-Saharan African population [25].

Abbreviations: Hb, hemoglobin; LBW, low birth weight; N/A, not applicable; SD, standard deviation.

[a]No. with outcome/total No. contributing.

[b]Gestational age, determined by Ballard score or where unavailable by last menstrual period, was missing from a variable number of individuals at each site; data completeness varied from 76% to 100%.

[c]Composite of maternal and placental impression smears.

[d]Active infection by placental histology, classified on a 5-point scale as described by Rogerson et al [30].

[e]Hemoglobin data were missing from a variable number of individuals at each site; data completeness ranged from 96% to 100% at each site, with the exception of Mali where 82% of participants had Hb data available.