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Evaluating artesunate monotherapy and dihydroartemisinin-piperaquine as potential antimalarial options for prevaccination radical cures during future malaria vaccine field efficacy trials

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Abstract

Background In malaria vaccine clinical trials, immune responses after vaccination may be compromised due to immunosuppression caused by concurrent *Plasmodium falciparum* infection. This has a direct effect on the protective efficacy of the vaccine being evaluated. Therefore, parasite clearance prior to vaccination is being considered. Drugs with good safety and efficacy profiles and a short posttreatment prophylaxis period should be used. Two antimalarial drugs, artesunate (AS) as monotherapy and dihydroartemisinin-piperaquine (DHAPQ), have been evaluated in order to identify the most suitable option for use in future trials.

Methods A cohort of children aged 1.5–12 years living in the Banfora Health District area was recruited. They were randomly assigned to receive supervised curative doses of AS monotherapy for 7 days or DHAPQ for 3 days. A polymerase chain reaction (PCR) was performed 21 days after treatment to confirm clearance of infection, and only those with a negative PCR were included in the study cohort for a 6-month longitudinal follow-up. Cohort children were actively visited fortnightly to collect blood samples for *P. falciparum* detection via microscopy and PCR. Passive surveillance was also conducted at the local health facility to record incident malaria episodes that occurred between two active visits.

Results A total of 513 children were treated. Among these patients, 458 (89.3%) were free of *P. falciparum* malaria infection on day 21: 87.3% (226/259) in the AS group vs 91.3% (232/254) in the DHAPQ group ($p = 0.053$). The mean time to first malaria infection by microscopy was 154.9 (2.9) days in the DHAPQ arm and 129.0 (3.9) days in the AS arm ($p < 0.01$). The incidence rates of clinical malaria episodes during the follow-up period were 0.507 (0.369–0.645) and 0.293 (0.190–0.397) in the AS and DHAPQ arms, respectively ($p < 0.05$).

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Conclusions These findings suggest that although both drugs are effective in clearing *P. falciparum* infections, AS is likely to cause no more than minimal interference with the evaluation of vaccine efficacy endpoints and could, therefore, be considered for use.

Trial registration: NCT04601714.

Keywords Malaria, Artesunate, Dihydroartemisinin-piperaquine, Radical cure, Infection, Incidence

Background

Malaria remains a major global public health problem, with ~ 40% of the world's population at risk of infection. In 2022, there were approximately 249 million cases of malaria and 608,000 deaths in 85 malaria-endemic countries [1]. Most mortality occurs among children under 5 years of age in sub-Saharan Africa.

In 2020, the Global Technical Strategy (GTS) called for a reduction in malaria case incidence and mortality rates of at least 40% by 2020, 75% by 2025 and 90% by 2030 from a 2015 baseline [1]. On the current trajectory, the GTS for the Malaria 2016–2030 milestone morbidity targets will not be met globally, and without accelerated change, the 2025 and 2030 targets will not be met as well. This alarming trend calls for new tools in the fight against malaria.

An effective malaria vaccine is critical in the face of persistently high malaria transmission, increasing drug and insecticide resistance, and inadequate coverage of current control interventions [2–5]. Among the numerous malaria vaccine candidates under development, only two (RTS,S and, more recently, R21) were prequalified by the World Health Organization (WHO) and are currently being deployed in malaria endemic countries [6]. However, a next generation with increased vaccine efficacy is still needed to significantly impact the malaria burden through elimination efforts.

In efforts to develop the next generation of more effective vaccines, there are increasingly some concerns that immune responses could be compromised by malaria-induced immunosuppression [7–13]. However, clearing existing parasitaemia may also affect the subsequent susceptibility of individuals to malaria infection, as shown in previous studies [14–16]. Indeed, persistent multiclonal infections carried during low malaria transmission contribute to maintaining protection against subsequent febrile malaria episodes, possibly through protective immune responses maintained by ongoing malaria parasite infections [17]. This finding has direct implications for the protective efficacy of the vaccine being evaluated. Therefore, parasite clearance prior to vaccination is being considered. In vaccine trials with the primary endpoint of time to infection, presurveillance clearance of asymptomatic infection is essential [19]. Pretreatment of malaria infection prior to vaccination eliminates blood-stage infections and reduces liver inflammatory responses due to the presence of malaria antigens, allowing the candidate vaccine to stimulate an optimal immune response without interference from nonvaccine-induced inflammatory mediators [18].

For parasite clearance, some considerations need to be taken into account. These include the drug's safety profile, gametocidal activity and half-life. Drugs with good safety and efficacy profiles and a short posttreatment prophylaxis period should be used to clear existing infections in phase IIb trials of candidate malaria vaccines. The drug should be able to clear parasites but not inhibit the acquisition of new infections during follow-up, as this would undermine the ability to detect vaccine efficacy [19–22].

In preparation for phase 2b malaria vaccine trials, a baseline study has been conducted to evaluate two options for prevaccination parasite clearance treatment regimens that could be used. The goal of this study was to identify which drug, artesunate (AS) as monotherapy with a short half-life (20–45 mn) [23] or dihydroartemisinin-piperaquine (DHAPQ) with a much longer half-life (22 days) [24], would be the best option for effectively clearing parasites while minimizing the post-clearance prophylactic period [25–27]. In addition, this study should provide updated data on the incidence of malaria in the context of the implementation of SMC to adequately inform the determination of the sample size for the vaccine trial.

Methods

Study site

The study was conducted in the Banfora health district, Cascades region of Burkina Faso, from September 2020 to April 2021. This region, located in the southwest of the country, is one of the best-watered regions. The study was carried out in a peri-urban area, specifically the Nafona and Bounouna peripheral health facilities areas. The two areas are nearly identical, with a small river running through the village of Nafona. The climate is characterized by intense seasonal malaria transmission during the rainy season from May to November [28].

Malaria is the main disease in the Banfora health district and is the result of complex environmental, human, entomological and parasitological factors. Malaria

transmission is stable throughout the year but peaks during the rainy season (May–November).

Plasmodium falciparum is responsible for > 90% of malaria cases [29]. On average, children aged 0–5 years suffer two episodes of malaria per year [29]. The overall entomological inoculation rate is estimated at 80.4 infectious bites per child over the six-month malaria transmission season. In terms of bed net use, 80.6% of children sleep under an insecticide-treated net (ITN) [30]. Artemether-lumefantrine, artesunate-amodiaquine and dihydroartemisinin-piperaquine are the officially recommended first-line treatments for uncomplicated malaria; artesunate, artemether or quinine are used to treat severe cases. Since 2014, children aged 3–59 months in the study area and throughout the country have been receiving seasonal malaria chemoprevention (SMC) four times during the transmission season via SP + AQ, as recommended by the WHO [31]. In the health district of Banfora, SMC is implemented from July to October.

Study participants

Children aged 1.5–12 years living in the area of the two health facilities in Bounouna and Nafona have been enrolled. During a community engagement meeting in the study area, a list of children whose parents were interested in the study was drawn. A public lottery method was used to select the subset needed for the screening process, as too many volunteer children were included if their parents or legal representatives did not plan to leave the area for the duration of the study and consented to the child's participation. The study team excluded children who were febrile (body temperature ≥ 38.0 °C) at the time of screening or who had clinical conditions that prevented oral treatment; children who had received a blood transfusion or immunoglobulins within 3 months; those with a known history of hypersensitivity/allergic reactions to the study drugs; those with current or previous participation in any malaria vaccine trials; and those with haemoglobin levels less than 8 g/dl.

Study design

A prospective cohort study was conducted in which participants aged 1.5–12 years were recruited. Baseline clinical and biological assessments were conducted on participants, who were then administered a curative dose of either artesunate or dihydroartemisinin-piperaquine to eradicate any existing parasitaemia. Parasite clearance was confirmed three weeks later via PCR, and only participants with negative PCR results were enrolled in the longitudinal follow-up. Both active and passive case detection methods were employed to ensure that a high proportion of infections in the cohort were captured.

Drugs for parasite clearance

Artesunate

Artesunate, a monotherapy derivative of artemisinin, is a highly safe and effective antimalarial drug that has been used for more than 30 years. Its pharmacodynamic effects are due to the rapid absorption and action of artemisinin against various stages of the parasite's life cycle, from young asexual forms (rings) to early sexual forms (gametocytes). Additionally, tolerance to this drug is excellent, making it a reliable choice for treatment [27]. However, artemisinin-based monotherapy is no longer recommended for the curative treatment of acute malaria episodes because of concerns over the development of drug resistance. Instead, it is now used in artemisinin-based combination therapy.

Dihydroartemisinin-piperaquine (DHAPQ)

DHA-PQ is a highly effective artemisinin-based combination therapy. It is a fixed combination of dihydroartemisinin (DHA) and piperaquine phosphate (PQ). This second compound ensures the long-term efficacy of DHAPQ, completing the total elimination of parasites from the body. In fact, PQ has a different mechanism of action and a much longer half-life [25, 26]. Pharmacologically, PQ is characterized by a large volume of distribution and reduced rates of excretion after multiple doses. This lipophilic drug is rapidly absorbed, with a time maximum (Tmax) (time to reach the highest concentration) of 2 h after a single dose [32]. Its efficacy in the treatment of uncomplicated *P. falciparum* malaria has been demonstrated in several large-scale clinical trials [33, 34]. DHAPQ provides a simpler dosage scheme (a single daily dose over 3 days) and is generally administered without specific food instructions. Since 2015, the WHO has recommended it as a first-line treatment for uncomplicated malaria episodes [35].

Randomization and treatment

The screening procedures included obtaining informed consent, performing clinical assessments, and measuring haemoglobin levels. Blood slides and filter papers were obtained from eligible children prior to treatment. The study had a two-arm design. Eligible children were randomly assigned to arms via a computer-generated code list; the first arm received supervised curative therapy with AS, and the second arm was treated with DHAPQ.

Artesunate monotherapy was given as oral therapy (4 mg/kg/day) for seven days. Artesunate tablets were administered orally by study personnel under direct observation. Artesunate tablets were administered on a daily basis, either in a single dose or in divided doses. The latter were administered within an hour of the former.

The administration was conducted under direct observation with at least 20 ml of liquid drink to ensure compliance. The participants were observed for 30 min after the last artesunate tablet was given to ensure that no medication had been vomited. If vomiting occurred within 30 min of administration, the artesunate treatment was repeated two times, and the product was taken within 30 min before vomiting.

Dihydroartemisinin-piperaquine was administered by the study staff once daily for three consecutive days, according to the participant's weight, under direct observation therapy. If vomiting occurred within 30 min of taking the drug, the full dose was given again; if vomiting occurred within 30–60 min, half a dose was given again. No dose was repeated more than twice.

Follow-up procedures

The study children were visited 21 days after the first dose of treatment, at which time blood samples were collected for blood smears and filter paper to check for parasite clearance. Only those with a negative parasite count by PCR were included in the follow-up study. The follow-up included a combination of active case detection (ACD) and passive case detection (PCD). Six qualified nurses, certified to administer treatments, were recruited and underwent training in preparation for their participation in the study. These nurses lived in the study area throughout the study period. The ACD consisted of field workers visiting the homes of the enrolled children fortnightly for 6 months. At each home visit, the axillary temperature was recorded, and symptoms consistent with malaria, preventive measures, and medication since the previous visit were recorded.

A blood smear and dried blood spots for PCR were systematically taken every 2 weeks to monitor for reappearance of malaria parasites. Children with fever (axillary temperature ≥ 37.5 °C) or a history of fever (within the last 24 h) were referred to the local health centre, where they were treated according to national guidelines.

If the child was not present at the time of the visit, the nurse tried again the following day. If the child was not available on the following day, he was recorded as absent for this visit. At enrollment and throughout the study, parents were encouraged to take their child to the nearest health facility if the child had a fever to comply with the PCD. The child was treated according to national guidelines. The study health facilities were supported by experienced nurses. The travel and treatment costs for sick children were reimbursed by the study. In the case of fever or a history of fever, a blood smear and a dried blood spot were taken for PCR.

At the first malaria infection detected by microscopy either during the first 2 weeks of blood smears or upon presentation to a health facility with malaria, biweekly visits at home were stopped, as the child was considered to have met the time to first malaria infection endpoint. The subject was subsequently monitored exclusively through the use of passive case detection at the health facility, with the objective of identifying any new clinical malaria episodes. To ensure that all field procedures were carried out correctly during the visits, a sample of each compound was revisited each week by a field supervisor.

Laboratory investigations

Blood smear processing procedures

Thick blood films were stained with Giemsa and examined at 1000× magnification by experienced microscopists at the Groupe de Recherche Action en Santé (GRAS). Parasitaemia was estimated on thick blood films using an average count of 8000 white blood cells per microlitre of blood. Each slide was read twice independently according to the manufacturer's procedures. If the ratio of the densities of the first two readings was < 1.5 or < 0.67 or if < 30 parasites were counted with a difference in the number of parasites of < 10, the slide was read a third time. The final result was the geometric mean of the parasite density. If there was a discrepancy in positivity, the slide was also read a third time, and the final result was based on the majority verdict for positivity. Dried blood samples were used for malaria parasite detection via PCR [36].

Blood spot analysis by PCR

PCR-dried blood spots on Whatman filter papers (Whatman 3 mm, GE Healthcare, Pittsburgh, PA, USA) were labeled and then air dried and placed individually in a plastic bag containing a desiccant, thus protecting them from humidity. Deoxyribonucleic acid (DNA) was extracted via the methanol method. *Plasmodium* species were identified via nested PCR amplification from two PCRs. Products obtained after the first PCR were amplified via specific primers for *P. falciparum*. The sequences of the primers and the PCR protocol are described in detail previously [36].

Ethical clearance

The study was approved by the National Ethics Committee of the Ministry of Health (under deliberation No. 2020-6-101 dated 10/jun/2020). Parents signed or fingerprinted the informed consent form before their child was screened. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practices, the Declaration of Helsinki, and the regulations in force in Burkina Faso.

Statistical analysis

The primary case definition of a malaria episode was both fever (axillary temperature ≥ 37.5 °C or a history of fever) and a positive slide (presence of *P. falciparum* trophozoite). The time to *P. falciparum* infection was defined as the first positive smear within 6 months of enrollment.

Descriptive statistics were obtained for all available indicators. The geometric mean parasite density in clinical cases of *P. falciparum* gametocyte was calculated. The comparison analyses processed data from the ACD and PCD parts. When normality conditions were fulfilled, Student's T test was used to compare both treatments for all the variables. Otherwise, a median test was preferred. All the frequency comparisons were carried out via a Chi^2 test if the application conditions allowed it. Otherwise, Fisher's exact test was used.

For example, when comparing several embedded categories successively, multiple tests had to be performed. Hence, Šidák's formula has been used to avoid an increase in type 1 error. The situation occurred several times in the analyses when comparing a specific variable among all the children and then, specifically, the child age categories ([1.5–5] or [5–12]) or sex categories (boys or girls). When the number of tests was equal to 2, the p value was compared to 0.0253 for an overall type 1 error equal to 0.05.

The incidence rate was estimated as the number of cases meeting the primary case definition divided by the total observation time in a group at risk. Two or more consecutive malaria episodes occurring within 3 weeks of the first episode were considered recrudescent infections and were treated as a single episode.

Survival analysis (Kaplan–Meier) was performed to analyze the time to reinfection and the time to first clinical episode. A log-rank test was used to estimate the treatment effect in the presence of covariates. A Nelson–Aalen curve was drawn to provide more detailed information about the progression of risk within the groups. Finally, to quantify how the risks differ between the two treatments, a Cox regression survival analysis was performed. The proportional hazards assumption was tested by including time-varying covariates in the model.

A p value less than 0.05 was considered statistically significant. Statistical analyses were performed via SAS System Version 9.4 software (SAS Institute, Cary, NC, USA).

Results

Baseline characteristics of the study population

A total of 513 children who met the eligibility criteria were screened and received treatment. A total of 259 children were randomized to the AS group, and 254 were randomized to the DHAPQ group. Directly observed treatment adherence was 100% for all participants in the current study. On day 21, 485 children were free of *P. falciparum* malaria infection, as confirmed by PCR. Finally, the parents of 458 eligible children agreed to allow their children to participate in the longitudinal follow-up cohort. This included 226 children who had previously received AS and 232 who had received DHAPQ. There were no significant differences in demographic characteristics between the treatment arms. Of those included in the 6-month longitudinal cohort, 53 did not complete the follow-up (dropouts or withdrawals) (Fig. 1). Among the enrolled children, 233 (50.9%) were girls. The mean age of the children was 6.40 (1.57–12.32) years. The baseline characteristics of the study participants are summarized in Table 1.

Efficacy of the study drugs in clearing parasites

At baseline, 78 (30.8%) and 86 (32.2%) children had parasites detectable by PCR in the DHAPQ and AS arms, respectively. Among those, 88.5% (69/78) and 83.7% (72/86) had cleared their infection on day 21 posttreatment in the DHAPQ and AS arms, respectively. The difference was not statistically significant ($p = 0.20$). Within 21 days, 5 and 8 children who were negative by PCR before treatment (baseline) were positive in the DHAPQ and AS arms, respectively, at the 21st timepoint.

Overall, the proportion of children who were free of infection 21 days post radical cure was 87.3% (226/259) in the AS group and 91.3% (232/254) in the DHAPQ group ($p = 0.053$).

Time to *Plasmodium falciparum* malaria reinfection

In total, 141 children were found to have microscopically detectable reinfections during the follow-up period. The percentages of reinfections were 21.5% (50/232) and 40.3% (91/226) in the DHAPQ and AS treatment arms, respectively. The difference was statistically significant ($p < 0.01$). The mean time to first or only reinfection with artesunate was 129.0 (3.9) days, whereas it was 154.9 (2.9) days with dihydroartemisinin-piperaquine treatment ($p = 0.02$). The difference between the treatment arms remained significant after age stratification (Table 2).

If PCR was considered, the overall proportion of reinfections was 42.6% (194/455). The percentages of reinfections were 37.5% (87/232) and 47.9% (107/223) in the DHAPQ and AS groups, respectively. The difference was statistically significant ($p = 0.01$). The mean time to first or only reinfection with artesunate was 120.2 (4.4) days, whereas it was 136.2 (3.8) days with DHAPQ. This difference was statistically significant ($p < 0.05$) (Fig. 2).

The proportional hazards assumption was satisfied for treatment, gender, age and village: all variables were nonsignificant. The Cox regression results summarized

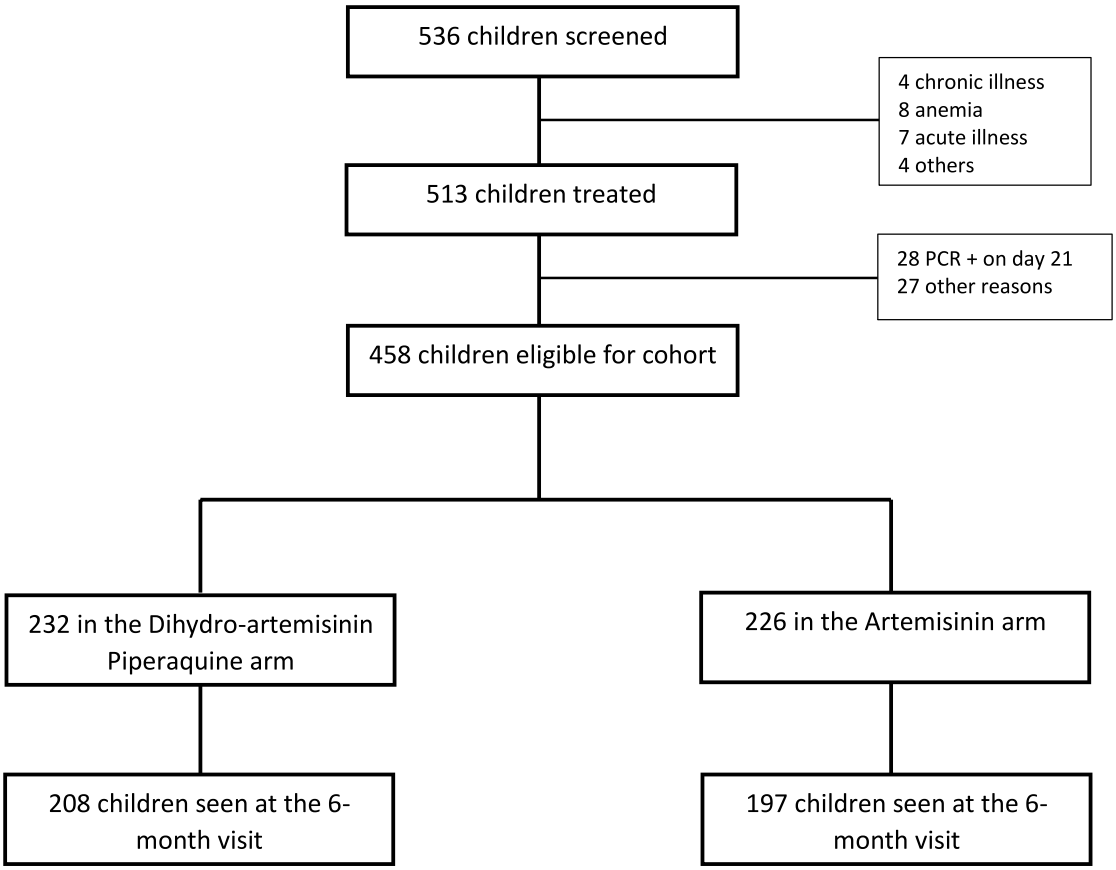

Fig. 1 Trial profile

in Table 3 show that reinfection was more likely in the artesunate group (HR = 1.55) than in the DHAPQ group.

Incidence of acute clinical malaria episodes

In total, 86 malaria episodes were recorded during the six months of follow-up.

The cumulative incidence of clinical malaria was 23.0% (52/226) in the AS arm compared with 14.0% (32/232) in the DHAPQ arm. The difference was statistically significant ($p = 0.01$). The mean time to first clinical episode was 133.1 (3.0) days in the AS group compared with 86.6 (0.9) days in the dihydroartemisinin-piperaquine arm. The difference was statistically significant ($p < 0.01$).

The overall incidence rate for all children was 4.02 episodes/1000 person-months at risk (3.16–4.88). The incidence rate was 5.07 episodes/1000 person-months at risk (3.69–6.45) in the AS group compared with 2.93 episodes/1000 person-months at risk (1.90–3.97) in the DHAPQ group. The difference was statistically significant ($p < 0.05$) (Table 4; Fig. 3).

A comparable pattern of elevated incidence was observed in the AS cohort relative to the DHAPQ arm when the analysis was limited to the SMC target population or school-aged children. Furthermore, an examination of the data reveals that the overall infection incidence is comparable between the two groups of SMC target and school-age populations (Table 4).

The geometric mean *P. falciparum* asexual parasite count in children presenting with fever during the follow-up visits was similar between the two groups. There were 10,373 trophozoites/µl (95% CI [5571–19,312]) and 12,719 trophozoites/µl (95% CI [5824–27,777]) in the AS and DHAPQ arms, respectively.

The geometric means of *P. falciparum* gametocytes were 79.0 gametocytes/µl and 4.0 gametocytes/µl (95% CI [4.0–4.0]) in the AS and DHAPQ arms, respectively. The number of participants carrying gametocytes was very low (3 participants), and the difference was not statistically significant ($p > 0.05$).

Discussion

The current study provides important information on the use of AS and DHAPQ as radical cures for apparently healthy children under 12 years of age during the high malaria transmission season in Africa. The study included a 180-day follow-up. The first outcome, the rate

Table 1 Characteristics of the study population

Parameters	Artesunate TTT N = 226	DHAPQ TTT N = 232	Total
Age (mean, range)	6.43 (1.57–12.32)	6.38 (1.57–12.32)	N = 458
Age group (n, %)			
[1.5–5]	88 (47.6%)	97 (52.4%)	N = 458
[5–12]	138 (50.6%)	135 (49.4%)	
Gender (n, %)			
M	111 (49.3%)	114 (50.7%)	N = 458
F	115 (49.4%)	118 (50.6%)	
Temperature (mean, range)			
All children	36.5 (36.0–38.8)	36.5 (36.0–38.7)	N = 458
[1.5–5]	36.5 (36.0–38.8)	36.5 (36.0–38.7)	N = 185
[5–12]	36.5 (36.0–38.6)	36.5 (36.0–37.6)	N = 273
M	36.5 (36.0–38.8)	36.4 (36.0–38.7)	N = 225
F	36.5 (36.5–38.6)	36.6 (36.0–38.7)	N = 233
Sleep under a bed net last night (n, %)			
All children	200 (88.5%)	206 (88.8%)	N = 458
[1.5–5]	83 (94.3%)	85 (87.6%)	N = 185
[5–12]	117 (84.8%)	121 (89.6%)	N = 273
M	100 (90.1%)	101 (88.6%)	N = 225
F	100 (87.0%)	105 (89.0%)	N = 233
Other malaria prevention measures (n, %)			
All children	45 (19.9%)	52 (22.4%)	N = 458
[1.5–5]	17 (19.3%)	16 (16.5%)	N = 185
[5–12]	28 (20.3%)	36 (26.7%)	N = 273
M	23 (20.7%)	29 (25.4%)	N = 225
F	22 (19.1%)	23 (19.5%)	N = 233

TTT treatment, *DHAPQ* dihydroartemisinin-piperaquine

When it was not mentioned, Student's t test was used (numerical data), or the Chi2 test was used (categorical data). Otherwise, a median test (Med) or Fisher's exact test (FE) was used

Multiplicity of tests: The alpha error was recalculated as follows: $1\text{-}(1\text{–}0.05)^{1/2} = 0.0253$ when the number of tests was equal to 2 (Šidák's formula)

of negative PCR results on day 21 posttreatment, was very similar between the artesunate and DHAPQ groups. Both AS and DHAPQ were effective at clearing malaria parasites. This could be related to the fact that directly observed treatment adherence was 100% for all participants in the current study. The cure rates were similar to, although slightly lower than, published rates for DHAPQ [37–39] and artesunate [40] at days 28 and 42 in the populations of African countries suffering from symptomatic malaria. No published data on 21-day cure rates found. In previous studies conducted in populations of mixed age in Africa, the efficacy of artesunate and DHAPQ in clearing malaria parasites was demonstrated in individuals with uncomplicated malaria [41–44].

Considering the time to reinfection, AS treatment provides protection against reinfection for approximately 17 weeks and has more than half the reinfection rate (107/195) during the season. AS did not reduce the number of reinfections during the season, as did DHAPQ. DHAPQ protected longer from the first infection (19 weeks). These findings could be explained by the pharmacology of both drugs. The half-lives of dihydroartemisinin (1–3 h) and piperaquin (20 days) are longer than the half-life of AS (1–3 h) and may influence the number and time to reinfection observed between the AS and DHAPQ groups [45]. Indeed, the more children are treated with short effective half-life drugs, the shorter the time to first malaria reinfection in an intense Malaria transmission area. Artesunate used as monotherapy has a short half-life and therefore does not have the prolonged preventive effects of longer-acting antimalarial drugs, such as DHAPQ [46, 47]. Piperaquine has a very long elimination half-life of two to three weeks, which allows for a long period of posttreatment prophylaxis [48].

During the 6-month follow-up, overall, 81.0% of the children did not develop any acute malaria episodes. This percentage was greater in the DHAPQ group (86.6%) than in the AS group (77.0%) ($p < 0.05$). Among those

Table 2 Time to first infection (only subjects with negative blood spots)

Group	Mean time to first infection Tf + (ACD and PCD)	Nb of children infected	Mean time to first infection PCR + (ACD and PCD)	Nb of children infected
All treatments				
All children	142.0 (2.5)	142[a]	128.4 (2.9)	195[a]
[1.5–5]	151.4 (3.6)	40	136.1 (4.5)	65
[5–12]	135.5 (3.4)	102	123.4 (3.8)	130
M	145.8 (3.4)	63	132.7 (4.1)	91
F	138.5 (3.6)	79	124.4 (4.2)	104
Artesunate treatment Group				
All children	129.0 (3.9)**	91	120.2 (4.4)*	107
[1.5–5]	138.7 (6.0)**	29	132.1 (7.0)*	33
[5–12]	118.6 (4.6)	62	112.5 (5.6)	74
M	136.1 (5.3)**	40	123.1 (6.0)*	53
F	118.6 (5.1)	51	118.1 (6.4)	54
Dihydroartemisinin-piperaquine Group				
All children	154.9 (2.9)	50	136.2 (3.8)	87
[1.5–5]	93.5 (1.8)	11	137.9 (5.6)	32
[5–12]	148.9 (4.0)	39	134.4 (5.0)	55
M	146.8 (3.4)	22	142.7 (5.5)	37
F	153.4 (4.1)	28	129.9 (5.2)	50

Mean time: Mean time (standard deviation). *Tf* + : *Trophozoite falciparum* positive; PCR + : positive PCR

[a] One treatment is missing

Methods: Kaplan–Meier survival estimates of infection by drug and Šidák's formula were used due to test multiplicity

Comparisons between treatments: **: $p < 0.01$; *: $p < 0.05$

who developed malaria, 85% had one episode. The burden of malaria appears to be lower than that reported in previous studies conducted in the same area without preseason radical cure [28, 29].

However, previous similar studies suggest that the clearance of asymptomatic infections increases an individual's risk of contracting a symptomatic malaria episode. A similar effect has been observed following the use of chemoprophylaxis in malaria-endemic areas [14, 15, 49, 50]. It has been proposed that asymptomatic carriage might be a form of tolerance to *P. falciparum* infection that protects against the development of clinical episodes [51]. A number of manuscripts have also indicated that while semi-immunity to malaria can be acquired in highly endemic areas by the age of 5 years, this immunity wanes rapidly without ongoing parasite exposure [52].

The low incidence posttreatment in our study could be explained by several factors. First, many malaria control strategies are being implemented in the study area. A universal coverage campaign distributed ITNs with permethrin or deltamethrin (Sumitomo Chemical, Vestergaard and BASF) in 2019, just a few months before this study. The proportion of study children who slept under a bed net the previous night was high, at 88.5% in the present study (Table 1). In addition, ~ 20% of the children used other malaria prevention methods in addition to ITNs (Table 1). Second, SMC is being implemented in the study area, and the study was conducted after 2 rounds of SMC, during which SP + AQ were given to children younger than five over 3 days, with a protective efficacy of over 50% against parasitaemia [53, 54]. Finally, the study drugs were administered during the high malaria transmission period, but half of the 6-month follow-up period occurred during the low transmission period.

In both arms, malaria incidence was similar in the SMC group and nontargeted SMC age groups (those aged 5–12), suggesting that these ages are now important reservoirs of infection, as previously reported in the same area [55]. These findings highlight the burden of malaria in school-age children. Therefore, expanding the age range eligible for SMC could be effective in reducing malaria further in Burkina Faso.

The greater incidence of reinfection in the AS group than in the DHAPQ group may also be because artesunate monotherapy has a short half-life and, therefore, does not prolong the preventive effects of longer-acting antimalarial drugs such as DHAPQ [46–48].

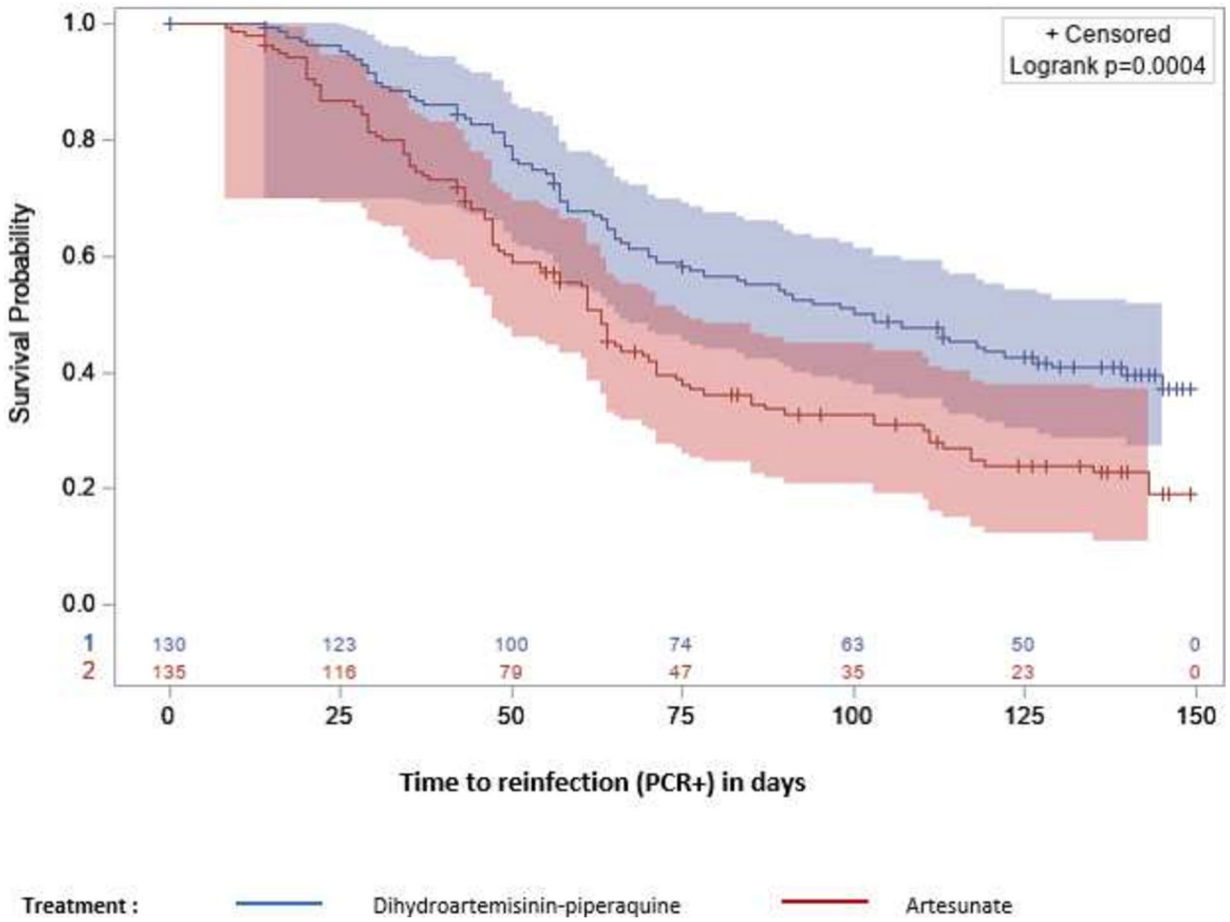

Fig. 2 Kaplan–Meier survival estimates by treatment arm by PCR [time to reinfection in the study cohort (PCR > 0)] with the number of subjects at risk and 95% Hall–Wellner bands

Table 3 Risk factors associated with malaria reinfection diagnosed via PCR via Cox regression analysis

Variable	Coefficient	Standard error	Hazard Ratio (95% CI)	P value
Treatment				
DHAPQ	0	–	1	–
Artesunate	0.439	0.149	1.551 (1.159–2.076)	0.003
Age, years				
[1.5–5]	0	–	1	–
[5–12]	0.350	0.160	1.419 (1.038–1.940)	0.028
Gender				
Male	0	–	1	–
Female	0.236	0.150	1.266 (0.943–1.698)	0.116

Adjusted for village status, BMI and the presence of bed net, anaemia, fever, and antimalarial drugs

DHAPQ dihydroartemisinin-piperaquine

Table 4 Incidence of clinical malaria episodes based on parasitaemia (*Tf* > 0) (ACD and PCD)

Age group	Number of children (%)	Number of incident malaria episodes	Total person-year at risk	Incidence rate (95% ci)	Comparison (Sidak)
All treatments					
All children	460 (100.0%)	84	209.00	0.402 (0.316–0.488)	
[1.5–5]	185 (40.2%)	34	83.24	0.408 (0.271–0.546)	
[5–12]	275 (59.8%)	50	125.75	0.398 (0.287–0.508)	
M	227 (49.4%)	40	103.74	0.386 (0.266–0.505)	
F	233 (50.6%)	44	105.26	0.418 (0.294–0.542)	
Artesunate treatment group					
All children	226 (100.0%)	52	102.51	0.507 (0.369–0.645)	*P < 0.05
[1.5–5]	88 (38.9%)	23	39.56	0.581 (0.344–0.819)	*P < 0.05
[5–12]	138 (61.1%)	29	62.95	0.461 (0.293–0.628)	NS
M	111 (49.1%)	27	50.97	0.530 (0.330–0.730)	*P < 0.05
F	115 (50.9%)	25	51.54	0.485 (0.295–0.675)	NS
Dihydroartemisinin-piperaquine group					
All children	232 (100.0%)	31	105.64	0.293 (0.190–0.397)	
[1.5–5]	97 (41.8%)	11	43.68	0.252 (0.103–0.401)	
[5–12]	135 (58.2%)	20	61.96	0.323 (0.181–0.464)	
M	114 (49.1%)	12	51.92	0.231 (0.100–0.362)	
F	118 (50.9%)	19	53.71	0.354 (0.195–0.513)	

Comparison (Sidak): Statistical comparison of each category between the artesunate treatment group and the dihydroartemisinin-piperaquine group via Šidák's formula (multiplicity of tests)

*P corresponds to the probability of the difference taking into account the multiplicity of tests (Šidák's formula)

There is one case where there is no treatment mentioned

The occurrence of artemisinin resistance has been documented in a limited number of African countries [56]. The use of artesunate (AS) as a monotherapy on a large-scale may facilitate the development of resistance in the *P. falciparum* parasite. This is due to the fact that a monotherapy regimen facilitates the parasite's development of resistance mechanisms. Conversely, the combination of artesunate with other antimalarial drugs has been demonstrated to reduce the risk of resistance, as the parasite must simultaneously develop resistance to multiple therapeutic agents, which is a much more challenging process. In the context of a pre-erythrocyte malaria vaccine trial with a limited number of asymptomatic participants, the use of artesunate monotherapy with a short half-life can provide evidence of vaccine efficacy without contributing to the further spread of artemisinin resistance. The advent of an efficacious malaria vaccine would represent a significant advancement in the global effort to eliminate malaria.

Conclusion

This study revealed that both AS and DHAPQ are effective drugs for clearing *P. falciparum* malaria infection and could, therefore, be used during the pretreatment phase before immunization. However, AS, which seems to provide relatively short-term protection against new infections, would be preferable for use, as it is unlikely to cause more than minimal interference with the evaluation of vaccine efficacy endpoints.

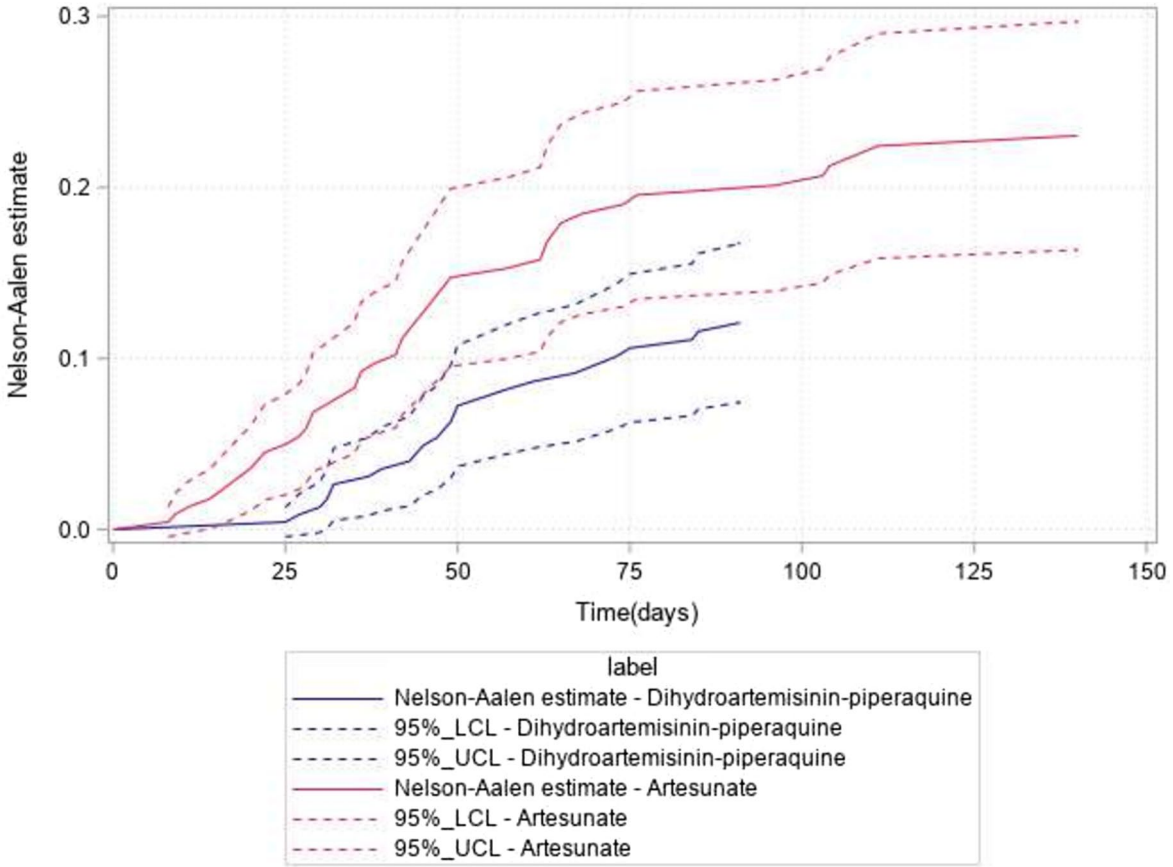

Fig. 3 Nelson–Aalen cumulative hazard estimates (and 95% confidence intervals) for clinical malaria episodes (Fever + *Trophozoite falciparum* > 0) between the treatment arms 95%_LCL: lower bound of the 95% confidence interval; 95%_UCL: upper bound of the 95% confidence interval

Abbreviations

ACD	Active case detection
AS	Artesunate
DHA	Dihydroartemisinin
DHAPQ	Dihydroartemisinin-piperaquine
DNA	Deoxyribonucleic acid
GRAS	Groupe de Recherche Action en Santé
GTS	Global technical strategy
ITN	Insecticide treated net
PCD	Passive case detection
PCR	Polymerase chain reaction
PQ	Piperaquine phosphate
SMC	Seasonal malaria chemoprevention
SP + AQ	Sulfadoxine-pyriméthamine amodiaquine
Tmax	Time maximum
WHO	World Health Organization

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Author contributions
A.O., A.B.T. and S.B.S: Designed the study A.Z.O: Curated the data; V.B., M.V.: Analysis of the data; D.O., S.M.O., A.D., E.S.B., A.H., D.H., E.C.B., I.N., A.O. and A.B.T.: Data collection and analysis; S.B.S., E.C.B.: Supervision of the study; A.O.: wrote the first draft of the manuscript; A.O., D.O., S.M.O., A.D., E.S.B., A.H., D.H., E.C.B., I.N., V.B., M.V., A.B.T. and S.B.S.: revised and edited the manuscript.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the National Ethics Committee of the Ministry of Health (under deliberation No. 2020-6-101 dated 10/06/2020).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. WHO. World malaria report 2023. Geneva: World Health Organization; 2023.
2. Greenwood BM, Targett GA. Malaria vaccines and the new malaria agenda. Clin Microbiol Infect. 2011;17:1600–7.
3. Guyant P, Corbel V, Guerin PJ, Lautissier A, Nosten F, Boyer S, et al. Past and new challenges for malaria control and elimination: the role of operational research for innovation in designing interventions. Malar J. 2015;14:279.
4. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, et al. Global malaria mortality between 1980 and 2010: a systematic analysis. Lancet. 2012;379:413–31.
5. Ouattara A, Laurens MB. Vaccines against malaria. Clin Infect Dis. 2015;60:930–6.
6. Prequalification of Medical Products: Prequalified Vaccines. https://extranet.who.int/prequal/vaccines/prequalified-vaccines.
7. Bejon P, Mwacharo J, Kai O, Todryk S, Keating S, Lowe B, et al. The induction and persistence of T cell IFN-gamma responses after vaccination or natural exposure is suppressed by *Plasmodium falciparum*. J Immunol. 2007;179:4193–201.
8. Calle CL, Mordmüller B, Singh A. Immunosuppression in malaria: do *Plasmodium falciparum* parasites hijack the host? Pathogens. 2021;10:1277.
9. Greenwood BM, Bradley-Moore AM, Bryceson AD, Palit A. Immunosuppression in children with malaria. Lancet. 1972;i:169–72.
10. Ho M, Webster HK, Looareesuwan S, Supanaranond W, Phillips RE, Chanthavanich P, et al. Antigen-specific immunosuppression in human malaria due to *Plasmodium falciparum*. J Infect Dis. 1986;153:763–71.
11. Morter R, Tiono AB, Nebie I, Hague O, Ouedraogo A, Diarra A, et al. Impact of exposure to malaria and nutritional status on responses to the experimental malaria vaccine ChAd63 MVA ME-TRAP in 5–17 month-old children in Burkina Faso. Front Immunol. 2022;13:1058227.
12. Cunnington AJ, Riley EM. Suppression of vaccine responses by malaria: insignificant or overlooked? Expert Rev Vaccines. 2010;9:409–29.
13. Illingworth J, Butler NS, Roetynck S, Mwacharo J, Pierce SK, Bejon P, et al. Chronic exposure to *Plasmodium falciparum* is associated with phenotypic evidence of B and T cell exhaustion. J Immunol. 2013;190:1038–47.
14. Ouedraogo A, Tiono AB, Diarra A, Nebie IO, Konate AT, Sirima SB. The effects of a pre-season treatment with effective antimalarials on subsequent malaria morbidity in under five-year-old children living in high and seasonal malaria transmission area of Burkina Faso. Trop Med Int Health. 2010;15:1315–21.
15. Tiono AB, Guelbeogo MW, Sagnon NF, Nebie I, Sirima SB, Mukhopadhyay A, et al. Dynamics of malaria transmission and susceptibility to clinical malaria episodes following treatment of *Plasmodium falciparum* asymptomatic carriers: results of a cluster-randomized study of community-wide screening and treatment, and a parallel entomology study. BMC Infect Dis. 2013;13:535.
16. Guinovart C, Aponte JJ, Sacarlal J, Aide P, Leach A, Bassat Q, et al. Insights into long-lasting protection induced by RTS, S/AS02A malaria vaccine: further results from a phase IIb trial in Mozambican children. PLoS ONE. 2009;4: e5165.
17. Sonden K, Doumbo S, Hammar U, Vafa Homann M, Ongoiba A, Traore B, et al. Asymptomatic multiclonal *Plasmodium falciparum* infections carried through the dry season predict protection against subsequent clinical malaria. J Infect Dis. 2015;212:608–16.
18. Cai J, Chen S, Zhu F, Lu X, Liu T, Xu W. Whole-killed blood-stage vaccine: is it worthwhile to further develop it to control malaria? Front Microbiol. 2021;12: 670775.
19. Bejon P, Mwangi T, Lowe B, Peshu N, Hill AV, Marsh K. Clearing asymptomatic parasitaemia increases the specificity of the definition of mild febrile malaria. Vaccine. 2007;25:8198–202.
20. Moorthy VS, Imoukhuede EB, Milligan P, Bojang K, Keating S, Kaye P, et al. A randomised, double-blind, controlled vaccine efficacy trial of DNA/MVA ME-TRAP against malaria infection in Gambian adults. PLoS Med. 2004;1: e33.
21. Bojang KA, Milligan PJ, Pinder M, Vigneron L, Allouecheé A, Kester KE, et al. Efficacy of RTS, S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. Lancet. 2001;358:1927–34.
22. Khaemba EN, Ogwang C, Kinyanjui S, Muindi JM, Koske JK, Kimani D, et al. Comparing drug regimens for clearance of malaria parasites in asymptomatic adults using PCR in Kilifi County, Kenya: an open-label randomised controlled clinical trial (MalPaC). Wellcome Open Res. 2020;5:36.
23. Morris CA, Duparc S, Borghini-Fuhrer I, Jung D, Shin CS, Fleckenstein L. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. Malar J. 2011;10:263.
24. WHO. Notes on the Design of Bioequivalence Study: Dihydroartemisinin/Piperaquine Tetraphosphate. Geneva: World Health Organization; 2024.
25. D'Alessandro U. Progress in the development of piperaquine combinations for the treatment of malaria. Curr Opin Infect Dis. 2009;22:588–92.
26. Raynes K. Bisquinoline antimalarials: their role in malaria chemotherapy. Int J Parasitol. 1999;29:367–79.
27. Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg. 2007;77:181–92.
28. Tiono AB, Kangoye DT, Rehman AM, Kargougou DG, Kabore Y, Diarra A, et al. Malaria incidence in children in South-West Burkina Faso: comparison of active and passive case detection methods. PLoS ONE. 2014;9: e86936.
29. Yaro JB, Ouedraogo A, Ouedraogo ZA, Diarra A, Lankouande M, Agboraw E, et al. A cohort study to identify risk factors for *Plasmodium falciparum* infection in Burkinabe children: implications for other high burden high impact countries. Malar J. 2020;19:371.
30. Yaro JB, Tiono AB, Sanou A, Toe HK, Bradley J, Ouedraogo A, et al. Risk factors associated with house entry of malaria vectors in an area of Burkina Faso with high, persistent malaria transmission and high insecticide resistance. Malar J. 2021;20:397.
31. WHO ()2012 Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for *Plasmodium falciparum* malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa. Geneva, World Health Organization
32. Liu C, Zhang R, Hong X, Huang T, Mi S, Wang N. Pharmacokinetics of piperaquine after single and multiple oral administrations in healthy volunteers. Yakugaku Zasshi. 2007;127:1709–14.
33. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menendez C, et al. Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. PLoS ONE. 2009;4: e7871.
34. Valecha N, Phyo AP, Mayxay M, Newton PN, Krudsood S, Keomany S, et al. An open-label, randomised study of dihydroartemisinin-piperaquine versus artesunate-mefloquine for falciparum malaria in Asia. PLoS ONE. 2010;5: e11880.
35. WHO. Guidelines for malaria. Geneva: World Health Organization; 2023.
36. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, Do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol. 1993;61:315–20.
37. Mairet-Khedim M, Nsango S, Ngou C, Menard S, Roesch C, Khim N, et al. Efficacy of dihydroartemisinin/piperaquine in patients with non-complicated *Plasmodium falciparum* malaria in Yaounde, Cameroon. J Antimicrob Chemother. 2021;76:3037–44.
38. Myint HY, Ashley EA, Day NP, Nosten F, White NJ. Efficacy and safety of dihydroartemisinin-piperaquine. Trans R Soc Trop Med Hyg. 2007;101:858–66.
39. Abuaku B, Boateng P, Peprah NY, Asamoah A, Duah-Quashie NO, Matrevi SA, et al. Therapeutic efficacy of dihydroartemisinin-piperaquine combination for the treatment of uncomplicated malaria in Ghana. Front Cell Infect Microbiol. 2022;12:1058660.
40. Barradell LB, Fitton A. Artesunate. A review of its pharmacology and therapeutic efficacy in the treatment of malaria. Drugs. 1995;50:714–41.
41. Kone A, Sissoko S, Fofana B, Sangare CO, Dembele D, Haidara AS, et al. Different *Plasmodium falciparum* clearance times in two Malian villages following artesunate monotherapy. Int J Infect Dis. 2020;95:399–405.
42. Assefa DG, Yismaw G, Makonnen E. Efficacy of dihydroartemisinin-piperaquine versus artemether-lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria among children in Africa:

a systematic review and meta-analysis of randomized control trials. Malar J. 2021;20:340.
43. Yeka A, Wallender E, Mulebeke R, Kibuuka A, Kigozi R, Bosco A, et al. Comparative efficacy of artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated malaria in Ugandan children. J Infect Dis. 2019;219:1112–20.
44. Dama S, Niangaly H, Djimde M, Sagara I, Guindo CO, Zeguime A, et al. A randomized trial of dihydroartemisinin-piperaquine versus artemether-lumefantrine for treatment of uncomplicated *Plasmodium falciparum* malaria in Mali. Malar J. 2018;17:347.
45. Jullien V. Pharmocokinetics of antimalarial drugs. La Lettre de l'Infectiologue; DOSSIER THÉMATIQUE novembre-décembre 2012, Tome XXVII:239–243.
46. Maiga AW, Fofana B, Sagara I, Dembele D, Dara A, Traore OB, et al. No evidence of delayed parasite clearance after oral artesunate treatment of uncomplicated falciparum malaria in Mali. Am J Trop Med Hyg. 2012;87:23–8.
47. Schwarz NG, Oyakhirome S, Potschke M, Glaser B, Klein Klouwenberg P, Altun H, et al. 5-day nonobserved artesunate monotherapy for treating uncomplicated Falciparum malaria in young Gabonese children. Am J Trop Med Hyg. 2005;73:705–9.
48. Zani B, Gathu M, Donegan S, Olliaro PL, Sinclair D. Dihydroartemisinin-piperaquine for treating uncomplicated *Plasmodium falciparum* malaria. Cochrane Database Syst Rev. 2014;2014: CD010927.
49. Konate AT, Yaro JB, Ouedraogo AZ, Diarra A, Gansane A, Soulama I, et al. Morbidity from malaria in children in the year after they had received intermittent preventive treatment of malaria: a randomised trial. PLoS ONE. 2011;6: e23391.
50. Aponte JJ, Menendez C, Schellenberg D, Kahigwa E, Mshinda H, Vountasou P, et al. Age interactions in the development of naturally acquired immunity to *Plasmodium falciparum* and its clinical presentation. PLoS Med. 2007;4: e242.
51. Farnert A, Rooth I, Svensson A, Snounou G, Bjorkman A. Complexity of Pl*asmodium falciparum* infections is consistent over time and protects against clinical disease in Tanzanian children. J Infect Dis. 1999;179:989–95.
52. Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clin Microbiol Rev. 2009;22:13–36.
53. Kirakoya-Samadoulougou F, De Brouwere V, Fokam AF, Ouedraogo M, Ye Y. Assessing the effect of seasonal malaria chemoprevention on malaria burden among children under 5 years in Burkina Faso. Malar J. 2022;21:143.
54. De Cola MA, Sawadogo B, Richardson S, Ibinaiye T, Traore A, Compaore CS, et al. Impact of seasonal malaria chemoprevention on prevalence of malaria infection in malaria indicator surveys in Burkina Faso and Nigeria. BMJ Glob Health. 2022;7: e008021.
55. Yaro JB, Tiono AB, Ouedraogo A, Lambert B, Ouedraogo ZA, Diarra A, et al. Risk of *Plasmodium falciparum* infection in south-west Burkina Faso: potential impact of expanding eligibility for seasonal malaria chemoprevention. Sci Rep. 2022;12:1402.
56. White NJ, Chotivanich K. Artemisinin-resistant malaria. Clin Microbiol Rev. 2024:e0010924.

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