

Surveillance of the efficacy of artemisinin-piperaquine for the treatment of uncomplicated *Plasmodium falciparum* among children under five years in Est Mono district, Togo, 2017

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Research

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Abstract

Background Malaria is a major public health problem in Togo. Guangzhou University of Chinese medicine of China, and the Ministry of Health and Social Security of Togo launched a nationwide artemisinin compound Mass Drug Administration Project in East Mono with a population of 150,000. Before launching the project, the sensitivity test of artemisinin piperazine tablet was conducted in Elawagnon general clinic. On this background, we evaluated the efficacy and safety of artemisinin piperazine in the treatment of uncomplicated falciparum malaria in children under five years of age.

Methods In this study, children aged 6-59 months without complications of falciparum malaria were observed, and the selected cases were treated with artemisinin piperazine. The patients were followed up for 28 days to observe the fever clearance time, parasitemia, gametocyte, cure rate, haemoglobin and msp-2 gene polymorphism. The primary end point was the 28-day cure rate, and PCR corrected reinfection and recrudescence. This research was conducted according to standardized WHO protocol for the assessment of the efficacy of anti-malarial treatment.

Results A total of 91 children participated in the study. The adequate clinical and parasitological response (ACPR) before PCR-corrected were 66 (72.52%) and 90 (98.90%) after PCR-corrected. The patient was well tolerated to artemisinin piperazine and no serious adverse reactions were observed. The average hemoglobin level increased by 0.05g/dl per day ($p < 0.0001$). The gametocyte doesn't declined at the beginning of treatment, however, 14 days later, it dropped ($D_{21}: p < 0.05$; $D_{28}: p < 0.01$). In the msp-2 gene polymorphism study of 24 children with positive parasite after treatment, 1 case of msp-2 with 3D7 haplotype and FC27 haplotype was reported, indicating that it's recrudescence, with a frequency of 4.2% (1/24); The others maybe reinfection, with a frequency of 95.8% (23/24).

Conclusion Artemisinin piperazine was effective in treating uncomplicated falciparum malaria in children under 5 years of age in Togo and well tolerated. Plasmodium falciparum in Togo remains sensitive to artemisinin piperazine, which could be used as a trial drug in the region. Trial Registration Trial registration: ECGPHCM No. B2017-054-01; MHSST AVIS N° 0001/2016/CBRS du 07 janvier 2016. Registered 17 March 2014, <http://www.chinadrugtrials.org.cn/eap/main>

Background

Malaria remains a major public health problem in Togo, where transmission rates are still high, with 2,141,700 cases and 5,199 deaths in 2017, and 7,698,500 people at risk. In Togo, more than 10% of children with fever go into the informal private sector, and most children with fever go untreated [1]. As the number of malaria cases increased, the Ministry of Health and Social Security of Togo decided to adopt a new antimalarial strategy and, with the help of various financial and technical donors, implemented chemopolytic treatment for seasonal malaria in children under five years of age in Grassland, Kara and Central regions [2]. In 2015, Guangzhou University of Chinese Medicine of China (GUCM) signed an agreement with the Ministry of Health and Social Security of Togo (MHSST) to

launch a mass drug administration(MDA) program with artemisinin piperaquine(AP) for about 1.5 million people in the Plateau Region, and selected about 150,000 people in Est-Mono province as a pilot area. Artemisinin-piperaquine were used for MDA, and latest clinical studies showed that it had 97% efficacy in treating uncomplicated falciparum malaria [3-6]. MHSST decided to conduct sensitivity tests for artemisinin piperaquine at the general clinic in Elawagnon county before launching the universal MDA. Among this background, we evaluated the efficacy and safety of artemisinin piperaquine in the treatment of uncomplicated falciparum malaria in children under five years of age in Elawagnon, Prefecture on Est-Mono province of Togo.

Methods

Study sites

Est Mono district covers an area of 2,474 km² with a population of 89,060 inhabitants and a population growth rate of 1.03% in 2010 [7]. Est Mono is one of the nine health districts in the Plateaux region, which is one of Togo's six regions. The district is situated in central Togo and has 17 health facilities run by nurses, auxiliary nurses, or non-qualified nurses. Each health unit has CHWs trained in health promotion and the management of uncomplicated malaria cases within their communities.

Est Mono district [7] and the other central and southern parts of Togo have two rainy seasons, the first starting in April and ending in July and the second starting in September and ending in November. The district receives on average 949 mm (± 37.4) of precipitation annually or 79 mm (± 3.1) each month. The climate in Togo is generally tropical, with average temperatures ranging from 27.5°C (81.5°F) on the coast to about 30°C (86°F) in the north. Malaria transmission is seasonal, with peaks related to rainfall. Two villages, EPT and RCPA, in Elawagnon, Prefecture on Est-Mono province of Togo, were selected for this study. The time span of MDA was from March to May, 2017.

Study design

The evaluation study of artemisinin piperaquine for treatment of uncomplicated falciparum malaria followed the drug sensitivity observation method developed by the World Health Organization(WHO) for 28 days [8]. Based on the studies that have been conducted, the proportion of failed treatments for artemisinin piperaquine is estimated at 5%. To ensure that 95% of the cases were treated successfully and about 5% couldn't participate, at least 73 people were selected for this study as a sample size and conducted at the study site. If 10% of the patients were lost and excluded, the sample size should be 80 persons [8,9]. Therefore, at least 80 people should be included as the target population for the study site, and the maximum number of patients lost should be avoided.

Inclusion criteria

Children 6 to 59 months old, the body temperature greater than or equal to 37.5 ° C, no danger signs or symptoms of severe malaria, without obvious symptoms of fever or other symptoms of severe malnutrition (e.g. upper arm length < 11 cm, height and weight ratio < 70%, systemic edema, to uncover her nakedness) were all included, at the same time, with single infection caused by plasmodium falciparum parasite, the population between 2000/μl and 250 000/μl, with the ability of oral drugs, received a parent or legal guardian of informed consent, convenient to clinic review, and with no history or allergic reaction of artemisinin piperazine contraindications.

Medicine and administration

Artemisinin piperazine tablets, containing 62.5mg of artemisinin and 375mg of piperazine each tablet, were administered 1/2 tablet every 24 hours over 2 days for children aged 6 to 24 months; 3/4 tablet every 24 hours over 2 days for children aged 25 to 59 months. The medicines were administered under supervision. Artemisinin piperazine was purchased from Artepharm, Co., Ltd (China), with batch number 20160601. All patients in the study were given acetaminophen treatment every 6 hours until the fever symptoms were completely resolved.

Laboratory inspection

Blood samples from finger tips were collected on D0, D2, D3, D7, D14, D21, and D28, respectively, to make thin and thick blood samples. The number of asexual bodies per cubic millimeter of blood was calculated before treatment. No asexual protozoa or gametophyte could be found in 500 fields of thick blood membrane as negative [8]. The amount of hemoglobin was measured on days D0, D7, D14, D21, and D28 using the Hemoglobin Analyzer URIT-12. The filter paper blood pieces were made on D0, D7, D14, D21 and D28, and thin and thick blood glass and filter paper blood pieces were made for laboratory testing in patients who were positive with parasite. At the same time, approximately 3mL of venous blood samples were taken at D0 and D28 of positive patients, using heparin vacuum tubes and stored at -20°C in the laboratory, for white blood cells (WBC) and PCR analysis of polymorphism of merozoite surface protein-2 (msp-2) of plasmodium falciparum to distinguish between recrudescence, and reinfection [10].

Outcomes evaluation

Clinical outcome evaluation was classified according to WHO [8] score:

Early treatment failure (ETF)

Danger signs or severe malaria on D1, D2 or D3, in the presence of parasitaemia; parasitaemia on D2 higher than on D0, irrespective of axillary temperature; parasitaemia on D3 with axillary temperature $\geq 37.5^{\circ}\text{C}$; and parasitaemia on D3 $\geq 25\%$ of count on D0.

Late clinical failure (LCF)

Danger signs or severe malaria in the presence of parasitaemia on any day between D4 and D28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure; and presence of parasitaemia on any day between D4 and D28 with axillary temperature $\geq 37.5^{\circ}\text{C}$ in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure (LPF)

Presence of parasitaemia on any day between D7 and D28 with axillary temperature $< 37.5^{\circ}\text{C}$ in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response (ACPR)

Absence of parasitaemia on D28, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

Every time after administration, ask whether there are any adverse reactions, such as mental disorder, insomnia, headache, tinnitus, deafness, nausea, vomiting, loss of appetite, abdominal pain, diarrhea, itching, rash and other symptoms, and make detailed records according to the records of adverse reactions.

Statistical analysis

All data was entered twice into the Excel spreadsheet. GraphPad PRISM (GraphPad software, USA) was used for all statistical analyses. The Shapiro-Wilk normality test was used to test the normality of the data. For normally distributed data, t-test was used for comparison between the two groups, while Welch's

correction was used at the same time; and One-way ANOVA and Tukey's test were used for multiple comparisons between multiple groups. For non-parametric data, Mann-Whitney test was used for comparison between two groups and Kruskal-Wallis and Dunn test was used for comparison between multiple groups. Bidirectional analysis of variance was used for two - dimensional grouping data.

Results

Study profile and baseline characteristics

Among 208 children, 6 to 59 months of age, with fever screened, 73(35.10%) was negative by microscopy, 7(3.36%) for mixed infections with plasmodium ovale and plasmodium malariae, 33(15.86%) for parasitaemia less than 2000 parasites/ mm³, the other 4 (1.92%) children were not included for the following reasons: 2 children for the difficulty in completing the 28-day follow-up because of the long journey; and of the 28-day follow-up, 1 child for receiving other antimalarial treatment on the first day, and 1 child for being failed to follow. And in the end, 91(43.75%) patients were included in this study (Fig. 1).

Baseline clinical and laboratory characteristics of the child subjects were shown in Table 1. Children (n = 91) had an average age of 31.41 months (6–59 months), among which 44 (48.35%) were females, with an average weight of 11.73 kg (8.73–14.73 kg) and an average body temperature of 38.5°C(37.6–39.4 °C). The mean hemoglobin concentration before treatment was 8.2 g/dl (6.6–9.8 g/dl), and the geometric mean of parasitemia was 36,184 (95% CI: 28249–44118).

Table 1
Baseline characteristics of enrolled children in Togo

Variable	Enrolled Children(N = 91)
Age in months, median	31.41(6–59)
Female, %	44(48.35%)
Weight,mean, kg	11.73(8.73–14.73)
Temperature at enrollment, median, °C	38.5(37.6–39.4)
Pre-treatment hemoglobin concentration, g/dl,median	8.2(6.6–9.8)
Parasitemia, parasites/ul, 95% CI	36184(28249–44118)

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Parasitemia and fever clearance time

After AP treatment, most of the patients experienced rapid remission of clinical symptoms (Fig. 2A), from an average body temperature of 38.5 °C on D0 to 36.5 °C on D2 ($p < 0.0001$), with 79 cases (87%) below 37 °C. On D2 after administration, 66 (73%) of the patients were observed clearance of parasites on blood smears, and complete clearance was observed on D3 (Fig. 2B).

Gametophyte and hematological parameters

In the gametophyte observation (Fig. 3A and 3B) it was found that the average gametocytaemia still increased before the D7, meanwhile the number of patients with gametophyte did not decrease significantly. Both of which decreased synchronously after D7, and significantly down on D21 ($p < 0.05$) and D28 ($p < 0.01$). While gametophytes were still observed on blood smears on D28 in 6 patients, with a mean value of 8/ul.

The hemoglobin level in the patient's was improved progressively from D0 to D14, D21 and D28 (Fig. 3C). Significant differences in haemoglobin levels were observed on D14, D21 and D28 when compared to D0 ($P < 0.01$; $P < 0.0001$; $P < 0.0001$), with an average increase of 0.05 g/dl per day

Cure rates

PCR-corrected ACPR was 90(98.90%)(Table 2) for AP, and treatment failure was only 1 for recrudescence. While Non-PCR-corrected ACPR was 66 (72.53%), including 4 (4.40%) for LCF, 21 (23.08%) for LPF.

Table 2

Efficacy outcomes and adequate clinical and parasitological response rates of artemisinin-piperaquine treatment by day 28 among the enrolled participants

Variable	Enrolled Children(N = 95)
Excluded(%)	2
Lost of follow-up (%)	2
Late parasitological failure-no(%)	27.47%
Recrudescence	1
Reinfection	24
Indeterminate or sample unavailable	0
PCR-uncorrected cure rate-%(95%CI)	
ACPR(%)	66(72.53%)
ETF	0
LCF	4
LPF	21
TF(%)	25(27.47%)
PCR-corrected cure rate-%(95%CI)	
ACPR(%)	90(98.9%)
ETF	0
LCF	0
LPF	1
TF(%)	1(1.10%)
Parasite clearance time, mean \pm SD hours	57.3 \pm 14.7
Fever clearance time, mean \pm SD hours	32.4 \pm 16.2
Adverse events	AP,N(%)
Cough	6 (6.32)
Diarrhoea	1 (1.05)
Loss of appetite	1 (1.05)

Table - 3 Summary of adverse events of children with uncomplicated malaria treated with Artemisinin-piperaquine in Togo.

Variable	Enrolled Children(N = 95)
Vomiting	2 (2.11)
Abdominal Pain	2 (2.11)
Insomnia	0
Irritability	0
Headache	1 (1.05)
Rash	1 (1.05)

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Polymorphism of msp-2 gene and clinical parameters of 24 positive children

Two alleles gene (3D7 and FC27) were detected in 24 children who were still positive after taking AP, using nested PCR to analyze the msp-2 gene of all the isolates before and after administration. Only 1 patient, whose 3D7 and FC27 haplotype of msp-2 were the same before and after taking the drug, suggested recrudescence, with a frequency of 4.2% (1/24). Other 23 patients may be reinfection with a frequency of 95.8% (23/24).

Meanwhile, the white blood cell levels of the 24 positive children were increased on D28 after taking AP ($p < 0.001$) (Fig. 4A). The parasitemia also decreased ($p < 0.05$) (Fig. 4B). Moreover, the gametophytes decreased from D21 ($p < 0.01$) to D28 ($p < 0.001$) (Fig. 4C).

Adverse events

In this study, the most common adverse reaction was cough, followed by diarrhea and rash of all the 91 children taking AP. Other notable adverse events included loss of appetite, vomiting and abdominal pain. No serious adverse reactions occurred and AP was well tolerated. The list of adverse events observed during the study was shown in Table 3.

Discussion

Malaria is the top public health problem in Togo, which was prevalent everywhere throughout the whole country and in 12 months of one year, moreover, the outbreak often occurs in the rainy season. According to national health authority data of Togo in 2014, there are approximately 11 million malaria cases each year, with children under the age of five most affected, accounting for 60.5%. In a secondary data analyse from the regional health information system on confirmed and suspected malaria cases[7], a total of 114,654 malaria cases ($19,109 \pm 6,622$ annually) were reported between January 2005 and December 2010, with all malaria cases increasing from 10,299 in 2005 to 26,678 in 2010 ($p < 0.001$). The prevalence of confirmed malaria cases increased from 23.1‰ in 2005 to 257.5‰ in 2010 ($p < 0.001$). Although the

implementation of the ACT and CHW strategies in Togo, the study showed an increase in malaria prevalence.

There are few studies on the efficacy of malaria treatment and resistance of Artemisinin Combination Therapies (ACTs) in Togo. Only one article on the effect of ACTs was found [3]. The therapeutic effect of AL (artemether - benflumetol) and ASAQ (artesunate - amodiaquine) were monitored in national malaria control program (NMCP) of Togo in 2013. It's suggested that PCR-corrected ACPR was 97-100% for AL, and PCR-corrected ACPR was 96.3-100% for ASAQ. However, adverse events was a bit high: AL was 2.68%, and ASAQ was 1.53%. In benin, a country bordering Togo, a 42-day AL efficacy study was conducted in 2014 [13]. On the 1st day of treatment, fever cleared apparently, meanwhile the clearance rate of parasites was about 90%; on the 2nd day, almost all parasites were cleared, and the hemoglobin concentration increased slightly with the clearance rate down of parasites. Non-PCR-corrected ACPR was 75.6% after 42 days of follow-up, and PCR-corrected ACPR was 100%, indicating that all treatment failures were due to refection, which of the above two papers was basically consistent with the results obtained in this study. WHO only recommends changing treatment policies when PCR corrected treatment failure is higher than 10%, which is not the case in Togo, ACTs are still sensitive there.

During this sensitivity test of artemisinin piperaquine in Elavagnon, fever reduced in most of the patients participating in the study on D1, while only 1 patients had a higher temperature than 37.5 on D2, and complete parasite clearance observed on D3. In addition, according to gametophyte, average gametophyte rose and gametophyte cases does not significantly reduced in the first 7 days. However, until D28, the average gametophyte falling faster. Which may be because that artemisinin piperaquine only effected on parasite in the blood stage, couldn't directly kill gametophyte that already born. Therefore, there was no obvious change in the early performance for patients carrying the gametophyte, even some mature gametes produced in some patients' bodies. Later a large number of parasites on the blood stage were killed, resulting in the late mature gametophyte have fallen sharply, Which, to a certain extent, inhibited the parasite transmission by mosquitoes [14]. Overall, parasite clearance reduction, gametophytic carrier rates reduction were consistent with improvements of hemoglobin levels in all 91 patients administrated. After PCR genotyping proofreading, the efficacy threshold of artemisinin piperaquine obtained in our study reached 98.90%, higher than the 95% efficacy threshold recommended by WHO for AL [11]. In addition, artemisinin piperaquine were well tolerated by local children in Togo, and no serious adverse events including diarrhea, weakness, anorexia, hemoglobinuria, itching, or eyelid edema were observed during the 28-day follow-up.

The results are similar to those of the ACTs studies conducted over the past 5 years in Sub-saharan Africa. This combination therapy has a high cure rate for falciparum malaria without complications. A

randomized study was [15] conducted in Kenya in 2014, which the PCR-corrected ACPR was 97.8% for AL, and 99.1% for dihydroartemisinin-piperaquine (DP). A single-arm prospective study was [16] conducted in Zambia in 2012, and the results showed that 98% of the patients participating in the study achieved complete clearance of parasitic diseases on D3, and the PCR-corrected ACPR was 100% for AL, that is, all the participating patients achieved ACPR. In a study conducted [17] in three provinces of Angola in 2015, the 28-day cure rate of AL after PCR-corrected was 88.1%-96.3%. No evidence of artemisinin resistance was found in this study. However, pfmdr1 haplotype was found in all AL treatment failures, which was associated with decreased sensitivity of phenylfluorene. A study was [18] conducted in Malawi in 2014, and the cure rate of AL was 69%-82.5% in Non-PCR-corrected, but PCR-corrected APCR was 98%-100%, which was also associated with high reinfection. The authors attributed this phenomenon to the short half-life of phenylfluorene (3-6 days). For the evaluation of the efficacy of ACT in six geographic regions of Nigeria, it was [19] found that AL and ASAQ were highly effective in Nigeria, with PCR-corrected cure rates 96.9% and 98.3%, respectively.

Trial Registration

Trial registration: ECGPHCM No. B2017-054-01; MHSST AVIS N° 0001/2016/CBRS du 07 janvier 2016. Registered 17 March 2014, <http://www.chinadrugtrials.org.cn/eap/main>

Conclusions

Artemisinin piperaquine was effective and well tolerated in treating uncomplicated falciparum malaria in children under 5 years of age in Elawagnon, Prefecture on Est-Mono province of Togo. Plasmodium falciparum in Togo remains sensitive to artemisinin piperaquine, which could be used as a trial drug in this region.

Abbreviations

ETF: Early treatment failure; ACPR: Adequate clinical and parasitological response; ACT: Artemisinin-based combination therapy; LCF: Late clinical failure; LPF: Late parasitological failure; NMCP: National Malaria Control Programme; WHO: World Health Organization; DP: Dihydroartemisinin-piperaquine; AL: Artemether-benflumetol; ASAQ: Artesunate-amodiaquine; AP: Artemisinin piperaquine.

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine (Approval No. B2017-054-01) and the Ministry of Health and Social Security of Togo (MHSST)

(AVIS N° 0001/2016/CBRS du 07 janvier 2016). The parents or guardians of all study participants provided informed written consent.

Consent for publication

All authors and institutes consent to publication.

Availability of data and material

Data are available upon reasonable request by an email to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

QW performed the statistical analysis and drafted the manuscript. CL, WY, ZZ, WW, GL, YL and GL collected and interpreted the data. HZ and ZP conducted the research and performed the PCR analysis. XH, WG, CL, MC, JG, YY and QX collected the data and conducted the research. JS conceived and designed the study, conducted the research. CD conducted the research. All authors read and approved the final manuscript.

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Figures

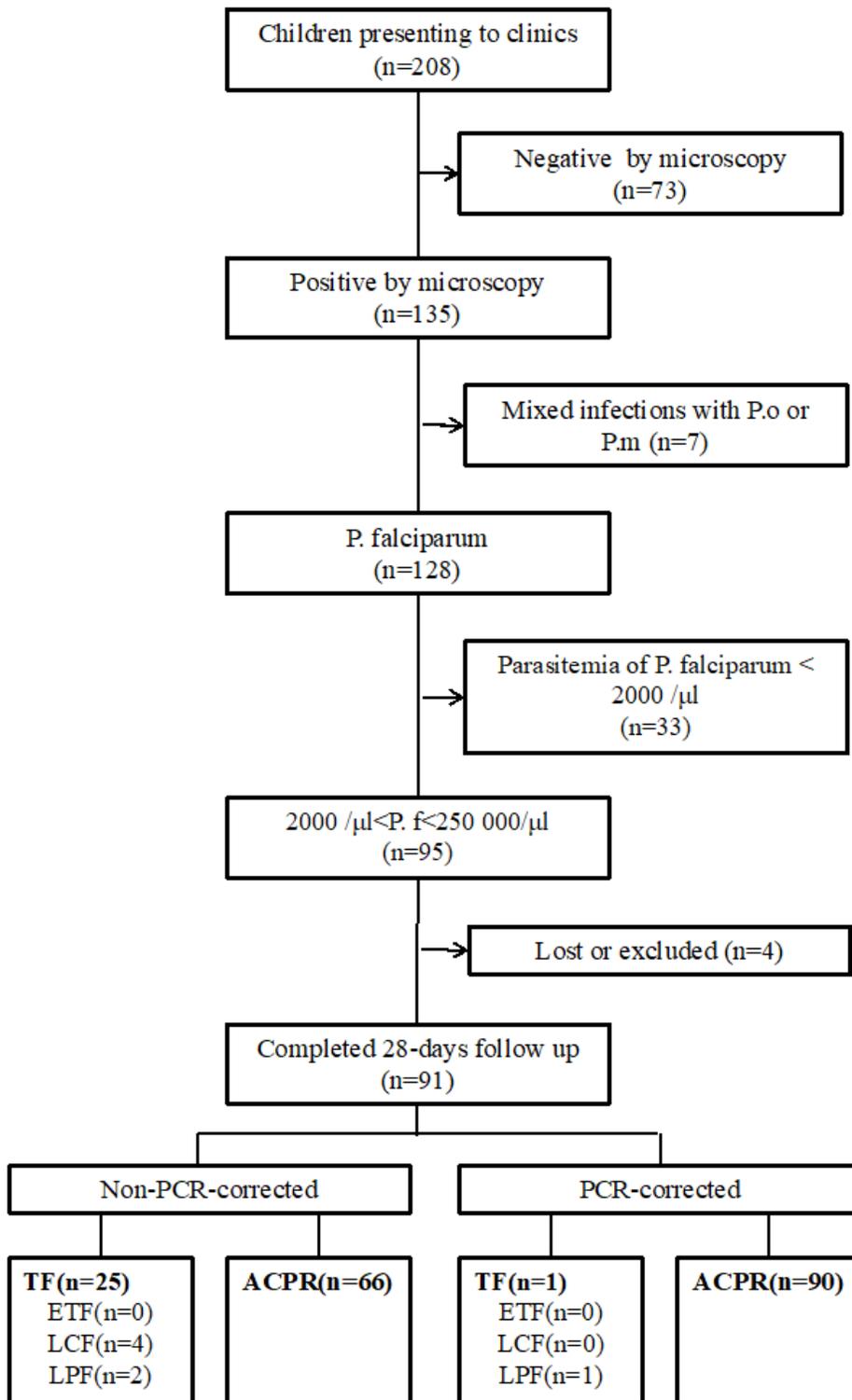


Figure 1

Patients flow chat in the clinical trial. ACPR=adequate clinical and parasitological response; ETF: early treatment failure;LCF: late clinical failure;LPF:

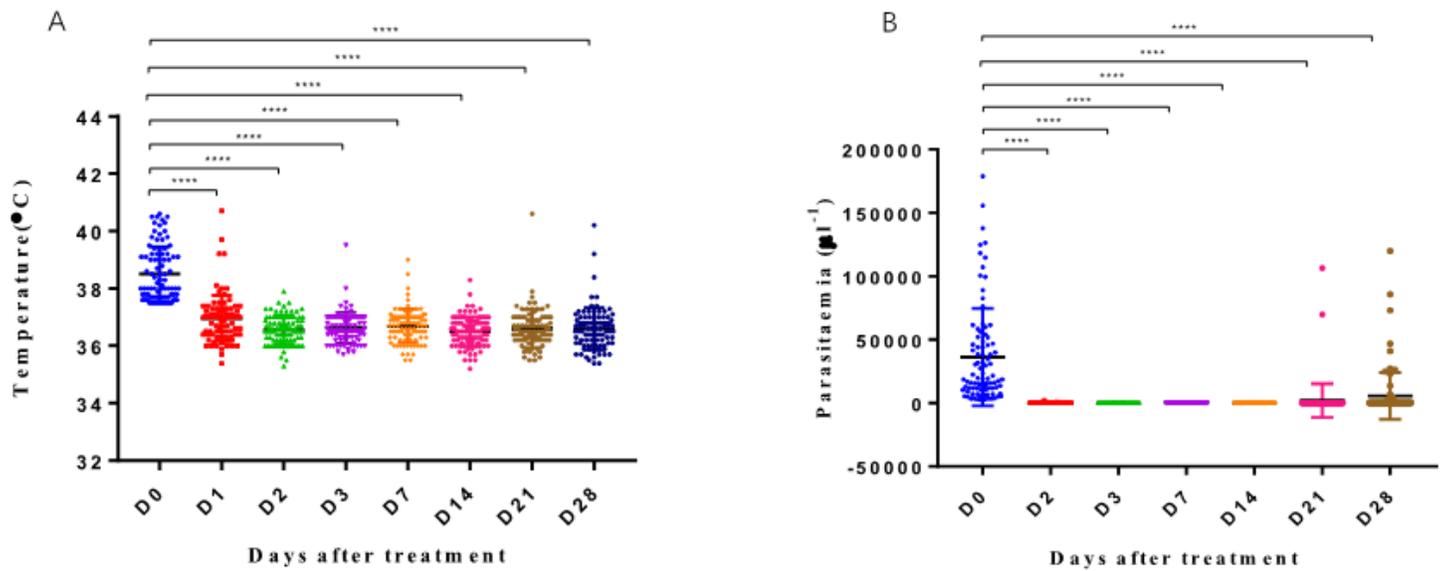


Figure 2

Temperature and parasitemia across days after treatment. Y axis represents the temperature(A) and parasitemia(B) of children. The significance levels were shown as: significant (****: $p < 0.0001$) and non-significant (NS) .

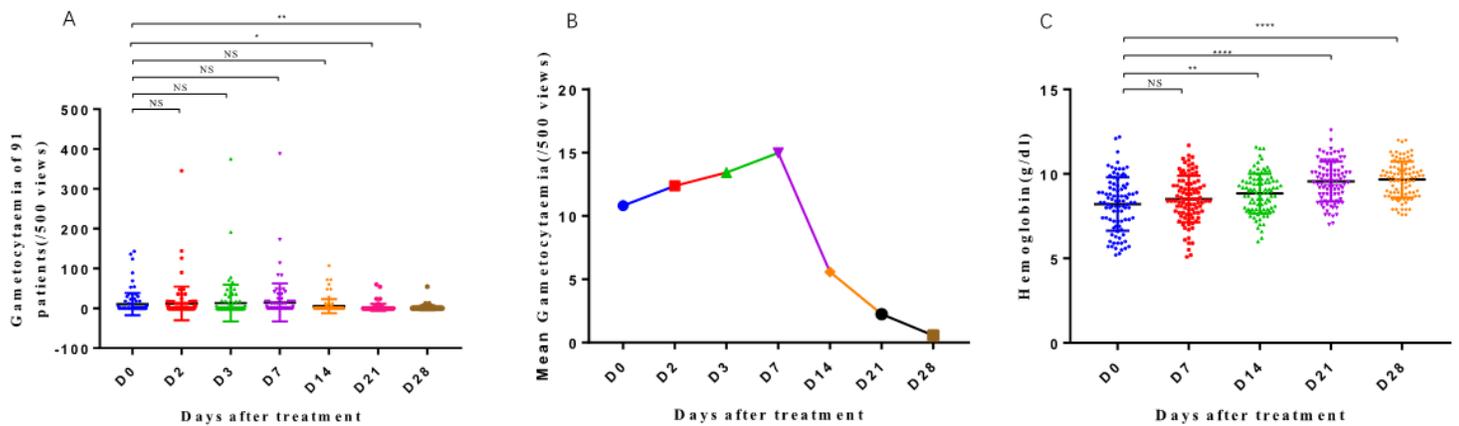


Figure 3

Gametocytaemia(A), mean Gametocytaemia(B) and Hemoglobin(C) of children from D0 to D28. The significance levels were shown as: significant (****: $p < 0.0001$; ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$) and non-significant (NS).

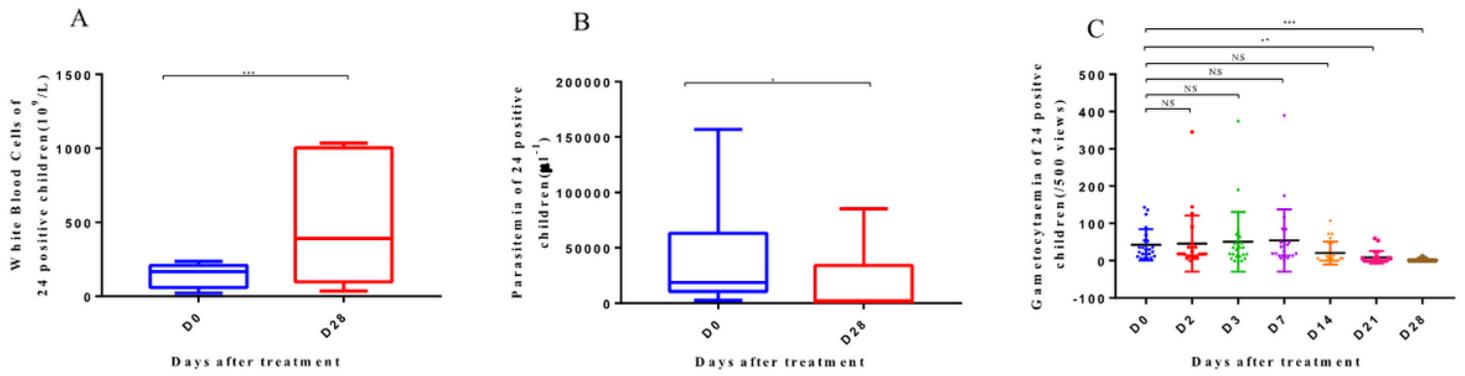


Figure 4

White blood cells(A), parasitemia(B) and gametocytaemia (C) of 24 children with positive parasite after treatment on D0 and D28. The significance levels were shown as: significant (****: $p < 0.0001$; ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$) and non-significant (NS).