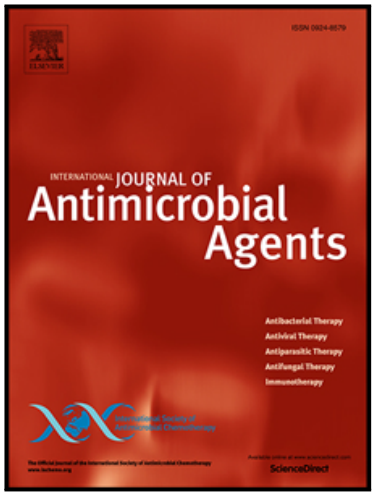
Editor: Dr Bruno Pradines

Journal Pre-proof



Artemisinin-Piperaquine versus Artemether-Lumefantrine for treatment of uncomplicated Plasmodium falciparum malaria in Grande Comore Island: an open-label, non-randomized controlled trial

Guoming Li , Yueming Yuan , Shaoqin Zheng , Chenguang Lu , Mingqiang Li , Ruixiang Tan , Hongying Zhang , Rahamatou Silai , Ruimei Liu , Kamal Said , Affane Bacar , Qin Xu , Jianping Song , Wanting Wu , Changsheng Deng

PII: S0924-8579(22)00179-0
DOI: https://doi.org/10.1016/j.ijantimicag.2022.106658
Reference: ANTAGE 106658

To appear in: *International Journal of Antimicrobial Agents*

Received date: 6 April 2022
Accepted date: 11 August 2022

Please cite this article as: Guoming Li , Yueming Yuan , Shaoqin Zheng , Chenguang Lu , Mingqiang Li , Ruixiang Tan , Hongying Zhang , Rahamatou Silai , Ruimei Liu , Kamal Said , Affane Bacar , Qin Xu , Jianping Song , Wanting Wu , Changsheng Deng , Artemisinin-Piperaquine versus Artemether-Lumefantrine for treatment of uncomplicated Plasmodium falciparum malaria in Grande Comore Island: an open-label, non-randomized controlled trial, *International Journal of Antimicrobial Agents* (2022), doi: https://doi.org/10.1016/j.ijantimicag.2022.106658

Highlights

- Both artemisinin-piperaquine and artemether-lumefantrine maintained their high efficacy during the treatment of falciparum malaria in Grande Comore Island.
- Artemisinin-piperaquine in combination with anti-gametocyte treatment was recommended for the rapid increase of the killing effect of the malaria gametocyte in areas with a high rate of carrying.
- Asymptomatic parasitaemia infections bring new challenges for malaria control in Comoros.

Title: Artemisinin-Piperaquine versus Artemether-Lumefantrine for treatment of uncomplicated *Plasmodium falciparum* malaria in Grande Comore Island: an open-label, non-randomized controlled trial

Running Title: Artemisinin-Piperaquine versus Artemether-Lumefantrine for treatment of falciparum malaria

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Abstract

Background. Malaria has rebounded significantly in 2018 in the Comoros. It posed an urgent need to conduct clinical trials to investigate the effectiveness of artemisinin and its derivatives there.

Methods. From June 2019 and January 2020, an open-label, non-randomized controlled trial of artemisinin-piperaquine (AP) and artemether-lumefantrine (AL) were conducted in Grande Comore Island. 238 uncomplicated falciparum malaria cases were enrolled and divided 1:1 into two treatments. The primary endpoint was the 42-day adequate clinical and parasitological responses (ACPR). Parasitemia and fever clearance at day 3, gametocyte, and tolerability were secondary endpoints.

Results. The 42-day ACPR before and after PCR-corrected were 91.43% [95% confidence interval (CI): 83.93%-95.76%] and 98.06% [95%CI: 92.48%-99.66%] for AP treatment, respectively, and 96.00% [95%CI: 88.17%-98.14%] and 98.97% [95%CI: 93.58%-99.95%] for AL treatment, respectively. Complete clearance of the parasitemia as well as of fever for both groups was detected on day 3. Gametocytes disappeared on day 21 in the AP group and on day 2 in AL group, respectively. Specifically, the adverse reactions were mild in both groups.

Conclusions: We discovered that AP and AL maintained their high efficacy and tolerance in the Comoros. Nonetheless, asymptomatic malaria infections bring new challenges to malaria control.

Keywords

artemisinin and its derivatives, artemisinin-piperaquine, artemether-lumefantrine, the Comoros, falciparum malaria

1. Introduction

Data retrieved from the World Health Organization (WHO) revealed that in 2020, 241 million malaria cases and 627,000 deaths were reported worldwide, which was an increase from 14 million reported malaria cases and 47,000 deaths since the outbreak of COVID-19 pandemics[1]. Since 2007, the Comoros gradually implemented rapid malaria control strategies including comprehensive preventive measures on the islands of Moheli, Anjouan, and Grande Comore [2][3][4]. The registered data showcased that malaria has been rapidly and effectively controlled. Concretely, in 2010 the malaria cases were 75.98 per 1,000 people, while in 2014 they dropped down to 0.14 per 1000 people [4]. Regardless of this reduced malaria incidence, data retrieved for 2018, 2019, and 2020 showed that 15,613, 17,599, and 4,546 cases, respectively, were reported, all of which were diagnosed as falciparum malaria and 440,086 people living in active foci[1]. As Anjouan and Moheli have been reached a pre-eradication state [5], most malaria cases distributed in Grand Comore.

The current strategies of the Comoros to antimalaria included long-lasting insecticide-treated nets, indoor residual spraying, intermittent presumptive treatment for pregnant women, medical care for malaria, and implementation of mass drug administration[5]. AL was recommended as the first-line drug for the treatment of uncomplicated *P. falciparum* malaria

currently, and quinine was recommended to treat severe *P. falciparum* malaria in the Comoros[1]. However, *plasmodium* resistance to antimalarial medicines is one of the key recurring challenges in fighting against malaria in recent years[6], as Angola [7], Rwanda [8], Burkina Faso [9] and other African countries had already been reported.

A large-scale mass drug administration of the AP therapeutic was reported during treatment approaches against malaria in Moheli[2], Anjouan[3], and Grande Comore (unpublished). Fortunately, in the region of Grande Comore, there was a 10-year follow-up of P. falciparum artemisinin-resistant genetic polymorphisms, which showed that its species there remain susceptible to artemisinin efficacy[10][11]. Additionally, seven clinical studies on the AP application in malaria treatments showed that it demonstrated comparable effectiveness and protection in comparison with other Artemisinin-based combination therapy (ACT) approaches [12]. However, for the duration of assessment for uncomplicated malaria clinical trial, Stepniewska et al. [24] recommended that a 28-day follow-up as the minimum standard for medicines with less than 7 days of elimination half-lives ($t_{1/2}$) to capture most failures, for mefloquine ($t_{1/2}$=16.1 days[25]) and piperaquine ($t_{1/2}$=11.7 days[26]), the minimum follow-up should be 42 days[23]. We consider that only Thanh and his colleagues have conducted a clinical study on AP during a 42-day follow-up[13]. It will be of great importance to gather more data necessary for explaining the AP application for falciparum malaria.

Therefore, a clinical controlled trial of AP and AL in the management of falciparum malaria was been conducted in Grande Comore. Our results highlighted two key points: (1) we verified the high efficacy of AP and AL for 42-day follow-up; (2) and further raised awareness on the

rebounding of malaria since 2018 in Grande Comore, raising an alert that malaria prevention and control will meet a new challenge in the Comoros.

2. Methods

2.1 Study design

The current study was permitted by the Ethical Board of Guangdong Provincial Hospital of Chinese Medicine (Registration Number: BF2019-169-01) and was performed in the period between June 2019 and January 2020 in Grande Comore.

As previous studies, the efficacy rate of AP and AL treatment were 95%-100% [13][14][15][16] and 82%-100% [15][17][18], respectively. Assuming a PCR-corrected ACPR of 95% and 82%, respectively, for AP and AL. At a confidence level of 95% with a two-sided z test that has a statistical power of 80% and a significance level of 5%, a minimum of 94 patients must be included. With a 20% increase to allow a loss to follow-up and withdrawals during the 42-day follow-up period, 112 patients should be included in the study per group. 119 had been chosen for our study.

The clinical trial was initially conducted in two hospitals: Centre Médicaux Chirurgicale de Mbeni (CMC Mbeni) and Hôpital pôle de Mitsamiouli (HP Mitsamiouli). However, the prevalence of malaria in the Comoros was low, and hard to find a large number of malaria patients in a short time, which made researchers decide to expand the pilot study and enroll eligible patients from six other medical facilities in Grande Comore. Meanwhile, to facilitate the management of the project, avoid confusion, and ensure the status of the treatment, the original randomized controlled trial was changed into a controlled clinical trial. Specifically, the enrollment of the patients for AP treatment occurred in the CMC Mbeni, Centre de Santé de District de Oichili Dimani (CSD Oichili-Dimani), Centre de Santé de District de Mitsoudjé (CSD

Mitsoudje) and Dzahani B/Programme National de Lutte Contre le Paludisme (PNLP), whereas the treatment of AL was realized in the HP Mitsamiouli, Maison de la Surveillance de Lutte Contre le Paludisme (MSLCP), Caritas and Malouzini B/PNLP. The geographical distribution of the study sites was shown in Figure 1.

2.2 Patients

Patients selected for the clinical trial covered the following criteria: 1) age - range between 0.5-60 years; 2) female patients, aged between 12 and 45 years, were confirmed without pregnancy (tested must be negative by the human chorionic gonadotropin hormone); 3) *Plasmodium falciparum* mono-infection after microscopic detection, indicating uncomplicated falciparum malaria; 4) parasitemia, presenting from 500 to 100000 parasites per μL of blood; 5) axillary temperature ≥ 37.5℃ or a medical history of infection 24 hours pre-enrollment; 6) capability to take medications orally; 7) competence and readiness to conform with the treatment procedure during the conduction of the trail; 8) written informed agreement (by the patient, a parent or guardian).

On the contrary, the exclusion criteria were: 1) patients aged less than 6 months or above 60 years; 2) pregnant or breastfeeding female patients; 3) patients with drug allergies; 4) febrile condition as a result of other infections, severe or chronic pathologies; 5) severe malnutrition [19]; 6) patients with signs of severe falciparum malaria [20]; 7) patients with impaired liver and renal function; 8) cases of patients taking regular treatments, which could affect the pharmacokinetics of the applied antimalarial treatment; 9) hemoglobin < 7g/dl.

2.3 Treatments

The patients in the AP group were treated with Artequick® pills (Artepharm, Co., Ltd), containing 62.5 mg artemisinin in combination with 375 mg piperaquine per tablet for 2 days. The patients in the AL group took Coartem® (Strides Shasun Ltd.), containing 20 mg artemether in combination with 120 mg lumefantrine for 3 days. Certain details on the age and bodyweight-dependent regimens for the two groups are listed in Appendix 1 (Table A.1 and Table A.2). However, patients with axillary temperatures higher than 38℃would be treated with paracetamol or acetaminophen. In cases of treatment failure, the artesunate injection would be applied, while patients with critical illness were admitted to the hospital.

2.4 Assessment

Laboratory inspection

Peripheral blood was obtained from the patient's finger. The blood smears were stained with Giemsa, after which thick and thin blood films were examined to recognize the parasite species and their concentration to approve the patient's inclusion in the clinical trial. The blood samples were obtained and examined on days 2, 3, weekly, and on any other day of clinical or parasitological failure. All of the smears were sent to the laboratory of the National Malaria Center of the Union of Comoros for examination by certified malaria microscopists. A negative smear was recorded if no parasite was observed in at least 200 fields with an oil immersion lens (100×). The negative smears were re-examined by a second microscopist to confirm the quality of the determinations. Any discrepancies were reviewed at a conference and resolved, if necessary, by the readings of a third microscopist.

We took 3 ml venous blood samples at the beginning of the trial as well as on any other day from the positive test for malaria. The blood samples were stored at −20°C. The samples were scheduled for DNA sequencing of the following *P. falciparum* genes: the merozoite surface proteins 1 and 2 (*map-1 and -2*), and the glutamate-rich protein (*glurp*). Detected them to discriminate between the incidence of recrudescence and reinfection [21][22]. The gene primers were retrieved from the Worldwide Antimalarial Resistance Network (www.wwarn.org) (Appendix 2).

Follow-up

Both drugs would be observed administration by medical staff and were not required to take with food. Those patients who vomited were additionally provided with the same dose of the drug again, and monitoring for 30 min. If the patients vomited twice, they would be discharged and treated with different medications.

All patients enrolled in the trial were requested to visit the clinic, where the study was held, once a week or at any moment when they felt ill for 42 days. For patients who were unsuccessful to do so, a visit by health specialists was appointed for assistance. Furthermore, the subjects were questioned weekly about preceding indications or signs that have arisen after the earlier stopover, and would be asked to make the blood smears and checked the axillary temperature. Blood routine examination would be done on day 0 and day 7. The incidence of any adverse event and abnormal laboratory index was documented in the report forms. The coordinator of the study site ensured the accuracy of the extracted data from the medical records.

Patients, who have completed the medication, do not return to the clinic for a follow-up visit at the appointed time, and are unable to be contacted by the health workers through three visits will be classified as a loss to follow-up. Subjects who meet any of the following will be classified as protocol violations: 1) withdrawal of consent, 2) Subjects, who vomit the study medication twice or get serious adverse events necessitating termination of treatment before the full course is completed, failure to complete treatment. 3) enrolment violation, 4) voluntary protocol violation to the administration of antimalarial activity drugs, and 5) involuntary protocol violation. Patients have been detected with a mono-infection with another malaria species, misclassified by laboratory error, or occurring with concomitant disease that would interfere with a clear classification of the treatment outcome.

Grouping patients' reactions to the two groups of applied cure

The classification of patients' responses to treatment was done following the generally accepted methods for surveillance of antimalarial drug efficacy [23]. Patients were classified as early treatment failure (ETF) if any of the following criteria were met: development of severe malaria in the first three days, accompanied with parasitemia on day 2 > day 0, presence of parasitemia on day 3 with axillary temperature ≥ 37.5 °C, or on day 3 parasitemia≥ 25% of day 0. Subjects who developed severe malaria between day 4 and day 42 with parasitemia or occurring fever were classified as late clinical failure (LCF). If patients did not cover the ETF,

nor the LCF criteria, but occurred parasitemia between day 7 and day 42 without fever were classified as late parasitological failure (LPF). If absence of parasitemia on day 42, who did not formerly run into any ETF, LCF, or LPF criteria was classified as ACPR.

2.5 Statistical analysis

Data quantitation was done by the SPSS 19.00 software. Calculation and statistical analysis of both qualitative (percentage) and quantitative (mean and standard deviation) indicators were conducted. The parameters of gender, outcomes, parasitemia, fever, gametocyte, and adverse reactions were analyzed by the chi-square test. The baseline parameters were analyzed by a two-sample t-test for two groups and were applied for the analysis of quantitative results. Intention-to-treat (ITT) analysis was performed by survival analysis for the efficacy outcomes. Kaplan-Meier curves were estimated for both 28 and 42 days of follow-up with two-side log-rank statistics. For ITT analysis, all withdrawals, losses to follow-up, and treatment failures were censored on the last day of follow-up. In all figures, the calculated p-values were given. P values ≤ 0.05 were considered statistically significant.

3. Results

3.1 Baseline information for patients enrolled in the clinical trial

In the beginning, the inclusion criterion was a parasitemia of 1000-100000 parasites per μL of blood. Surprisingly, with the increase in the screening number, only a few patients met the requirements, which made us adjust by lowering the concentration of parasites to 500-100000 per μL of blood. In the end, 6,295 people were screened, aged 2-60 years and a total of 238 uncomplicated falciparum malaria patients met the aforementioned inclusion criteria, with an equal amount of cases (n=119) in the two studied groups. Among them, 105 cases in the AP group and 100 cases for the AL group were finished 42 days of follow-up. Patients' data and

enrollment details in any of the studied groups are shown in Figure 2. In the initial medical physical characteristics of the patients in the two groups, only temperature and total bilirubin showed significant statistical difference ($p<0.05$), others with no significant statistical difference ($p>0.05$). See Table 1.

3.2 Therapeutic responses

Primary endpoint

On day 42, analysis was completed for 105 patients in the AP group and 100 patients in the AL group. ACPR before and after PCR-corrected were 91.43% [95%CI: 83.93%-95.76%] and 98.06% [95%CI: 92.48%-99.66%] for AP treatment, respectively, and 96.00% [95%CI: 88.17%-98.14%] and 98.97% [95%CI: 93.58%-99.95%] for AL treatment, respectively. There was no significant difference between the two groups ($p>0.05$). See Table 2.

No ETF or LCF were observed in two treatments for 42 days of follow-up. LPF before and after PCR-corrected showed were 8.57% (9/105) and 1.94% (2/103) in the AP group, respectively, and 4.00% (4/100) and 1.03% (1/97) in the AL group, respectively. The reinfected patients were seven patients in the AP group and two patients in the AL group. Two cases in the AP group and three cases in the AL group were not corrected by PCR for missing the blood samples.

The survival analysis using the ITT definition demonstrated a non-PCR-corrected 28-day ACPR of 96.19% for AP and 98.00% for AL ($p =0.106$). And at day 42, the non-PCR-corrected ACPR were 91.43% for AL and 96.00% for AL ($p = 0.183$). Ses Figure 3.

Secondary endpoints

Regarding outcomes like parasitemia and fever, both groups showcased a quick clearance and disappeared on day 3 (Table 2). Only two cases in the AP group (1.71%, 2/117) and five

cases in the AL group (4.39%, 5/114) were diagnosed with parasitemia after microscopic detection on day 2. Meanwhile, 14 cases (11.76%, 14/119) in the AP group and 21 cases (17.65%, 21/119) in the AL one, whose axillary temperature maintained above 37.5℃ on day 1, though these results were not statistically significant (p=0.200). Only one case (0.85%, 1/117) in the AP group and four cases (3.51%, 4/114) in the AL group had a fever (≥37.5℃) on day 2.

The gametocyte carriage in the two groups was low. However, there were 13 cases (10.92%, 13/119) in the AP group and 4 cases (3.36%, 4/119) in the AL one, which showed a significant statistical difference (p=0.023) at the initial. In the AP group, a substantial alteration in the gametocyte carriage between the two-time points was detected between day 7 (0.93%, 1/108) and the initial time point, with a statistically significant difference (p=0.002). But the gametocyte did not disappear completely until day 21 in the AP group. Interestingly in the AL group, the gametocyte carriage turned to zero on day 2 (Figure 4).

Patients safety analysis

There were two cases in AP group and five cases in AL group who vomited the study drug twice and gave for alternative treatment. All of them were children under 6 years old.

Two cases with adverse reactions to the applied treatment in the AP group were diagnosed, including headache (0.85%, 1/117) and fatigue (0.85%, 1/117). Three cases with adverse reactions to the treatment in the AL group, including anorexia (0.88%, 1/114), fatigue (0.88%, 1/114), and insomnia (0.88%, 1/114). Statistical data provided no statistical difference between the two groups (p=0.630), regarding these adverse reactions to the applied therapeutic approach.

4. Discussion

In previous studies, the cure rate for uncomplicated falciparum malaria treated with AP and AL ranged from 95% to 100%[14][15][16] [13] and from 82% to 100%[15][17][18], respectively. We conducted our clinical trial to study the efficacy of AP and AL drugs during a 42-day follow-up. The results showed that the effectiveness of the two artemisinin-based drugs reached 98% by PCR-corrected with mild adverse reactions. This means that the two artemisinin-based drugs remained high efficacy for *P. falciparum* malaria and well tolerance in Grande Comore. However, to avoid the *P. falciparum* resistance to the current therapeutic protocols we provided two suggestions: 1) to standardize the treatment of malaria cases and provide approaches for choosing the most effective dose and complete the treatment until the parasitemia is completely cleared from the human body; 2) aggressive responses to study the comprehensive control measures, complete regional eradication of malaria before resistance sets, especially in countries like the Comoros.

Under our clinical trial, it was obvious that the gametocyte carriage in the AP group was greater than that in the AL group for the baseline ($p<0.05$), which means that the gametocyte prevalence level for the study sites of the AP group was higher than AL. It might be one reason for more cases of re-infection diagnosed among the AP group during the 42-day follow-up, as the 28 and 42-day ACPR between the two sets of treatment groups was no significant difference ($p>0.05$). Data showed that artemisinin has the ability to kill gametocytes [27]. The AP treatment affected the gametocyte, and the statistically important alteration was detected on day 7 ($p=0.002$). This result was similar to our clinical study in Togo [12]. We also noted that in the present study, gametocyte was undetectable at day 2 in AL, which appeared to AL had an advantage over AP in gametocyte clearance. This was consistent with most of the previous data evidence that gametocyte clearance was faster in the AL compared to the dihydroartemisinin-

piperaquine[28][29] (as the pharmaceutical composition of AP is similar to dihydroartemisinin-piperaquine). Therefore, recommendations exist for the application of AP in combination with anti-gametocyte treatment on the first day of *Plasmodium* infection to rapidly achieve gametocyte killing effect in areas with a high gametocyte prevalence level.

Our clinical trial found another challenge things. We found that all cases for recrudescence and reinfection were found in weekly follow-up visits without obvious symptoms, which indicated that they belonged to asymptomatic parasitemia infections. Papa Mze et al[30] recently found that among *P. falciparum* a great genetic diversity was observed in Grande Comore. These may bring major challenges to the discovery and treatment of asymptomatic malaria cases, and even influence the steps of malaria control in the Comoros.

Although this study has various limitations, including that the drug-resistant study was not implemented and the clinical trial was not carried out as randomized controlled trial due to insufficient project management intensity. However, the controlled clinical research data obtained are of great significance for guiding local malaria prevention and control drugs in the Comoros regions in the next stages, to control the rebound rates of malaria there.

Acknowledgments

We would like to thank the patients who participated in this trial, and all medical staff followed the study. The authors are grateful to the reviewers who participated in the review, as well as MJEditor (www.mjeditor.com) for providing English editing services during the preparation of this manuscript.

Declarations

Funding: This work was supported by Natural Science Foundation of China [Grant Number 81873218 and 82074301], Science and Technology Project of Guangdong Province [Grant Number 2021A0505030060 and 2020A0505090009], and the Chinese Medicine Cooperation Special Program of the National Administration of Traditional Chinese Medicine (Grant Number GZYYGJ2021).

Competing interest: The funding agencies had no role in study design, data collection, and analyses, decision to publish, or preparation of the manuscript.

Ethical Approval: Guangdong Provincial Hospital of Chinese Medicine (Registration Number: BF2019-169-01).

Sequence Information: Not applicable

Author Contributions:

G. Li and Y. Yuan contributed equally to this study. Q. Xu, J. Song, W. Wu and C. Deng designed, organized, and directed the trial. G. Li, C. Lu, R. Liu, R. Silai, S. Kamal and A. Bacar carried out the fieldwork. Y. Yuan, S. Zheng, M. Li, H. Zhang, R. Tan, C. Lu, and R. Silai collected and analyzed the data. G. Li, Y. Yuan, W. Wu and C. Deng wrote and revised the manuscript. All authors read and approved the final manuscript.

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Table 1. Baseline characteristics

Characteristics	AP(n=119)	AL(n=119)	p-value
Age-years	15.6±15.2	15.8±12.0	0.917
Males(%)	63（52.94）	68（57.14）	0.515
Temperature (℃)	38.3±0.8	38.5±0.8	0.018
Parasites(/μL)	4405.2±5644.6	4213.6±4922.4	0.780
Hemoglobin-g/L	103.62±17.52	103.52±19.56	0.967
Hematocrit (%)	34.86±6.47	33.72±7.15	0.198
leukocyte (×10^9/L)	6.14±1.83	6.58±2.52	0.124
Erythrocyte (×10^12/L)	4.13±0.58	4.25±0.77	0.168
Blood platelet count	200.54±112.52	190.31±129.87	0.517
Creatinine -μmol/L	9.88±2.31	10.43±3.10	0.119
Glutamic oxalacetic transaminase (U/L)	26.97±12.68	26.37±15.14	0.742
Glutamic-pyruvic transaminase (U/L)	25.99±13.90	27.36±14.69	0.461
Direct bilirubin (μmol/L)	0.98±0.88	1.08±1.03	0.429
Total bilirubin (μmol/L)	3.66±2.46	2.96±1.81	0.014

*AP= Artemisinin-Piperaquine. AL= Artemether- Lumefantrine.

Table 2. Therapeutic responses

Therapeutic response	AP %[95%CI]	AL %[95%CI]	p-value
Early treatment failure	0%[0%-3.96%](0/117)	0%[0%-4.06%](0/114)	1.000
Day 3 fever clearance	100%[96.04%-100%](117/117)	100%[95.94%-100%](114/114)	1.000

Day 2 parasite clearance	98.29%[93.35%-99.70%](115/117)	95.61%[89.56%-98.37%](109/114)	0.235
Day 3 parasite clearance	100%[96.04%-100%](117/117)	100%[95.94%-100%](114/114)	1.000
Day 28 non-PCR-corrected ACPR	96.19%[89.97%-98.77%](101/105)	98.00%[92.26%-99.65%](98/100)	0.442
Day 28 PCR-corrected ACPR	98.10%[92.62%-99.67%](103/105)	98.99%[93.70%-99.95%](98/99)	0.596
Day 42 non-PCR-corrected ACPR	91.43%[83.93%-95.76%](96/105)	96.00%[88.17%-98.14%](96/100)	0.179
Day 42 PCR-corrected ACPR	98.06%[92.48%-99.66%](101/103)	98.97%[93.58%-99.95%](96/97)	0.596

*AP= Artemisinin-Piperaquine. AL= Artemether-Lumefantrine. PCR= Polymerase Chain Reaction. ACPR=Adequate clinical and parasitological response.

Figure 1. Study site.*AP= Artemisinin-Piperaquine. AL= Artemether-Lumefantrine.CMC Mbeni=Centre Médicaux Chirurgicale de Mbeni.HP Mitsamiouli=Hôpitalpôlede Mitsamiouli. CSDOichili-Dimani=Centre de Santéde District de OichiliDimani.CSD Mitsoudje=Centre de Santé de District de Mitsoudjé. PNLP =Programme National de Lutte Contre le Paludisme. MSLCP=Maison de la Surveillance de Lutte Contre le Paludisme.

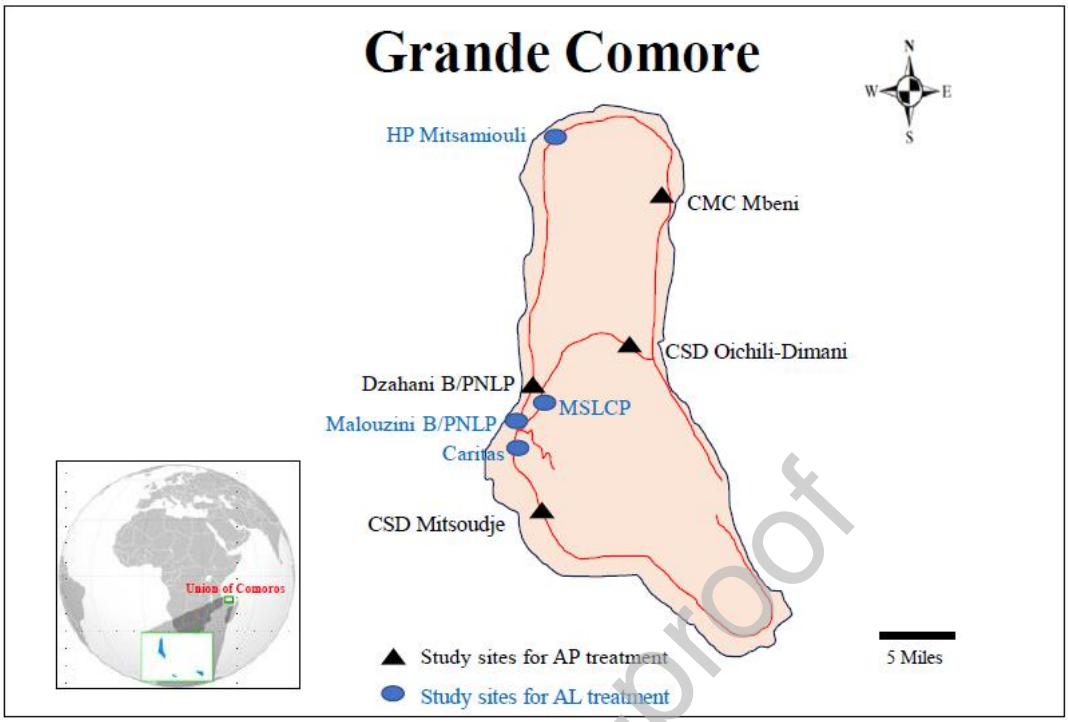

Figure 2. Study profile *AP= Artemisinin-Piperaquine. AL= Artemether- Lumefantrine. PCR= Polymerase Chain Reaction. RDT= Rapid Diagnostic Tests.

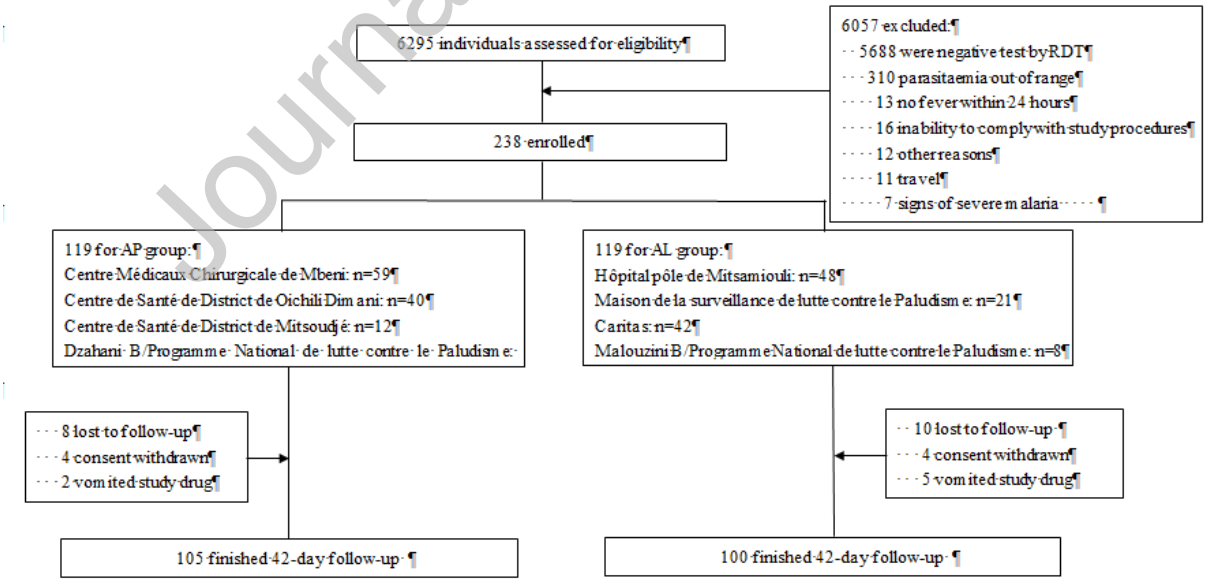

Figure 3. Survival curve of enrolled patients by non-PCR-corrected data (This graph is using intention to treat (ITT) analysis.)

*AP= Artemisinin-Piperaquine. AL= Artemether- Lumefantrine.

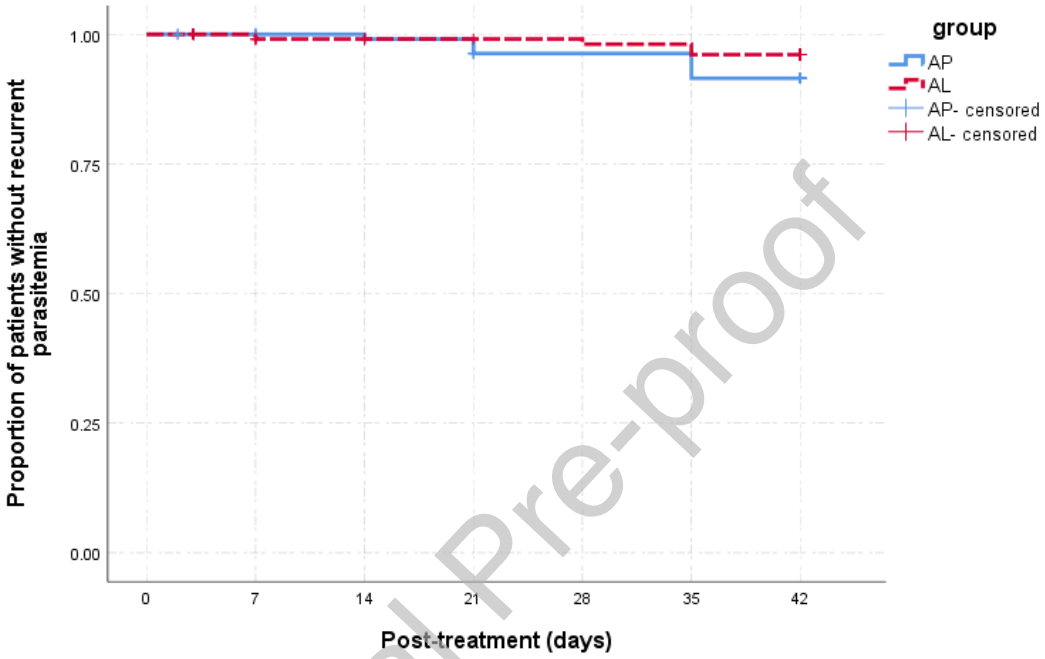

Figure 4. Gametocyte parameters

*AP= Artemisinin-Piperaquine. AL= Artemether- Lumefantrine.