Comparison of Artemether-Lumefantrine with Sulfadoxine-Pyrimethamine for the Treatment of Uncomplicated Falciparum Malaria in Eastern Nepal

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Abstract. Because available data suggest that resistance of Plasmodium falciparum to sulfadoxine-pyrimethamine (SP) is increasing in Nepal, an open-label, parallel-group efficacy/safety study was conducted in 99 Nepalese patients with uncomplicated falciparum malaria randomized 2:1 to artemether-lumefantrine (AL) or SP. Efficacy was assessed from clinical and microscopic evidence of treatment failure. Four SP-treated patients (12.1%; 95% CI, 4.0–29.1%) redeveloped parasitemia during the 28-day follow-up versus 0% (95% CI, 0–6.9%) in the AL group (P = 0.011), a difference that was confirmed by polymerase chain reaction (PCR) analysis of parasite DNA. PCR detected an additional six patients (two SP and four AL) with sub-microscopic gametocytemia or breakthrough parasitemia between Days 14 and 28, suggesting that AL efficacy was lower than estimated by microscopy. Dhfr and dhps mutations were not associated with outcome. AL is more effective than SP for uncomplicated malaria in Nepal, but regular monitoring of its efficacy should be carried out if this combination therapy is introduced.

Although mosquito vectors cannot survive at high altitude in mountainous regions of Nepal, malaria transmission occurs in the lower-lying areas of the Terai in the south of the country.1 Over the last 20 years, the annual incidence of malaria in Nepal has varied between 0.4 and 3.6/1,000 people, with Plasmodium falciparum accounting for up to 20% of cases.2 Approximately 80% of total cases and >90% of those of falciparum malaria occur in 12 districts that share a border with India.3 These districts contain ~6 million people, a figure approaching one quarter of the country’s population.3

Parasite resistance to conventional antimalarial drugs has emerged in Nepal. High levels of chloroquine resistance resulted in a change in first-line antimalarial therapy to sulfadoxine-pyrimethamine (SP) in 1989, but limited data suggest that SP resistance now varies between 56% and 87%.3 The most detailed studies have been carried out in the Jhapa district in the southeast of the country, 1 of the 12 districts with relatively high transmission, including a significant proportion of cases of falciparum malaria. In a clinical study conducted in Jhapa in 2003–2004,4 the failure rate for SP treatment of falciparum malaria was 21% at 28 days by World Health Organization (WHO) criteria.5,6 This finding is consistent with an in vitro study done in the same area in 2002, which showed that the C59R and S108N mutations in P. falciparum dihydrofolate reductase (dhfr) were nearly universal, whereas triple mutations (N51I, C59R, and S108N) were found in 10% of isolates.7

As a response to the antimalarial drug resistance situation, the WHO now supports the use of artesinin-based combination therapy (ACT) in countries such as Nepal.8 The recommended first-line ACT is artemether-lumefantrine (AL).8 In light of this recommendation, failing SP effectiveness in the Terai, and the need for data to inform national antimalarial drug policy in Nepal and other countries in a similar epidemiologic situation, we conducted a detailed study of SP and AL therapy in the Jhapa District with the primary aim of comparing the efficacy of the two treatments. Secondary aims were to 1) assess whether there are age-associated differences in the response to either regimen, 2) relate clinical and parasitologic treatment failure after SP therapy to parasite dhfr and dihydropterote synthase (dhps) mutations, 3) document the effect of the two regimens on gametocyte carriage and thus their potential influence on transmission, and 4) determine, through the use of polymerase chain reaction (PCR) analysis of parasite DNA in all follow-up blood samples, whether there are submicroscopic recrudescences that might be the forerunners of clinically significant parasite resistance.

MATERIALS AND METHODS

Study design, timelines, approval, and registration. This study was an open-label, randomized, parallel-group efficacy and safety study that was carried out during the rainy season months of August, September, and October 2005. Given the availability of recent SP efficacy data from the Jhapa district4,6 and the lack of AL efficacy data, treatment was by sequential allocation in the ratio of two AL cases to one SP case. Under this design, a total of at least 99 patients was needed to show a 28-day cure rate for SP (75%)7,9 that was 20% less than that for AL (95%)10 at 80% power and P < 0.05.9 The study was approved by the Nepal Health Research Council, registered with the Australian Clinical Trials Registry (ACTRN012605000551695), and conducted in accordance with the Helsinki Declaration.

Patients. We recruited patients > 5 years of age and of either sex who had 1) uncomplicated falciparum or mixed falciparum/vivax malaria infection based on absence of WHO criteria for severity,10,2 a P. falciparum asexual parasite density > 500/μL whole blood, 3) no history of antimalarial treatment within the previous month, and 4) an axillary temperature > 37.5°C and/or history of fever within the previous 3 days. Exclusion criteria included 1) body weight < 10 kg, 2) pregnancy, and 3) co-incident severe non-malarial illness. All
recruited patients gave informed consent or, in the case of children, consent was provided by a parent or guardian with the child assenting to study procedures.

**Clinical procedures.** Initial clinical assessments were carried out in district clinics and health posts, where a rapid diagnostic test (AccessBio, Monmouth Junction, NJ) or blood smear for microscopy was performed. After an explanation of study procedures had been provided, and informed consent was obtained, eligible patients were transferred promptly (usually within 2 hours) to Mechi Zonal Hospital, where a standard detailed history and physical examination were performed and blood was taken by finger prick for a confirmatory blood smear. Capillary blood was also taken for measurement of hematocrit and preparation of filter paper blood spots for subsequent PCR analysis (see below).

SP (Pyralin; Lupin, Aurangabad, India; 500 mg sulfadoxine and 25 mg pyrimethamine) was administered as a single dose of between one and three tablets to the nearest half tablet by body weight (range: one tablet for patients 10–14.9 kg to three tablets for those ≥50 kg). Coartem (Novartis Pharma, Beijing, China; 20 mg artemether and 120 mg lumenfane) was also given by body weight but to the nearest whole tablet as six doses of between one and four tablets over 3 days with food (at 0 and 12 hours on Day 0 and twice daily on Days 1 and 2). Doses ranged between one tablet per dose for patients 10–14.9 kg to four tablets per dose for those ≥37 kg. In contrast to Coartem, the SP used in this study was not produced under Good Manufacturing Practice standards. Six SP tablets from the batch used were assayed for content using previously validated methods.11 The mean sulfadoxine content was 86.6% (range, 75.2–93.3%) of stated and that for pyrimethamine was 117.4% (107.0–124.7%).

All eligible subjects were hospitalized for ≥24 hours, and all doses were supervised. Subjects vomiting within 30 minutes of dosing were withdrawn from the study and treated with the alternative drug regimen. Antipyretic, antiemetic, and other supportive therapy was given as needed. Monitoring of pulse, blood pressure, axillary temperature, and parasitemia was performed every 6 hours during the first 24 hours. In the AL group, additional assessments were carried out in the morning and evening of Days 1 and 2 and on the morning of Day 3. If SP-treated patients were eating, drinking, and improving clinically on Day 1, they were discharged and asked to return on the mornings of Days 2 and 3 for reassessment. In both groups, the same clinical procedures, together with collection of blood smears and filter blood spots, were carried out on Days 7, 14, 21, and 28. Side effects and their severity were recorded on standard forms. Patients developing fever and/or other symptoms of malaria between assessments were requested to reattend promptly for treatment with the alternative regimen if the blood slide was positive. Patients with gametocytemia during follow-up received primaquine phosphate 15 mg daily for 3 days for falciparum and 15 mg daily for 5 days for vivax gametocytes.

All blood slides were assessed by two experienced microscopists who were blinded to therapeutic allocation. There was good agreement between parasite densities (Pearson $r = 0.95$; $N = 663$ slides, $P < 0.001$), minimal systematic between-microscopist error on a Bland-Altman plot, and agreement on negative slides in 97.7% of cases ($N = 557$).

**Outcome measures.** The primary outcome was response to treatment assessed from the 28-day WHO in vivo test.5,6 Parasite resistance was categorized as RI (prompt, sustained parasite clearance to Day 7 but reappearance before Day 28), RII (>75% fall in parasitemia by 48 hours but no clearance, plus persistent parasitemia on Day 7), or RIII (<25% fall in parasitemia by 48 hours, plus persistent Day 7 parasitemia); a sensitive (S) response was recorded otherwise.3 Response at 28 days was also categorized for a low to moderate transmission area as 1) early treatment failure (ETF; danger signs/severe malaria within 3 days, rise in parasitemia by Day 2, or fever ≥37.5°C or parasitemia ≥25% on Day 3), 2) late clinical failure (LCF; presence of parasitemia with fever between 4 and 28 days), 3) late parasitologic failure (LPF; presence of parasitemia without fever between 7 and 28 days), or 4) adequate clinical and parasitologic response (ACPR). Secondary outcomes were 1) parasite clearance time (PCT), the time to the first of two consecutive negative blood smears, 2) the time taken to clear 50% of asexual parasites from the blood (PCT$_{50}$), and 3) fever clearance time (FCT), the time to the first of two consecutive axillary temperatures ≤37.0°C.

**Molecular studies.** Field microscopy was assessed against *P. falciparum* and *P. vivax*-specific nested PCR using parasite DNA extracted from blood spots on filter paper collected on Day 0 and at each follow-up visit.12,13 Recrudescences were distinguished from re-infections using multilocus genotyping14,15 with polymorphic regions of the *P. falciparum* genes encoding merozoite surface proteins MSP-1 (three allelic families) and MSP-2 (two allelic families), and glutamate-rich protein (GLURP) amplified by nested PCR. The presence of identical multilocus genotypes in Day 0 and recrudescence samples indicated true recrudescence.

Mutant alleles on the dhfr and dhps genes were detected using direct PCR sequencing. *P. falciparum* DNA extracted from blood spots using InstaGene was used to amplify a 594-bp dhfr fragment with polymorphic sites at positions N51L, C59R, S108N, and I164L (the single-letter amino acid codes for wild and mutant alleles are before and after each position, respectively). Extracted DNA13 (15 µL) was amplified in 50-µL nest-1 reactions containing 200 µmolar/µL of each of dNTP, 0.5 U Phusion DNA polymerase (Finnzymes, Espoo, Finland) in 1× Phusion HF Buffer, and 250 nmol/L of each primer of the pair PfdhfrNIF-5’ ATGATGGAACAAGTCTCGAC and PfdhfrNIR-5’ CGTTGATCATCTTTGTTATTTTC at 98°C (30 seconds), and then 30 cycles at 98°C (5 seconds), 62°C (10 seconds), 72°C (5 minutes). Nest-1 products (2 µL) were amplified in 20-µL nest-2 reactions with 0.4 U of Phusion DNA polymerase (Finnzymes) and the primer pair PfdhfrN2F-5’ CATGTTGTAAGGTTGAAG and PfdhfrN2R-5’ CTAGTATAACATCGCTAACAGY as follows: 98°C (30 seconds) and then 35 cycles of 98°C (10 seconds), 56°C (10 seconds) and then 30 cycles at 98°C (5 minutes), 56°C (30 seconds) and then a final extension at 72°C (5 minutes). A 711-bp dhps fragment incorporating polymorphic sites at amino acid positions S436A, A437G, K540E, A581G, and A613T/S was also amplified. Extracted DNA (5 µL) was amplified in 50-µL nest-1 reactions containing 200 µmolar/µL of each dNTP, 0.5 U Phusion DNA Polymerase (Finnzymes) in 1× Phusion HF Buffer, and 250 nmol/L of PdhdpsNIF-5’ GATTCTTTTCTCAGATGGAGG and PdhdpsNIR-5’ TTCTTCATGTAAATCTGTA at 98°C (30 seconds), and then 30 cycles of 98°C (5 minutes), 57°C (10 seconds), 72°C (12 seconds), and a final extension at 72°C (5 minutes).
Nest-1 products (2 μL) were amplified in 20-μL nest-2 reactions with 0.4 U of Phusion DNA polymerase (Finnzymes) and the primer pair PfdhpsN2F-5′/AACCTAAACGTGCTGTTCAA and PfdhpsN2R-5′/AATTTGTGATTTGTCCA-AA at 98°C (30 seconds), and then 30 cycles of 98°C (5 seconds), 60°C (10 seconds), 72°C (11 seconds), and a final extension at 72°C (5 minutes). Nest-2 PCR products were prepared for direct PCR sequencing using BigDye Terminator DBTV.3 cycle sequencing (Applied Biosystems, Foster City, CA) and the relevant nest 2 forward and reverse primers. Forward and reverse sequence was generated per gene per isolate (ABI Prism 3100 version 3.7; Genetic Analyzer, GMI Inc., Ramsey, MN). The sequences were assembled to give one forward sequence per gene per isolate for alignment and dhfr and dhps haplotype construction (MegAlign DNA*STAR Version 5; DNASTAR, Madison, WI).

Data analysis. Statistical analysis was performed using SPSS for Windows (SPSS, Chicago, IL). Data are presented as proportions, percentages, mean ±SD, geometric mean (SD range), or for non-normally distributed variables, median (range). Two-sample comparisons were by Fisher exact test, Student t test, or Mann-Whitney U test as appropriate. Associations between variables were assessed using Pearson product moment or Spearman correlation coefficients. P < 0.05 was considered significant.

RESULTS

Study sample. We recruited 102 eligible patients (Figure 1). Three (2.9%) were excluded from analysis, one patient vomiting after taking SP on Day 0 and two AL-treated patients defaulting on Days 3 and 28, respectively. Complete follow-up was, therefore, available for 99 patients (97.1% of the recruited cohort), of whom 33 were allocated to SP and 66 to AL. Details of these 99 patients are summarized in Table 1. There were no significant differences between the two groups for a range of demographic, anthropometric, and infection-related variables.

Initial response to treatment. Changes in parasitemia in the two groups during the first 3 days are plotted in Figure 2, and measures of initial parasite and fever clearance are summarized in Table 2. PCT, PCT₅₀, and FCT were all ~50% shorter in the AL group, the latter despite similar use of antipyretic treatment in the two groups (data not shown). Similar significant differences in these variables were also seen when the treatment groups were divided into adults and children < 16 years of age (data not shown). Most (~88%) of the AL-treated patients took each dose with food as requested, and there was no relationship between measures of parasite and fever clearance and whether AL was taken on an empty stomach (data not shown).

WHO efficacy assessment. Assessed by microscopy, 4 of 33 patients or 12.1% (95% CI, 4.0–29.1%) of SP-treated patients redeveloped parasitemia during the 28-day follow-up period compared with 0 of 66 patients or 0% (95% CI, 0–6.9%) in the AL group (Table 2). These four treatment failures were made up of one RII and three RI by the conventional WHO classification and one ETF, two LPFs, and one LCF by contemporary methods. The RII/ETF case was a 30-year-old man with no past history of malaria or previous treatment who remained slide-positive until Day 3, when his parasitemia was 268% of that on Day 0. He was treated with AL on Day 4 and recovered uneventfully. Two of the RI patients were LPF cases (parasitemia without fever on Day 21) and the third was a LCF (parasitemia with an axillary temperature of 39.0°C on Day 28). All three were treated with AL and responded uneventfully.

Gametocyte carriage. There was a significant rise in the percentage of patients who were slide positive for P. falci-
parum gametocytes after SP therapy to a peak on Day 7 (median gametocyte density, 88/μL; range, 26–394/μL), whereas, in contrast, the prevalence of gametocytemia fell progressively after treatment with AL (Day 7 median, 0/μL; range, 0–0/μL; Figure 3). The cumulative requirement for primaquine therapy had reached 84.8% of patients by Day 28 in the SP group compared with only 22.7% in the AL group (P < 0.001).

Molecular assessment of efficacy. Of the four treatment failures identified by microscopy, no PCR was performed in the RII/ETF case and, in another, genotyping data were incomplete. Nevertheless, the three RI/LTF/LCF cases were interpreted as recrudescences by PCR. However, multi-locus genotyping showed that, in one of these cases, the Day 21 blood spot contained original and new alleles from a dhps-resistant haplotype (SGKAA to AGEAA).

The PCR results for the remaining 95 patients from Day 7 to Day 28 inclusively are shown in Figure 4. Consistent with the gametocyte data, and because the techniques used do not differentiate between sexual and asexual forms, most of the SP group was positive at Day 7 by slide and/or PCR. Six of the 95 patients (6.3%) were PCR positive at some time between Days 14 and 28 but were microscopy negative for asexual and gametocyte stages. Four of these were in the AL treatment group. Although genotyping was not possible for the follow-up sample in one of these AL patients, no new alleles were detected in the other three.

Multilocus genotypes using MSP-1, MSP-2, and GLURP alleles were constructed for 48 pre-treatment (Day 0) samples (48.5% of the total). These patients were made up of 1) 15 who had detectable parasite DNA on Day 7 or beyond and 2) a randomly selected group of the remaining patients. The values of all the baseline variables listed in Table 1 for this group were similar to those of the 51 patients who were not selected for multilocus genotyping (P > 0.17 in each case). Of the Day 0 samples, 33 (68.8%) complete and 9 (18.8%) partial genotypes were obtained. The remaining six patients (12.5%) had mixed genotype infections at Day 0. Only 10 different genotypes were detected in the 33 complete single genotype infections, and 17 (51%) of these were one of two clones occurring at high frequency in the study sample. Nine participants were infected with genotype designated G6 and another eight with G5. The two genotypes were distributed between the two treatment groups, but all patients infected with G6 responded to treatment (four in the SP group and five in the AL group). For 15 of the 48 patients with detectable parasite DNA during follow-up, 8 were infected with G5 (5 SP and 3 AL) and 5 had parasite DNA detectable beyond Day 7 (3 SP and 2 AL).

### Table 1

Details of the patients in the two treatment groups at study entry

<table>
<thead>
<tr>
<th></th>
<th>Sulfadoxine-pyrimethamine</th>
<th>Artemether-lumefantrine</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>33</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.5 ± 13.0</td>
<td>26.5 ± 13.8</td>
<td>0.30</td>
</tr>
<tr>
<td>Age ≥ 16 years (%)</td>
<td>32.3</td>
<td>31.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>72.7</td>
<td>53.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>42 ± 14</td>
<td>42 ± 11</td>
<td>0.99</td>
</tr>
<tr>
<td>Previous malaria (%)</td>
<td>12.1</td>
<td>12.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Duration of fever (d)</td>
<td>5.0 (3.0–6.5)</td>
<td>5.0 (4.0–7.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Axillary temperature (°C)</td>
<td>37.5 ± 0.8</td>
<td>37.8 ± 1.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Pulse rate (/min)</td>
<td>102 ± 13</td>
<td>102 ± 17</td>
<td>0.92</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td>107 ± 15</td>
<td>101 ± 14</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)*</td>
<td>65 ± 11</td>
<td>64 ± 11</td>
<td>0.58</td>
</tr>
<tr>
<td>Splenomegaly (%)</td>
<td>9.1</td>
<td>12.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37 ± 6</td>
<td>36 ± 7</td>
<td>0.70</td>
</tr>
<tr>
<td>*P. falciparum parasitemia (μL)</td>
<td>2,997 (1,225–7,987)</td>
<td>6,492 (1,463–23,601)</td>
<td>0.18</td>
</tr>
<tr>
<td>Gametocytemia (μL)</td>
<td>0 (0–0)</td>
<td>0 (0–19)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

* Data are percentage, mean ± SD, or median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Sulfadoxine-pyrimethamine</th>
<th>Artemether-lumefantrine</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment failure by 28-day test</td>
<td>4/33 (12.1%)</td>
<td>0/66 (0%)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>(4–0.9–29.1)</td>
<td>(0–6.9%)</td>
<td></td>
</tr>
</tbody>
</table>

* Data are mean ± SD, median (95% CI), or percentage (95% CI).

† There were one RII and three RI failures, or one ETE, two LPFs, and one LCF that were all confirmed by PCR (see text).
Dhfr and dhps haplotypes. Dhfr haplotypes were determined in 36 Day 0 samples including the 15 recrudescent/re-infection cases. Of these, 23 (63.8%) had the NRNI dhfr two-point mutation haplotype coupled with wild-type dhps. These parasites would be expected to respond adequately to SP treatment with or without prolonged gametocytemia. Fifteen (65.2%) patients infected with these parasites were in the SP group and, although all but one adequately cleared asexual stage parasites, only five (33%) were completely free of parasite DNA during follow-up. The dhfr NRNI haplotype was coupled with mutant dhps alleles in 10 (27.7%) patients, and only 3 of these were treated with SP. Of these three patients, one was coupled with the dhps SGKGA double mutation and had R1 resistance. The remaining patients were infected with parasites with three-point dhfr mutations (two NRNL and one IRNI), both of which are associated with reduced response to SP. Two of these patients were in the SP group and one had R1 resistance. Two patients in the SP group had different multilocus genotypes on follow-up. Both of these were associated with selection for mutant dhps haplotypes from the SAKAA wild-type to SGKAA or AGEAA.

Plasmodium vivax infections. There were no mixed infections at study entry by microscopy, but 5/98 (5.1%) were positive for both P. falciparum and P. vivax by PCR. All these patients were allocated to AL, and all were PCR negative by Day 3 or Day 7 with no recrudescence. A further 18/98 patients (18.4%) became PCR positive for P. vivax at one time-point from Day 3 onward. Nine of these 18 patients (50%) were allocated to SP (representing 27.2% of the SP group) and 9 were AL treated (13.6% of the group). The median times to P. vivax PCR positivity were 21 and 28 days, respectively, with a range of between 3 and 28 days in each group. One patient, who was treated with AL, developed PCR positivity for P. vivax on Day 3 and remained positive at all subsequent time-points except Day 21.

Adverse effects. Apart from fever, the most frequent symptoms at presentation were headache (97% and 88% in AL and SP groups, respectively), nausea (42% and 64%, respectively), and vomiting (39% and 46%, respectively). Other gastrointestinal, neurologic, musculoskeletal, respiratory, and dermatologic complaints were much less frequent. On direct questioning during treatment, < 12.5% of patients had one or more symptoms, they were rated as mild in the majority of cases, and there were no significant differences in symptom reporting between the two treatment groups (data not shown).

There were no group-specific differences in changes in pulse or systolic or diastolic blood pressure during initial therapy (data not shown; P > 0.20 in each case). Electrocardiographs were taken from a subset of 18 patients (10 in the AL and 8 in the SP group), from which the rate-corrected QT interval (QT/√RR; QTc) was measured manually by a trained observer who was blind to treatment allocation. There was no change in QTc during treatment with AL (0.425 ± 0.028 seconds at baseline versus 0.421 ± 0.028 seconds at 72 hours) or SP (0.422 ± 0.024 and 0.452 ± 0.059 seconds, respectively; P > 0.5 in each case).

DISCUSSION

This study has, reflecting other reports from a variety of geo-epidemiologic contexts, confirmed a high cure rate associated with AL therapy for uncomplicated falciparum malaria in adults and children in Eastern Nepal. Initial parasite clearance was rapid, and we detected no failures by clinical assessment or microscopy during the 28 days of follow-up. Consistent with previous studies, gametocytes were detected by microscopy in the minority of patients (< 25%) during this time, and only one AL-treated patient had a blood film positive for gametocytes beyond Day 7. AL therapy was, as expected, well tolerated. Most of the symptoms reported
during the first 3 days of therapy were mild and corresponded with those of acute malaria per se, and there was no clinical or electrocardiographic evidence of cardiotoxicity.

Despite these findings, analysis of the PCR data raises some concerns. There was evidence of subclinical, submicroscopic recrudescence between Days 14 and 28 inclusive in 6% of the AL-treated patients. This trend deserves study, because such breakthrough parasites have survived ACT, regardless of whether low-density asexual and/or sexual forms were present, and sets the scene for selection of resistance to the component drugs in an area in which artemisinin derivatives, lumefantrine, and related compounds have not been used previously. Mixed-species submicroscopic infections were present at baseline, as has been described previously, but late post-treatment emergence of PCR-detected \textit{P. vivax} was more common than that of \textit{P. falciparum}. Vivax malaria developing in the aftermath of treatment of \textit{P. falciparum} is recognized even with artemisinin combination therapy, but one of our patients had evidence of a low-density \textit{P. vivax} parasitemia or gametocytemia that emerged on the last day of AL therapy and persisted throughout the 28-day follow-up period, an observation consistent with low-grade resistance.

Reflecting the specificity of SP therapy for mature rather than young parasite forms, initial parasite clearance in this group was relatively slow. The treatment failure rate by microscopy was significantly higher than that in AL-treated patients, and the 95% confidence intervals spanned the level at which WHO recommends that alternative therapies are considered (10%). As has been reported previously, \textit{P. falciparum} gametocytemia developed or emerged in a substantially greater proportion of patients allocated to SP than AL therapy, with >80% requiring primaquine treatment during follow-up and approaching 50% blood-smear positive for gametocytes at Day 14. Nevertheless, SP therapy was well tolerated, with the frequency and severity of adverse effects similar to those in the AL group.

It is possible that the low sulfadoxine tablet content (mean, 87%) compared with International Pharmacopoeia requirements (90–110% of that stated on the packaging) may have contributed to the relatively high treatment failure rate in the SP group, especially when all parasites in a subgroup had at least two \textit{dhfr} point mutations, and there was evidence of drug-related selection of resistant breakthrough parasites in two patients in the SP group. Indeed, one of the RVLTF cases had the \textit{dhfr} haplotype IRNI, which has been associated with a poor response to SP in African populations, and two patients were infected with parasites exhibiting the triple mutation NRNL, which is associated with SP treatment failure, particularly in Asia. These observations are of concern.

In a study conducted in 2003, which involved 102 patients from the Jhapa district treated with SP for uncomplicated falciparum malaria, ETF occurred in 6.9%, and 14.7% had LTF compared with 3.0% and 9.1%, respectively, in this study. This provides some evidence that local parasite resistance has not worsened over recent years. In addition, there was a similar distribution of \textit{dhfr} two- and three-point mutations to those in this study in a field survey done in the same area in 2002. Indeed, molecular characterization revealed a limited number of parasite clones circulating at the time of this study. One of these (G6) was associated with prompt and sustained parasite clearance, whereas the second predominant clone (G5) appeared to be associated with prolonged gametocyte presence or emergence during follow-up. Evidence of restricted parasite diversity on a background of seasonal malaria transmission suggests importation of falciparum malaria into the Jhapa district (perhaps from neighboring districts of Nepal or from India) followed by local expansion. Thus, although drug sensitivity should reflect that in the area of origin, the increasingly global distribution of SP resistance makes it unreliable as first-line therapy. A corollary of the local predominance of clones G5 and G6 is that one or more of the recrudescences in the SP arm may have been re-infections. Notwithstanding the development of strain-specific immunity, lumefantrine has a similar half-life (4–6 days) to those of sulfadoxine and pyrimethamine. The fact that there were no re-infections in the larger AL group of patients is consistent with all the late treatment failures being recrudescences.

This study had limitations. We had a relatively small sample of patients, especially for molecular studies, but they were well-characterized and came from sub-districts that were representative of the pattern of malaria transmission during the previous rainy season (Figure 1). In addition, we achieved near-complete follow-up and data collection, and our results were consistent with those in previous, albeit less comprehensive, studies done in the Jhapa district several years earlier.

In countries such as Nepal, the choice is between inexpensive, widely available therapies that have declining efficacy such as SP and new forms of ACT including AL that are more effective but also relatively expensive and currently more difficult to procure. The cost-benefit equation will, however, include other considerations. In the case of SP, the cost of increased malaria transmission and primaquine use (which should involve pre-treatment testing for glucose-6-phosphate dehydrogenase activity) and management of recrudescences would need to be estimated. The need for a 3-day, six-dose AL regimen taken with food to improve bioavailability may be associated with reduced compliance compared with single-dose SP, with implications for increased recrudescence and transmission rates. Concerns have been raised as to the emergence of resistance to AL.

Our follow-up PCR data for both \textit{P. falciparum} and \textit{P. vivax} add to these concerns and underscore the need for regular in vivo and in vitro monitoring if AL therapy is introduced when conventional therapies fail.

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