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Single-dose sulfadoxine–pyrimethamine plus artesunate–pyronaridine for malaria: a multicentre, randomized, controlled, assessor-blinded clinical trial

Ghyslain MOMBO-NGOMA
ghyslain.mombo-ngoma@bnitm.de
Bernhard-Nocht Institute für Tropenmedizin https://orcid.org/0000-0003-2658-8706
Alex Zinsou
CERMEL
Johanna Keuschnig
Bernhard-Nocht Institute für Tropenmedizin
Madeleine Eunice Betouke Ongwe
CERMEL
Wilfrid Ndzebe Ndoumba
CERMEL
Lumeka Kabwende
CERMEL
Cany Flamand Mucani Nsiengui
CERMEL
Alexia Charlotte Ovenga Nzelapanga
CERMEL
Lars Matthies
Bernhard-Nocht Institute für Tropenmedizin https://orcid.org/0009-0002-1956-2946
Claude Bernard Ako'o Mve
CERMEL https://orcid.org/0009-0009-7845-6044
Benjamin Mbadinga Ngoma
CERMEL
Lionel Mombo Nzamba
CERMEL
Saidou Mahamoudou
CERMEL
Bertrand Lell
CERMEL
Rella Zoleko Manego
CERMEL
Mario Recker
University of Tübingen
Ayola Akim Adegnika
University of Tübingen
Sanjeev Krishna
St George's, University of London

Michael Ramharter
Bernhard Nocht Institute for Tropical Medicine https://orcid.org/0000-0002-9259-1885
Peter Kremsner
Institut für Tropenmedizin, Universität Tübingen, Germany, and Centre de Recherches Medicales de Lambarene, Gabon.
https://orcid.org/0000-0002-3926-4113

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Abstract

First-line artemisinin-based combination therapies retain efficacies of >90% in the treatment of malaria in clinical trials. In everyday practice their effectiveness is substantially lower, and sub-optimal treatment-adherence with multidose 3-day regimens contributes to treatment failures. Poor adherence also increases risks of selecting for drug resistances. Simplified therapeutic regimens with high efficacy can reinvigorate progress towards malaria elimination. Ideally, a single dose regimen will be most useful as it allows supervision of drug intake by healthcare personnel. Developing single-dose treatments using existing antimalarials has many potential advantages over new drug combinations. Efficacy and safety of these treatments is established, and access and cost limitations are largely addressed compared with those of new entities. We assessed a single dose therapy of two established antimalarial combinations consisting of sulfadoxine-pyrimethamine plus artesunate-pyronaridine (SPAP) in a pragmatic, non-inferiority, multi-centre, randomised, controlled clinical trial in patients with uncomplicated malaria in Gabon. The primary efficacy outcome was day 28 adequate clinical and parasitological response (ACPR). 1021 patients received SPAP (54%, 555/1021) or artemether-lumefantrine (AL) (46%, 466/1021). The modified intention to treat day 28 uncorrected ACPR were 92% (95% CI: 90, 93) in the SPAP treatment arm and 89% (95% CI: 88-90%) in the AL treatment arm (OR 1.35, 95% CI: 0.85-2.15), demonstrating non-inferiority of SPAP to AL. PCR-corrected day 28 ACPR rates were 98% (95% CI: 97-99%) in the SPAP treatment arm and 97% (95% CI: 96-98%) in the AL treatment arm (OR 1.41, 95% CI: 0.55, 3.58). The robustness of these findings was confirmed in the per protocol and sensitivity analyses. SPAP was associated with more episodes of vomiting within one hour of administration 19/555 (3.4%) than AL 3/466 (0.6%). Overall safety was comparable between regimens. Single dose SPAP is as safe and efficacious as three-day AL. Efficacy in settings with different patterns of SP resistance and optimising formulation for dosing and tolerability could accelerate development of SPAP as a readily available and affordable single-dose regimen for treating uncomplicated malaria. Registration: PACTR202405571736678. Funding: Centre de Recherches Médicales de Lambaréné (CERMEL).

Main

Malaria remains a major global health challenge, with an estimated 282 million cases and 610,000 deaths in 2024[1]. *Plasmodium falciparum* causes about 95% of malaria cases and most malaria related deaths in Africa. Early effective treatment prevents progression to severe disease and chronic complications of infection such as anaemia. New tools and better use of existing ones are urgently needed to better combat malaria.

The World Health Organization (WHO) recommends artemisinin-based combination therapies (ACTs), which are given over three days. Their efficacy in clinical trials remains >90%, but their effectiveness in practice has poorer outcomes, with the global effectiveness of ACTs estimated at 72% between 2016 and 2019[2], mainly because of challenges with adherence to a three-day, multidose treatment course[3,4]. While treatment adherence with ACT regimens varies between studies, it may fall to as low as 40% [5,6].

Simplified therapeutic regimens that enhance adherence could play a critical role in reinvigorating progress toward malaria elimination. Over the past decade, substantial progress has been made in the discovery and development of new chemical entities for malaria, although the most advanced candidates still require a three-day dosing regimen. Shortening treatment courses requires multiple drug combinations to achieve high cure rates and to prevent the selection of drug resistant isolates. Multi-drug regimens, such as artemether-lumefantrine-amodiaquine are well tolerated[7], but these aim to address emerging resistance to lumefantrine and not to improve treatment adherence[8–10].

The current malaria pipeline has encouraging candidates that may eventually address some of these issues[11]. However, registration of new chemical entities and uptake into global malaria programmes may take another 5-10 years and may deliver products that still need to address the challenges of access and affordability. Future antimalarial treatment strategies must improve adherence, reduce the risk of selecting for drug resistance, minimise costs, and preserve safety

and efficacy. One solution is to make best use of existing, approved drugs by assessing new ways to combine them[12]. These approaches can also have immediate impact in vulnerable populations such as pregnant women and young children.

Sulfadoxine-pyrimethamine (SP) has well defined mechanisms of antiparasitic action that are distinct from ACTs and well-established safety and favourable cost profiles[13]. Artesunate-pyronaridine (AP) was identified as our best choice, to combine with SP aiming to achieve a single-dose cure because it has a good safety and tolerability record, emerging data in early pregnancy, no significant food effect, and no confirmed resistance[12]. In addition it has been shown to be highly efficacious in single dose treatment of asymptomatic infections[14].

We conducted a randomised controlled clinical trial to assess the new combination of single-dose SPAP against the most widely used ACT, twice-daily three-day artemether-lumefantrine (AL). The primary objective was to compare the efficacy of single-dose SPAP with standard twice-daily three-day AL for the treatment of uncomplicated malaria. The main secondary objective was to describe the safety and tolerability profile of SPAP.

Results

Study design

This was a pragmatic, single-country, multicentre, open-label, assessor-blinded, non-inferiority, randomized controlled clinical trial. Directly observed single-dose SPAP was compared with standard twice-daily three-day AL treatment. The first dose of AL was directly observed and subsequent doses were unsupervised. A 1:1 randomisation ratio was used for treatment allocation of participants into the clinical trial. In case of a subsequent malaria episode after the last day of follow-up, patients were eligible for re-screening and were allocated to the SPAP treatment group to increase the safety and efficacy data for the SPAP regimen. Follow-up assessments were on day 0, day 7, day 28, and day 42 and also at unscheduled visits. The primary efficacy endpoint was uncorrected and PCR-corrected ACPR at day 28 in the modified intention-to treat (mITT) population and secondarily in the per protocol ACPR at day 28, both PCR-uncorrected and corrected. The main secondary safety endpoint was the incidence of adverse events including serious adverse events and adverse events of special interest.

All patients with suspected uncomplicated malaria (according to the WHO criteria) underwent a rapid diagnostic test or microscopy of a blood smear. If malaria was confirmed and the patient (or legal representative in case of children) provided informed consent, they were included in the study if they could take oral medication and weighed ≥ 5 kg. Exclusion criteria included signs or symptoms of hepatic injury or known liver disease, known allergy to any of the study drugs, pregnancy in the first trimester, severe malaria, and patients that investigators considered would be at risk if receiving an anti-malarial or if participating in the study. Further details of study design and study population are reported in the Methods section.

Patients disposition and characteristics

Overall, 944 individuals with uncomplicated malaria provided their consent and were screened for eligibility from 25 May 2024 to 31 December 2025. Eleven of these patients did not meet the selection criteria because of severe anaemia (n=4), hepatic injury (n=1), parent's refusal (n=1), and investigator's decision (n=5). 933 individuals were randomized to SPAP (n=467) and AL (n=466). During the study period and as indicated above, there were 77 subsequent malaria episodes (E01) outside of the follow-up window, 42 from the AL group and 35 from the SPAP group. They were re-screened and allocated to SPAP, and of these 11 had a second subsequent malaria episode (E02) that was again allocated to SPAP, making it a total of 1021 treated malaria episodes (AL: 466 (46%), SPAP: 555 (54%)) to be evaluated for safety and efficacy. The re-treatment of a new malaria episode after the follow-up period was chosen to be SPAP, to provide more safety and efficacy data for this experimental regimen. This added an extra 88 malaria episodes to the SPAP arm, explaining the eventually unbalanced sample size in Table 1.

Study sites recruited as follows: 107 (10%) in Four-place, 325 (32%) in Lambaréné, 214 (21%) in Sindara, and 375 (37%) in Tchibanga (Table 1). Details of patients' allocation into groups, treatment, follow-ups and populations of analysis are shown in Fig.1.

868 (85%) of 1021 patients completed the day 28 follow-up visit, 398 (85%) in the AL arm and 470 (85%) in the SPAP arm, of which 833 were evaluable with a valid blood slide reading and visit within -3/+7 days range of the scheduled date.

For the mITT day 28 PCR-uncorrected efficacy analysis, a total of 188 individuals with either missing outcome data or whose day 28 visit was outside the -3/+7 days range were imputed using multiple imputation (see Methods). In the PCR-corrected efficacy analysis, only slide-positive samples with a definite PCR interpretation as either a new infection (n=39) or recrudescent infection (n=17) were considered alongside slide-negative samples; 219 individual outcomes were imputed including those with failed PCR results (n=5) or undetermined results (n=26). The day 28 per protocol (PP) efficacy population included 833 individuals in the uncorrected analysis and 802 individuals in the PCR-corrected analysis.

782 (77%) of the 1021 malaria patients enrolled completed the day 42 follow-up visit, 362 in the AL arm and 420 in the SPAP arm. The day 42 efficacy analyses of 1021 malaria episodes in the mITT PCR-uncorrected analysis included 268 imputed individual outcomes, and PCR-corrected imputed 376 individual outcomes. The PP population at that time-point included 753 patients in the uncorrected analysis and 645 in the PCR-corrected analysis.

The safety population included all 1021 participants that took at least one dose of the study drugs. Details of the safety, mITT and PP populations for day 28 and day 42 for both uncorrected and PCR-corrected analyses where applicable are presented in Fig. 1.

Baseline characteristics of all enrolled and treated individuals, as well as those considered for the various endpoint analyses, were comparable between the two treatment arms (Table 1).

Compliance with study treatment

Of 1021 treated patients, 22 vomited within the hour following study drug administration. Of these, 20 were re-dosed successfully. One patient in the control group (AL) was unwilling to take the re-administered dose and one patient in the intervention group (SPAP) had repeated vomiting and was treated parenterally. Both patients received rescue medication for malaria according to national guidelines.

In the AL treatment group, out of 466 enrolled and treated, 227 returned their study drugs' boxes and only 2/227 (0.9%) had tablets left suggesting a relatively high compliance with the instructions of the study team.

Efficacy Results

Because of the pragmatic design of the study, the primary efficacy analysis was performed on the mITT population for both uncorrected and PCR-corrected ACPR at day 28. The main secondary efficacy analysis was the per protocol efficacy both uncorrected and PCR-corrected. Therefore, all results presented below refer to the mITT analysis unless specified otherwise.

Overall, for the day 28 efficacy, the PCR-uncorrected ACPR was 89% for AL, and 92% for SPAP, giving an OR 1.35 (95% CI: 0.85-2.15). The PCR-corrected ACPR was high for both AL (97%) and SPAP (98%), OR 1.41 (95% CI: 0.55-3.58). For both the PCR-uncorrected and corrected ACPR at day 28, therapeutic non-inferiority of SPAP to AL was established as the odds ratio estimates were above the pre-specified non-inferiority margin (0.474) (Table 2). The analyses in the per protocol population (Table 2) and all sensitivity analyses (Extended Data Table S1) confirmed the robustness of these results.

When looking at individual sites, PCR-uncorrected efficacy of SPAP as compared to AL showed a tendency to be higher in rural sites Four-place and Sindara, for both the mITT and PP populations. At urban sites it was similar (Fig.2 and Extended

Data Table S2). Further analysis of the uncorrected efficacy by age, body weight and parasite density showed that SPAP had similar efficacy to AL in all ages, including very young children, and high parasite densities (Extended Data Table S3).

The mITT uncorrected day 42 ACPR rates were 78% for AL and 83% for SPAP, OR 1.36 (95% CI: 0.95-1.93) (Table 3), showing no statistically significant difference between the two treatment groups, although there was a trend towards better performance of SPAP. The same trend was observed at site level, as well as for age groups, weight, and parasite density (Extended Data Table S4). After PCR-correction, day 42 ACPR was the same for both AL and SPAP (96%) (Table 3). The PP uncorrected day 42 ACPR rates were 77% in the AL group and 83% in the SPAP group, OR 1.45 (95% CI: 1.01-2.08). The PP PCR-corrected ACPR rate was 97% in both AL and SPAP groups (Table 3).

Safety Results

All the 1021 patients who received any study drugs were included in the safety analysis. There were 235 adverse events reported, 96 in the AL treatment group and 139 in the SPAP treatment group. The most commonly reported adverse events were vomiting (14%), common cold (11%), fever (10%), drop of haemoglobin > 2g/dl from baseline (7%), and some others as shown in Table 4. Most adverse events were mild (35%) to moderate (62%). Most adverse events (86%) were judged not to be related to the study drugs. Treatments were generally well tolerated with 22 patients (2.2%, 95% CI: 1.4-3.2%) vomiting within an hour after drug administration, 3/466 (0.6%, 95% CI: 0.4-2.0%) in the AL treatment arm and 19/555 (3.4%, 95% CI: 2.1-5.3%) in the SPAP treatment arm. Later episodes of vomiting were not considered related to study drugs.

There were 10 serious adverse events (SAEs) reported in this study, four were in the AL treatment arm and six were in the SPAP treatment arm. Most SAEs were unrelated to study drugs but were classified as serious because they required hospitalisation (Extended Data Table S6). All but one patient with SAEs recovered. A 12 years old male patient allocated to AL with sickle cell disease was hospitalised on the day following inclusion in the study for a vasoocclusive crisis complicated by severe anaemia He subsequently died. Two SAEs were classified as adverse events of special interest (AESI) as prespecified in the study protocol. One was a patient in the SPAP treatment arm presenting with transient and clinically asymptomatic grade 3 elevation of liver enzymes that was reported around four weeks after drug intake and normalized within a week. The other AESI was pruritus in a 42 years old female patient that occurred on the third study day following treatment with SPAP.

Discussion

In this malaria treatment trial, a single-dose of SPAP was non-inferior to the 3-day multidose AL in all analyses including the PCR-uncorrected and -corrected mITT and the per protocol analyses. For all endpoints, SPAP performed as well as or better than AP (ORs > 1 in all cases), with statistically significant superiority demonstrated in the per protocol day 42 PCR-uncorrected ACPR. Safety was similar for the two treatments for uncomplicated malaria in Gabonese patients. These findings were consistent in all age groups, irrespective of parasite density, and in rural and urban populations in areas of perennial and high transmission in Gabon. Both AL and SPAP were highly efficacious with PCR-corrected ACPRs > 95%. These exceed the WHO threshold of 90% for acceptable outcomes[15]. Vomiting within an hour after dosing was more common in the SPAP group, although repeated dosing was well tolerated.

The increased risk of vomiting with SPAP (3%), which is still relatively low compared with other multidrug combinations, may in the future be reduced by developing a fixed-dose combination in granules that will also allow easier administration. In the literature, early vomiting (within one hour of dose administration) is reported in 3%-11% of adults, and can rise to > 15% in children with malaria, particularly those who are breastfeeding[16,17]. Mefloquine or amodiaquine containing ACTs have been associated with a modest increase in vomiting[18]. In our study, all re-treatable patients vomiting within the first hour were successfully re-administered the study drugs. In health facility settings, SPAP treatment could be administered under direct observation for one hour to ensure successful re-dosing after early vomiting.

A few patients in the SPAP group developed slightly elevated liver enzymes transiently, but these were short lived self-limited findings as previously reported with AP treatment[19]. A previous, small safety study that included patients with uncomplicated malaria, across all ages allowed a more detailed safety assessment. Clinical and biological data including for haematology and biochemistry were collected at days 3, 7, 28, and 42. Results from this safety cohort study will be reported elsewhere; in brief, no related serious adverse events were seen and all measured parameters were within acceptable ranges.

Single-dose treatments for uncomplicated malaria have several challenges to overcome: their pharmacological profiles have to cover at least 3 cycles of replication for *P. falciparum* (6 days); they have to retain effectiveness in individuals with variable degrees of antimalarial immunity, including very young children, and they should work when parasite counts are high. For a sensitivity-analysis we set an arbitrary parasite density threshold of 5000 / µl to evaluate treatment outcomes of low and high parasitaemic patients, respectively. Our finding that SPAP was similarly effective in low and high parasiteemias suggests that the previously observed potential of AP to clear parasites when administered as a single dose[14], has been enhanced by the combination with SP.

The widespread use of SP in chemoprevention is likely to maintain, rather than reduce, resistance levels in parasite populations in Africa[20], in contrast to the re-emergence of chloroquine-sensitive parasites following withdrawal of drug pressure[21,22]. Despite this, SP shows notable clinical resilience as a drug combination, which may in part reflect the matched pharmacokinetics of its components and their synergistic activity[23].

In the present study, the rapid parasite clearance associated with artesunate–pyronaridine, together with the relatively long half-life of pyronaridine, is likely to complement the prolonged activity of SP, providing a pharmacological basis for the observed efficacy of the SPAP combination. Consistent with this, very few recrudescences were identified by PCR, and these were evenly distributed between treatment arms. Genotyping of resistance markers in recrudescent infections is planned.

The relationship between mutations in the target genes *dihydrofolate reductase* (*dhfr*) and *dihydropteroate synthase* (*dhps*) and clinical efficacy is not straightforward, as SP is clinically relevant even in the presence of multiple resistance mutations[24,25]. Host immunity, drug metabolism, and infection multiplicity are likely to contribute to this discordance. In chemoprevention studies, SP-based regimens remain effective even in settings with a high prevalence of quintuple *dhfr/dhps* mutations[26].

In this context, SP appears to contribute to the overall efficacy of the SPAP combination despite the presence of established resistance markers. This is particularly relevant in settings such as Gabon, where a high prevalence of quadruple mutations and increasing frequencies of quintuple mutants have been reported[27,28].

Although estimated costs for SPAP suggest the loose-dose combination is approximately US$0.95[29] and in the same range as the three-day adult course of AL priced at US$0.60, more detailed pharmacoeconomic modelling that accounts for all variables such as ease of use, adherence, efficacy and other variables will be needed to fully characterise the advantages and cost effectiveness of single-dose cures.

Our trial may have several limitations by its design, which may *pari passu* be advantageous. It was designed to reflect real-world settings with patients diagnosed with malaria based on rapid diagnostic test (RDT) or blood film microscopy as it is done in malaria endemic settings. Follow-up visits were deliberately limited to day 7 after treatment to record events that could have occurred at home following study drugs administration, and visits on days 28 and 42 to evaluate the efficacy as recommended by WHO. This real-world design has the advantage of being less biased as it includes virtually all malaria patients with the maximum of participants in the analysis. It was inspired from a validated protocol for an artesunate-pyronaridine study that included 8,560 malaria patients[19]. Our trial had an open label design. The inherent risk of bias was managed in several ways. Strict randomisation ensured comparability of baseline characteristics between groups. The

higher number of patients in the SPAP group was through allocation of patients with recurrent malaria episodes to the SPAP treatment arm, enabling collection of additional safety and efficacy data for re-treated patients. In addition, a randomisation method was used that allows for chance imbalances as explained in Methods. Allocation was concealed from investigators and those assessing blood films. Although 15% of patients were lost to follow-up at day 28 - the proportion of drop-outs was similar in both treatment arms. The numbers remained sufficient for evaluation of the primary outcome. Though the study was an open-label design with the patient and healthcare provider knowing the assigned treatment, assessor blinding was implemented to ensure that the lab personnel including the microscopists evaluating efficacy outcomes were unaware of which treatment a participant received[30].

The findings of this study can be generalised to the extent that all malaria patients, adults and children of both sex have been included. Although conducted in a single country, these findings support further evaluation in multinational trials – including regions with high prevalence of quintuple mutations as biomarkers of SP resistance where suitable drug formulations may further improve tolerability and ease of use of SPAP.

The single-dose combination of SPAP had efficacy and a safety profile comparable to AL. A single dose SPAP regimen therefore offers a simple and readily deployable treatment for uncomplicated malaria. A granule formulation of SPAP has been developed and will be evaluated in a phase III trial soon.

Methods

Study settings

This clinical trial took place in four sites of the Centre de Recherches Médicales de Lambaréné (CERMEL), located in Gabon. The recruitment sites, well distributed over Gabon and representing urban and rural settings were Lambaréné, an urban site in the Moyen-Ogooué province[31], Sindara, a rural site in Ngounié province[32], Four-place, a rural site in the province of Estuaire, and Mighoma/Tchibanga, a semi-urban site in the Nyanga province.

Clinical trial design and oversight

This study was designed as a pragmatic, single country, multi-site, open-label, assessor-blinded, randomized, controlled, non-inferiority study. All the patients for whom a diagnosis of uncomplicated malaria (according to the WHO criteria) was suspected, underwent a malaria test. If malaria was confirmed by either rapid test or thick blood smear microscopy and the patient (or parent/ guardian in case of children) provided his/her informed consent, he/she was assigned a unique study number and was enrolled in the study. Blood was taken for a dried blood spot for PCR analysis, and for full blood count and clinical chemistry. The single dose of SPAP and the first dose of AL were given under direct observation, but patients took their subsequent doses of AL without direct supervision under usual conditions at home. Patients were also required to attend the health care facilities if they had any adverse events.

Patients were visited at home at day 7 ±1 day, by a community health worker, in order to capture the adverse events experienced since study drug administration. Blood was taken for thick blood smear and a blood spot. In the case of adverse events that could reflect any organ toxicity, the patients were referred to the health facilities and blood was taken on this occasion for biological assessment. If patients could not be directly reached, the community health worker organised an appointment with this patient within a -3/+7 days range. Special procedures were followed in case of serious adverse events and/or severe adverse events and adverse events of special interest.

At day 28 ±2 days, all patients were seen at the research centres for the primary outcome assessment. At this occasion, blood was also taken for thick blood smear and blood spot as well as full blood count and clinical chemistry. Adverse events occurring since the previous day 7 visit were monitored and recorded.

At day 42 ±2 days, all patients were seen by a community health worker for a final assessment. At this occasion, blood was only taken for thick blood smear and blood spot. In the case of adverse events occurring since the previous day 28 visit, the patient was referred to the health facilities and blood taken for safety assessment.

Ethical approval

This clinical trial complies with all relevant ethical regulations and was conducted in accordance with the trial protocol and the ethical principles originating in the Declaration of Helsinki and guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH-GCP). The clinical trial protocol was approved by the Scientific Review Committee (SRC-2024/03) and the Institutional Ethics Committee of CERMEL (CEI-005/2024). Each patient or their legally accepted representative signed an informed consent form before any study procedure was initiated.

Trial population and patient selection

Inclusion criteria included uncomplicated malaria diagnosed as per national policies and in line with WHO recommendations (fever or history of fever in the previous 24 hours; confirmed malaria by a parasitological diagnosis, rapid test or thick blood smear), body weight ≥ 5 kg, ability to take oral medication, ability and willingness to participate based on a signed informed consent. Exclusion criteria were clinical signs or symptoms of hepatic injury or known severe liver disease, known allergy to any of the study drugs, known pregnancy in the first trimester, severe malaria, patients that the investigator considered would be at particular risk if receiving an anti-malarial or if participating in the study.

A patient was included in the study when he/she met the selection criteria of the protocol and agreed to participate. In particular, to enter the study, the patient had to accept: (i) that a small amount of blood was taken for the preparation of a blood spot, as well as for a safety assessment, (ii) that the community health worker visits him/her at home, (iii) that the data collected during those visits are registered and analysed and finally (iv) that, in case of adverse events classified as severe he/she had to contact the community health worker immediately.

Randomisation and masking

After informed consent was signed, participants were attributed a study number and the corresponding sealed envelope was opened to reveal the assigned treatment group. Participants were randomly assigned (randomisation ratio 1:1) to single dose SPAP or twice-daily AL for three days. Allocation of participants to study arms was done centrally by the clinical sponsor using R software, generating site specific randomisation lists. The method employed was randomisation that assigned participants to treatment arms within specific trial sites without enforcing rigid, predefined block sizes[33]. This method avoids predictable treatment sequences, allowing each patient an independent probability of assignment while accounting for site-specific variations. Instead of maintaining a strict sequential balance (such as used in permuted block-randomisation approaches), this design typically relies on complete/unrestricted randomization or biased-coin algorithms at the individual site level. The advantages of the method include the allocation concealment that eliminates the predictability of upcoming treatment assignments inherent to block randomization, and logistical flexibility as it is highly adaptable for trials where sites are added mid-study. A consequence of this randomisation method is the absence of identical group sizes. Study number allocation for each participant was concealed in sealed opaque envelopes that were opened only after recruitment.

The investigators were not formally blinded but the study drugs were allocated and given by the pharmacists away from the investigators. The strict blinding was not done because SPAP was given as a single dose while AL was given twice daily for three days. However, the microscopists who determined the efficacy results were kept masked from the study drug allocation. We can therefore say that the study was assessor-blinded.

As this study was designed as a real-world assessment, to mimic reality, patients could be included more than once in the study in case of any new malaria episode beyond the 42-day follow-up period. Subsequent malaria episodes were allocated

to the SPAP treatment group to collect additional data on re-treated subjects for the experimental treatment arm even for patients that were initially allocated to the AL group.

Study drugs prescription and dosing

In the intervention arm, SPAP was given as a loose combination of SP and AP administered simultaneously. A fixed-dose of AP (Pyramax® Shin Poong Pharmaceutical, Seoul, South Korea) was administered once according to the bodyweight, with two formulations to facilitate the dosing in young infants, children and adults: 60:20mg granules and 180:60mg tablets of pyronaridine and artesunate, respectively. A fixed-dose of SP (500:25mg) tablets were given orally once, according to bodyweight. 25mg/kg sulfadoxine and 1.25mg/kg pyrimethamine as single dose.

In the control arm, a fixed-dose of artemether-lumefantrine (AL) (20:120mg and 80:480mg) (COARTEM®, Novartis, Basle, Switzerland) tablets were given orally, twice daily. The patients were instructed to take one AL dose twice-daily over a period of three days, i.e. at day 0 (initial dose at hour 0 and then after 8 hours), then at day 1 (after 24 hours and 36 hours) and then on day 2 (after 48 hours and then after 60 hours). Only the first dose was administered under supervision in the clinic, the subsequent doses were taken by the subject at home.

Data collection

Demographic data and vital signs

Demographic data were collected at baseline including age (reported or deduced from the date of birth), sex, weight, height and vital signs (pulse and blood pressure), body temperature. Vital signs and body temperature were further collected at each scheduled and unscheduled visit.

For further statistical analysis, age was categorised into five groups including infants (≤ 2 years), young children (3 – 5 years), school-aged children (6 – 10 years), adolescents (11 – 20 years), and adults (>20 years). Considering that very small bodyweight is associated with drug formulation issues, the bodyweight was dichotomised into ≤10 kg and >10 kg. Body temperature was categorised to fever (axillary temperature >37.5°C) and no fever. Parasite density was set into two categories, ≤5000 parasites per µl and above.

Collection of and blood smears and spots

At each scheduled trial visit and at all unscheduled illness visits at which malaria was suspected, a finger prick blood (or venipuncture) sample was collected from which a Giemsa stained thick blood smear was obtained, as well as dried blood spots onto Protein Saver 903 DBS cards (Whatmann). Blood smears were prepared according to the Lambaréné method[34,35] and were read according to the standard operating procedures at CERMEL.

Blood smear microscopy

Blood smears were read by two independent readers who were unaware of the trial group assignments. A third reader was involved in case of discrepancies between the two initial readers.

Extraction of genomic DNA

Genomic DNA was extracted from dried blood spots using the QIamp DNA mini-kits (Qiagen GmbH, Hilden, Germany), according to the manufacturer's recommendations. Shortly, two pieces of each dried blood spot sample were cut with unicore punch (Lot: 178042009; Ref: WB100039) with sterilization using 70% ethanol between samples. The cut portions of dried blood spots were used for DNA extraction. The DNA extracted was eluted in 100 µL of elution buffer, aliquoted, labelled and stored at -80^{o} C until further analyses.

Genotyping of the msp1, msp2 and TA60

The msp1, msp2 and TA60 fragments of *P. falciparum* were amplified by conventional PCR according to a modified protocol[36]. In summary, the PCR reactions used a set of primers corresponding to the conserved regions of block 2 for msp1, block 3 for msp2 and a microsatellite TA60. The template-free control was used in all reactions and the genomic DNA from laboratory strains 3D7, HB3 and 7G8 were used as a positive control. Dilutions were performed on highly concentrated DNA samples and all PCR products were analysed by electrophoresis on 1.5% agarose gel. PCR products were denaturated in Hi-Di ^{TM} and then transferred to a genotyping plate for DNA fragments sizing by capillary electrophoresis using Seq Studio (Seqstudio™ Genetic Analyzer, A33770, SN: 232001229). Fragment size was measured using Gene Mapper software Version 6.0. The height limit for minor clones was set at more than 30% of the height of the dominant peak, and the threshold was 500 relative fluorescence units (RFU). Samples that successfully genotyped on day 0 (screening, before treatment) and on day of the recurrent infections for the all markers (msp1, msp2, and TA60) were considered for the analysis to distinguish recrudescence from new infection according to WHO recommendations (WHO, 2021). This molecular analysis was carried out at the Research Laboratory of Immunology and Molecular Biology of the CERMEL in Gabon.

Study data were collected and managed using REDCap electronic data capture tools hosted at CERMEL[37,38]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Analysis of primary efficacy outcomes

There were two co-primary clinical outcome measures:

1. PCR uncorrected ACPR up to day 28: treatment failure detected during the active follow-up period, regardless of genotyping or Plasmodium species.
2. PCR-corrected ACPR up to day 28: early failure before day 7 plus recurrent parasitaemia detected at day 7 or later provided they are classified by genotyping as recrudescence. Thus, patients presenting PCR results as new infection were considered success while those presenting PCR results as recrudescence were considered failure. In case where there was recurrent malaria following treatment, post-treatment samples could contain parasite that were present in pre-treatment samples as well as new strains not present in pre-treatment samples, defined as mixed genotype results. These were considered as recrudescent infections. If PCR results showed that the same parasite strains were found in both pre- and post-treatment samples the outcome was defined as recrudescence.

To provide a pragmatic comparison of the policies of the different drug treatments, the principle of intention-to-treat, as far as is practically possible, was the main strategy of analysis adopted for the two primary endpoints. These analyses were conducted on all patients assigned to the treatment groups SPAP, or AL as randomised, regardless of the study treatment or non-study treatment received.

As recommended for non-inferiority trials, an analysis on a per-protocol approach was conducted to assess the outcomes of treatments when applied to the protocol defined study population following the study protocol. Protocol deviations and violations were classified as minor and major where minor deviations could be included in the PP analysis population and major violations were excluded.

The main study hypothesis was that the single dose multidrug combination with SPAP is non-inferior to the standard 3-day AL as measured by the proportion of patients with PCR corrected and uncorrected adequate clinical and parasitological response (ACPR) up to day 28. The limit of clinical non-inferiority was set at an absolute difference in efficacy between treatments of no more than 5%. The expected success rate in the AL (control) group was 95%, meaning the success rate in the SPAP (intervention) group was not to be worse than 90%. The planned analysis strategy resulted in estimates of

treatment differences on an odds ratio scale, which under these assumptions equated to 0.474. If the true odds ratio for treatment success was higher than the limit of non-inferiority on the odds ratio scale, SPAP would be therapeutically non-inferior to AL. If the true odds ratio for treatment success was similar than the limit of non-inferiority, SPAP would be inferior to AL. The hypothesis was tested by calculating the two-sided 95% confidence interval for the odds ratio from the observed data. If the two-sided 95% confidence interval for the odds ratio in success rates lied entirely above the odds ratio limit then therapeutic non-inferiority of SPAP to AL was concluded; if it contained the odds ratio limit then therapeutic non-inferiority could not be established; if it lied entirely below the limit, then SPAP was judged inferior to AL. In addition, statistical significance was assessed using a one-sided Wald test of the pooled treatment effect against the pre-specified non-inferiority margin on the odds-ratio scale (0.474). The pooled log odds ratio and its variance were obtained using Rubin's rules across the multiply imputed datasets (see below). The test statistic was calculated as the difference between the pooled log odds ratio and the logarithm of the non-inferiority margin, divided by the pooled standard error. The one-sided p-value was obtained from a *t*-distribution using the degrees of freedom derived according to Rubin's rules.

For the efficacy analysis, both a modified intention-to-treat (mITT) and a per-protocol (PP) approach were adopted, with the mITT analysis being the primary approach, to provide a pragmatic comparison of the policies of the different drug treatments. In the mITT analysis, missing efficacy outcomes, defined as absence of parasitological outcome data within the pre-defined time window (day 28 -3/+7 days and day 42 -3/+7 days), were handled using multiple imputation by chained equations under the assumption that data were missing at random (MAR) using the mice R package[39]. The binary primary endpoint was imputed using logistic regression including age, baseline parasitaemia, and treatment group as predictors. A total of 1,000 imputed datasets were generated to account for uncertainty associated with the imputation of missing outcomes. Each imputed dataset was analysed separately according to the pre-specified analysis model, and estimates were combined across imputations using Rubin's rules. Efficacy outcomes were expressed as odds ratios (ORs) with corresponding 95% confidence intervals; note, since the imputation procedure constitutes a probabilistic prediction on a group level, no prediction of individual treatment outcomes was performed but rather prediction of the unobserved group. Imputation was performed separately for day 28 and day 42 analysis and for PCR-uncorrected and corrected results.

Sensitivity analysis were performed as worst-case scenario (all unobserved outcomes classified as treatment failure – sensitivity analysis 1) and best-case (all unobserved efficacy outcomes classified as success – sensitivity analysis 2) and depicted as point estimates and 95% confidence intervals. In the Per Protocol (PP) analysis, patients with missing efficacy outcomes were excluded from analysis and no imputation or sensitivity analysis were performed.

Other explorative analyses were performed using fixed effects logistic regression models with treatment outcomes (success/failure) as dependent variable and treatment arm, study site and their interaction as fixed effects. Other baseline characteristics, such as age, weight and initial parasitaemia were considered individually alongside treatment arm. Results are provided as point estimates and associated CIs on the OR scale and presented as forest plots.

All analyses were performed using R version 4.6.1 (R Core Team, 2026)[40].

Sample size and Power considerations

With an absolute non-inferiority margin of 5% under the assumption of 95% success rate (ACPR), equating to a non-inferiority limit of 0.474 on the odds ratio scale, a one-sided significance level of 2.5% ($\alpha = 0.025$), 80% power ($\beta = 0.2$), adjusting for 10% loss to follow-up, and a 1:1 randomization ratio, the required sample size per group is 329 participants, making a total sample size (both groups) of 658 participants. To allow for a more comprehensive safety evaluation of the new treatment regimen, the statistical analysis plan defined to enrol at least 1,000 patients leading at the same time to higher precision for the treatment outcomes. ACPRs are presented as n/N (%, 95% CI). Treatment difference is expressed as odds ratio and 95% CI.

Description of safety outcomes

For the analysis of safety outcomes, all patients who received any study drug were included in the safety analysis dataset in the treatment group they actually received (all-patients-treated approach). All adverse events (AEs) and serious adverse events (SAEs) reported were presented, identified by treatment group. Malaria was not reported as an AE since it was an efficacy outcome and was reported as such.

Analysis of secondary efficacy outcomes

The analysis of secondary efficacy outcomes (the per protocol efficacy, both uncorrected and PCR-corrected) followed the same approach as detailed for the primary efficacy outcome analysis, with the main difference that missing outcome data was excluded, rather than imputed. Efficacy outcomes are expressed as odds ratios (ORs) with corresponding 95% confidence intervals. Statistical significance of non-inferiority was assessed by comparing the inferred effect against the pre-specified non-inferiority margin, with the one-sided p-value calculated from the Wald test statistic using the standard normal distribution.

Declarations

Data availability

Requests for access to the individual deidentified trial participant dataset can be submitted to corresponding author GMN for a period up to 24 months after publication of this article. Upon reasonable request (outlined proposal for the intended analysis and a list of variables required), the corresponding author will respond within a maximum of 4 weeks following a review and subject to approval by a committee from CERMEL. In addition, reported results from this trial are publicly available at https://pactr.samrc.ac.za/TrialDisplay.aspx?TrialID=30503. Supporting clinical documents (protocol and statistical analysis plan) are provided as Supplementary Information.

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Author information

Authors and Affiliations

Centre de Recherches Médicales de Lambaréné, Lambaréné BP 242, Gabon

Ghyslain Mombo-Ngoma, Alex Hounmenou Zinsou, Johanna Keuschnig, Madeleine Eunice Betouke Ongwe, Wilfrid Ndzebe Ndoumba, Lumeka Kabwende, Cany Flamand Mucani Nsiengui, Alexia Ovenga Nzelapanga, Lars Matthies, Claude Bernard Akoo Mve, Benjamin Mbadinga Ngoma, Lionel Mombo Nzamba, Saidou Mahamoudou, Bertrand Lell, Rella Zoleko Manego, Mario Recker, Ayola Akim Adegnika, Sanjeev Krishna, Michael Ramharter, Peter G. Kremsner

Drug Implementation Research Group, Bernhard Nocht Institute for Tropical Medicine, & I. Dept. of Medicine, University Medical Centre Hamburg-Eppendorf, Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany

Ghyslain Mombo-Ngoma, Alex Hounmenou Zinsou, Madeleine Eunice Betouke Ongwe, Wilfrid Ndzebe Ndoumba, Lumeka Kabwende, Cany Flamand Mucani Nsiengui, Alexia Ovenga Nzelapanga, Claude Bernard Akoo Mve.

German Centre for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Hamburg-Lübeck-Borstel-Riems, Germany

Ghyslain Mombo-Ngoma, Rella Zoleko Manego, Michael Ramharter

Center for Tropical Medicine, Bernhard-Nocht Institute for Tropical Medicine & I. Dept. of Medicine, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

Johanna Keuschnig, Lars Matthies, Rella Zoleko Manego, Michael Ramharter

Institut de Recherches en Ecologie Tropicale, Centre National de la Recherche Scientifique et Technologique, Libreville, Gabon.

Madeleine Eunice Betouke Ongwe,

Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, 1090 Vienna, Austria

Bertrand Lell

Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany

Mario Recker, Ayola Akim Adegnika, Sanjeev Krishna, Peter G. Kremsner

Centre for Ecology and Conservation, University of Exeter, Penryn, UK

Mario Recker

German Centre for Infection Research, Partner Site Tübingen, Tübingen, Germany

Ayola Akim Adegnika, Sanjeev Krishna, Peter G. Kremsner

Clinical Academic Group, Institute for Infection and Immunity, St. George's University Hospitals NHS Foundation Trust-St. George's University of London, London, UK.

Sanjeev Krishna

University Malaya, Department of Pharmacology, Faculty of Medicine 50603 Kuala Lumpur

Sanjeev Krishna

Contributions

G.M.N., M.R., S.K., A.A.A. and P.G.K. designed the clinical trial. G.M.N, R.Z.M and A.H.Z supervised the clinical trial. B.M.N. and L.M.N. coordinated the operational conduct of the clinical trial. J.K., W.N.N., L.K., C.F.M.N., L.M., A.O.N. enrolled and clinically followed-up participants into the clinical trial. S.M. and B.L. developed the database and managed the clinical trial data. G.M.N. accessed and verified the data. M.E.B.O. generated and supervised the genotyping data. M.Re. performed the statistical analysis. G.M.N. wrote the first version of the manuscript. M.R., S.K., M.Re. and P.G.K gave the first input, then all authors provided input into the manuscript and approved its final version to be submitted for publication.

Corresponding author:

Correspondence to Ghyslain Mombo-Ngoma at Ghyslain.mombo-ngoma@bnitm.de

Ethical declarations

Competing interests: None of the authors have a conflict of interest.

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Tables

Table 1 | Patients characteristics at baseline

Characteristics	Overall	Treatment arm	
	N=1021	AL (N = 466)	SPAP (N = 555)
Sites			
Four-place, n (%)	107 (10)	50 (11)	57 (10)
Lambaréné, n (%)	325 (32)	145 (31)	180 (32)
Sindara, n (%)	214 (21)	100 (21)	114 (21)
Tchibanga/Mighoma, n (%)	375 (37)	171 (37)	204 (37)
Age in years, median (IQR)	11 (6, 21)	11 (6, 21)	11 (6, 21)
Age groups			
≤ 2 years, n (%)	77 (8)	31 (7)	46 (8)
3-5 years, n (%)	175 (17)	83 (18)	92 (17)
6-10 years, n (%)	229 (22)	109 (23)	120 (22)
11-20 years, n (%)	278 (27)	122 (26)	156 (28)
>20 years, n (%)	262 (26)	121 (26)	141 (25)
Female sex, n (%)	528 (52)	237 (51)	291 (52)
Weight kg, median (IQR)	32 (18, 55)	30 (18, 54)	34 (18, 55)
Weight categories			
≤10 kg	36 (4)	17 (4)	19 (3)
>10 kg	985 (96)	449 (96)	536 (97)
Temperature °C, median (IQR)	36.9 (36.4, 37.7)	36.9 (36.4, 37.6)	36.9 (36.4, 37.6)
Fever (axillary temperature³37.5°C), n (%)	309 (30)	141 (30)	168 (30)
Haemoglobin g/dL, median (IQR)	10.8 (9.8, 12.3)	10.7 (9.8, 12.0)	10.9 (9.8, 12.4)
WBC count x10^3/mm^3, median (IQR)	6.4 (4.8, 8.5)	6.5 (4.9, 8.4)	6.3 (4.7, 8.5)
AST UI, median (IQR)	22.6 (16.7, 31.0)	22.7 (16.9, 31.0)	22.5 (16.5, 31.0)
ALT UI, median (IQR)	13.0 (8.3, 19.0)	13.2 (8.5, 18.0)	12.9 (8.2, 19.5)
Creatinine µmol/L, median (IQR)	57.0 (44.0, 75.0)	57.0 (44.0, 74.0)	57.0 (43.5, 76.0)
Gametocytes present, n (%)	31 (3)	14 (3)	17 (3)
Parasite density per µl, geometric mean (95% CI)	4031 (556, 16864)	3844 (599, 15312)	4488 (532, 19445)

AL=artemether-lumefantrine. SPAP=sulfadoxine-pyrimethamine+artesunate-pyronaridine. IQR=interquartile range. CI=confidence interval.

Table 2| PCR-uncorrected and corrected Day 28 adequate clinical and parasitological response (mITT and per protocol populations)

		AL treatment arm	SPAP treatment arm	Treatment difference†	Non-inferiority p-value*
Modified intention to treat analysis					
Uncorrected Day 28 ACPR	*N=1021*	89.0 (87.8-90.2)	91.6 (90.4-92.9)	1.35 (0.85-2.15)	<0.001
PCR-corrected Day 28 ACPR	*N=1021*	97.1 (96.2-98.0)	97.9 (97.1-98.7)	1.40 (0.55-3.59)	0.012
Per protocol analysis					
Uncorrected Day 28 ACPR	*N=833*	88.9 (85.4-91.7)	91.9 (89.0-94.1)	1.42 (0.89-2.26)	<0.001
PCR-corrected Day 28 ACPR	*N=802*	97.3 (95.0-98.5)	98.2 (96.4-99.1)	1.48 (0.58-3.91)	0.009

Data are N, % (95% CI). †Treatment difference is expressed as odds ratio (95% CI). In the modified intention to treat analysis, all individuals were considered, those with missing data were imputed using multiple imputation. Non-inferiority p-values were obtained from the one-sided Wald test. AL = artemether-lumefantrine. SPAP = sulfadoxine-pyrimethamine + artesunate-pyronaridine. ACPR = adequate clinical and parasitological response. PCR = polymerase chain reaction. *One-sided p-value based on Wald test using pre-specified non-inferiority margin of 0.474 (OR).

Table 3| PCR-uncorrected and corrected Day 42 adequate clinical and parasitological response (mITT and per protocol populations)

		AL treatment arm	SPAP treatment arm	Treatment difference†	Non-inferiority p-value*
Modified intention to treat analysis					
Uncorrected Day 42 ACPR	*N=1021*	77.9 (76.0-79.8)	82.7 (80.8-84.6)	1.36 (0.95-1.93)	<0.001
PCR-corrected Day 42 ACPR	*N=1021*	96.1 (94.4-97.7)	96.2 (94.8-97.6)	1.04 (0.44-2.44)	0.036
Per protocol analysis					
Uncorrected Day 42 ACPR	*N=753*	77.0 (72.3-81.0)	82.9 (78.8-86.3)	1.45 (1.01-2.08)	<0.001
PCR-corrected Day 42 ACPR	*N=645*	96.6 (93.8-98.2)	96.9 (94.4-98.3)	1.08 (0.44-2.60)	0.032

Data are N, % (95% CI). †Treatment difference is expressed as odds ratio (95% CI). In the modified intention to treat analysis, all individuals were considered, those with missing data were imputed using multiple imputation. Non-inferiority p-values were obtained from the one-sided Wald test. AL = artemether-lumefantrine. SPAP = sulfadoxine-pyrimethamine + artesunate-pyronaridine. ACPR = adequate clinical and parasitological response. PCR = polymerase chain reaction. *One-sided p-value based on Wald test using pre-specified non-inferiority margin of 0.474 (OR).

Table 4| Most common adverse events, safety population

Preferred term	Overall N=1021	AL treatment arm N=466	SPAP treatment arm N=555
Total number of adverse events	**235**	**96**	**139**
Vomiting	32	7	25
Common cold	26	15	11
Fever	24	12	12
Cough	19	7	12
Drop of haemoglobin >2g/dl	17	8	9
Abdominal pain	10	4	6
Skin infection	10	3	7
Bronchitis	9	5	4
Asthenia	9	3	6
Headache	8	0	8

AL=artemether-lumefantrine. SPAP=sulfadoxine-pyrimethamine+artesunate-pyronaridine.

Figures

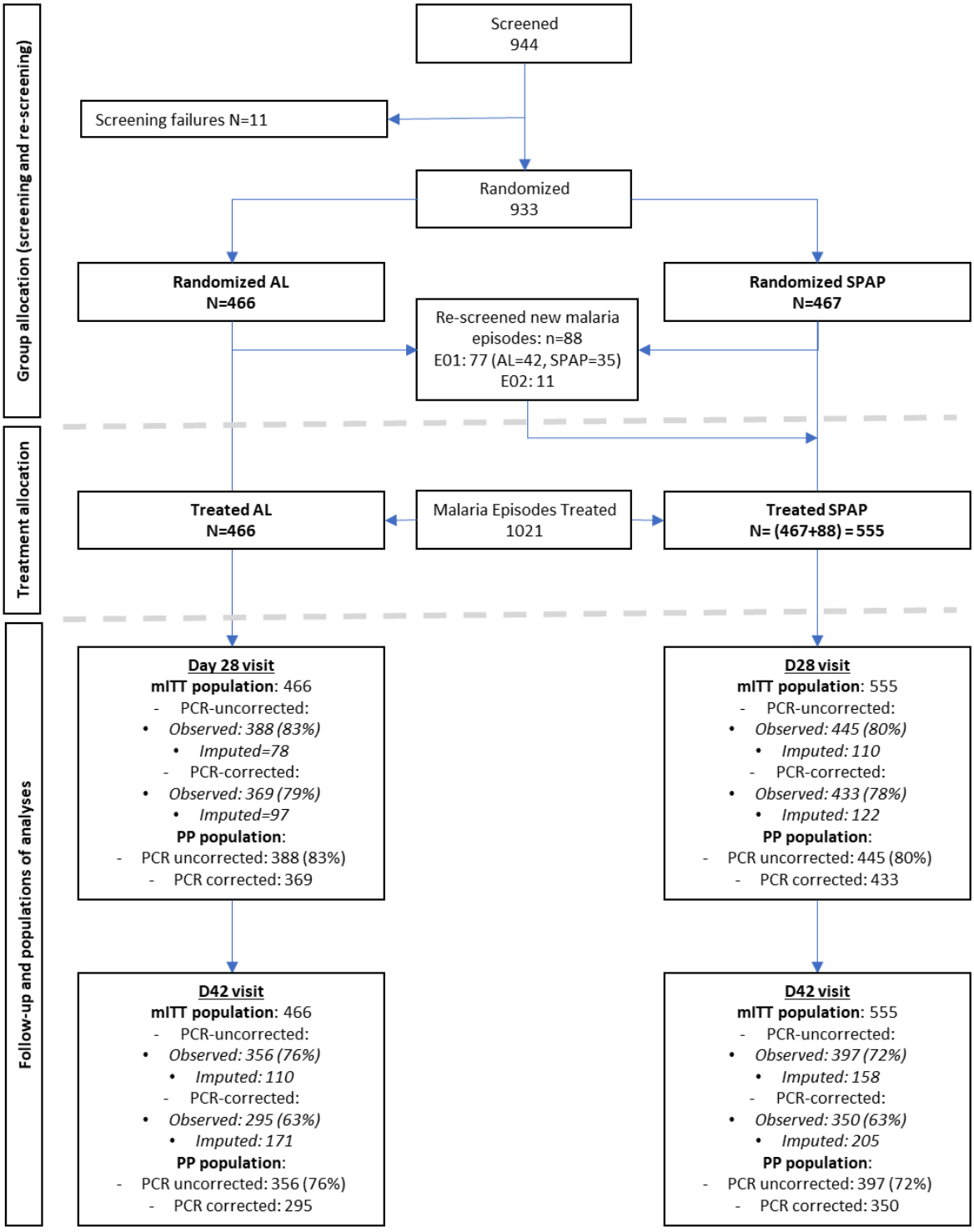


Figure 1

Consort Diagram.AL=artemether-lumefantrine, SPAP=sulfadoxine-pyrimethamine+artesunate-pyronaridine, mITT = modified intention-to-treat population. PP = per protocol population. The safety population is the same as the mITT population.

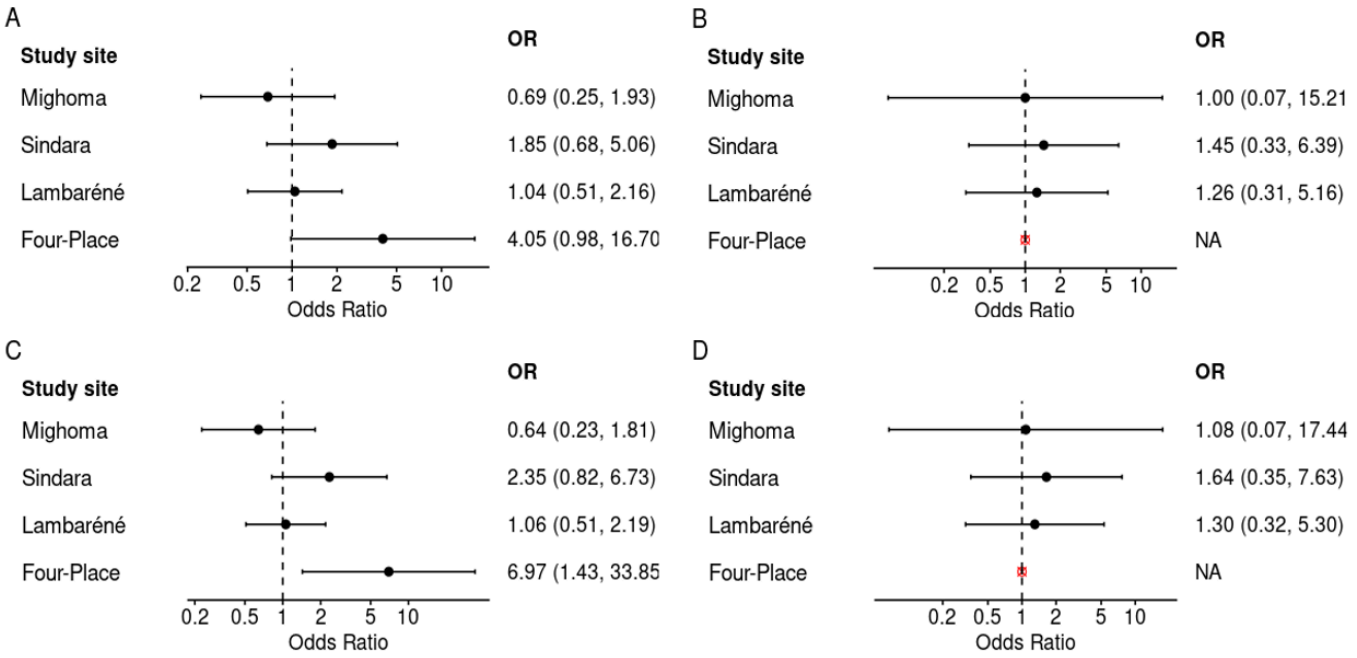


Figure 2

Day 28 efficacy analysis by study site on the odds ratio scale. The dot represents the odds ratio (OR) estimate, the horizontal line represents the 95% confidence interval. A: PCR-uncorrected ACPR in modified intention to treat (mITT) population; B: PCR-corrected ACPR in mITT population; C: PCR-uncorrected ACPR in the per protocol (PP) population; D: PCR-corrected ACPR in the PP population.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2025editablechecklistMomboNgomaetal.2026.docx
- NMEDA155777RSF.pdf
- ExtendedData.docx