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Recurrence of *Plasmodium malariae* and *P. falciparum* Following Treatment of Uncomplicated Malaria in North Sumatera With Dihydroartemisinin-Piperaquine or Artemether-Lumefantrine

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Background. We assessed the efficacy of artemisinin-based combination therapies for treatment of uncomplicated *falciparum* malaria, with or without co-infecting *Plasmodium* spp., in Sumatera, Indonesia.

Methods. Febrile patients aged >6 months with uncomplicated *P. falciparum* were randomized to receive dihydroartemisinin-piperaquine or artemether-lumefantrine, plus single-dose primaquine, and were followed for 42 days. Mixed *Plasmodium* infections were included; *P. vivax* infections received 14 days of primaquine. We retrospectively restricted the analysis to cases with polymerase chain reaction (PCR)–confirmed parasitemia. Recurrent parasitemia in follow-up was identified by species-specific nested PCR.

Results. Of the 3731 participants screened, 302 were enrolled and randomized. In the dihydroartemisinin-piperaquine arm, *P. falciparum* infections were confirmed by PCR in 59 participants, with mixed infections in 23 (39.0%). In the artemether-lumefantrine arm, *P. falciparum* infections were confirmed by PCR in 55 participants, with mixed infections in 16 (29.0%). Both regimens were well tolerated, and symptoms improved rapidly in all treated participants. In the dihydroartemisinin-piperaquine arm, 1 *P. falciparum* recurrence (on day 7) and 6 *P. malariae* recurrences (1 had a mixed infection with *P. falciparum*) were identified during days 3–42 of follow-up. In the artemether-lumefantrine arm, 1 *P. falciparum/P. malariae/P. vivax* recurrence occurred on day 35. Submicroscopic persistence occurred during follow-up in 21 (37%) of 57 receiving dihydroartemisinin-piperaquine and 20 (39%) of 51 receiving artemether-lumefantrine.

Conclusions. In Sumatera, both regimens effectively cleared initial parasitemia, but *P. falciparum* and *P. malariae* persisted in some individuals. Molecular species detection should be deployed in antimalarial efficacy trials in Indonesia.

Trial registration. NCT02325180.

Keywords. artemisinin combination therapy; in vivo drug efficacy; multispecies malaria infections.

Artemisinin-based combination therapy (ACT), co-formulating a short-acting artemisinin with a long-acting partner drug, is currently recommended for uncomplicated *falciparum* malaria. The widespread deployment of ACT, together with other control strategies, has reduced malaria morbidity and mortality, particularly in Africa [1]. ACT efficacy is threatened by *Plasmodium falciparum* with reduced susceptibility to both artemisinin and its partner drugs in the Greater Mekong subregion (GMS) [2–5]. Declining ACT efficacy against *P. falciparum* to ACT in this region raises concern that multidrug resistance may emerge in other malaria-endemic countries [6, 7].

Indonesia lies to the south and east of mainland Southeast Asia, and nearly 70 million of its population are at risk of malaria, including multispecies co-infections [8–10]. The ACT artesunate-amodiaquine, plus a single dose of primaquine to clear gametocytes, has been used for the treatment of uncomplicated *falciparum* malaria in Indonesia since 2004 [11]. Efficacy <90% was reported, and this combination was subsequently replaced by dihydroartemisinin-piperaquine (DP), plus a single dose of primaquine [12, 13]. For non-*falciparum* malaria, the standard 3-day course of DP is also recommended in combination with 1–14 days of primaquine for certain infecting species [14]. Published estimates of ACT efficacy

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against the non-*falciparum* parasite species in Indonesia exist only for *P. vivax*, which commonly occurs either alone or in co-infections with *P. falciparum* [8, 15], but no studies have adequately assessed the in vivo susceptibility of *P. malariae* or *P. knowlesi*, which co-circulate in Western Indonesia [8].

DP efficacy throughout Indonesia is reported to be excellent, and there are no reports of delayed clearance of *P. falciparum*, a sign of artemisinin resistance [13, 15–18]. However, a potential threat remains of artemisinin-resistant parasites being introduced from the GMS [6], adjacent to West Indonesia, or from Niugini (PNG), with a land border to East Indonesia [7]. Thus, there is a need to evaluate *P. falciparum* populations in Indonesia for susceptibility to current regimens in vivo. We aimed to compare the efficacy of DP, the first-line treatment for *falciparum* malaria, with artemether-lumefantrine (AL), also licensed in Indonesia, for treatment of uncomplicated *falciparum* malaria as single- and multispecies infections. The work was carried out in the North Sumatera province, which is a short sea crossing from Southern Thailand.

METHODS

Study Site and Participants

We conducted a prospective, open-label, randomized comparison of DP and AL for the treatment of uncomplicated *falciparum* malaria in the Batubara, Langkat, and South Nias regencies in the North Sumatera province, Western Indonesia (Supplementary Figure 1). Patients with uncomplicated *P. falciparum* malaria (including mixed infections with non-*falciparum* species) were eligible if they were aged >6 months, had fever/fever history (temperature ≥37.5°C) in the past 48 hours, and parasitemia >250 asexual parasites per μL, as determined by microscopic examination. Exclusion criteria were signs and symptoms of severe or complicated malaria, severe malnutrition, hemoglobin <7 g/dL, nonmalaria febrile illness, pregnancy or lactation, known hypersensitivity or allergy to study drugs, any antimalarial drug use in the previous 2 weeks, and any study drug use in the past 4 weeks.

The study was registered at ClinicalTrials.gov with the identifier NCT02325180 and approved by the Universitas Sumatera Utara (USU; August 2014; identifier 401/KOMET/FK USU/2014) and London School of Hygiene and Tropical Medicine (LSHTM; October 2014; Ref: 8504) research ethics committees. Consent forms were prepared in English and translated into Bahasa Indonesia. All adult participants and guardians of those <18 years old provided written informed consent.

Procedures

DP (Duo-Cotecxin, Holley Pharmaceutical, Hangzhou, Zhejiang Province, China; 40 mg dihydroartemisinin, 320 mg piperaquine) was given at doses of 2.25 mg/kg/dose and 18 mg/kg/dose, administered at enrollment, 24 hours, and 48 hours. AL (Coartem, Novartis Pharma, China; 40 mg artemether, 240 mg lumefantrine) was given according to weight, with 1 half-tablet for every 5 kg of body weight, at enrollment and hours 8, 24, 36, 48, and 60. DP was given on an empty stomach, and AL was given with biscuits or milk. Administration of all doses was supervised by study staff. Re-administration of a full dose or half-dose occurred if the patient vomited within 30 minutes or 30–60 minutes after treatment, respectively. As set out in Indonesian Government Treatment Guidelines, an additional single dose of primaquine at 0.75 mg/kg was given at enrollment for *P. falciparum* infection, and 14-day treatment with 0.25 mg/kg of primaquine per day was administered for all participants who were microscopy-positive for *P. vivax* at enrollment, commencing on day 42 [11].

Signs, symptoms, and adverse events were assessed and recorded before and during treatment and at all follow-up visits. Finger-prick blood samples provided blood films for microscopic assessment and dried filter paper blood spots for post hoc molecular analysis. Parasitological assessments were made from Giemsa-stained thick and thin blood smears and were single-read by a study team microscopist.

Parasite DNA was isolated from filter paper, and polymerase chain reaction (PCR) amplification of 18S rRNA genes for molecular identification of *Plasmodium* spp., *pfmdr1* amplification for *P. falciparum,* and *pksicavar* amplification for *P. knowlesi* was performed as previously described [8]. Parasite clearance at days 0, 1, 2, and 3 was measured using microscopy and by quantitative PCR (qPCR) for amplification of genus-specific *pgmet* normalized to the human β-tubulin gene *humtubb,* as previously described [19, 20]. Hemoglobin levels were assessed using HemoCue at enrollment and day 28.

Recurrent *P. falciparum* infections, identified by microscopy during routine follow-up (days 1, 2, 3, 7, 14, 21, 28, 35, 42), were treated with quinine (10 mg of salt/kg given 3 times a day for 7 days) and doxycycline (100 mg twice a day for 7 days), except for children aged <8 years and pregnant women, who were treated with quinine and clindamycin. Patients who developed a non-*falciparum Plasmodium* infection were re-treated according to the national guidelines [11]. Recurrent infections identified by microscopy were confirmed post hoc by species-specific nested PCR amplification of 18S rRNA or merozoite antigen genes [8, 21]. Subpatent *P. falciparum* recurrences on days 28 and 42, or the latest available sample, were identified post hoc by PCR amplification of the *pfmdr1* gene [22].

Outcomes

The primary end point defined in the study protocol was 42-day adequate clinical and parasitological response (ACPR) following DP and AL treatment for *P. falciparum* infection, as defined by the World Health Organization (WHO) [21]. Secondary and exploratory end points included qPCR parasite clearance over days 0 to 3; the proportion of patients with parasitemia at day 3 measured by microscopy and qPCR; efficacy of treatment against PCR-confirmed non-*falciparum Plasmodium*

spp.; and the proportion of patients with subpatent *Plasmodium* spp. recurrences at days 28 and 42.

Statistical Analysis

A sample size of 150 participants in each group would provide 80% power to show the noninferiority of AL compared with DP at day 42, with an α of .05 and δ of 5%.

Data were double-entered and analyzed using Stata IC (version 15; StataCorp, TX, USA). Those lost to follow-up or who withdrew from the study were censored as having treatment failure. Only those outcomes determined in the PCR-defined population are presented in this report. Odds ratios and 95% confidence intervals were estimated for testing associations; *P* values of ≤.05 were considered statistically significant.

RESULTS

A total of 3731 participants were screened from January to June 2015 in the Regencies of in Batubara, Langkat, and South Nias (Supplementary Figure 1), as previously described [8]. Three hundred two individuals, comprising 263 *P. falciparum* infections and 39 mixed *P. falciparum/P. vivax,* as identified by microscopy, were eligible for enrollment and randomly assigned to receive DP (n = 153) or AL (n = 149) (Figure 1). Both regimens were well tolerated, and symptoms improved rapidly in all treated subjects. A single *P. falciparum* infection failed to clear by day 3 by microscopy in a patient receiving DP; post hoc PCR confirmed *P. falciparum* DNA (alone) at the day of enrollment. *P. malariae* and *P. falciparum* DNA were detected together at day 3 in this individual (Figure 1), and *P. falciparum* gametocytes were observed in the blood-film on days 1, 2, and 3, suggesting a chronic infection.

We performed species-specific nested PCR retrospectively on DNA from enrollment samples of all 302 randomized individuals. Surprisingly, *Plasmodium* spp. DNA was successfully detected in only 195 individuals (Figure 1), indicating that lack of accuracy in microscopic diagnosis had resulted in the inappropriate recruitment of individuals without a detectable malaria infection. Further, *P. falciparum* parasite DNA was specifically amplified in just 117 randomized patients (38.8%), in 40 of whom evidence was found of co-infecting *Plasmodium* spp. (Table 1). As a result of these findings, and excluding 5 individuals who did not complete 3 days of ACT treatment, we established a PCR-defined cohort (n = 190) and performed an exploratory efficacy assessment for each treatment group on a modified per-protocol basis, in which both enrollment and follow-up parasite detection was performed by PCR (Figure 1). Comparison of baseline characteristics for the 114 evaluable *P. falciparum*–infected individuals included in this analysis is given in Table 2.

Within the PCR-defined cohort, microscopically identified recurrent *P. falciparum* infections during follow-up were

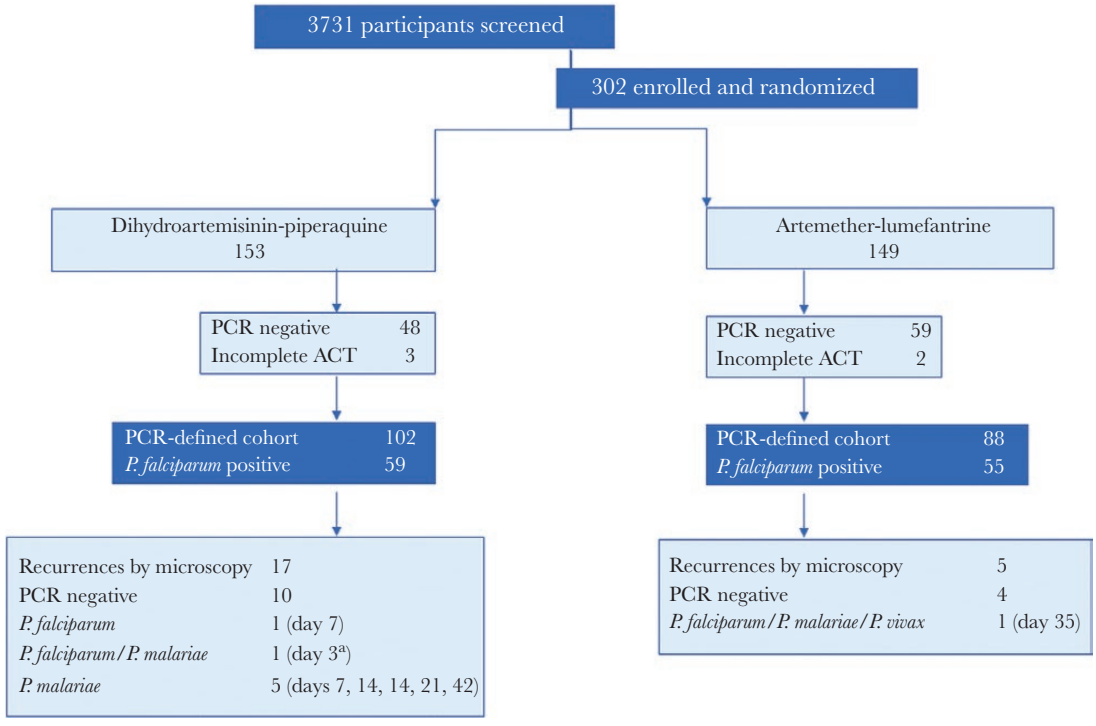

Figure 1. Trial profile. PCR-defined cohorts were identified in each treatment arm as shown. Lower cells show PCR outcomes for 22 follow-up blood samples identified as recurrent infections by microscopy. [a]Denotes a single early treatment failure identified by microscopy and PCR-confirmed. Abbreviations: ACT, artemisinin-based combination therapy; PCR, polymerase chain reaction.

Table 1. Species-Specific PCR Detection Frequency of Parasites in All 302 Randomized Individuals, From Peripheral Blood Sampled at Day of Enrollment, by Amplification of 18S rRNA and *sicavar* Genes

	Additional Species							
Species 1	None	Pv	Pk	Pm	Pm/Pk	Pv/Pk	Pv/Pm	Total (%)
Negative	107							107 (35.4)
Pf	77	14	18	2	3	2	1	117 (38.7)
Pv	28		16	4	3			51 (16.9)
Pk	19			2				21 (7.0)
Pm	6							6 (2.0)
Total								302

The table is arranged as a hierarchy based on prevalence in the data set, Pf>Pv>Pk>Pm, and does not necessarily reflect relative density in peripheral blood in mixed-species infections. Thus, all results are qualitative, so it cannot be assumed that, in a mixed infection, the species denoted by row is circulating at higher density than the species denoted by column in any particular individual.

Abbreviations: PCR, polymerase chain reaction; Pf, *Plasmodium falciparum*; Pk, *Plasmodium knowlesi*; Pm, *Plasmodium malariae*; Pv, *Plasmodium vivax*.

reported for 22 patients (17 for DP, 5 for AL). Species-specific PCR on the follow-up samples failed to detect parasite DNA in 10 and 4, respectively (Figure 1). Of the remainder, the majority harbored *P. malariae* alone (at days 7, 14, 14, 21, and 42) or mixed (day 3, 35), with only a single PCR-confirmed case of recurrent *P. falciparum* mono-infection (day 7). *P. vivax* DNA was detected in a single recurrence in the AL-treated group of the PCR-defined cohort at day 35, mixed with *P. falciparum* and *P. malariae*. Therefore, the specificity of microscopic identification of recurrent parasitemia was poor in our study, as judged by post hoc PCR.

An exploratory analysis of drug efficacy was then performed on both treatment arms of our PCR-defined cohort. First, we investigated parasite clearance dynamics of PCR-confirmed *P. falciparum* infection. We generated qPCR data for all enrolled study subjects for which DNA samples were available at days 0, 1, 2, and 3. Of the 114 confirmed *P. falciparum* patients, 60 were evaluated by qPCR at all 4 time points, 36 in the DP-treatment group and 24 in the AL group. Over 70% of patients in both groups cleared qPCR-detectable *Plasmodium* DNA within 24 hours, and only 4 (11%) and 3 (12%) receiving DP and AL, respectively, remained qPCR-positive at day 3 (Figure 2). Ninety percent or greater reduction in parasite density within 24 hours was achieved in 97% and 96% of patients treated with DP and AL, respectively. These data are consistent with continuing susceptibility to artemisinin in this *P. falciparum* population.

Table 2. Baseline Characteristics of Patients With PCR-Confirmed *P. falciparum* Infection

Characteristic	PCR-Confirmed *P. falciparum* Infection	
	Dihydroartemisinin-Piperaquine Group	Artemether-Lumefantrine Group
No. of evaluable patients	59	55
Infecting species at enrollment,[a] No. (%)		
P. falciparum	36 (61.0)	39 (70.9)
P. falciparum and *P. vivax*	9 (15.3)	5 (9.1)
P. falciparum and *P. malariae*	2 (3.4)	0 (0)
P. falciparum and *P. knowlesi*	8 (13.6)	10 (18.2)
P. falciparum, P. vivax, P. malariae	1 (1.7)	0 (0)
P. falciparum, P. vivax, P. knowlesi	1 (1.7)	1 (1.8)
P. falciparum, P. malariae, P. knowlesi	2 (3.4)	0 (0)
Median *P. falciparum* enrollment density (IQR), μL^{-1}	320 (272–1160)	400 (272–1520)
Female sex, No. (%)	35 (59.3)	25 (45.5)
Age		
Median (25%–75% range), y	11.8 (7–21)	22 (8–39)
<5 y, No. (%)	10 (16.9)	5 (9.1)
5–14 y, No. (%)	30 (50.9)	20 (36.4)
≥15 y, No. (%)	19 (32.2)	30 (54.6)
Temperature ≥37.5°C, No. (%)	9 (15.3)	8 (14.6)
Mean hemoglobin at enrollment (SD), g/dL	11.7 (1.9)	11.9 (1.7)
Mean hemoglobin at day 28 (SD), g/dL	11.8 (1.5)	11.7 (1.9)
No. of anemic patients (Hb <10.0 g/dL) (%)	8 (13.6)	4 (7.3)

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

[a]Identification and measurement by microscopy.

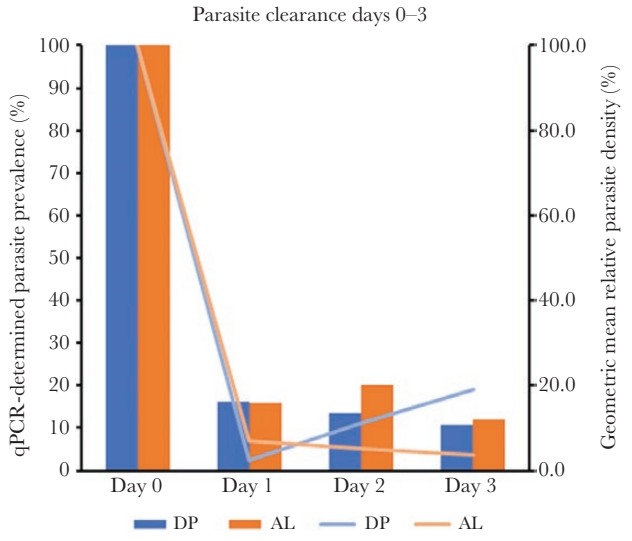

Figure 2. Parasite prevalence and relative parasite density as determined by qPCR at days 0, 1, 2, and 3. Prevalence of detectable *Plasmodium* DNA at each time point is shown as a percentage of tested individuals in each treatment group (vertical bars). Parasite density relative to day 0 in the same individual is presented as the geometric mean of all DNA-positive samples at each time point (colored lines). Data are shown for all evaluable patients confirmed PCR-positive for *P. falciparum* at day 0 (n = 37 for DP [blue]; n = 25 for AL [orange]). Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine; qPCR, quantitative polymerase chain reaction.

Second, with PCR-confirmed *P. falciparum* infections at enrollment, we identified parasite recurrence by nested PCR amplification of the *pfmdr1* locus in all available 4- and 6-week follow-up samples. *P. falciparum* DNA was detected on days 28 or 42 in 41% of evaluable patients in the DP group (n = 59), and 35% in the AL group (n = 55; odds ratio, 0.833; 95% confidence interval, 0.365–1.89; P = .636) (Figure 3). Remarkably, all 24 patients from Langkat harbored PCR-detectable *P. falciparum* DNA at day 28 or day 42, whereas in Batubara and Nias the proportion was much lower, at 6/30 (20%) and 13/60 (22%), respectively.

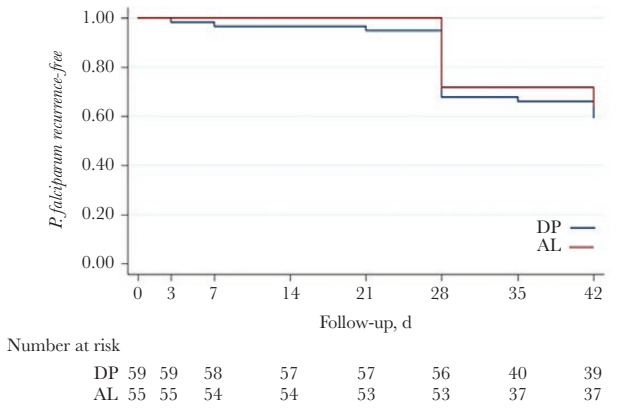

Figure 3. Survival rate of *P. falciparum* recurrences detected by nested PCR of *pfmdr1*. Post hoc exploratory efficacy assessment of patients with PCR-confirmed *P. falciparum* infection at enrollment, with recurrent *P. falciparum* parasitemia after treatment with DP or AL detected by nested PCR of the *pfmdr1* gene fragment encompassing codons 86 and 184. Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine.

Third, we explored ACT efficacy in patients harboring only non-*falciparum* malaria parasites, as measured by parasite clearance in the first 72 hours of treatment. Of 76 evaluable patients not harboring *P. falciparum*, 50 were PCR-positive for *P. vivax* at day 0, including 28 where this was the sole species (Table 1). Parasite clearance was measured in 30 of these individuals: All cleared qPCR-detectable parasitemia within 48 hours, except for 1 individual treated with DP who cleared parasitemia within 72 hours and 1 individual treated with AL who harbored persistent day 3 parasitemia, estimated as 0.3% of the starting parasite density. Three of 17 AL-treated patients with *P. vivax* (alone or mixed with *P. knowlesi* and/or *P. malariae*) were qPCR-positive at day 28 or day 42, equivalent to PCR-detected treatment failure of 17.6%, and 1 of 31 DP-treated patients with *P. vivax* (alone or mixed) was qPCR-positive at day 28, equivalent to a PCR-detected treatment failure of 3.2%. Of 19 *P. knowlesi* mono-infections, 8 generated parasite clearance data, of which 1 was qPCR-positive on day 3; this AL-treated individual was again qPCR-positive on day 28. Four individuals with *P. malariae* monoinfections at enrollment and 1 with both *P. malariae* and *P. knowlesi* were all qPCR-negative within 24 hours of commencing treatment with AL (2) or DP (3). Our qPCR detection target is specific only to the genus level, and further identification of the species present was unsuccessful in post-treatment isolates, which were of a low density. However, later recurrence of *P. malariae* did occur in 7 individuals originally treated for PCR-confirmed *P. falciparum*. In conclusion, this off-protocol analysis demonstrates the potential for qPCR to provide estimates of post-treatment parasite clearance dynamics and to detect recrudescence in non-*falciparum* malaria.

DISCUSSION

We report the results of an open-label efficacy comparison of DP (n = 153) vs AL (n = 149) for the treatment of uncomplicated malaria in 3 Regencies of the North Sumatera province, where *P. falciparum* co-circulates with *P. vivax, P. knowlesi,* and *P. malariae*. This is the Indonesian region closest to the GMS, where *P. falciparum* with reduced susceptibility to artemisinin, associated with slow clearance of ACT-treated infections, first emerged. We sought information on the current efficacy of ACT in these settings. Poor diagnostic accuracy of clinic microscopy led to enrollment and randomization of many individuals who did not meet the enrollment criteria, thus hampering any meaningful analysis on an intention-to-treat basis. Similarly, post hoc species-specific PCR indicated that many microscopically identified recurrent infections in follow-up did not harbor *Plasmodium* spp. DNA and had been misclassified. We therefore deviated from our original study protocol and restricted

analysis to a PCR-defined per-protocol cohort comprising 102 and 88 PCR-confirmed infections and assessed drug efficacy using both qualitative and quantitative DNA detection end points.

Clearance of PCR-confirmed *P. falciparum* infections over the first 72 hours of treatment (measured by qPCR for 62 individuals) was rapid for both regimens, with only 11% and 12% remaining qPCR-positive at day 3. This suggests that *P. falciparum* in Western Indonesia retains susceptibility to artemisinin, although the occurrence of 1 DP-treated individual with microscopy-positive (PCR-confirmed) early treatment failure on day 3 is of concern. Genotyping of this recrudescent day 3 infection at resistance-associated loci has been reported elsewhere [22]; it was found to carry a wild-type *pfkelch13* gene, *pfcrt* 72–76 haplotype SVMNT, *pfmdr1* 86/184/1246 haplotype YYD, and multiple copies of the *pfmdr1* gene. The *pfcrt* genotype is known to be an important modulator of parasite response to a variety of current antimalarials, including artemisinin, lumefantrine, and piperaquine [23, 24], but no studies have systematically examined the impact of the SVMNT haplotype on *P. falciparum* susceptibility to these drugs. We recently identified isolates from the China-Myanmar border region with the SVMNT haplotype of *pfcrt* also harboring *pfk13* propeller-domain mutations [25].

Two elements of our study provide reasons to be cautious regarding any firm conclusions about *P. falciparum* artemisinin susceptibility. First, our study was planned as a treatment trial for *falciparum* malaria patients, with and without other co-infecting species, expected to be *P. vivax* (https://clinicaltrials.gov: NCT02325180), but randomization between the 2 treatment arms was seriously compromised because of the large number of uninfected enrollees (Figure 1, Table 1). Randomization may also have potentially been compromised by the ad hoc screening procedure, in which participants were identified by different means across the 3 sites [8]. For this reason, we do not present any comparative statistical analysis between the drug arms, but present descriptive statistics only. Second, as demonstrated by our community-wide study, carried out simultaneously during recruitment, there is a complex mix of 4 *Plasmodium* species circulating in our study areas [8]. The use of DNA amplification methods to confirm the species present and to verify cases of putative treatment failure, was therefore important in determining the real (per-protocol) denominator of treatment failure in each treatment group, but is not a recognized approach to therapeutic efficacy studies.

A minority of microscopically detected parasite recurrences were confirmed to be PCR-positive for *Plasmodium* spp., and surprisingly the majority of these were due to *P. malariae,* despite *P. vivax* (68 individuals) and *P. knowlesi* (65 individuals) being much more prevalent at enrollment (Table 1). Our results therefore suggest that artemisinin is effective at rapidly clearing the majority of *P. vivax* and *P. knowlesi* infections and that *P. malariae* can rebound to microscopy- or PCR-detectable levels 2–6 weeks after ACT treatment. This is consistent with reports of *P. malariae* recurrence after ACT treatment in Ghana and Uganda [26, 27], with suggestions that the 72-hour intra-erythrocytic cycle of *P. malariae* provides an opportunity for this species to survive 3-day artemisinin exposure [28, 29]. This supports the argument that extended artemisinin regimens may be required for radical cure [30, 31], but these findings must be interpreted cautiously, given the problems with parasite identification at screening and enrollment.

Estimates of drug efficacy against PCR-confirmed *P. falciparum* infection were determined in each group at days 28 and 42. Here we found evidence of submicroscopic parasite persistence at day 28 and/or day 42 in 41% and 36% of the DP and AL groups, respectively (Figure 3). We conclude that, although both regimens seem effective at clearing initial parasitemia by qPCR, *P. falciparum* recurrence was a common occurrence with both regimens. Unfortunately, these presumably subpatent infections were not of sufficient parasite density to permit strain genotyping, but it is safe to say that neither 3-day ACT regimen provided protection against persistent submicroscopic *P. falciparum* for treated individuals at 4–6 weeks. As previously argued, longer sequential ACT treatment regimens over 6 days may provide both effective initial parasite clearance and prevention of *P. falciparum* recurrence [30], a strategy also likely to prove beneficial against *P. malariae* in North Sumatera [31].

Our results present a confusing picture of apparent good artemisinin efficacy, evidenced by satisfactory parasite clearance of all infecting *Plasmodium* species by day 3, overlaid by complex patterns of multispecies parasite recurrence manifested as PCR-detectable *P. falciparum* and microscopy-detectable *P. malariae*. Parasite DNA detection is not a recognized measure of antimalarial treatment outcome, and only microscopically detected parasitological treatment failure is recognized by current WHO protocols. This remains the appropriate current standard, as there are uncertainties as to the exact meaning of persistent parasite DNA in the treated individual. For example, in the case of *P. falciparum,* gametocyte emergence occurs late in infection and can provide a DNA biomarker in a treated individual despite effective clearance of all replicating asexual parasites [20]. It can also be argued that drug-killed parasites bequeath sufficient circulating DNA in the host that a positive PCR result does not necessarily mean viable, replicating parasites are still present. However, the lack of evidence that *P. vivax* DNA persisted in our treated individuals and work in both rodent malaria [32] and ACT-treated human infections where even hyperparasitemic patients rapidly become PCR-negative [19] strongly suggest this is not the case. Nevertheless, the results presented here suggest that work toward a new protocol for therapeutic efficacy studies for antimalarial drugs, in which molecular detection methods are intrinsic to enrollment as well as to outcomes, is needed.

The complexity of malaria in our study areas may be the result of human communities being isolated with poor access to diagnosis and treatment for malaria, as well as a tendency for low-density, multispecies chronic infections to develop among individuals exposed to regular mosquito challenge in these settings [8, 26]. Such established infections elicit immune responses from the host and can enter a steady state maintained by antigenic variation, and therefore may be more difficult to treat than acute infections [27]. This provides an additional rationale for the evaluation of extended ACT regimes as a means to reduce persistent parasitemia in treated individuals [30]. The complexity of malaria infections in Sumatera is a challenge for point-of-care diagnosis as well as for patient recruitment for antimalarial efficacy trials, as recently noted in neighboring Aceh Province [10], suggesting that further diagnostic and surveillance capacities are needed in the region [33].

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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