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Efficacy and Safety of Pyronaridine-Artesunate Versus Artemether-Lumefantrine in the Treatment of Acute Uncomplicated Malaria in Children in South-West Nigeria: An open- labelled randomized controlled trial

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Abstract

Background: Declining responsiveness to artemether-lumefantrine (AL), the ACT of choice since 2005, has been reported in Nigeria. Pyronaridine-artesunate (PA) is a newer fixed-dose ACT recently included in the WHO list of prequalified medicines for the treatment of uncomplicated falciparum malaria. However, data from the Nigerian paediatric population is scarce. Therefore, we compared the efficacy and safety of pyronaridine-artesunate with artemether-lumefantrine using the WHO 28-day antimalarial efficacy protocol in Ibadan, southwest Nigeria.

Method: In an open-labelled randomized controlled clinical trial children aged 3 to 144 months with a history of fever and microscopically confirmed *Plasmodium falciparum* malaria were enrolled. Enrolees were randomly assigned to receive pyronaridine–artesunate or artemether–lumefantrine at standard dosages according to bodyweight, for 3 days. Venous blood was obtained for haematology, blood chemistry and liver function tests on days 0, 3, 7, and 28 as part of safety evaluation.

Results: One hundred and sixty-five (95.9%; 165/172) enrolees completed the study. About half [52.3%; 90/172] of enrolees were male. Eighty-seven (50.6%) received artemether-lumefantrine while 85 (49.4%) received pyronaridine-artesunate. Day 28 adequate clinical and parasitological response for pyronaridine-artesunate was 92.7% [(76/82) 95% CI: 83.1, 95.9] and 71.1% [(59/83) 95% CI: 60.4, 79.9] for artemether-lumefantrine ($\rho<0.001$). PCR-corrected cure rates were similar for PA and AL as all parasite recurrences [PA = 6 (7.3%); AL = 24 (28.9%)] were re-infections. Haematological recovery at day-28 was significantly better among pyronaridine-artesunate treated patients (34.9% ± 2.8) compared to those treated with artemether-lumefantrine (33.1% ±3.0) [$\rho = 0.002$]. Adverse events in both treatment arms were fever, chills and rigors, anorexia, cough and headache, similar to symptoms observed during malaria infection. Blood chemistry and liver function tests were mostly within normal limits, with an occasional marginal rise. There was no serious adverse event in both arms of the study.

Conclusion: The two ACTs were well-tolerated. Pyronaridine-artesunate has a higher ACPR than artemether-lumefantrine in the per-protocol-population and a non-inferiority response after PCR correction in the treatment of uncomplicated malaria in Nigerian children. The results of this study support the inclusion of pyronaridine-artesunate in the antimalaria treatment guidelines in Nigeria.

Retrospective Trial Registration in Clinicaltrials.gov: NCT05192265

Background

Despite efforts to control and eliminate malaria, it remains a major public health concern in sub-Saharan Africa. As documented in successive editions of the World Malaria Report, malaria continues to cause unacceptably high levels of disease and death in sub-Saharan Africa. According to the 2021 WHO malaria report, there were an estimated 241 million malaria cases and 627,000 malaria deaths in 2020, representing about 14 million more cases in 2020 compared to 2019, and 69,000 more deaths [1]. Artemisinin-based combination therapy (ACT), the current WHO global standard for the treatment of acute uncomplicated malaria [1], has contributed to a significant reduction in malaria prevalence in recent years [2]. This progress is endangered, however, by the emergence of *P. falciparum* strains with reduced *in-vivo* susceptibility to artemisinin, which was first reported in Western Cambodia [3, 4], as well as reduced political commitment and funding for malaria control [2].

Since the first report of reduced *in-vivo* susceptibility in the late 2000s, the prevalence of drug-resistant infections has increased and spread to other parts of Southeast Asia and the rest of the world [2, 3, 5]. Artemisinin resistance, which is strongly linked to a mutation in the propeller region of the *P. falciparum* Kelch protein gene on chromosome 13 (Kelch13), was first discovered in western Cambodia and has since spread to the majority of malaria-endemic regions around the world, including Africa and India [6–9]. Previous research found a lack of selection for Kelch13 in southwest Nigeria [10]. Another study also recorded low polymorphism in samples from Kano, Nigeria, indicating a lack of selection for the Kelch 13 gene [10, 11].

Artemether-lumefantrine (AL), the most commonly used treatment for uncomplicated malaria in several African countries including Nigeria, has an impressive safety and efficacy record [12, 13]. However, it must be taken twice daily, must be consumed with a fatty meal for optimal absorption, and the relatively short half-life puts patients at risk of reinfection [14].

There is the need to expand options for antimalarial treatment, first to delay resistance, contribute to resistance management where this is already a problem and reduce drug pressure on AL. Pyronaridine-artesunate does not have the limitations of AL and could reduce the pressure on AL thus making it a viable option to evaluate.

Pyronaridine-artesunate (PA) [Pyramax™; Shin-Poong Pharma Ltd. South Korea] first received a positive scientific opinion from the European Medicines Agency under Article 58 for the tablet formulation in 2012. Pyramax™ granules (special paediatric formulation] was subsequently granted a positive scientific opinion through its Article 58 in 2015 [15, 16]. This regulatory authority approval confirms that Pyramax™ can be used in malaria patients, particularly African children. The safety and efficacy of pyronaridine-artesunate in adults and children in Africa and Asia have been demonstrated in many randomised controlled clinical trials [17–22]. Consequently, PA was recommended by the WHO in 2019 as a "safe and efficacious artemisinin-based combination therapy for the treatment of uncomplicated malaria in adults and children weighing 5 kilograms or more in malaria-endemic areas" [23]. As a result, PA was included in Nigeria's national treatment guidelines [13]. However, more research is needed to provide Nigeria with in-country data on the safety and efficacy of PA, based on the good safety and efficacy observed in the Phase III program and the CANTAM Study 2021 [24].

Furthermore, Sowunmi et al. [25] recently reported a decline in the responsiveness of childhood *Plasmodium falciparum* infections to ACTs (artemether-lumefantrine and artesunate-amodiaquine) in Nigeria. This underscores the importance of evaluating another effective and user-friendly antimalarial with good efficacy and tolerability in the country. This clinical trial resulted from efforts to improve efficacy, tolerability, cost, and treatment regimens. As the country is endemic for malaria, it is also critical to continue assessing the efficacy of AL, the current first-line ACT of choice in Nigeria, in order to detect early loss of efficacy. There is currently no data on the safety and efficacy of pyronaridine-artesunate in the Nigerian population. The goal of this study is to compare the safety and efficacy of pyronaridine-artesunate and artemether-lumefantrine in children aged 3 months to 12 years (144 months) with symptomatic acute uncomplicated malaria in south-west Nigeria.

Methods

Study design and setting

This comparative, randomized, open-label, parallel-group clinical trial followed WHO guidelines [12]. Study drugs were pyronaridine-artesunate (PA) granules or tablets (Pyramax™; 60mg pyronaridine/20mg artesunate per sachet of granules, 180/60 respectively per tablet) supplied in aluminium sachets (Shin Poong Pharmaceutical Company, Ltd., Ansan, Korea), and artemether-lumefantrine (AL) tablets (Coartem™; Novartis SA, Basel, Switzerland). supplied in blister packs. Each tablet of dispersible Coartem contains 20 artemether and 120 mg lumefantrine. Patients were enrolled from the Kola Daisi Primary Healthcare Centre (PHC), University College Hospital, Ibadan and Ikereku PHC in Akinyele Local Government Area, southwest Nigeria.

Study participants

Children aged 3 to 144 months with clinical features consistent with acute uncomplicated malaria and microscopically confirmed *Plasmodium falciparum* malaria were enrolled if they had a minimum asexual parasite density of 1000/μL, an axillary temperature of ≥ 37.5°C or a history of fever within 24 hours of presentation, lived within fifteen kilometres of the study centre, and were capable of taking drugs orally. Additional inclusion criteria included the absence of artemisinin-based combination therapy (ACT) intake in the two weeks preceding enrolment and signed informed consent from prospective enrolees' parents or guardians to participate in the study. Children with a history of allergy to any of the study drugs, including artemisinin, lumefantrine, or pyronaridine, were excluded from the study. Children with concurrent illnesses that could impair response evaluation, such as bacterial or viral infections, were also excluded. Additionally, children with clinical evidence of severe malaria were excluded from the study [26]. Children with severe malnutrition or known chronic diseases such as chronic liver disease, heart failure, or sickle cell anaemia were also excluded from the study. Children of parents/guardians who, in the investigator's opinion, will not adhere to the study protocol were not permitted to participate in the study.

Randomization and blinding

Patients were allocated to either PA or AL group following a pre-generated randomization table in ascending order. Different clinical personnel performed clinical assessments and drug administration.

Treatments

The study drugs were administered orally for three days (days 0, 1, and 2). Pyronaridine-artesunate (Pyramax™ - Shin Poong Pharmaceutical Company, Ltd., Ansan, Korea) was given once daily in the clinic by the research nurse supervised for three days. Pyramax™ contains 60mg pyronaridine/20mg artesunate per sachet of granules, 180/60 respectively per tablet supplied in aluminium sachets. Dosing was based on body weight: 5 – <8kg: one sachet; 8 – <15kg: two sachets; 15 – <20kg; three sachets; 20 – <24kg: one tablet and 24 – <45kg: two tablets. All PA doses were administered with a milk drink under the supervision of a research nurse. AL was administered as dispersible tablets (Coartem™, Novartis Pharma Basil Switzerland). Each dispersible tablet contains 20mg of artemether/120mg of lumefantrine. Study participants were dosed as follows: 5 – <15Kg; one tablet, 15 – <25Kg two tablets, 25 –<35Kg three tablets, and 35Kg and above; four tablets. AL was administered at 0, 8, 24, 36, 48, and 60 hours respectively. The first, third, and fifth doses of AL were administered supervised in the clinic with milk drinks while the second, fourth, and sixth doses of AL were administered with milk drinks at home by the parent or guardian. Mothers of children who received AL were encouraged to breastfeed very young babies who were still on the breast soon after the drug was administered. At the end of Days 0, 1, and 2 visits, parents and guardians were given a sachet of full cream powdered milk each daily for the 2nd, 4th, and 6th doses of AL and were also reminded by phone 30 minutes before the next drug dosing. Although a fatty meal was not required for optimal absorption of PA unlike for AL, all enrolees received study drugs with milk drinks because the children who were randomised to receive PA felt disadvantaged because they did not receive milk drinks, and this could lead to poor compliance with follow up visits.

Each enrolee irrespective of drug group was observed in the clinic for 1 hour after drug administration for vomiting. If vomiting occurred within 30 minutes of drug administration, the full treatment drug dose (PA or AL) was re-administered. If vomiting occurred between 30–60 minutes of drug administration, half the treatment dose was re-administered. Any enrolee who vomits the repeat dose was withdrawn from the study and treated with artesunate-amodiaquine as rescue medication as per the Nigeria national malaria treatment guidelines [13].

Data collection procedures

Enrolees who met the inclusion criteria were given a detailed explanation of the study and were only enrolled after the accompanying parent or guardian signed an informed consent form. The information gathered from each participant was entered into case record forms (CRF) created specifically for the study. In the individual CRF, socio-demographic information, contact information for each enrolee, past medical history, as well as clinical and laboratory details for all enrolees in the study, were recorded. Each enrolee's parent or guardian provided a detailed medical history, which included a history of the presenting symptoms as well as a list of current medications. In 2019, an electronic thermometer was used to measure axillary temperature, while an infrared thermometer (beamed at the forehead) was used in 2020 because of the SARS-CoV-2 pandemic.

A few drops of capillary blood were obtained via finger prick for thick and thin blood smears and some into a capillary tube for haematocrit determination, and blood spots on filter paper for parasite genotyping at enrolment. For malaria parasite detection, thick and thin blood films were prepared from finger prick blood samples of each enrolee on Day 0, whereas only thick blood films were prepared at all other contact times and stained with fresh 10% Giemsa stain at pH 7.2. Dry stained blood films were viewed at a magnification of x1000 using a light microscope in accordance with the WHO guideline [27]. At enrolment and on all contact days, gametocyte carriage was specifically observed and counted in the thick films. At enrolment and at each follow-up visit, blood spots on filter paper were obtained for DNA analysis using the polymerase chain reaction (PCR) to differentiate re-infection from recrudescence [28]. At each point of contact, blood was drawn into capillary tubes for haematocrit determination. A five-millilitre venous blood sample was also obtained via venepuncture for the evaluation of haematological and biochemical parameters (urea and creatinine, as well as a liver function test) on days 0, 3, 7, and 28. Blood samples were collected from study participants and analysed at the chemical pathology and haematology laboratories of the University College Hospital Ibadan, Nigeria.

All enrolees were followed up on out-patient basis daily from days 0 to 3 and then on days 7, 14, 21 and 28. Enrolees were also seen whenever a participant fell ill or a parent or guardian was concerned about their children's health. The day before follow-up visits, parents and guardians of enrolees were reminded by phone. Enrolees were provided transportation refunds to encourage compliance with follow-up. Evaluation at each visit included a brief clinical history and a physical examination to assess new complaints and medication side effects. A finger prick blood sample was taken on days 1, 2, 3, 7, 14, 21, 28, and any other (unscheduled) visit day for the preparation of thick blood films for the identification and quantification of asexual stages of the malaria parasite while we collected filter paper blood samples on days 0, 3, 7, 14, 21, and 28 for parasite genotyping from the same capillary blood samples.

DNA Isolation and Amplification of Plasmodium falciparum

Paired samples of blood spots on filter paper collected from patients with parasite recurrence on or before D28 (D0 and Day of parasite recurrence) were analysed using PCR techniques for parasite DNA. Genomic DNA extraction was performed with QIAamp DNA Mini kit blood and tissue (QIAGEN Hilden Germany) kit, according to manufacturer's instructions. Eluted genomic DNA was stored at -20^{o}C until further PCR analysis. The small sub-unit ribosomal RNA (ssrRNA) gene of *P. falciparum* was amplified in a nested PCR using a primary genus-specific primer and a secondary *P. falciparum* species-specific primer following the previously described standard protocol [29, 30]. Paired samples with confirmed parasitaemia were further genotyped to distinguish recrudescence from new infection using merozoite surface protein-1 (*msp-1*), merozoite surface protein-2 (*msp-2*) and glutamate-rich protein (*glurp*) genes respectively.

In a 20μL reaction volume, PCR mixture containing 5X PCR Master Mix (Solis Biodyne, Estonia), 0.5mM each of forward and reverse primers, nuclease-free PCR grade water, and 5 μL of extracted genomic DNA as template was prepared for the primary reaction. In the Nested reaction, 2μL of the primary PCR product was used as a template in 18μL PCR mixture, containing the same reagents as the primary reaction except with a species-specific forward and reverse primers. The PCR thermal cycling conditions were previously described [29]. All amplification of DNA was performed in an Eppendorf AG 22331 thermal cycler (Hamburg, Germany).

Genotyping of msp-1, msp-2 & Glurp antigenic loci

PCR amplification targeting the polymorphic regions of *msp-1* (block 2), *msp-2* (block 3) and glurp (R2) regions were genotyped with allelic specific primers as previously described [30, 31]. The antigenic allelic families of *msp-1* (K1, RO33 and MAD20), *msp-2* (FC27 & 3D7) were amplified in a final reaction volume of 20μL (25μL for glurp), containing 5X PCR Master mix (Solis Biodyne, Estonia), primers (forward and reverse) 0.5mM (0.75mM for glurp) each and 5 μL of extracted genomic DNA as template in the primary reaction. 2μL of the primary PCR product was used as template in the nested reaction. The PCR cycling conditions for both primary and nested reactions were as previously described [30, 31]. Ten (10μL) of the nested PCR products (amplicon) were electrophoresed on 1.5% agarose gel pre-stained with EZ-vision blue light DNA dye (EZ-Vision® Blue light DNA Dye, VWR Chemicals USA) and was allowed to migrate at 100volt for 40–60 minutes for fragment size differentiation. The gel was visualized and photographed under a UV trans-illuminator documentation system (UVP® DigiDoc-It™, USA). Amplified fragments were paired for base-pair sizing and comparison. Fragments with the same base pair sizes (paired samples) in the amplified loci were considered recrudescence while un-identical base pair sizes were considered new infections. Rrecrudescence was defined as when a subsequent sample from follow up contained identical alleles or a subset of the alleles present in the day 0 sample. Reinfection was defined as when a follow up sample contained only new alleles that are un-identical with day 0 sample.

Study endpoints

The WHO 2009 treatment outcome criteria were used to assess efficacy [27]. Early treatment failure (ETF) was defined as danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia; parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; parasitaemia on day 3 with an axillary temperature ≥ 37.5 ºC or parasitaemia on day 3 ≥ 25% of count on day 0. Late clinical failure (LCF) was defined as danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who had not previously met any of the criteria for early treatment failure; or the presence of parasitaemia on any day between day 4 and day 28 (day 42) with an axillary temperature of ≥ 37.5 ºC (or history of fever) in patients who had not previously met any of the criteria for early treatment failure. Late parasitological failure (LPF) is

defined as the presence of parasitaemia on any day between days 7 and 28 (day 42) and an axillary temperature of < 37.5ºC in patients who did not previously meet any of the criteria for early treatment failure or late clinical failure. The main end point for the study was adequate clinical and parasitological response (ACPR), defined as the absence of parasitaemia on day 28, irrespective of axillary temperature, in patients who had not previously met any of the criteria of early treatment failure, late clinical failure, or late parasitological failure.

Recrudescence was defined as the recurrence of asexual parasitaemia within 28 days of receiving antimalarial treatment that contained the same genotypes that caused the initial illness. At the time of enrolment, PCT was defined as the time from the first dose of ACT until the first total and continued disappearance of asexual parasite forms for at least 24 hours, whereas fever clearance time (FCT) was defined as the time from the first dose of ACT until the first time the body temperature (for those with a raised temperature at enrolment) drops to below 37.5 ºC and stays below 37.5 ºC for at least 24 hours.

In this study, safety was evaluated in terms of adverse events and severe adverse events by examining symptoms, clinical signs, and laboratory parameters. All study participants who took at least one dose of either of the study drugs were evaluated for safety. The evaluation of liver enzymes was a special category that was evaluated due to previous reports of transaminitis [22]. Alanine transaminase (ALT)/ aspartate transaminase (AST) greater than three times the upper limit of normal (ULN) plus peak total bilirubin greater than two times the ULN in the absence of a significant alkaline phosphatase increase were considered serious adverse events (SAE). An ALT and AST level greater than 5 times the ULN were considered an adverse event of special interest (AESI).

Ethics statement

The University of Ibadan/University College Hospital Ethics Committee (EC approval No - UI/EC/19/0114) and the Oyo State Ministry of Health Ethics Committee (EC approval No - AD 13/479/206) both approved the study protocol. Individual written informed consent was obtained from eligible participants' parents or guardians. Our study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki [32], as well as applicable regulations.

Sample size calculation

We calculated the required minimum number of enrolees using the sample size formula for test of non-inferiority as written by Chow and colleagues [33], by assuming a difference of 2.5% in cure rate between PA and AL (98.9% vs 96.4%), 140 patients are required to attain a power of 90% at 95% level of confidence to demonstrate non-inferiority of PA versus AL with a non-inferiority limit of 5%. However, allowing for 20% loss to follow up, the total number of patients required would be 168 to give 84 patients per arm.

Data analysis

All study participants who received any amount of the study drugs were included in the intent-to-treat population. The per-protocol-population included children who received the entire course of study medication, had a known day-28 efficacy end point, and had no major protocol deviations. The data was analysed for efficacy in both the intention-to-treat and per-protocol populations. The efficacy endpoints were summarized by age. Safety outcomes were adverse events and abnormalities of laboratory results including liver function test and haematological indices. SPSS IBM Statistic Software version 20 was used to analyse the data at $\rho = <0.05$.

Results

Enrolees’ baseline characteristics and symptoms at presentation

The study was conducted between May and December 2019 and between July and December 2020. A total of 1,204 febrile children were screened for malaria parasitaemia during the study period; 550 (45.7%) had patent parasitaemia, with 172 eligible patients randomly assigned to PA (n = 85) and to AL (n = 87). Figure 1 shows the details of the reasons for the exclusion of ineligible patients.

Baseline demographic and clinical characteristics were similar between the two treatment groups (Table I).

Table 1
Baseline Characteristics of children suffering from acute uncomplicated malaria treated with artemether-lumefantrine or pyronaridine-artesunate in Ibadan SW Nigeria

Characteristics	Drug Group		Total	ρ-value
	AL	PA		
Sex	45 (50.0%)	45 (50.0)	90 (100)	0.880
• Male	42 (51.2%)	40 (48.8%)	82 (100)	
• Female				
Age (months)	79.93 ± 37.16	82.39 ± 38.63	81.15 ± 39.80	0.671
• Mean ± sd	8–144	4–143	4–144	
• Range				
• 3–11 months	3 (3.5)	5 (5.9)	8 (4.7)	0.703
• 12–59 months	20 (23.0)	21 (24.7)	41 (23.8)	
• 60 and above	64 (73.6)	59 (69.4)	123 (71.5)	
Weight (kg)	18.59 ± 6.49	18.67 ± 6.74	18.63 ± 6.59	0.938
• Mean ± sd	7.0–36.0	5.5–40.0	5.5–40.	
• Range				
Temperature (^{o}C)	37.7 ± 1.06	37.4 ± 1.07	37.6 ± 1.08	0.074
• Mean ± sd	35.1–40.2	35.9–39.9	35.1–40.2	
• Range				
Parasite density (/µl)	32,755	28,211	30,424	0.815
• Geomean	1035–758,958	1600–1,652,505	1035–1,652,505	
• Range				
Haematocrit (%)	32.14 ± 5.06	32.14 ± 5.48	32.14 ± 5.26	0.997
• Mean ± sd	18–41	18–48	18–48	
• Range				
Anaemic at DO	19 (21.8)	21 (24.7)	40 (23.3)	0.396
(PCV < 30%)				
Height (cm)	110.65 ± 21.31	110.65 ± 28.90	110.38 ± 25.36	0.892
• Mean ± sd	18–143	18–186	18–186	
• Range				

The four most common symptoms at presentation were fever (100%), headache (69.8%), chills and rigors (62.6%), and loss of appetite (57.2%) (Table 2). Table 2also shows that there were statistically significant differences in the frequencies of only chills and rigors and loss of appetite which were higher in the AL group than in the PA group (ρ = 0.033 and 0.014, respectively). Five

patients (5.9%) in the PA compared with 3 (3.4%) in the AL group (ρ = 0.347), vomited the first dose of the study drug and had to be re-dosed. After redosing, one of the children that received AL vomited and was withdrawn from the study.

Table 2
Distribution of children with acute uncomplicated malaria according to symptoms other than fever at presentation by treatment groups

Presenting complaint	Total population N = 172 (100)	Artemether-lumefantrine N = 87 (100%)	Pyronaridine-artesunate N = 85 (100%)	ρ-value
Headaches	120 (69.8)	61 (70.1)	59 (69.4)	1.000
Chills & rigors	117 (68.0)	66 (75.9)	51 (60)	0.033
Loss of appetite	101 (58.7)	59 (67.8)	42 (49.4)	0.014
Vomiting	82 (47.7)	44 (50.6)	38 (44.7)	0.450
Abdominal Pain	68 (39.5)	33 (37.9)	35 (41.7)	0.642
Nausea	54 (31.4)	30 (34.5)	24 (28.6)	0.417
Cough	45 (26.2)	21 (24.1)	24 (28.6)	0.603
Diarrhoea	32 (18.6)	18 (20.7)	14 (16.5)	0.558
Irritability	33 (19.2)	13 (14.9)	20 (23.5)	0.178
Insomnia	17 (9.9)	10 (11.5)	7 (8.3)	0.611
Palpitations	10 (5.8)	5 (6.0)	5 (5.7)	1.000

Results of Efficacy Evaluation

Intention-to-treat population analysis

Of the 172 randomized to the study groups, 165 (95.9%) completed the study, and the primary efficacy endpoint of the study was attained. The distribution of the participants in the two study groups by treatment outcomes based on intention-to-treat and per-protocol analyses is shown in Table 3. There were no significant differences in the number of patients who completed the study (ρ = 0.682), those who were lost to follow-up (p = 1.000) and those who withdrew (p = 1.000) between the two study groups.

Table 3
Treatment outcome among patients with uncomplicated malaria treated with Artemether-lumefantrine or Pyronaridine-artesunate in Ibadan, southwest Nigeria

Treatment Outcome	Artemether-lumefantrine N (%)	Pyronaridine-artesunate N (%)	Total N (%)	ρ-value
TOTAL Enrolled	**87 (100)**	**85 (100)**	**172 (100)**	
• Completed study.	83 (95.4)	82 (96.5)	165 (95.9)	.535
• LTFU	3 (3.4)	2 (2.4)	5 (2.9)	
• Withdrawn	1 (1.2)	0 (0.0)	1 (0.6)	
• Withdrawal of consent	0 (0.0)	1 (1.2)	1 (0.6)	
ITT- Uncorrected	**N = 87 (100)**	**N = 85 (100)**	**N = 172 (100)**	
• ACPR – D28	59 (67.8)	76 (89.4)	135 (78.5)	.002
• LPF	21 (24.1)	8 (9.4)	29 (16.9)	
• LCF	7 (8.1)	1 (1.2)	8 (4.7)	
ITT – PCR Corrected	**N = 87**	**N = 85**	**N = 172**	
• **Cured**	83 (95.4)	82 (96.5)	165/172 (95.9)	1.000
• **Failed**	4 (4.6)	3 (3.5)	7/172 (4.1)	
PP – Uncorrected	**N = 83**	**N = 82**	**N = 165**	
• ACPR – D21	73/83 (88.0)	82/83 (98.8)	135/166 (93.4)	.016
• ACPR – D28	59/83 (71.1)	76/82 (92.7)	135 (81.8)	.001
• LPF – D28	17 (20.5)	5 (6.1)	22 (13.3)	
• LCF – D28	7 (8.4)	1 (1.2)	8 (4.8)	
PPP- PCR Corrected	**N = 83**	**N = 82**	**N = 165**	
• Cured - D28	83 (100)	82 (100)	165 (100)	NA
Day of Failure	**24 (100)**	**6 (100)**	**30 (100)**	
• Day 14	2 (8.3)	0 (0.0)	2 (6.7)	.405
• Day 21	9 (37.5)	1 (16.7)	10 (33.3)	
• Day 28	13 (54.2)	5 (88.3)	18 (60.0)	
• % Failed before D28	11 (45.8)	1 (16.7)	12 (40.0)	.358
• % Failed on D28	13 (54.2)	5 (83.3)	18 (60.0)	
Parasite clearance time (days)				
• Mean ± sd	2.15 ± 0.68	2.08 ± 0.64	2.12 ± 0.66	.430
• Range	1–4	1–3	1–4	
Fever clearance time (days)				
• Mean ± sd	1.11 ± 0.32	1.13 ± 0.44	1.13 ± 0.38	0.664
• Range	1–2	1–3	1–3	
Haematocrit at D28 (in %)				

Treatment Outcome	Artemether-lumefantrine N (%)	Pyronaridine-artesunate N (%)	Total N (%)	ρ-value
• Mean ± sd	33.3 ± 2.95	34.9 ± 2.79	34.13 ± 2.95	.002
• Range	23–40	28–42	25–42	
• Haematocrit < 30% D28	1 (1.4)	1 (1.4)	2 (1.4)	1.000

Among the intention-to-treat population, the percentage of patients with adequate clinical and parasitological responses (ACPR) on day-28 was significantly higher among patients in the PA group than the AL group (ρ = <0.002), but the difference was no longer significant after PCR-correction (ρ = 1.000) as shown in Table 3. The total number of patients who experienced treatment failure in the AL group (28/87 (32.2%) was significantly higher than those who had treatment failure in the PA group [9/85 (10.6%)]. The risk of failure was also higher in the AL group than in the PA group (RR = 1.80; 95% CI: 1.39, 2.33), ρ = <0.001. Similarly, patients randomised to the AL group had an increased risk of experiencing treatment failure before day-21 compared with those in the PA group (RR = 1.87; 95% CI: 1.44, 2.43).

Although haematological recovery was excellent for both study drugs, the mean haematocrit on day 28 was significantly higher in the patients randomised to the PA group (34.9 ± 2.8%) than in those in the AL group (33.3 ± 3.0%); ρ < 0.002. Conversely, there were no significant differences in the mean parasite and fever clearance times as well as the number of anaemic patients between the PA group and the AL group (Table 3). The graphs (Figs. 2 and 3) from the Kaplan-Meier survival analysis also corroborate the lack of differences in the fever and parasite clearance times between patients randomised to the PA group and those in the AL group.

Per-protocol population analysis

There was no early treatment failure during the study. Response to treatment was prompt in all study participants except in an enrolee among AL treated children who had recurrent vomiting and had to be withdrawn on day 0. By day 1, 11.6% (10/86) and 12.9% (n = 11/85) of patients treated with AL and PA respectively were free of patent parasitaemia while corresponding values for day 2 were 70.9% (n = 61/86) and 72.9% (n = 62/85) for AL and PA, respectively. Two (2.3%) of the study participants who received AL had patent parasitaemia at day 3, but they both recorded adequate clinical and parasitological responses at day 28. All participants were free of patent parasitaemia by day 7. However, two children among those who received AL failed treatment at day 14; one each recorded LCF and LPF.

In contrast to those treated with AL, treatment failure was first seen among children who received PA on day 21. The ACPR in the per-protocol population at day 21 was significantly higher for PA than for AL treated children (98.8% versus 88.0%, respectively, ρ = 0.009). The chances of ACPR were significantly lower in the AL than PA (RR = 0.52; 95% CI: 0.40, 0.67). Similarly, ACPR (unadjusted cure rate) at day 28 was 92.7% for PA and 71.1% for AL, with PA having significantly higher chances of attaining ACPR than AL (RR = 2.81; 95% CI: 1.36, 5.85), ρ = 0.001.

The efficacy outcomes observed by age in the per-protocol population are as shown in Table 4. The ACPR was significantly higher in the PA group than in the AL group (ρ = 0.008) among children under five-year-old (< 60 months) and over 5-year-old (≥ 60 month) old children (RR = 1.64; 95% CI: 1.19, 2.26), ρ = 0.016, similar to what was recorded in the entire study population.

All the 30 children with parasite recurrence among PP population - [6/82 (7.3%) for PA versus 24/83 (28.9%) for AL; ρ < 0.001] were treated with artesunate-amodiaquine (ASAQ). Response to ASAQ was prompt, leading to rapid parasite clearance and resolution of symptoms where there were symptoms.

Table 4
Treatment outcome among children (PP) from southwest Nigeria with acute uncomplicated malaria treated with artemether-lumefantrine or pyronaridine-artesunate in different age groups.

Treatment Outcome	Drug group		Total	ρ-value
	AL	PA		
Age < 12 months [N = 8]	3	5	8	
• ACPR	2 (66.7)	5 (100)	7 (87.5)	0.375
• LCF	1 (33.3)	0 (0)	1 (12.5)	
• PCR Corrected ACPR	3/3 (100)	5/5 (100)	8/8 (100	Nil
Age < 60 months [N = 47]	N = 22	N = 25	N = 47	
• ACPR	14 (63.6)	24 (96.0)	38 (80.9)	0.008
• LPF	4 (18.2)	1 (4.0)	5 (10.68)	
• LCF	4 (18.2)	0 (0)	4 (8.5)	
Age ≥ 60 months [N = 118]	N = 61	N = 57	N = 118	
• Cured (ACPR)	45 (73.8)	52 (91.2)	97 (82.2)	0.016
• Failed (LCF + LPF)	16 (26.2)	5 (6.1)	21 (17.8)	
• ACPR	45 (73.8)	52 (91.2)	97 (82.2)	0.046
• LPF	13 (21.3)	4 (7.0)	17 (14.4)	
• LCF	3 (4.9)	1 (1.8)	4 (3.4)	
Total PPP [N = 165]	83	82	165	
• ACPR	59/83 (71.1)	76/82 (92.7)	135/165 (81.8)	0.001
• LPF	17/83 (20.5)	5/82 (6.1)	22 (13.3)	
• LCF	7/83 (8.4)	1/82 (1.2)	8 (4.8)	
• Cured (ACPR)	59/83 (71.1)	78/82 (92.7)	135/165 (81.8)	< 0.0001
• Failed (LCF + LPF)	24/83 (28.9)	6/82 (7.3)	30/165 (18.2)	
• PCR Corrected cure rate	83/83 (100)	82/82 (100)	163/165 (98.8)	NA
• PCR corrected treatment failure rate	0/83 (0.0)	0/82 (0.0)	0/165 (0.0)	

Molecular analysis

Molecular biology analysis of paired samples for enrolees with parasite recurrence was carried out with *msp-1, msp-2* and *glurp*. This showed that all six cases of parasite recurrence among children treated with PA were re-infections. In a similar manner, all the 24 cases among AL treated children were also re-infection giving PCR corrected cure rates of 100% for PA and AL for the PP sampled populations.

Safety of pyronaridine-artesunate compared with artemether-lumefantrine

Complaints and physical examination.

The ITT population was considered as the safety population. Recorded adverse events were similar to the symptoms and signs seen during malaria infection. There was no incidence of serious adverse event throughout the study. The type and prevalence of the various adverse events were similar for the two study drugs. The five most often recorded adverse events were fever, chills and rigors, anorexia, cough, and headache. These are clinical symptoms that occur during malaria infection. Further details are provided in Table 5. There was no clinical evidence of jaundice, intravascular haemolysis, hepatic dysfunction, or renal impairment. No death was recorded during the study.

Table 5
Summary of Adverse Events among children (PP) from southwest Nigeria with acute uncomplicated malaria treated with artemether-lumefantrine or pyronaridine-artesunate

Event	All patients N = 172	Artemether-lumefantrine N = 87	Pyronaridine-artesunate N = 85	p
Adverse event from any cause,	53 (30.8)	27 (31.0)	26 (30.6)	0.949
Fever	58 (34.1)	24 (27.9)	34 (40.5)	0.106
Chills and rigors	33 (19.6)	16 (18.6)	17 (20.5)	0.847
Anorexia	29 (17.1)	16 (18.6)	13 (15.5)	0.685
Cough	28 (16.6)	14 (16.3)	14 (16.9)	1.000
Headache	27 (16.0)	12 (14.1)	15 (17.9)	0.536
Fatigue	22 (12.9)	11 (12.6)	11 (13.1)	1.000
Pallor	17 (10.1)	9 (10.6)	8 (9.6)	1.000
Abdominal pains	12 (7.1)	5 (5.8)	7 (8.3)	0.563
Vomiting	11 (6.4)	6 (7.0)	5 (5.9)	1.000
Diarrhea	11 (6.4)	4 (4.6)	5 (6.0)	0.744
Palpitations	6 (3.5)	3(3.4)	3 (3.6)	1.000
Nausea	6 (3.5)	3 (3.5)	3 (3.6)	1.000
Rashes	1 (0.6)	0 (0.00)	1 (1.2)	1.000

Laboratory results were mostly within normal ranges, with occasional marginal increases as (shown in Supplementary file 1 – Tables 6a – 6h, Tables 7a to 7h and Figs. 4–11). There were no records of blood urea, creatinine, random blood glucose, or total bilirubin levels that were more than twice the upper limit of normal after receiving either study drugs. Both PA and AL had no deleterious effect on the transaminases. On day-0 (before antimalarial administration), two study participants who received AL had alanine transaminase (ALT) levels that were twice the upper limit of normal. By day 3, the elevated ALT levels had returned to normal. On day 0, one of the two children who received AL and had ALT more than twice the upper limit of normal also had aspartate aminotransferase (AST) more than twice the upper limit of normal, which returned to normal by day 3.

On day-3, one participant in the PA group had AST level that was twice the upper limit of normal. By day 7, the elevated AST level had returned to normal. There was no instance of any adverse event of interest. Changes in blood levels of various enzymes and other parameters measured among participants in the two drug groups were statistically significant on Days 7 and 28 with ALT and Day 28 with AST and alkaline phosphatase (ALP). The observed increases in liver enzymes, bilirubin, urea, creatinine, and random blood sugar levels were not clinically significant (Supplementary file 1).

Gametocyte carriage

The number of participants who received PA with gametocyte carriage was consistently higher than that of those who received AL, except on day 28, when only one of the PA-treated children and two of the AL-treated children had gametocytes. However, the differences were only statistically significant on day 7. Supplementary file 2 - Table 8a contains further information on gametocyte carriage. The number of participants with gametocytes was not only higher in children treated with PA, but the gametocytes densities were also consistently higher (supplementary file 2 - Table 8b). One participant in particular stood out because he had gametocytes from day 0 to day 28. The gametocyte counts were also high, ranging from 728/µL to 3,088/µL. Two other participants did not clear their gametocytes until day 21, and another participant had gametocytaemia until day 14. All four participants who had delayed gametocyte clearance had received PA.

Discussion

During this study, we evaluated the comparative efficacy and safety of pyronaridine-artesunate [Pyramax™; Shin Poong Pharm Co., Ltd, Seoul, South Korea] and artemether-lumefantrine [Coartem™; Novartis Pharma; Switzerland] in the treatment of acute uncomplicated falciparum malaria among Nigerian children. The two ACTs were found to be efficacious and well tolerated by the study participants. There was no record of early treatment failure in any arm of the study, and only one participant who received AL was withdrawn as a result of recurrent vomiting.

The day-28 PCR-corrected cure rate in children with uncomplicated falciparum malaria treated with PA granules or tablets was 100.0% in the per-protocol population and 96.5% in the intention-to-treat population. The primary efficacy outcome for pyronaridine–artesunate was greater than 95% in all age groups, be it among children under one year (< 12 months) of age, under-five years old (< 60 months) or 60 to 144 months old. The efficacy of pyronaridine–artesunate against falciparum malaria was generally consistent with previous studies on the use of pyronaridine–artesunate [17–24, 34, 35].

A highly significant efficacy finding in this study is that pyronaridine–artesunate had a significantly higher ACPR than artemether–lumefantrine in both the intent-to-treat (89.4% versus 67.8%) and per-protocol populations (92.7% versus 71.1%). However, because recurrent parasites were re-infections, there was no significant difference in the PCR-corrected cure rates of pyronaridine–artesunate and artemether–lumefantrine at day 28. This finding raises concern about the post treatment prophylaxis of AL compared with PA. Our findings are in keeping with reports of pyronaridine–artesunate and artemether–lumefantrine efficacy in the treatment of acute uncomplicated malaria among Gabonese children [35], Kenyan children [34], and children from Burkina Faso, Guinea, and Mali [21]. Although there are no reports on the efficacy and safety of PA in Nigeria, numerous studies have reported on the efficacy of AL, the comparator drug in our study in the management of acute uncomplicated malaria in Africa in general and Nigeria in particular [36–39]. It is particularly noteworthy that PA demonstrated a statistically significant higher efficacy among under 5-year-olds during this study [96.0% vs 63.6% for PA and AL, respectively, as shown in Table 4 ($\rho = .008$). PA also recorded a better and statistically significantly higher efficacy among children ≥ 60 months than AL [91.2%vs 73.8% for PA vs AL; $\rho = 0.016$]. Details are shown in Table 4. It can be assumed that PA's significant superiority among children ≤ 59 months of age reflects its true efficacy since this group of children are generally believed to be relatively immuno-naïve and the chemotherapeutic effect of AL was not augmented by immunity.

There was no difference in parasite clearance time between pyronaridine–artesunate and artemether–lumefantrine treated groups, and our findings are consistent with Roth and colleagues' findings in Kenyan children [34]. In contrast, three previous studies demonstrated that pyronaridine–artesunate group cleared *P. falciparum* more rapidly than artemether–lumefantrine [17, 40]. On day 3, all enrolees were fever-free, and no difference in fever clearance time was observed, which is consistent with a previous study [20], though another study found that pyronaridine–artesunate caused faster fever clearance [17]. The occurrence of one LCF and one LPF on D14 among participants who received AL is worrisome. This suggests declining efficacy of AL in the treatment of malaria in the study area. Sowunmi *et al.* [25] previously reported a decline in the responsiveness of uncomplicated malaria to Nigeria's preferred antimalarial drugs – artemether-lumefantrine and artesunate-amodiaquine. This was stated in a report detailing a post-hoc analysis of clinical trials in south-west of Nigeria among 360 children under 16 years at 5-year intervals in 2009–2010, and 2014–2015, and 1341 children under 5 years in studies from six-geopolitical zones from the Nigerian national antimalaria efficacy studies conducted at 2-year intervals in 2009–2010 and 2012–2015, respectively, after deployment in 2005.

In our study, both artemether-lumefantrine and pyronaridine-artesunate had good safety profiles. The commonly observed adverse events were fever, chills and rigors, anorexia, and headache. These symptoms are similar to those seen in malaria patients and resolve as the infection is cleared. These observations are similar to reports by previous workers [22, 24, 35, 37, 40]. There was no record of clinical hepatic impairment for PA or AL. Laboratory evaluation of liver transaminases, alkaline phosphatase, and bilirubin only revealed marginal increases with very few records of twice the upper limit of normal in both treatment arms (Supplementary file 1 – Tables 6a – 6h, Tables 7a – 7h and Figs. 4–11). The alterations in transaminases were similar in both treatment arms and were not clinically significant. This contrasts with the findings of Sagara *et al*. [41], who found that 13 out of 996 (1.3%) patients had transaminases increase after the first cure, including one possible Hy's law case, and two out of 311 (0.32%) after a retreatment of a second bout of malaria with PA. The lack of any cases of significant transaminase increases could be attributed to the relatively small number of participants (85) in our study compared to 996 in the study reported by Sagara *et al.* [41]. Our observation is, however, supported by the report of Lutete *et al.* [24] in their cohort event monitoring study in five African countries involving over 7,000 malaria patients in a protocol resembling real-world clinical practice.

Another notable finding from the present study is that from days 0 to 21, gametocyte carriage was consistently higher in PA-treated children compared to AL-treated children, but the observed differences were not statistically significant -(Supplementary file 2 - Tables 8a and 8b). The significance of this relatively higher gametocyte carriage is unclear, as the presence of gametocytes does not always indicate infectivity [41–44]. Gametocytes are the stage in the plasmodial life cycle that is infectious to mosquitoes; the insect in which sexual multiplication of malaria parasites occurs, leading to the development of sporozoites, which in turn infect humans. However, only mature gametocytes are infectious to mosquitos after ingestion. Furthermore, microscopy cannot distinguish between viable living gametocytes and dead or drug-affected gametocytes. Nonetheless, the high gametocyte carriage suggests that pyronaridine does not have particularly effective gametocidal properties. The pyronaridine nucleus is derived from mepacrine (a 9-aminoacridine) and contains an amodiaquine-like side chain. The WWARN study group reported clear differences in gametocyte clearance between various ACTs in a systematic review [45]. The WWARN study group reported that ACTs with 4-aminoquinoline partner drugs, such as dihydroartemisinin-piperaquine and artesunate amodiaquine, clear gametocytes at a much slower rate than those with aryl-amino alcohol and related structures (artesunate-mefloquine and artemether lumefantrine).

This study's findings should be viewed with a few caveats in mind. The current study included only eight children under the age of 12 months, of which only five were randomly assigned to the pyronaridine-artesunate group. Despite the fact that the ACPR for pyronaridine-artesunate group was 100 percent on day 28, the number of enrolees is insufficient to draw any conclusions about efficacy and safety in that age group. As a result, more information on this patient population is needed for pyronaridine-artesunate.

Conclusion

The efficacy and safety of PA among Nigerian children is both impressive and important as Nigeria is one of the highest burden countries for malaria in sub-Saharan Africa especially as this is being recorded at a time that AL has started to show declining efficacy. This study provides empirical evidence for the inclusion of PA in the National Treatment Guidelines and it is hoped that findings from routine TES will provide relevant updates on the efficacy of ACTs in the country for their sustained recommendation.

Declarations

Conflicts of interests

The authors declare that they have no conflict of interest

Authors’ contribution

COF, OEA and OAM designed the study. COF, FIO, OSM, ADA, AEO and BEO conducted the studies, RIF ran the molecular analysis, COF and AEO analysed the data and drafted the manuscript. All authors read and approved the manuscript,

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

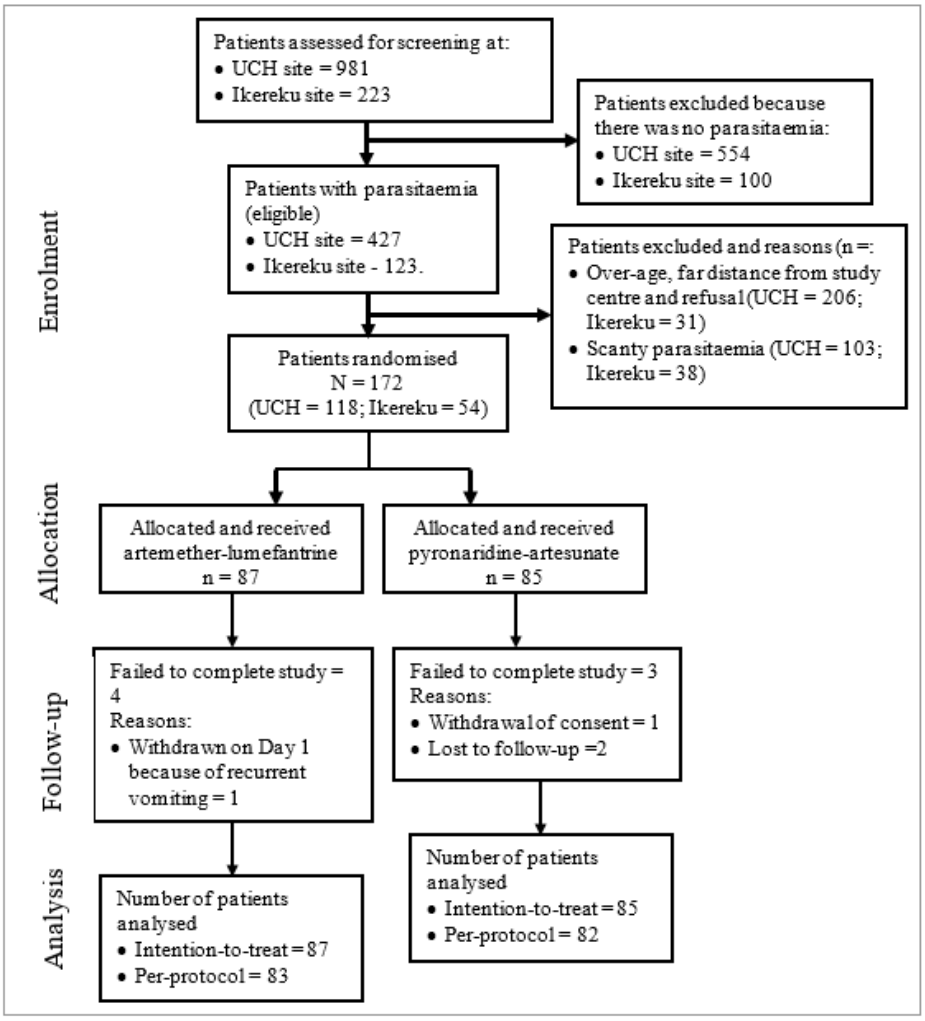


Figure 1

Study design and patient flow among children from southwest Nigeria with acute uncomplicated malaria treated with artemether-lumefantrine or pyronaridine-artesunate

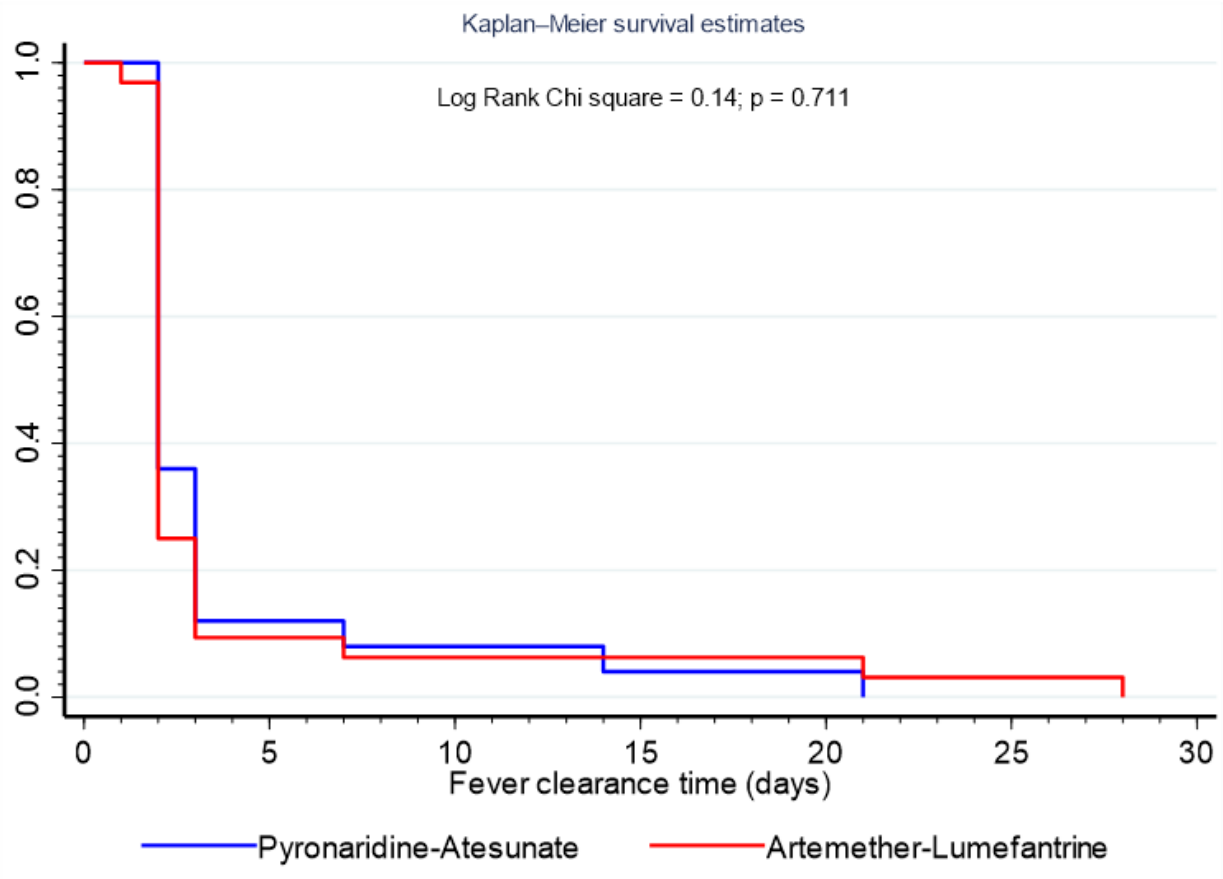


Figure 2

Kaplan–Meier analysis (intent-to-treat population) comparing fever clearance time between Pyronaridine-Artesunate and Artemether-Lumefantrine

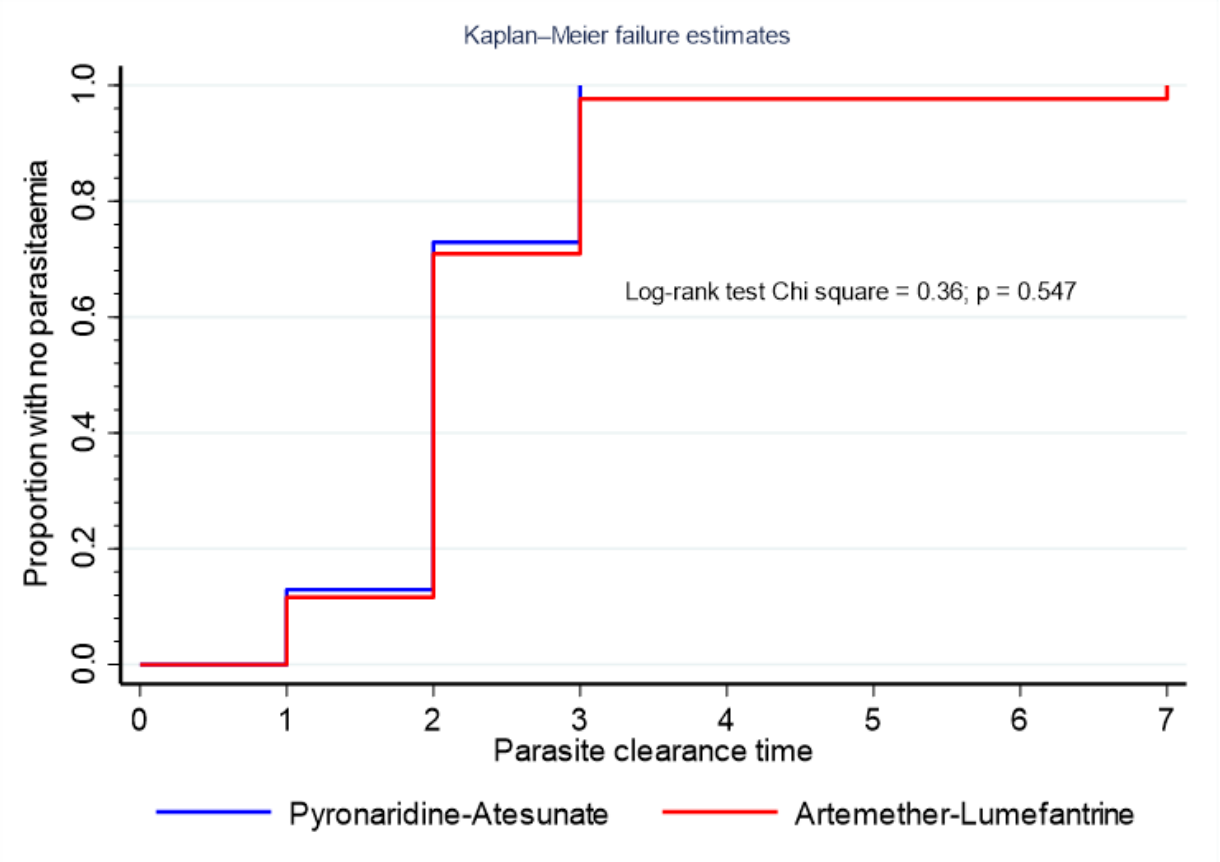


Figure 3

Kaplan–Meier analysis (intent-to-treat population) comparing parasite clearance time between pyronaridine-artesunate and artemether-lumefantrine

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