Low Efficacy of Amodiaquine or Chloroquine Plus Sulfadoxine-Pyrimethamine against *Plasmodium falciparum* and *P. vivax* Malaria in Papua New Guinea

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Abstract. Because of increasing resistance to 4-aminoquinolines in Papua New Guinea, combination therapy of amodiaquine (AQ) or chloroquine (CQ) plus sulfadoxine-pyrimethamine (SP) was introduced as first-line treatment against uncomplicated malaria in 2000. The purpose of this study was to monitor in vivo efficacy of the current standard combination therapy against *Plasmodium falciparum* and *P. vivax* malaria. Studies were conducted between 2003 and 2005 in the Simbu, East Sepik, and Madang Provinces in Papua New Guinea according to the revised protocol of the World Health Organization (WHO) for assessment of antimalarial drug efficacy. Children between six months and seven years of age with clinically overt and parasitologically confirmed *P. falciparum* or *P. vivax* malaria were treated according to the new policy guidelines (i.e., AQ plus SP given to patients weighing < 14 kg and CQ plus SP given to patients weighing ≥ 14 kg). Children were monitored up to day 28 and classified according to clinical and parasitological outcome as adequate clinical and parasitological response (ACPR), early treatment failure (ETF), late clinical failure (LCF), or late parasitological failure (LPF). For *P. falciparum* malaria, polymerase chain reaction (PCR)-corrected treatment failure rates up to day 28 ranged between 10.3% and 28.8% for AQ plus SP and between 5.6% and 28.6% for CQ plus SP, depending on the region and the year of assessment. Overall treatment failure rate with AQ or CQ plus SP for *P. vivax* malaria was 12%. Our results suggest that the current first-line treatment in Papua New Guinea is not sufficiently effective. According to the new WHO guidelines for the treatment of malaria, a rate of parasitological resistance greater than 10% in the two dominant malaria species in the country justifies a change in treatment policy.

INTRODUCTION

Malaria is a serious health problem in Papua New Guinea and access to safe and effective treatment still remains the mainstay in the control of the disease. The 4-aminoquinoline drugs amodiaquine (AQ) and chloroquine (CQ) have been first-line treatment against uncomplicated malaria until the late 1990s. However, resistance of *Plasmodium falciparum* to CQ was first documented in 1976 and numerous studies in different provinces at different times showed the problem to be widespread. Within two decades, resistance to the 4-aminoquinolines AQ and CQ increased gradually with a slow shift from RI to RII and RIII types.1–4 The first documented evidence for *P. vivax* resistance in Papua New Guinea was reported in 1989 and showed a similar increasing trend as for *P. falciparum*.5

Although pyrimethamine in combination with CQ has been used in mass drug administration campaigns in the 1960s,6 the combination of sulfadoxine-pyrimethamine (SP) was not previously part of the standard treatment against uncomplicated malaria and was used only in combination with quinine to treat severe or treatment failure malaria in Papua New Guinea. Despite the low use of SP, resistance of *P. falciparum* to this drug combination was first described in Papua New Guinea in 1980.7 Thereafter, *P. falciparum* resistance to SP as well as reduced efficacy of SP against *P. vivax* have been reported in Madang Province.8–15

In view of the low efficacy of the 4-aminoquinolines used as first-line regimen against malaria, health authorities in Papua New Guinea were prompted to revise the antimalarial treatment policy in 1997. Combination therapy for uncomplicated malaria has been advocated for some years to improve clinical effectiveness and to delay the development and spread of resistance to individual drugs.16–19 Although evidence for success of the combination regimen of AQ or CQ plus SP was scarce at that time and there was evidence for in vivo resistance against either of the drugs in Papua New Guinea, the decision to investigate the possible change to this combination regimen was made. Based on efficacy studies conducted between 1998 and 1999 that showed that the combinations were efficacious with treatment failure rates below 5%,20 the Papua New Guinean Department of Health chose the combination of AQ plus SP for young children and CQ plus SP for others to replace monotherapy with AQ or CQ as the standard first-line treatment against uncomplicated malaria in 2000.

Since the introduction of the new drug policy, little data has been collected to monitor the efficacy of the new standard treatment. Two studies conducted at health facilities in Maprik and Madang in 2001 recorded treatment failure rates up to day 14 of 3% and 8%, respectively (Reeder JC, unpublished data). These data showed some clinical resistance to the combination regimen one year after its introduction. Further molecular studies substantiated these findings by showing a high prevalence of mutations in CQ resistance–associated marker genes (*P. falciparum* chloroquine resistance transporter gene and *P. falciparum* multidrug-resistance gene 1) and appearance of mutations in the gene encoding *P. falciparum* dihydrofolate reductase, which is known to confer resistance to SP.21–23

The purpose of our study was to monitor the clinical efficacy of the current first-line regimen of AQ or CQ plus SP against *P. falciparum* and *P. vivax* malaria in Papua New Guinea after its official implementation in 2000. Within the framework of a project for the clinical and molecular monitoring of drug resistant malaria in Papua New Guinea, we conducted in vivo studies according to the revised protocol of the World Health Organization (WHO) for assessment of
antimalarial drug efficacy under current standard first-line policy in three different areas in Papua New Guinea between 2003 and 2005.

MATERIALS AND METHODS

Patients and study sites. The studies were conducted at the Sigimaru health center in the Karimui area (Simbu Province), the Kunjingini health center in the South Wosera area (East Sepik Province), and the Mugal health center in the North Coast area of Madang Province. Studies were conducted in the Karimui area between October and April in 2002, 2003, and 2004. Studies were conducted in the South Wosera area between December and June in 2003 and 2004. Studies were conducted in the North Coast area between April 2004 and February 2005. Although all three study sites are rural areas endemic for malaria, they differ with regard to malaria epidemiology, level of health care provision, and history of drug use.25–30

Scientific approval and ethical clearance for the study was obtained from the Medical Research and Advisory Committee of the Ministry of Health in Papua New Guinea. Informed consent was obtained from parents or legal guardians prior to recruitment of each patient.

Assessment of drug efficacy. In vivo drug efficacy studies were conducted according to the standardized WHO protocol for low-to-moderate transmission areas.31 Briefly: children between 6 months and 7 years of age were enrolled if they came to a health center with a microscopically confirmed Plasmodium infection. (P. falciparum density > 1,000 asexual parasites/μL of blood or a P. vivax density > 250 asexual parasites/μL of blood) and clinically overt malaria (axillary temperature ≥ 37.5°C or history of fever during the last 24 hours for P. falciparum, or fever during the last 48 hours for P. vivax). Cases with P. falciparum malaria were enrolled regardless of whether they had a concomitant infection with any other Plasmodium species, whereas a mixed infection with another species was an exclusion criterion for the P. vivax group. However, in mixed P. falciparum plus P. vivax infections, drug action was evaluated against both parasites species.

Further inclusion criteria were the absence of danger signs for severe or complicated malaria and no signs of any other disease, malnutrition or anemia. Standard AQ or CQ plus SP first-line treatment (10 mg of amodiaquine or chloroquine/kg on days 0, 1, and 2, and 25 mg of sulfadoxine/kg plus 1.25 mg of pyrimethamine/kg on day 0) was administered under supervision over the first three days. Treatment was given according to the official manual for Standard Treatment for Common Illnesses of Children in Papua New Guinea (Seventh edition, released in 2000) which prescribes SP plus AQ plus SP first-line treatment (10 mg of amodiaquine or chloroquine/kg on days 0, 1, and 2, and 25 mg of sulfadoxine/kg plus 1.25 mg of pyrimethamine/kg on day 0) was administered under supervision over the first three days. Treatment was given according to the official manual for Standard Treatment for Common Illnesses of Children in Papua New Guinea (Seventh edition, released in 2000) which prescribes SP plus AQ plus SP (for children weighing < 14 kg and SP plus CQ for children weighing ≥ 14 kg). Follow-up visits were scheduled on days 1, 2, 3, 7, 14, and 28. On every visit, patients were clinically examined and a Giemsa-stained blood slide was taken for the microscopic assessment of parasitemia.

Patients were advised to come to the health center on any other day if symptoms occurred. Whenever a child was diagnosed as treatment failure, standard second-line treatment (5 mg of artesunate/kg on day 1, followed by 2.5 mg of artemesunate/kg on days 2–7, and a single dose of 25 mg of sulfadoxine/kg plus 1.25 mg of pyrimethamine/kg on day 3) was given.

A patient was withdrawn from the study when any of the following occurred during the follow-up period: development of a concurrent infection requiring treatment, consumption of other antimalarial drugs, or loss of follow-up because of refusal of consent or failure to trace a patient on a follow-up visit.

Molecular analyses. Blood samples were taken on day 0 (pre-treatment sample) and on days 14 and 28 or any day of treatment failure for molecular genotyping purposes. Differentiation between recrudescence and new infection with P. falciparum was achieved by comparing polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP)–generated genotype patterns of the merozoite surface protein 2 (msp2) in pairs of samples obtained at enrollment and at the day of reappearance of parasitemia, as described elsewhere.33,34

Data analysis. Data were double entered in EpiData software version 3.02 (EpiData Association, Odense, Denmark) and analysis was performed using STATA software version 8.2 (Stata Corporation, College Station, TX). Patients in the P. falciparum group were classified according to their clinical and parasitologic responses as follows: early treatment failure (ETF; parasitemia on day 2 higher than on day 0 or parasitemia on day 3 with an axillary temperature ≥ 37.5°C), late clinical failure (LCF; parasitemia with an axillary temperature ≥ 37.5°C or a history of fever from days 4 to 28), late parasitologic failure (LPF; parasitemia from days 7 to 28 without an axillary temperature ≥ 37.5°C or a history of fever), or adequate clinical and parasitologic response (ACPR; absence of parasitemia on day 28 without meeting any of the previously described criteria for early or late treatment failure).31 For the P. vivax group, a patient was classified as a treatment failure when 1) clinical deterioration caused by P. vivax malaria in the presence of parasitemia, or 2) parasitemia between days 3 and 28 with an axillary temperature ≥ 37.5°C, or 3) parasitemia between days 7 and 28, irrespective of clinical conditions, was observed.35

Logistical regression analysis was used for the investigation of possible risk factors for treatment failure. Frequencies were compared by using chi-square tests or Fisher’s exact tests as applicable.

RESULTS

In vivo drug efficacy against P. falciparum. Of 687 children eligible for the P. falciparum group (pool of children enrolled in six in vivo efficacy studies), 38 (5.5%) were excluded from the analysis population. There were no exclusions due to concomitant infectious disease during the study period. All losses were due to withdrawal of consent of parents or guardians during the follow-up period (3.6%) or migration and/or absence of families after day 3 (1.9%). Therefore, clinical and parasitologic monitoring up to day 28 was accomplished for a study group of 649 (94.5%) children. The baseline characteristics of the children at the day of enrollment were similar for the three study sites and the corresponding years, except for mean parasite density at day 0 (Table 1). Mean parasite densities were higher in the lowland areas of the Wosera (t = 2.80, P ≤ 0.01) and the North Coast (t = 3.10, P ≤ 0.01) regions than in the highland region of Karimui.

At enrollment, 555 children (85.5%) had a monoinfection with P. falciparum and 94 (14.5%) had a mixed infection.
Among the mixed infections, 86 (91.5%) were simultaneously infected with *P. vivax*, 7 (7.4%) with *P. malariae*, and 1 (1.1%) with both of these species.

Overall, 521 (80.3%) children were treated with AQ plus SP and 128 (19.7%) children were treated with CQ plus SP. As expected from the treatment policy that gave AQ plus SP to children weighing <14 kg and CQ plus SP to the others, the median age was different in the two cohorts (i.e., 4 years for AQ plus SP and 6 years for CQ plus SP).

In our study, none of known risk factors (i.e., age, fever, or parasite density at day of enrollment) or the combination regimen (SP plus AQ or CQ, respectively) were associated with an increased risk of treatment failure (Table 2).

In the Karimui area, treatment failure rates with AQ or CQ plus SP up to day 28 decreased over the three-year period from 30% to 25% and 18%, respectively. This trend remained even after correction by PCR (28%, 18%, and 16%, respectively), which identified 11%, 26%, and 9% of recurrences to be new infections ($\chi^2 = 4.81$, degrees of freedom $[df] = 2$, $P = 0.09$). The overall decreasing trend in treatment failure rates over the study period was especially pronounced because of a decrease in clinical failures. In the South Wosera area, the overall failure rate tended to increase from 2003 to 2004 ($\chi^2 = 1.19$, $df = 2$, $P = 0.28$), from 19% in 2003 to 28% in 2004, and after genotyping correction from 16% to 22%, respectively, with 24% of recurrences in 2003 and 33% in 2004 being new infections. The treatment failure rate up to day 28 in 2004 was 16.4% in the North Coast area of Madang, 11.5% after correction by PCR with 29% of recurrent parasitemias being new infections.

Of 120 (18.5%) treatment failures in the *P. falciparum* group, 97 (80.8%) had a monoinfection with *P. falciparum*, 20 (16.7%) a mixed infection with *P. vivax*, and 3 (2.5%) a mixed infection with *P. malariae* at day 0. A mixed infection with *P. vivax* and/or *P. malariae* at day 0 showed a slightly increased risk of *P. falciparum* treatment failure (odds ratio = 1.53), but this effect did not reach statistical significance ($P = 0.11$).

Recurrent parasitemia with other species was seen in 36 (5.5%) of all cases, in 34 (5.2%) with *P. vivax*, and in 2 (0.3%) with *P. malariae*. Ten (11.6%) of the 86 patients with a mixed infection with *P. vivax* at day 0 had a recurrence with *P. vivax*, which represented *P. vivax* failure cases (Table 3). Of patients with a *P. falciparum* monoinfection at day 0, 24 (4.3%) had recurrence with *P. vivax* and 1 (0.2%) with *P. malariae*. Of the two patients with recurrent *P. malariae*, one had a monoinfection with *P. falciparum* and one had a mixed infection with *P. falciparum* and *P. vivax* at day 0. The patient with a mixed infection with all three species at enrollment had no recrudescence parasitemia during the follow-up period.

**In vivo drug efficacy against *P. vivax***: To maximize the sample size for analysis, data from the *P. vivax* groups enrolled at all study sites between 2004 and 2005 were pooled. Of 106 children with a *P. vivax* monoinfection at admission day, 2 were lost because of withdrawal of consent. At baseline, the analysis population had a mean age of 3 years (95% confidence interval [CI] = 2.7–3.4), a mean axillary temperature of 37.7°C (95% CI = 37.4–38.0°C), a mean hemoglobin level of 10.1 g/dL (95% CI = 9.7–10.5 g/dL), and a mean parasite density of 4,182 asexual parasites/µL of blood (range = 40–50,640/µL). A total of 98 (94.2%) children were treated with AQ plus SP and 6 (4.8%) children were treated with CQ plus SP.
Table 2

Treatment outcomes for chloroquine (CQ) plus sulfadoxine-pyrimethamine (SP) versus amodiaquine (AQ) plus SP against *Plasmodium falciparum* malaria

<table>
<thead>
<tr>
<th>Study site</th>
<th>Karimui area (Simbu Province)</th>
<th>South Wosera area (East Sepik Province)</th>
<th>North Coast area (Madang Province)</th>
<th>P†</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment groups</td>
<td></td>
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<tr>
<td>CQ or AQ plus SP</td>
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<tr>
<td>Mean age, years (95% CI*)</td>
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<tr>
<td>CQ plus SP</td>
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<tr>
<td>Mean age, years (95% CI)</td>
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<tr>
<td>AQ plus SP</td>
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<tr>
<td>Mean age, years (95% CI)</td>
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<tr>
<td>Follow-up to day 14, PCR-corrected rates, x/n (%)</td>
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</tr>
<tr>
<td>ACPR (all)</td>
<td>80 (82.47)</td>
<td>83 (89.25)</td>
<td>126 (98.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (all)</td>
<td>17 (17.53)</td>
<td>13 (10.75)</td>
<td>2 (1.56)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>ACPR (CQ plus SP)</td>
<td>14 (82.35)</td>
<td>16 (94.12)</td>
<td>32 (96.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (CQ plus SP)</td>
<td>3 (17.65)</td>
<td>1 (5.88)</td>
<td>1 (3.03)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>ACPR (AQ plus SP)</td>
<td>66 (82.50)</td>
<td>67 (88.16)</td>
<td>94 (98.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (AQ plus SP)</td>
<td>14 (17.50)</td>
<td>9 (11.84)</td>
<td>1 (1.05)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>TF†</td>
<td>0.61</td>
<td>0.42</td>
<td>0.45</td>
<td>0.07</td>
<td>0.20</td>
</tr>
<tr>
<td>0.61</td>
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<tr>
<td>Follow-up to day 28, PCR-corrected rates, x/n (%)</td>
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</tr>
<tr>
<td>ACPR (all)</td>
<td>70 (72.16)</td>
<td>76 (81.27)</td>
<td>107 (83.59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (all)</td>
<td>27 (27.84)</td>
<td>17 (18.28)</td>
<td>21 (16.41)</td>
<td>0.09</td>
<td>18 (16.07) 25 (21.74) 0.28 12 (11.53)</td>
</tr>
<tr>
<td>ACPR (CQ plus SP)</td>
<td>13 (76.47)</td>
<td>16 (94.12)</td>
<td>27 (81.82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (CQ plus SP)</td>
<td>4 (23.53)</td>
<td>1 (5.88)</td>
<td>6 (18.18)</td>
<td>0.35</td>
<td>1 (5.56) 8 (22.22) 0.12 2 (28.57)</td>
</tr>
<tr>
<td>ACPR (AQ plus SP)</td>
<td>57 (97.25)</td>
<td>60 (78.95)</td>
<td>80 (94.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (AQ plus SP)</td>
<td>23 (28.75)</td>
<td>16 (21.05)</td>
<td>15 (15.79)</td>
<td>0.12</td>
<td>17 (18.09) 17 (21.52) 0.57 10 (10.31)</td>
</tr>
<tr>
<td>TF†</td>
<td>0.46</td>
<td>0.13</td>
<td>0.47</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>0.19</td>
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</tbody>
</table>

* CI = confidence interval; ACPR = adequate clinical and parasitological response; TF = treatment failure.
† By chi-square test for trend of TF rates at respective sites.
‡ By Fisher’s exact test for differences in TF rates between treatment groups.

*Plasmodium vivax* treatment failure, defined as recurrent parasitemia after day 3 irrespective of clinical symptoms, was seen in 13 (12.5%) of the children (Table 3). There was a significant difference of failure rates between sites ($\chi^2 = 13.95, df = 2, P = 0.001$): 10 (29.4%) of 34 *P. vivax* infections in the North Coast area of Madang and 3 (6.5%) of 46 in the Karimui area failed treatment, whereas all 27 infections were successfully cleared in the Wosera area. Recurrent para-

Table 3

Treatment outcomes for chloroquine (CQ) plus sulfadoxine-pyrimethamine (SP) versus amodiaquine (AQ) plus SP against *Plasmodium vivax* malaria

<table>
<thead>
<tr>
<th>Study site</th>
<th>Karimui area (Simbu Province)</th>
<th>South Wosera area (East Sepik Province)</th>
<th>North Coast area (Madang Province)</th>
<th>P†</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em> monoinfections, no. (%)</td>
<td></td>
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<tr>
<td>CQ or AQ plus SP</td>
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<td></td>
</tr>
<tr>
<td>ACPR (all)</td>
<td>40 (93.0)</td>
<td>27 (100)</td>
<td>24 (70.6)</td>
<td></td>
<td>91 (87.5)</td>
</tr>
<tr>
<td>TF (all)</td>
<td>5 (7.0)</td>
<td>0 (0)</td>
<td>10 (29.4)</td>
<td>0.001</td>
<td>13 (12.5)</td>
</tr>
<tr>
<td>ACPR (CQ plus SP)</td>
<td>3 (75.0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td></td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>TF (CQ plus SP)</td>
<td>1 (25.0)</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>0.27</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>ACPR (AQ plus SP)</td>
<td>37 (94.9)</td>
<td>26 (100)</td>
<td>24 (72.7)</td>
<td></td>
<td>87 (88.8)</td>
</tr>
<tr>
<td>TF (AQ plus SP)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>9 (27.3)</td>
<td>0.001</td>
<td>11 (11.2)</td>
</tr>
<tr>
<td>TF†</td>
<td>0.26</td>
<td>–</td>
<td>0.29</td>
<td>0.16</td>
<td></td>
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<tr>
<td>Mixed <em>P. vivax</em> plus <em>P. falciparum</em> infections, no. (%)</td>
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<tr>
<td>CQ or AQ plus SP</td>
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<td></td>
</tr>
<tr>
<td>ACPR (all)</td>
<td>33 (91.7)</td>
<td>29 (90.6)</td>
<td>14 (77.8)</td>
<td></td>
<td>76 (88.4)</td>
</tr>
<tr>
<td>TF (all)</td>
<td>3 (8.3)</td>
<td>3 (9.4)</td>
<td>4 (22.2)</td>
<td>0.29</td>
<td>10 (11.6)</td>
</tr>
<tr>
<td>ACPR (CQ plus SP)</td>
<td>4 (100)</td>
<td>4 (80.0)</td>
<td>0 (0)</td>
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<td>8 (80.0)</td>
</tr>
<tr>
<td>TF (CQ plus SP)</td>
<td>0 (0)</td>
<td>1 (20.0)</td>
<td>1 (100)</td>
<td>0.09</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>ACPR (AQ plus SP)</td>
<td>29 (90.6)</td>
<td>25 (92.6)</td>
<td>14 (82.3)</td>
<td></td>
<td>68 (89.5)</td>
</tr>
<tr>
<td>TF (AQ plus SP)</td>
<td>3 (9.4)</td>
<td>2 (7.4)</td>
<td>3 (17.7)</td>
<td>0.54</td>
<td>8 (10.5)</td>
</tr>
<tr>
<td>TF†</td>
<td>0.70</td>
<td>0.41</td>
<td>0.22</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

* ACPR = adequate clinical and parasitological response; TF = treatment failure.
† By chi-square test for trend of TF rates between sites.
‡ By Fisher’s exact test for difference of TF rates between groups.
sitemia with *P. falciparum* was observed in two (1.9%) patients who had both successfully cleared their *P. vivax* infection. As with *P. falciparum* malaria, age, fever, parasite density at day of enrollment, or the combination regimen (SP plus AQ or CQ, respectively), were not associated with an increased risk of treatment failure (Table 3).

**DISCUSSION**

In Papua New Guinea, standard first-line therapy with AQ or CQ against uncomplicated malaria was replaced with the combination regimen of AQ or CQ plus SP in 2000. The current studies conducted between 2003 and 2005 were the first to monitor therapeutic efficacy of the newly introduced combination regimen against *P. falciparum* and *P. vivax* malaria using the revised WHO standard protocol. In our studies conducted in three different areas over a period of three consecutive years, we observed overall PCR-corrected treatment failure rates of up to 29% with both combination regimens (SP plus AQ or CQ, respectively) for *P. falciparum* malaria. Overall treatment failure rate with AQ plus SP for *P. vivax* malaria was 11%.

There is strong advocacy for artemisinin-based combination therapy (ACT). However, for economic reasons, many countries have decided on combination regimens including more affordable options, such as AQ or CQ plus SP. Papua New Guinea replaced 4-aminooquinoline monotherapy with AQ or CQ plus SP in 2000, a decision that was based on efficacy levels above 95% assessed in hospital-based studies between 1998 and 1999 using the 14 day follow-up protocol. When we restricted the analysis in our studies to the day 14 outcomes on the basis of clinical and parasitological criteria only, we measured treatment failure rates between 2% and 18%. As expected, failure rates up to day 28 were higher, with PCR-corrected values between 12% and 28%, depending on the area and the year. In concordance with previous data, our results show that in vivo studies with a follow-up period of 14 days are not sensitive enough to assess the therapeutic efficacy of the current first-line regimen in areas moderately to highly endemic for malaria. Assessment up to day 14 clearly underestimates the true failure rate because in most patients, recurrent parasitemia appeared after day 14. Furthermore, late recurrences (i.e., appearing after day 14) have to be expected for regimens including drugs with long elimination half-lives, such as SP.

Our results show moderate efficacy of AQ or CQ plus SP only three years after successful implementation of the new first-line regimen. Although clinical failure rates were still low (<10% at all three sites), resistance levels exceeded 12% in all three sites. It is commonly accepted that parasitological response should be used as an additional indicator for the in vivo efficacy of drugs. Parasitological failure rates are likely to translate into clinical failure rates, either within a short term in the infected individual depending on the immunological status, or within a long term on population level as parasite resistance increases. Moreover, according to the new WHO guidelines, which recommend that a policy change should be seriously considered when efficacy of a combination regimen up to day 28 is less than 90%, these high levels of in vivo resistance are worrisome.

Surprisingly, the dynamics of drug efficacy over time in the Karimui and Wosera areas showed contrasting trends. Whereas treatment failure rates showed an increasing trend over two years in Wosera, the trend was decreasing over three years in Karimui. The question remains whether our observations are the product of intrinsic regional variations or reflect real trends in the dynamics of resistance in these areas. We are aware that sample sizes and time intervals between the studies might not have been sufficient to detect real trends. Supplementation of the in vivo results with additional molecular data, the most important being the level of resistance in the circulating parasite population (Marfurt J and others, unpublished data), and ongoing monitoring activities will give further insight on the level and dynamics of drug-resistant *P. falciparum* malaria in these areas.

In view of the history of drug use in Papua New Guinea, the observed failure rates with AQ or CQ plus SP were not surprising. In the face of increasing CQ resistance, many countries in Africa and Asia had adopted SP as first-line antimalarial treatment between the 1960s and the 1980s. Thereafter, several countries facing increasing levels of SP resistance had introduced the cheap and safe combination of AQ or CQ plus SP as an interim option for antimalarial therapy. Whereas SP combined with AQ had shown a reduction in clinical as well as total failure rates up to day 28, the combination with CQ has not been associated with much benefit over monotherapy with SP. More recently, non-artemisinin-based combination therapy (NACT) with AQ plus SP has been shown to be equally or more efficacious than ACT with SP in northern Ghana and Uganda, the main reason being that resistance levels to SP and AQ were still low in these regions. Therefore, in certain regions where previous AQ and SP use was low, NACTs can still be considered as cost-effective interim options before full implementation of ACTs. In contrast, previous drug history in Papua New Guinea was clearly different (i.e., constant AQ pressure for more than 20 years and sporadic use of SP) and the prospect for the combination of AQ plus SP to work better than CQ plus SP was therefore low. An added benefit of combination therapy is heavily dependent on pre-existing efficacy of the partner drugs. In view of the high levels of resistance to AQ and CQ reported in Papua New Guinea, it was therefore unlikely that these drugs would have had sufficient capacity to significantly curb the development of resistance to SP and therefore prolong its useful therapeutic life.

Known risk factors, such as age, parasite density, and temperature at admission day, were not associated with an increased risk of treatment failure with AQ or CQ plus SP. Although the issue of a difference in efficacy between the two treatment regimens (i.e., SP plus AQ or CQ) has been addressed by age-stratified analysis of data, which did not show a difference in treatment failure rate between the two treatment categories, one might speculate that CQ plus SP is less efficacious than AQ plus SP because the proportion of children who were able to clear CQ-resistant parasites might have been larger in the CQ plus SP group (i.e., on average older children). However, it was the principle aim of our in vivo studies to determine treatment failure rates under the current first-line policy in Papua New Guinea, and not to compare clinical efficacy of AQ plus SP versus CQ plus SP.

Chloroquine resistance in *P. vivax*, the second dominant species in Papua New Guinea, was first described in 1989 and treatment failure rates up to 20% for CQ and 8% for AQ
were reported from Maprik in the late 1990s. Reduced sensitivity of *P. vivax* malaria to SP has been observed in Madang. When we evaluated the therapeutic efficacy of AQ or CQ plus SP in 190 patients with a *P. vivax* infection, we measured a total treatment failure rate with AQ or CQ plus SP up to day 28 of 12%.

Relapse is an important aspect of *P. vivax* malaria and refers to clinical malaria caused by reappearing parasites that originate from the dormant liver stages called hypnozoites. Therefore, circulating asexual stages after blood schizonticidal therapy might either originate from asexual parasites that survived therapy from activated hypnozoites, which lead to a relapse, or from a new infection. Unlike *P. falciparum* infections, where true recrudescences can be distinguished from new infections by the use of genotyping methods, current molecular methods used for the genetic analysis of *P. vivax* do not enable unambiguous classification of recurrent parasitemia, in particular the distinction between a relapse originating from an antecedent infection and a newly acquired infection during the follow-up period, which is critical in the analysis of the therapeutic response. However, recent work on the establishment of standard protocols, similar to those developed for *P. falciparum* including multiple polymorphic gene look promising and might be included in future drug efficacy studies. Although patients in our study were exposed to the risk of a new infection during the follow-up period and parasite genotyping methods were not applied, we assume that our data represent true *P. vivax* resistance to treatment. Studies demonstrating that no *P. vivax* relapses occurred until day 36 after full compliance to treatment with the long half-life drug CQ, most probably due to minimal effective concentrations of the drug preventing a first relapse to become patent in the blood, led to the proposition that parasitemia recurring within 28 days after initiation of CQ therapy reflects resistance to the drug. This concept might be even more relevant with a combination regimen containing CQ and a second long half-life drug, such as SP.

It has long been thought that SP is less active against *P. vivax* malaria, an assumption that was mainly based on clinical studies failing to demonstrate SP efficacy against this species. Accordingly, SP has never been recommended for treatment of *P. vivax* malaria. Nevertheless, increasing levels of resistance of *P. vivax* to CQ led to the introduction of SP in many countries in southeast Asia, Central and South America and other parts of Oceania, where both species are endemic, and resistance had developed rapidly in many areas within only a few years after its initial deployment as monotherapy. It has been shown recently that the mechanisms of *P. vivax* resistance to antifolates are similar to those of *P. falciparum*. Several studies have reported an association between single nucleotide polymorphisms in *P. vivax* dihydrofolate reductase and reduced sensitivity to SP. Moreover, in vivo studies conducted in areas with a previous history of SP use against *P. falciparum* have shown that SP resistance of *P. falciparum* was paralleled by the development of resistance of *P. vivax*. These findings further argue for a similar mechanism of antifolate resistance in both species, one that is driven by exertion of selective drug pressure and progresses rapidly. It does therefore not come as a surprise that despite the addition of SP, *P. vivax* failure rates increased from 8% with AQ monotherapy to 12% with the combination of AQ or CQ plus SP. However, high levels of *P. vivax* resistance is mainly restricted to the Madang area, where *P. vivax* resistance even exceeds that seen in *P. falciparum*. *Plasmodium falciparum* parasitemia was seen in two (2%) of *P. vivax* patients. Cryptic coinfections with *P. falciparum* after treatment against *P. vivax* malaria have been described in other areas where both species are endemic. Because appearance of *P. falciparum* in both cases was seen after two weeks of treatment, it may reflect acquisition of a new infection. However, a more plausible explanation is that a concomitant *P. falciparum* infection was not recognized at the day of admission, the most likely reason being that the *P. falciparum* infection was in its hepatic stage. In contrast, *P. vivax* in patients with a *P. falciparum* monoinfestation at admission day was seen in 24 (4.3%) of all patients. *Plasmodium vivax* parasites appeared between days 7 and 28, which suggests that patients had a concomitant infection with both species at presentation, either as patent infection, which was not detected by microscopic diagnosis because of low *P. vivax* parasitemia, or as relapse from intra-hepatic infection shortly after initiation of treatment against *P. falciparum*. In both cases, recurrent parasitemia would represent resistance because circulating drug levels should have eliminated drug-sensitive parasites.

In conclusion, the high parasitologic failure rates of *P. falciparum* and *P. vivax* to the combination therapy with AQ or CQ plus SP only after a short time of successful implementation suggest that the current first-line regimen in Papua New Guinea is not sufficiently effective and that a policy change needs to be considered. Although further monitoring assessing molecular markers for parasite resistance to AQ/ CQ and SP, and also other drugs, such as the artemisinin derivates, is ongoing in Papua New Guinea, clinical trials to test the safety and efficacy of alternative replacement regimens are urgently needed so that a policy change can be rapidly initiated. *Plasmodium falciparum* and *P. vivax* malaria are both endemic in Papua New Guinea and in most health facilities, antimalarial therapy is given on the basis of presumptive clinical diagnosis. Therefore, apart from safety, tolerability, practicability, and cost, efficacy to both of the prevailing species is an important aspect to consider in the evaluation of any future combination regimen.

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