Sulfadoxine-Pyrimethamine, Chlorproguanil-Dapsone, or Chloroquine for the Treatment of Plasmodium vivax Malaria in Afghanistan and Pakistan

A Randomized Controlled Trial

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Context In areas where Plasmodium falciparum and Plasmodium vivax coexist and treatments for the 2 species differ, misdiagnosis can lead to poor outcomes in either disease. A unified therapy effective against both species would reduce reliance on species-specific diagnosis, which in many areas is difficult to maintain. The antifolates are an important and affordable antimalarial class to which it is often assumed P. vivax malaria is intrinsically resistant.

Objective To test the relative efficacy and safety of 2 antifolate drugs against P. vivax malaria and compare each with chloroquine.

Design, Setting, and Patients An open-label randomized controlled trial comparing chloroquine, sulfadoxine-pyrimethamine, and chlorproguanil-dapsone for the treatment of P. vivax malaria was conducted in eastern Afghanistan and northwestern Pakistan, areas in which P. vivax malaria predominates. A total of 20,410 patients older than 3 years were screened; 767 patients (315 in Pakistan and 452 in Afghanistan) with confirmed P. vivax malaria were enrolled and followed up daily for 4 days, then weekly for 28 days, between March 2004 and June 2006.

Main Outcome Measures Complete clearance of parasites with no recrudescence by day 14. Secondary outcomes included being parasite-free by day 28, clinical failure, and anemia.

Results By day 14, only 1 patient in the sulfadoxine-pyrimethamine group had parasitemia. By day 28, failure rates were found in 2 of 153 patients (1.3%) in the chloroquine group, 5 of 290 patients (1.7%) in the sulfadoxine-pyrimethamine group, and 27 of 272 patients (9.9%) in the chlorproguanil-dapsone group. Chlorproguanil-dapsone was less effective than sulfadoxine-pyrimethamine (adjusted odds ratio [OR], 6.4; 95% confidence interval [CI], 2.4–17.0; P < .001) and chloroquine (adjusted OR, 8.4; 95% CI, 2.0–36.5; P = .004). Chloroquine and sulfadoxine-pyrimethamine were equivalent in efficacy at day 28 (adjusted OR, 1.3; 95% CI, 0.3–7.0; P = .73). Chloroquine cleared gametocytes and asexual parasites more rapidly than sulfadoxine-pyrimethamine or chlorproguanil-dapsone did. All drugs were well tolerated.

Conclusions Although chloroquine remains the drug of choice, antifolates are effective against P. vivax malaria in South Asia. These drugs may be appropriate for unified treatment where species-specific diagnosis is unavailable, most likely in combination with other drugs.

Trial Registration clinicaltrials.gov Identifier: NCT00158561

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(Reprinted) JAMA, May 23/30, 2007—Vol 297, No. 20  2201
TREATMENT OF PLASMODIUM VIVAX MALARIA IN AFGHANISTAN AND PAKISTAN

There are, however, considerable operational problems where *P vivax* malaria constitutes the great majority of cases but diagnostic services are not reliable at species differentiation. This is the situation in many heavily populated areas of South Asia, including those in our trial, and presents a major public health challenge. Until recently, both forms of malaria could be treated with the same widely available drug, chloroquine, affordable both in the public and private sectors; mistakes in species differentiation were therefore unlikely to result in adverse clinical outcome. In the prevailing situation of widespread chloroquine-resistant *P falciparum* malaria, if this species is mistakenly diagnosed as *P vivax*, fatalities may occur if treated with chloroquine. The reverse is also problematic: if *P vivax* is misdiagnosed as *P falciparum*, the patient would be treated with artemisinin combination therapy, which, although safe, is substantially more expensive and no more effective.

Current options are to improve diagnosis so that species differentiation is reliable, to treat all malaria with artemisinin combination therapies, which are substantially more expensive than chloroquine, or to identify relatively affordable alternatives to chloroquine that work against both species. Improving diagnosis in areas of limited health services has been a consistent challenge; misdiagnosis occurs even in well-resourced laboratories. Rapid diagnostic tests that reliably detect both *P falciparum* and *P vivax* malaria are expensive and often unobtainable. A policy of treating *P falciparum* malaria with the same drug as *P vivax* malaria in areas of mixed endemicity may therefore be easier to implement than relying on improved diagnosis, provided cost can be contained. A unitary treatment would simplify protocols for informally trained health workers, treat mixed infections, and reduce risks associated with misdiagnosis. A unitary treatment that can be used in areas affected by war, conflict, or natural disaster would be particularly useful in situations in which population movement can rapidly increase transmission rates or public health systems become unstable or collapse completely. Antifolates are an important class of drugs that currently provide some of the cheapest alternatives to chloroquine. These drugs can be used alone or in combination. There is a widespread assumption dating back to the 1950s that *P vivax* malaria is intrinsically resistant to antifolates as a class, at least in settings in which antifolates are widely used. If this is so, it precludes an entire drug class from use in unitary treatment in many areas of the world. In Southeast Asia, there is longstanding, if limited, evidence that *P vivax* malaria is resistant to sulfadoxine-pyrimethamine, which may be associated with areas in which sulfadoxine-pyrimethamine use is common. Sulfadoxine-pyrimethamine resistance to *P falciparum* malaria has developed rapidly in some areas of low to moderate transmission (eg, Thailand). There is no reliable evidence regarding the current efficacy of antifolates against *P vivax* in South Asia, and specifically Afghanistan and its border regions, where *P vivax* malaria is predominant (>90% of malaria) and diagnostic services are limited. The most widely used antifolate is sulfadoxine-pyrimethamine. More recently, chlorproguanil-dapsone has been developed for low-resource settings. Chlorproguanil-dapsone has shown efficacy against uncomplicated *P falciparum* malaria, including sulfadoxine-pyrimethamine-resistant strains, in Africa and is well tolerated. Its safety has been explored in Africa and found to be comparable with sulfadoxine-pyrimethamine in terms of adverse event frequency, although concerns have been raised about its effect in G6PD (glucose-6-phosphate dehydrogenase)-deficient African children. This is potentially important in Afghanistan and Pakistan, because studies in the general population have shown high prevalence of G6PD deficiency in certain ethnic groups and Asian variants of G6PD deficiency can lead to significant hemolysis with some antimalarials. Antifolate resistance in *P falciparum* malaria is limited compared with Southeast Asia. The policy for confirmed *P falciparum* malaria recently changed to sulfadoxine-pyrimethamine in combination with artemesunate, but outside the formal sector where a high proportion of malaria is treated, antifolate monotherapy with sulfadoxine-pyrimethamine is the norm and, during the early period of the trial, almost all *P falciparum* malaria in the region was being treated with either chloroquine or sulfadoxine-pyrimethamine monotherapy. If antifolates are effective against *P vivax* malaria in South Asia, it would open up a range of possibilities for a unified *P vivax* and *P falciparum* malaria treatment policy.

Our goal was to examine the relative efficacy and safety of chlorproguanil-dapsone, sulfadoxine-pyrimethamine, and chloroquine in a South Asian population.

METHODS

Study Sites and Patient Enrollment Our study was conducted at 2 sites: the Malaria Reference Center, Jalalabad, Afghanistan, and Adizai Refugee Village, Peshawar, Pakistan. This area of long-standing conflict and political instability has more than a million Afghan refugees still living in Pakistan. The patients recruited at the Pakistan site were Afghan refugees, and the patients recruited at the Afghan site were Afghan residents of Jalalabad City and the surrounding area. Both study sites were situated in a similar epidemiological setting; malaria is seasonal with *P vivax* transmission peaking in the summer months and *P falciparum* in the autumn.

This was an open-label trial in which patients with microscopically confirmed *P vivax* malaria were randomized into 3 groups: chlorproguanil-dapsone, sulfadoxine-pyrimethamine, or chloroquine. All patients aged 3 years or older were eligible to be enrolled and were screened for malaria in the general outpatient departments at both facilities on the basis of history of febrile illness. Inclusion criteria were microscopically confirmed *P vivax* malaria, age...
3 years or older, written or witnessed verbal consent (by parents in the case of minors), available for the duration of follow-up, and willingness to be tested for G6PD deficiency at admission. Exclusion criteria were general condition requiring hospital admission, evidence of any concomitant infection or disease likely to mask treatment response, known allergy to any classes of the study drugs, known methemoglobin reductase deficiency, treatment within the past 7 days with any drug with known antimalarial properties or with an investigational drug within 30 days, severe anemia (hemoglobin <7 g/dL), mixed infection (P vivax and P falciparum), and pregnancy (confirmed by testing), lactation, or both.

If written informed consent was given, patients were enrolled in the study and assigned consecutive patient numbers. The treatment group was reassigned to patient numbers using randomization in blocks of different sizes (6-10) (STATA version 7; STATA Corp, College Station, Tex) in London, England. These numbers were stored in opaque envelopes and opened by the enrolling clinic staff at the time of enrollment; opening the envelope constituted admission to the trial and intention-to-treat analysis was conducted on the basis of that allocation. Randomization to chloroquine, sulfadoxine-pyrimethamine, and chlorproguanil-dapsone was by a ratio of 1:2:2. This ratio was chosen because knowledge of local failure rates of chloroquine are known (>95% efficacy against P vivax malaria), allowing for precise estimates, and the difference in failure rates, if any, between the antifolates was considered likely to be smaller than between chloroquine and either antifolate.

**Treatment and Follow-up Procedures**

Patients were administered either chloroquine (250-mg base) in divided doses over 3 days, chlorproguanil-dapsone (2.0 and 2.5 mg/kg per day, respectively) at 24-hour intervals over 3 days, or sulfadoxine-pyrimethamine (25 and 1.25 mg/kg, respectively) in single dose on day 0 followed by 2 days of placebo pills (not exactly identical to sulfadoxine-pyrimethamine tablets in appearance). Use of primaquine in a 5-day course for radical therapy was abandoned as national policy several years before our study on the basis of poor efficacy and, therefore (following local policy), patients did not receive this drug during the follow-up period. All doses were taken under supervision and patients were observed for 30 minutes after dosing. Vomiting within this time was noted as an adverse event and the patient received a new dose.

Patients were assessed on days 1, 2, 3, 7, 14, 21, and 28, and any intervening day they were unwell for 3, 7, 14, 21, and 28, and any intervening day they were unwell for 7 g/dL), mixed infection (P falciparum and P falciparum), and pregnancy (confirmed by testing), lactation, or both.

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Patients were assessed on days 1, 2, 3, 7, 14, 21, and 28, and any intervening day they were unwell for 3, 7, 14, 21, and 28, and any intervening day they were unwell for

**Outcome Measures**

The primary outcome was parasitological failure at day 14 read blind to treatment allocation. The main secondary outcome was parasitological and clinical failure by day 28. Clinical failure was defined as the presence of parasites and a temperature of at least 37.5°C anytime between day 3 and day 28. Other major secondary outcomes were the proportion with anemia (hemoglobin <10 g/dL) and proportion with gametocytes. Safety outcomes were adverse event frequency up to day 14 and hemoglobin changes in patients with G6PD deficiency as potential evidence of hemolysis. Seventeen-day efficacy was selected as the primary outcome because recrudescence due to drug failure and relapse from hypnozoites cannot be differentiated by molecular means. Relapses are likely to mask true recrudescence after the first 2 weeks posttreatment.

**Statistical Analyses**

The study was designed to detect superiority of chlorproguanil-dapsone over sulfadoxine-pyrimethamine or vice versa and the superiority or otherwise of chloroquine over either antifolate. Sample size was calculated to detect a difference of 85% compared with 70% cure rate in the 2 antifolate groups and between 99% efficacy in the chloroquine group and 91% efficacy in either of the antifolate groups (on a 1:2:2 randomization ratio) with \( \alpha = .05 \) and \( \beta = .80 \). A total sample size of 750 allowed for loss to follow-up. The analysis plan was agreed upon by a data and safety monitoring board before data collection was completed. Data were double-entered and compared using Microsoft Excel XP (Microsoft Corp, Redmond, Wash) and analyzed using STATA version 7 (STATA Corp).

Outcomes were measured using proportions, and a logistic regression model was constructed, with odds ratios (ORs) calculated uncorrected and adjusting for potential confounding factors. Association between the outcome variable and the predetermined factors of age, sex, presence of fever, anemia, parasite level at enrollment, and study site was investigated. Those factors independently associated with the outcomes at the 90% confidence level were included in the final multivariate model. All patients who were enrolled were classified as treat-
ment successes, treatment failures, losses to follow-up, or withdrawals. Withdrawals were further defined by reason for withdrawal (adverse event, protocol violation, or consent withdrawn). An additional safety analysis was performed classifying all withdrawals and losses to follow-up as failures.

Ethical Approval
Ethical approval was given by the Pakistan Medical Research Council National Bioethics Committee, the Ministry of Health of the Transitional Government of Afghanistan, and the London School of Hygiene and Tropical Medicine Ethics Committee. Permission was also given by the United Nations High Commissioner for Refugees. A data and safety monitoring board reviewed safety data, assessed serious adverse events, and determined preset stopping rules. The trial was pre-registered on an open-access database (http://www.clinicaltrials.gov).

RESULTS
At the 2 sites, 20,410 patients were screened (9705 in Pakistan and 10,705 in Afghanistan), with 2722 patients positive for P vivax. Of these patients, a total of 767 patients were enrolled (315 at the site in Pakistan and 452 at the site in Afghanistan) between March 2004 and June 2006. A total of 159 patients were randomized to chloroquine, 306 to sulfadoxine-pyrimethamine, and 302 to chlorproguanil-dapsone. The main reasons for patients with P vivax not being included were not consenting, inability to attend follow-up, or concurrent disease. The flow of patients through the trial is shown in Figure 1. A total of 716 of 767 patients (93.4%) completed treatment and follow-up to day 28 (90.4% in the chlorproguanil-dapsone group, 94.8% in the sulfadoxine-pyrimethamine group, and 96.3% in the chloroquine group). Fifty-one patients were either lost to follow-up (n = 31) or excluded/were withdrawn from the trial (n = 20). Of these patients, 9 withdrew consent, 10 were withdrawn due to adverse events, and 1 was withdrawn due to protocol violation. Baseline characteristics were similar between groups (Table 1).

The results of the primary and major secondary outcomes are shown in Table 2, with time to failure by study drug in Figure 2. All patients in all 3 groups completely cleared parasites by day 14, with 1 patient in the sulfadoxine-pyrimethamine group having parasites on day 14. By the primary efficacy variable, all 3 study drugs were therefore equally and highly effective. By day 28, chlorproguanil-dapsone was less effective than sulfadoxine-pyrimethamine and chloroquine, with 27 of 273 patients (9.9%) with disease refractory to treatment. Chloroquine failure rates (2 of 153 patients [1.3%]) and sulfadoxine-pyrimethamine failure rates (5 of 290 patients [1.7%]) were similar. Unadjusted and adjusted ORs for failure at day 28 in the 3 groups are shown in Table 3. Clinical failure rates were comparable between treatment groups up to days 14 and 28 (Table 2).

Age group and study site were independently associated with treatment outcome at the 90% confidence level and were included in multivariate logistic regression to calculate adjusted ORs. Sex, anemia on enrollment, fever on enrollment, and level of asexual parasitemia on enrollment were not included. Chlorproguanil-dapsone was less effective than sulfadoxine-pyrimethamine (adjusted OR, 6.4; 95% confidence interval [CI], 2.4-17.0; P < .001) and chloroquine (adjusted OR, 8.4; 95% CI, 2.0-36.5;
P = .004). In both cases, this is significant even with strict adjustment for 6 comparisons (chloroquine vs sulfadoxine-pyrimethamine, chloroquine vs chlorproguanil-dapsone, sulfadoxine-pyrimethamine vs chlorproguanil-dapsone at both day 14 and day 28). For public health purposes, chloroquine and sulfadoxine-pyrimethamine were equivalent in efficacy at day 28 (adjusted OR, 1.3; 95% CI, 0.3-7.0; P = .73).

There was more loss to follow-up in the chlorproguanil-dapsone group than in the other 2 groups. Because this is a potential bias, an additional analysis was undertaken classifying all loss to follow-up and withdrawals as failures. This gave a failure rate at day 28 of 5.3% for chloroquine, 7.4% for sulfadoxine-pyrimethamine, and 23.3% for chlorproguanil-dapsone (a worst-case scenario). Odds ratios for parasitological failure at day 28 were 3.2 (95% CI, 1.9-5.3) for chlorproguanil-dapsone vs sulfadoxine-pyrimethamine and 4.4 (95% CI, 2.0-9.5) for chlorproguanil-dapsone vs sulfadoxine-pyrimethamine, and 23.3% for chloroquine vs sulfadoxine-pyrimethamine, chloroquine vs sulfadoxine-pyrimethamine and chloroquine vs sulfadoxine-pyrimethamine, and sulfadoxine-pyrimethamine vs chlorproguanil-dapsone.

It is not possible to differentiate recrudescence from relapse, and differentiation of new infections is of limited use in *P. vivax* malaria. In a secondary subgroup analysis, almost all the difference between chlorproguanil-dapsone and the other groups was due to patients aged 3 to 10 years in the chlorproguanil-dapsone group in Jalalabad (Table 4). Stratified by sex, hemoglobin at enrollment was significantly lower in 3- to 10-year-old patients in Jalalabad than in Adizai (males: mean, 11.6 vs 12.3 g/dL; by t test, P < .001; and females: mean, 11.7 vs 12.2 g/dL; by t test, P = .005; respectively), which may suggest a difference in the nutritional status of participants between the 2 sites.

Clearance of asexual blood stage parasites was achieved more rapidly in the chloroquine group than either sulfadoxine-pyrimethamine or chlorproguanil-dapsone (Figure 3) with chloroquine clearing 100% of patients on day 7 and 94% of patients on day 14. Parasites were cleared in 100% of patients on day 14 and day 28. For public health purposes, chloroquine and sulfadoxine-pyrimethamine were equivalent in efficacy at day 28 (adjusted OR, 1.3; 95% CI, 0.3-7.0; P = .73).
day 2, and chlorproguanil-dapsone and sulfadoxine-pyrimethamine by day 7. Chloroquine also cleared gametocytes more rapidly than sulfadoxine-pyrimethamine or chlorproguanil-dapsone did (Figure 3).

Anemia varied by study drug over time, with chloroquine associated with the lowest rates of anemia (TABLE 5). Only 6 patients with G6PD deficiency were recruited into the trial (1 in chloroquine, 3 in sulfadoxine-pyrimethamine, and 2 in chlorproguanil-dapsone), making robust statistical comparison of safety difficult. In the chlorproguanil-dapsone group, 1 patient’s hemoglobin decreased from 10.7 g/dL to 8.3 g/dL over 24 hours, accompanied by abdominal pain, pallor, and headache, and the patient was withdrawn from the study as a precaution. The other patient with G6PD deficiency who was treated with chlorproguanil-dapsone had no decrease in hemoglobin. The maximum hemoglobin decrease after treatment in the other patients who were G6PD deficient was 1.1 g/dL (1 patient in the chloroquine group) and 2.0 g/dL, 0.5 g/dL, and 1.4 g/dL (3 patients in the sulfadoxine-pyrimethamine group).

There were no serious adverse events recorded during the trial and no patients required hospitalization. All study drugs were well tolerated, although there were observable differences in adverse event frequency between treatment groups (TABLE 6). Chloroquine was associated with the lowest frequency of any adverse events. The frequency of nausea and vomiting was higher in the chlorproguanil-dapsone group, but this was not associated with clinical failure in the 3- to 10-year-old age group.

**Table 3. Unadjusted and Adjusted ORs for Parasitological Failure at Day 28, Adjusted for Age Group and Study Site**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Unadjusted OR (95% CI)</th>
<th>P Value</th>
<th>Adjusted OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorproguanil-dapsone vs sulfadoxine-pyrimethamine</td>
<td>6.3 (2.4-16.6)</td>
<td>&lt;.001</td>
<td>6.4 (2.4-17.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sulfadoxine-pyrimethamine vs chloroquine</td>
<td>1.3 (0.3-6.9)</td>
<td>.73</td>
<td>1.3 (0.3-7.0)</td>
<td>.73</td>
</tr>
<tr>
<td>Chlorproguanil-dapsone vs chloroquine</td>
<td>8.3 (2.0-35.5)</td>
<td>.004</td>
<td>8.4 (2.0-36.5)</td>
<td>.004</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

**Table 4. Treatment Failures by Day 28, Stratified by Age Group and Study Site**

<table>
<thead>
<tr>
<th>Study Site by Age</th>
<th>Chloroquine</th>
<th>Sulfadoxine-Pyrimethamine</th>
<th>Chlorproguanil-Dapsone</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-10 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adizai</td>
<td>1/43 (2.1)</td>
<td>0/65</td>
<td>2/56 (3.6)</td>
</tr>
<tr>
<td>Jalalabad</td>
<td>1/47 (2.1)</td>
<td>3/91 (3.3)</td>
<td>22/93 (23.7)</td>
</tr>
<tr>
<td>11-20 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adizai</td>
<td>0/16</td>
<td>1/37 (2.7)</td>
<td>1/29 (3.5)</td>
</tr>
<tr>
<td>Jalalabad</td>
<td>0/19</td>
<td>0/53</td>
<td>0/44</td>
</tr>
<tr>
<td>&gt;20 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adizai</td>
<td>0/6</td>
<td>1/17 (5.9)</td>
<td>0/16</td>
</tr>
<tr>
<td>Jalalabad</td>
<td>0/22</td>
<td>0/27</td>
<td>2/34 (5.9)</td>
</tr>
</tbody>
</table>

**Figure 3. Proportions of Patients With Asexual Parasites and Gametocytes on Days of Follow-up by Treatment Group**

No. at Risk
Chlorproguanil-Dapsone 302 298 292 292 288 302 298 292 292 288
Sulfadoxine-Pyrimethamine 306 305 304 304 299 306 305 304 304 299
Chloroquine 159 157 157 157 155 159 157 157 157 155

Error bars represent 95% confidence intervals.

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concluded that chloroquine, sulfadoxine-pyrimethamine, and chlorproguanil-dapsone are all effective against acute stage \( P \) \( \text{vivax} \) malaria, and the antifolate class therefore may have a much greater potential use in areas of mixed infection than previously thought. Although chloroquine should remain the drug of choice in proven cases of \( P \) \( \text{vivax} \) malaria, this study raises the possibility of a unified drug policy for \( P \) \( \text{falciparum} \) and \( P \) \( \text{vivax} \) malaria using antifolates where reliable species differentiation is not possible or mixed infections are found. In cases in which sulfadoxine-pyrimethamine monotherapy is used for presumed cases of \( P \) \( \text{falciparum} \) malaria (as is common among private sector health care providers), it will treat \( P \) \( \text{vivax} \) malaria if there is misdiagnosis or a mixed infection. With the overdue move to combination therapy, the combination of sulfadoxine-pyrimethamine plus artesunate is now the recommended treatment for \( P \) \( \text{falciparum} \) malaria in Afghanistan, and antifolate use is increasing in Pakistan. The results of our trial demonstrate that where misdiagnosis occurs, \( P \) \( \text{vivax} \) will be adequately treated using this policy. Because the artemisinins have anti-\( P \) \( \text{vivax} \) activity, artemisinin combination therapies that include an antifolate are likely to be more effective against \( P \) \( \text{vivax} \) than an antifolate used alone. 23,30

Although all study drugs provided more than 85% cure rate when assessed parasitologically during the 28-day follow-up period, sulfadoxine-pyrimethamine was as effective as chloroquine, and somewhat more effective than chlorproguanil-dapsone. The losses to follow-up were higher in the chlorproguanil-dapsone group and, therefore, introduce a potential bias. But even taking the most pessimistic interpretation, that all losses and withdrawals constituted treatment failure, the relative differences between the groups remained similar. In reality, most of the losses to follow-up were considered to be random (eg, patient travel), and withdrawals were for relatively minor adverse events; therefore, analysis treating these results as missing are likely to be a more accurate reflection of reality.

The half-lives of the 2 components of sulfadoxine-pyrimethamine are 116 and 81 hours vs 12 to 20 hours and 20 to 30 hours for chlorproguanil-dapsone. It is possible that chlorproguanil-dapsone is intrinsically less active than sulfadoxine-pyrimethamine, but it seems likely that the known prophylactic effects of sulfadoxine-pyrimethamine and chloroquine are a

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factor. Relapse may (and often does) occur in the first month after infection, although in this area the first episode of malaria after treatment with chloroquine generally occurs at 45 to 90 days posttreatment. The temporal pattern of relapse is complicated by the hypnozoite reservoir from infections, which predate enrollment in this study. Hypnozoites may produce acute episodes for years after initial infection; therefore, in some patients the treatment failure may be related not to the current acute episode but to prior infections. Current molecular markers do not reliably differentiate between relapse and recrudescence making firm conclusions impossible.

The trial was not powered for subgroup analysis, but the difference between efficacy of chlorproguanil-dapsone in children at Afghan and Pakistani sites is striking. A number of possibilities have to be considered. A systematic error in dosing in the 3- to 10-year-old age group of patients was considered but records revealed no difference in recorded dosage between the 2 study sites, and all patients were monitored for 30 minutes for vomiting. At both sites, drugs were administered identically with a small amount of food and with water. In Jalalabad, dosages were crushed and administered in water; whereas, in Adizai, whole (or part of) tablets were administered. It seems unlikely that crushing tablets would have led to a reduction in bioavailability (rather the reverse), but it is possible some tablet particles were lost in the crushing process or left at the bottom of water beakers. However, this seems unlikely to have been unique to chlorproguanil-dapsone. There were no differences in reporting of gastrointestinal disturbances on any day of treatment in this age group between successes and failures, which discounts vomiting as a factor in dosage. Anecdotally, socioeconomic status in Jalalabad (and in Afghanistan in general) is worse than in the refugee population across the border in Pakistan. Nutritional factors (also linked to socioeconomic status) may affect pharmacokinetics, immunity, and susceptibility to disease.

The safety of chlorproguanil-dapsone in patients with G6PD deficiency cannot be assessed from these data because fewer patients with the condition presented to the study than would have been anticipated in the general population (3%-15% prevalence in Pashtun ethnic groups). This raises the possibility that G6PD is protective against \textit{P vivax} malaria, an effect which has been described for \textit{P falciparum} in Africa, and would have implications for drugs (especially the anti-hypnozoite drugs primaquine and tafenoquine), which are contraindicated in patients with G6PD deficiency. G6PD was measured because of safety concerns on the interaction of G6PD deficiency and chlorproguanil-dapsone. The effect of dapsone on patients with G6PD deficiency has been described. The African variant strains of G6PD are regarded as being generally less likely to lead to serious hemolysis than either Asian or Mediterranean variants. Data showing that 1 patient with G6PD deficiency treated with chlorproguanil-dapsone had a decrease in hemoglobin of more than 2 g/DL over 24 hours requiring withdrawal from the trial is not conclusive (decreases in hemoglobin occurred also with chloroquine and sulfadoxine-pyrimethamine), but is compatible with an interaction between Asian variants of G6PD deficiency and chlorproguanil-dapsone. Further safety data in South Asian individuals with G6PD deficiency may be required.

This trial suggests that antifolate resistance in \textit{P vivax} malaria is regional, and probably associated with specific drug resistance alleles (like \textit{P falciparum} malaria), rather than intrinsic resistance to the drug class. Sulfadoxine-pyrimethamine resistance is associated with successive specific multiple mutations on the \textit{P vivax} dihydrofolate reductase gene (\textit{Pvdhfr}). Sulfadoxine-pyrimethamine failures in resistant \textit{P vivax} malaria were associated with nonclearance of parasitemia up to 7 days posttreatment in one in vivo study, but in our study all patients cleared parasites by day 7. Chlorproguanil-dapsone has been shown to select multiple mutations on the \textit{dhfr} gene in African \textit{P falciparum} in one study, contradicting the findings of another study. There is currently no data on the activity of chlorproguanil-dapsone and \textit{Pvdhfr} mutations. The sulfone components of antifolate drugs act on dihydropteroate synthase, and mutations of this gene have been demonstrated in the presence of \textit{Pvdhfr} multiple mutations and are associated with treatment failure. Proguanil resistance has been reported in \textit{P vivax} malaria, but there are inadequate data on the action of chlorproguanil in \textit{P vivax}. It seems likely that if sulfadoxine-pyrimethamine continues to be widely used as monotherapy, \textit{Pvdhfr} mutations may render \textit{P vivax} malaria resistant to antifolate drugs and combinations as they have with \textit{P falciparum} malaria.

Our trial demonstrates that in South Asia where antifolate resistance is limited in both \textit{P falciparum} and, based on our trial, in \textit{P vivax} antifolates could be used for a unified treatment policy in areas of mixed infection, ideally in combination with an artemisinin.

**Author Contributions:** Mr Leslie had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Leslie, Whitty, Rowland.

**Acquisition of data:** Leslie, Mayan, Hasan, Safi, Whitty.

**Analysis and interpretation of data:** Leslie, Klinkenberg, Whitty, Rowland.

**Drafting of the manuscript:** Leslie, Mayan, Hasan, Safi, Whitty, Rowland.

**Critical revision of the manuscript for important intellectual content:** Leslie, Klinkenberg, Whitty, Rowland.

**Statistical analysis:** Leslie, Whitty.

**Obtained funding:** Leslie, Whitty, Rowland.

**Administrative, technical, or material support:** Leslie, Mayan, Hasan, Safi, Klinkenberg.

**Study supervision:** Leslie, Mayan, Hasan, Safi, Whitty, Rowland.

**Financial Disclosures:** Mr Leslie reported attending a GlaxoSmithKline-sponsored 1-day meeting and was paid an honorarium. Drs Whitty and Rowland reported they are supported by the Gates Malaria Partnership with funds from the Bill & Melinda Gates Foundation. No other authors reported financial disclosures.

**Funding/Support:** The study was funded by GlaxoSmithKline as an independent investigator-initiated trial. Drs Whitty and Rowland are supported by the Gates Malaria Partnership. HealthNet TPO Malaria and Leishmaniasis Control Programme is funded by the European Commission, Global Fund for AIDS, TB, and Malaria, United Nations High Commissioner for Refugees, and World Health Organization Special Programme for Research in Tropical Disease. None of these donors contributed directly to the study.
Role of the Sponsors: GlaxoSmithKline had no role in design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript. Before implementation, GlaxoSmithKline and the sponsor (London School of Hygiene and Tropical Medicine) provided minor comments on the protocol. HealthNet TPO implemented the project in the field, with technical support from the London School of Hygiene and Tropical Medicine. Data analysis was conducted by Drs Whitty and Rowland, and Mr Le- slie according to a preset analysis plan approved by all authors and the data and safety monitoring board.

Acknowledgment: None of these donors necessarily agree with the views expressed herein, which are solely those of the authors.

We thank the data and safety monitoring board for permission to report the results. We thank the data and safety monitoring board for ... in Afghanistan, Pakistan. HealthNet TPO implemented the project in the field, with technical support from the London School of Hygiene and Tropical Medicine. Data analysis was conducted by Drs Whitty and Rowland, and Mr Leslie according to a preset analysis plan approved by all authors and the data and safety monitoring board.

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