AAC Accepts, published online ahead of print on 7 July 2014
Antimicrob. Agents Chemother. doi:10.1128/AAC.02682-14

A randomized comparison of the efficacy and tolerability of three artemisinin-based combination treatments for children with acute falciparum malaria in The Democratic Republic of Congo

Running title: Antimalarials efficacy and tolerability in DRC

Onyamboko A. M. [a, c], Fanello C.I. [a, b, #], Wongsaen K. [d], Tarning J. [a, b], Cheah P.Y. [a, b], Tshefu K. A. [c], Dondorp A.M. [a, b], Nosten F. [b, d], White N. J. [a, b] and N. P. J. Day [a, b]

[#]**Corresponding author:** C. Fanello caterina@tropmedres.ac

[a] *Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand*

[b] *Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, United Kingdom*

[c] *Kinshasa School of Public Health, University of Kinshasa, Kinshasa, DRC*

[d] *Shoklo Malaria Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand*

Abstract

An open label, randomized controlled trial was carried out in 2011-12 in the Democratic Republic of Congo to test the efficacy, safety and tolerability of the artemisinin-based combination treatments dihydroartemisinin-piperaquine, amodiaquine-artesunate and artemether-lumefantrine. 684 children aged 3 to 59 months with uncomplicated *Plasmodium falciparum* malaria were randomly allocated to each study arm. Children were hospitalized for three days, given supervised treatment and followed-up weekly for 42 days. All regimens were well tolerated and rapidly effective. The median parasitemia clearance half-life was 2.2 hours and similar between arms (p=0.19). The PCR-uncorrected cure rates by day 42 were 73.0% for amodiaquine-artesunate, 70.2% for artemether-lumefantrine and 86.3% for dihydroartemisinin-piperaquine (p=0.001). Early treatment failure occurred in three patients (0.5%), one in each arm. The PCR-corrected cure rates were 93.4% for

amodiaquine-artesunate, 92.7% for artemether-lumefantrine, and 94.3% for dihydroartemisinin-piperaquine (p=0.78). The latter provided a longer post-treatment prophylactic effect compared with the other two treatments. The day 7 plasma concentration of piperaquine was below 30 ng/mL in 47% of the children treated with dihydroartemisinin-piperaquine and the day 7 lumefantrine concentration was below 280 ng/mL in 37.0% of children who received artemether-lumefantrine. Thus although cure rates were all satisfactory, they could be improved by increasing the dose (Trial Registration ISRCTN20984426).

Introduction

Background

The Democratic Republic of Congo (DRC) is one of the five countries with the greatest malaria burdens in the world (1). The current national policy for the treatment of uncomplicated *Plasmodium falciparum* malaria consists of amodiaquine-artesunate or artemether-lumefantrine, although artemether-lumefantrine, which was introduced in 2010, has very limited availability in the public sector. Amodiaquine-artesunate remains the most widely distributed antimalarial therapy in DRC. It was introduced in 2006, replacing sulphadoxine-pyrimethamine which is now only used as Intermittent Preventive Treatment in pregnancy. The distribution and access of antimalarials in the rural areas of the country is organized through the public sector, whereas in the urban setting the private sector is predominant. Due to the civil unrest that has affected the country for many years there is a paucity of data concerning the efficacy of antimalarial drugs in DRC. Available studies show substantial geographic variation in therapeutic efficacy, with similar variation in the prevalence of polymorphic alleles in *P. falciparum* genes associated with parasitological failure (2-4). This reflects the vast geographical area of the country.

Dihydroartemisinin-piperaquine is an artemisinin-based combination therapy (ACT) with a good safety and tolerability profile which is as effective as other ACTs in endemic areas of Asia and Africa (5). Piperaquine is a bisquinoline with a chemical structure similar to chloroquine and amodiaquine. The long terminal elimination half-life (~23 days) provides lengthy post-treatment chemoprophylaxis and the simple once daily dosage regimen facilitates adherence (6). Dihydroartemisinin-piperaquine efficacy in Africa has so far been good, although no data are available for DRC.

The aim of this trial was to assess the efficacy of amodiaquine-artesunate for the treatment of uncomplicated *P. falciparum* malaria in children in Kinshasa, DRC, five years after its introduction as a first line treatment, and to compare this with the efficacies of potential alternatives, dihydroartemisinin-piperaquine and artemether-lumefantrine, the latter recently added to the first-line treatment policy.

Methods

Study area

The study was carried out in a health centre located in an urban district of Kinshasa (DRC). Malaria transmission in the area is intense and perennial, with two annual peaks corresponding to the rainy seasons.

Patient population

Patients attending the health centre with suspected clinical malaria were screened and enrolled in the study if they met the following inclusion criteria: age 3 to 59 months; weight ≥ 5 kg; mono-infection with *P. falciparum;* parasitemia density between 2,000 and 200,000 asexual parasites/µL; axillary body temperature ≥37.5°C, or history of fever in the preceding 24 hrs; hemoglobin ≥5.0 g/dL and able to take oral medication. Patients with severe malaria (7), mixed species malaria infection, any other significant concomitant illness, underlying disease, malnutrition, known allergy to any of the study drugs, a clear history of adequate antimalarial treatment with drugs in the previous 72 hrs, or taking prophylaxis with drugs having antimalarial activity were excluded. All cases excluded from the trial were referred to the hospital for diagnosis and treatment.

Trial design

This was an individually randomized, open label study; comparing three fixed-dose oral artemisinin-based combination therapies: dihydroartemisinin-piperaquine (DP), artemether–lumefantrine (AL) and amodiaquine-artesunate (AA).

Treatment

Patients were randomly allocated to receive one of the three study treatments.

AA (Artesunate-Amodiaquine Winthrop® Sanofi, Kenya) was administered once a day for 3 days according to body weight at a mean dosage of 3.8 mg/kg/day of artesunate and 10.2 mg/kg/day of amodiaquine. Three types of fixed-dose tablets were used, containing artesunate 25 mg, 50 mg or 100

mg, plus amodiaquine 67.5 mg, 135 mg or 270 mg. Tablets were administered according to the manufacturer's instructions: 4.5 -8.9 kg, 1 tablet 25/67.5; 9 - 17.9 kg, 1 tablet 50/135; 18-35.9 kg. 2 tablets 100/270.

DP (DARTEPP, Guilin Pharmaceutical China; 40 mg dihydroartemisinin and 320 mg piperaquine each tablet) was administered once a day for 3 days according to body weight with the following scheme: 5 - 7.9 kg, ½ tablet; 8-9.9 kg, 0.75 tablet; 10-14.9 kg, 1 tablet; 15-20.9 kg, 1.5 tablets and 21-29.9 kg, 2.0 tablets. This dosage scheme was different from the one recommended by the manufacturer (5 - 10 kg, ½ tablet; 11-20 kg, 1 tablet; and 21-35 kg, 2.0 tablets). We used 5 intervals of weight instead of 3 to improve the therapeutic dose of dihydroartemisinin at the upper limit of the range for each interval .The mean dosage was 3.3 mg/kg/day of dihydroartemisinin and 26.6 mg/kg/day of piperaquine.

AL (CoartemTM, Novartis, Switzerland) was administered in 6 doses over 3 days (0, 8, 24, 36, 48, and 60 hours). Each tablet contained 20 mg artemether and 120 mg lumefantrine and the mean dose was 2.0 mg/kg of artemether and 12.7 mg/kg of lumefantrine for each dose. Tablets were administered according to the manufacturer's instructions: 5-14.9 kg, 1 tablet; 15-24.9 kg, 2 tablets; 25-34.9 kg, 3 tablets; >35 kg,4 tablets.

Tablets were administered under medical supervision and with 100 mL of milk (the fat in the milk improves drug absorption). Patients were observed for 1 hour after drug ingestion. The full dose was repeated if the patient vomited within 30 minutes and half-dose if the patient vomited within 1 hour. Patients who failed treatment were treated with IV quinine if severe (20 mg salt/kg body weight loading dose followed by 10 mg/kg 8 hourly) or oral quinine if uncomplicated (10 mg/kg three times daily for seven days) according to national policy. Parenteral artesunate was not available.

All children were hospitalized for 3 days and followed-up actively once a week for 42 days after treatment. Caretakers were invited to come back to the centre or contact the study nurse in case the child was unwell. If the patient did not report for the scheduled visits, every effort was made to locate him or her at the home address. At each visit the medical history, clinical signs and symptoms, body temperature and a blood sample for parasitemia were collected.

Sample size

For the calculation of the sample size we assumed a cure rate of 95% with AL, 99% with DP and 85% with AA. A sample size of 621 patients would have been adequate to detect a 10% difference between the standard treatment (AA) and AL or DP at the 5% level and with 90% power. The sample size was increased by 10% to allow for loss to follow-up (final sample size N=684).

Randomization, sequence generation, type, allocation concealment mechanism, implementation

The randomization sequence, in blocks of fifteen, was computer-generated and numerically sequenced. Opaque envelopes containing the study drug name were prepared at the Mahidol Oxford Tropical Medicine Research Unit (MORU), Bangkok. Patients were enrolled by the study physician and assigned to treatment by the study nurse who opened the next consecutively numbered envelope. Once an envelope was opened, the patient was considered included in the study.

Outcome measurements

The primary outcome measure was the PCR corrected cure rate by day 42. Secondary outcome measures were parasite and fever clearance and occurrence of adverse events (AE). Treatment outcome was established according to standard WHO classification (8). Early treatment failure (ETF) was defined as: (1) danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitemia; (2) parasitemia on day 2 higher than on day 0, irrespective of axillary temperature; (3) parasitemia on day 3 with axillary temperature $\geq$ 37.5 °C; and (4) parasitemia on day 3 $\geq$ 25% of count on day 0. Late clinical failure (LCF) was defined as: (1) danger signs or severe malaria in the presence of parasitemia on any day between day 4 and day 42 in patients who did not previously meet any of the criteria of early treatment failure; (2) axillary temperature $\geq$ 37.5 °C in the presence of parasitemia on any day between day 4 and day 42 in patients who did not previously meet any of the criteria of early treatment failure. Late parasitological failure (LPF) was defined as presence of parasitemia between day 7 and day 42 with a temperature < 37.5°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) was defined as absence of parasitemia on day 42, 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.

Safety reporting was performed according to ICH Harmonized Tripartite Guideline for Good Clinical Practice (9).

Laboratory Methods

Asexual and sexual malaria parasites were identified and counted on Giemsa-stained thick films and reported per 200 WBC, assuming a total WBC count of 8,000/µL (10). Slides were declared negative after examination of at least 100 high-power microscopy fields. Parasite species was determined on the thin film. The laboratory technicians were blinded to the treatment received by individual patients. The blood film prepared during the screening was considered the admission slide.

Blood films were prepared at baseline, 6 and 12 hours and then repeated every 12 hours until 2 consecutive negative blood films were observed. Parasite clearance was assessed i) as the time for the parasite count to reduce to 50% of its initial value (PC50) and ii) as parasite clearance rate derived from the log-linear section of the log parasitemia–time curve and expressed as the parasite clearance half-life ($PCt_{½}$; $\log_e 2$/ parasite clearance rate).

To compare the $PCt_{½}$ measured in this study with the more recent data collected in 2013 during the Tracking Resistance to Artemisinin Collaboration (TRAC) project, all slides were read a second time after the study was terminated by the same microscopists team using a different counting technique: if more than 20 parasites were seen on the thick smear after 10 fields, parasitemia per 1,000 RBC was counted on the thin smear. Below that threshold, parasites were counted on the thick smear per 500 WBC.

Hemoglobin was measured on admission using a portable photometer (HemoCue Hb201+, Angelholm, Sweden). Thereafter the hematocrit was measured at baseline, daily during the hospitalization and at days 7 and 14 of the follow-up by microhematocrit centrifugation (Hawksley Haematospin 1400, Hawksley & Sons, Ltd. UK).

Total and differential WBC counts were assessed daily during the hospitalization and at day 7 and 14 of the follow-up (Sysmex® automated hematology analyzer).

Liver function tests, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were measured from plasma at the hospital laboratories (SEAC-Screenmaster) at baseline and 48 hours.

A dried blood spot (DBS) was prepared at admission, daily during the hospitalization and at each follow-up visit for further molecular analysis.

Drug analysis

A random sample of tablets of DP was analyzed for content and quality at the Department of Pharmacology of MORU and 2 mL of venous blood were taken at day 7 from 246 consecutive patients to measure plasma concentrations of lumefantrine and piperaquine.

Molecular analysis

Paired filter paper samples from enrollment and the follow-up day on which parasites were detected by microscopy were analyzed at SMRU to distinguish between recrudescence and re-infection. Parasite DNA was purified (QiaAmp DNA Micro kit; Qiagen, UK) and the three polymorphic markers MSP-1, MSP-2 and GLURP genotyped. A recrudescent infection was defined as one that matched in size at least one allele of each marker between the first and second samples. If any pair of alleles of a polyclonal primary infection was detected during a second episode, this was considered a recrudescence.

Ethical approval

The study was approved by Oxford University Research Ethic Committee (OXTREC), the Institutional Review Board of Kinshasa School of Public Health (KSPH) and the Ministry of Public Health of DRC. A verbal consent was obtained from caretakers before screening children for malaria and anemia. A written consent form was obtained from caretakers whose children fulfilled all inclusion criteria before enrolling the patient in the study.

The study was monitored regularly by a qualified internal monitor (MORU Clinical Trials Support Group) for adherence to GCP regulations. Serious adverse events (SAEs) were notified to all Investigators and the REC of KSPH.

The study was registered with International Standard Randomized Controlled Trial Number Register (www.isrctn.org) ISRCTN20984426.

Statistical analysis

Data were double entered in Microsoft Access 2007 and validated using Epi Info 6.4b (CDC, Atlanta, GA, USA). Statistical analyses were performed using STATA v.11 (College Station, TX: StataCorp LP). Descriptive statistics were used to summarize demographic data and baseline values. For the per protocol analysis, χ^2 was used to compare proportions. ANOVA was used for normally distributed continuous data and the non-parametric Kruskal—Wallis test to analyze continuous data with a non-normal distribution.

For the intention-to-treat analysis, the logrank test was used to test the equality of the survivor function across groups and Cox regression to estimate hazard ratio of infections post-treatment. The overall fractional reduction in hematocrit was defined as the difference between the patient's lowest level of hematocrit and that at baseline (i.e., pre-treatment) divided by the hematocrit at baseline. The percentage of patients whose hematocrit fell >20% or 25% was compared between groups. No interim analyses for efficacy or futility were done.

Results

Intention to treat analysis, deviations from study protocol

Between September 2011 and November 2012, 684 patients were included in the study, 228 in each treatment group. Forty-two patients (6.1%) discontinued the study: 5 children were withdrawn during the hospitalization because the families changed their mind and 37 were lost to follow-up between day 7 and 42 (DP=16; AL=10; AA=16). One patient, in the AA group, died at day 29 from causes unrelated to malaria or the study drug. These cases were not included in the per protocol analysis, and

they were censored on the last day they were visited by the doctor and tested for malaria in the intention to treat analysis (ITT). The flow of patients through the study is outlined in the patient flow diagram (Figure 1).

Baseline Characteristics and treatment

At enrollment, patients had similar demographic, clinical and parasitological characteristics (Table 1). The tablets of DP contained an average of 35.6 mg dihydroartemisinin (89%) and 306 mg piperaquine (95.5%). This was compared to Eurartesim (Sigma Tau) product, which contained an average of 40.7 mg dihydroartemisinin (102%) and 300 mg piperaquine (94%).

Drug efficacy

Per protocol analysis: The cure rate by day 42 (primary outcome), PCR uncorrected, was similar in patients treated with AA (73.5%) or AL (70.6%), whereas it was significantly higher in patients treated with DP (86.8%) (p=0.001); Table 2. In the follow-up period, 145 children were diagnosed with a second episode of malaria (starting as early as day 16); most of these cases were new infections and only 30 (21%) were confirmed by PCR as recurrent infections. Among the new infections there were 12 cases of *P. malariae* and 1 *P. ovale*. For 9 patients the PCR was unsuccessful; as we could not ascertain if these 9 cases were new or recurrent infections we excluded them from the PCR-corrected analysis. After correcting the results for the new infections, the cure rates were comparable in the three groups: 93.4% for AA (95% CI 89.1% - 96.3%), 92.7% for AL (95% CI 88.4% - 95.7%), and 94.3% for DP (95% CI 90.3% - 97.0%), p=0.76.

Early treatment failure occurred in three patients (0.5%), one in each arm. Data are reported also using day 28 cure rates as the endpoint (Table 3).

Intention to Treat Analysis: The ITT analysis showed similar results (logrank test for equality of survivor functions PCR-uncorrected χ^2 =18.83, p = 0.0001 and PCR-corrected χ^2 =1.00, p=0.61; Figure 2). The risk (hazard ratio) of having a second episode of malaria (either new or recurrent) in the follow-up period was 1.5 times higher in the AA arm and 2.4 higher in the AL arm compared to the DP arm (p>0.0001). The results were not affected by age or initial parasitemia.

Fever Clearance

On admission 26.8% of patients had fever (axillary temperature ≥ 37.5°C). For the other patients the parent or guardian reported a history of fever in the preceding 24 hours as the main reason for seeking a doctor at the health centre. After 24 hours, 97% of children were afebrile and there was a significantly higher proportion of children with fever in the AL group at day 2 ($p = 0.003$; Table 4).

Parasitemia Clearance

All treatments were associated with a rapid clearance of parasitemia. The parasite positivity rate (proportion of children with a positive slide at day 2) was significantly higher in the AL arm ($p<0.001$; Figure 3). Accordingly, the median PC50 was significantly longer for AL (8.4 hours, range 0.2 to 23.9 hours, N=214) than for AA (5.7 hours, range 0.1 to 24.3 hours, N=204) and DP (6.5 hours, range 0.1 to 34.4 hours, N=212), $p<0.001$ (Table 5). The median $PCt_{½}$ was 2.2 hours (range 1.0 to 6.3 hours, N=657) with no significant differences between arms indicating a similar efficacy of the three different artemisinin derivatives, $p=0.08$ (Table 6).

Gametocytaemia

On admission 28.5% (N=195/684) of patients were gametocytaemic with no significant differences between groups. Treatment with AL resulted in lower gametocyte carriage rates than the other two treatments in the follow-up period, days 7-21 ($p<0.001$; Figure 4). In 7 children gametocytaemia was microscopically detectable from admission until day 35 (AA=4, DP=2 and AL=1) and in 2 children treated with DP until day 42. In a number of children gametocytes were not detected on admission blood smears, but became apparent in the first 72 hours of treatment with no significant differences between arms. New appearance of gametocytes was however uncommon from day 7 onward.

Hematology

The mean PCV at admission was 30.1% (95% CI 29.8%-30.5%; range 13%-42%). In the first week there was an overall mean fractional reduction in the PCV of 10% (SD 8.3) with no differences between arms ($p=0.65$; Table 8). Hyperparasitemic children (≥ 150,000/µL) were those most affected,

with a mean fractional reduction of 15.1% (95% CI 7.4-8.7) compared to 8.1% (95% CI 13.8-16.3) in those not hyperparasitemic ($p<0.001$).

A reduction of >25% of the initial PCV value was observed in 14.9% of hyperparasitemic children and 2.3% of non-hyperparasitemic children ($p<0.001$).

Ten patients developed decompensated anemia within 4 days of recruitment and required a blood transfusion; 3 in the AA group, 4 in the AL group and 3 in the DP group ($p=0.62$). The risk of receiving a blood transfusion was 6.5 times higher in the hyperparasitemic children (CI 95% 2.90 - 22.3; $p=0.005$). By day 14 the levels were comparable to admission in all patients.

The median WBC count was similar between the treatment groups on recruitment and at each day of follow-up with an increase from day 0 to day 7 to normal values (Table 7). Neutrophil counts decreased gradually from baseline values until day 14 with no differences between groups on any day. Mild neutropenia (neutrophils <1000/ µl) was observed in 2.5% of patients at enrollment and between day 1 and 7 the neutrophils count fell below 1000/µl in 18% of patients (123/684) with no differences between groups (n=40 in AA, 40 in AL, and 43 in DP; $p=0.92$). Fourteen of these patients developed severe neutropenia (neutrophils <500/µl) (2, 4, and 8 in the AA, AL and DP groups, respectively; $p=0.13$).

Hepatotoxicity

Mean serum levels of aspartate aminotransferase (U/L), alanine aminotransferase (U/L) and creatinine (mg/dL) were similar at baseline among groups. With minor fluctuations (a trend toward a reduction for AST and ALT) the mean levels at day 2 remained similar to those of day 1 with no statistical differences between groups (data not shown).

Tolerability

During the first day, children treated with DP vomited within 1 hour of the first dose significantly more often (N=21; 9.2%) than children treated with AA (N=10; 4.4%) or AL (N=5; 2.2%), $p=0.03$. The second dose of AL - administered after 8 hours – was vomited in seven cases, and taking this into account the overall difference between treatments during the first day was not significant ($p=0.06$).

These children were all given a second dose of the drug. On the second day there was no difference in vomiting post-dose between children treated with AA (15; 6.6%) or DP (17; 7.5%), whereas in the AL arm only 2 (0.9%) cases vomited after the first dose and none after the second dose (p=0.02).

Adverse Events

At least one adverse event was reported in 37.4% of patients during the post-treatment period that was not present on admission or increased in intensity and was classified as possibly or probably related to the study drug. Most AEs were graded as of minor or moderate intensity. The most frequent AEs were weakness, anorexia and gastrointestinal disorders (nausea, vomit, abdominal pain and diarrhea). However these symptoms overlap with known malaria symptomatology. Anorexia and weakness were reported more frequently in children treated with AA than those treated with DP and AL during the second and third day of treatment (day 1: 15.2% (34/224) for AA, 5.3% (12/227) for AL, and 5.8% (13/228 for DP ; p=0.0001, and day 2: 8.2% (17/207) for AA, 2.3% (5/220) for AL and 4.0% (9/224) for DP; p=0.013). There were otherwise no other differences in the number of AEs between treatment groups. In 17 cases the adverse event was graded as severe or life-threatening. All cases were classified as unlikely to be related to the study treatment. Ten patients developed decompensated anemia during the hospitalization and required a blood transfusion (described above). There was one case of severe skin eruption 18 days post-treatment (DP), one case of chicken-pox 12 days post-treatment (AL), 1 case of abscess 11 days post-treatment (DP), 1 case of leukocytosis 2 weeks post treatment (AL), and 3 cases of asthenia (2 AL and 1 AA). One child in the AA group died in a different hospital at day 29. The cause of death was unknown but was considered unlikely to have been caused by either malaria or the drug treatment.

Plasma lumefantrine levels at day 7

121 samples of venous blood were collected at day 7 from patients who received AL (Table 9). The median concentration of lumefantrine in the blood was 377 ng/mL (range 57.1-1150 ng/mL). Drug levels were positively correlated with body weight (r=0.22; test for trend p=0.005). The plasma level was significantly lower in children weighing < 15 kg (median 309 ng/mL; range 57.1-1080, N=87)

compared to those weighing ≥ 15 kg (median 473 ng/mL, range 108-1150, N=34, p=0.01). Accordingly, 43.7% of children weighing <15 kg had a plasma level ≤ 280 ng/mL considered the cut-off for therapeutic efficacy, (11) compared to 20.6% in those weighing ≥ 15 kg (p=0.018). The 7 children in this sub-sample with a PCR-confirmed recrudescence had a median level of 429 ng/mL (range 147 to 703 ng/mL), not significantly different from those who successfully cleared the infection (376 mg mL; range 57.1-1150, p=0.8, N=114).

Plasma piperaquine levels at day 7

125 samples were collected from venous blood at day 7 from patients who received DP (Table 10). The median concentration of piperaquine was 31.4 ng/mL (range 10.9-189.0), and drug levels were positively correlated with body weight (r= 0.22; test for trend p=0.04). In 47.2% (59/125) of patients the plasma level was below 30 ng/mL, the previously published threshold associated with therapeutic efficacy (12), and the 3 patients with a PCR-confirmed recrudescence had a piperaquine level of 16.3, 31.4 and 33.1 ng/mL, respectively.

Discussion

The efficacy of the three combination therapies tested in this trial was similar. The proportion of children with an Adequate Clinical and Parasitological Response was 93% for AL and 94% for DP, both rarely used in the area. These cure rates are similar to that of AA (93%), which has been extensively used in DRC since its introduction in 2006.

The PCR-corrected day 28 cure rate for AL in the present study was 96.8%, which is comparable to the 97.9% day 28 cure rate observed in the same area 3 years previously (13).

In the subsample analyzed the drug level of piperaquine at day 7, reflecting the concentration of drug to which residual parasites are exposed and thus predictive of outcome, was suboptimal in 47% of patients. This confirms previous results (6, 14) showing that as small children have a higher body-weight normalized oral clearance they need a higher dose than the one currently recommended. Of the children who received artemether-lumefantrine 37% had a sub-optimal day 7 lumefantrine level, with the smaller children having the lowest levels. These results are comparable to those observed in Uganda (15). As there was no evidence of delayed parasitemia clearance suggestive of artemisinin resistance, the PCR-confirmed treatment failures observed in the current study are likely caused by either low drug exposure, as suggested by the PK results, or to parasite resistance to the non-artemisinin partner drug. The former is much more likely. Moreover in high transmission areas, the chances that the recurrent infection contains a parasite with the same genotype as the primary infection are higher than in low transmission areas. This, along with the persistence of gametocytes in the blood can lead in some cases to a misclassification of recurrent infections as recrudescences (16).

In this clinical trial we measured efficacy of treatments administered under supervision, with a glass of milk, and retreatment was given if the first dose was vomited. Drug exposure, in a non-trial setting (generally unsupervised) is expected to be lower (15). Dose optimization and schedule changes are a priority for the ACT treatment especially in small children to ensure adequate drug exposure (14, 17).

Although the efficacy in terms of ACPR rates of the three ACTs was comparable, children treated with DP were at lower risk of having a second episode of malaria during the follow-up period because of the longer post-treatment prophylactic effect of DP related to the longer plasma half-life of piperaquine. This chemoprophylactic effect is important in endemic areas - such as the study area –

and makes this drug a good candidate for replacing sulphadoxine pyrimethamine for intermittent preventive treatment in pregnant women and children (18; 19).

This study population was characterized by hyperparasitemia and the initial high levels of parasitemia affected, as expected, the recovery of hematocrit after the initial episode of malaria, but not the treatment efficacy. The initial level of gametocytemia was also high (30%) and AL was significantly more effective in clearing the sexual stages compared to the other ACTs. Data in literature on the gametocytocidal properties of the different ACT are conflicting and the effect, if any, on malaria transmission is unclear (20).

The median $PCt_{½}$ was 2.2 hours (range 1.0 to 6.3 hours, N=657) and comparable to the results observed in 2013 during the TRAC project: 2.2 hours (range from 1.2 to 4.6 hours, N=60) (21). The difference we observed between PC50 with the three therapies (but not with the $PCt_{½}$,) could be attributed to the relatively slow conversion of artemether to dihydroartemisinin (artemether half-life of 2.0 hours) compared to artesunate (artesunate half-life of 0.84 hours) and dihydroartemisinin in the acute phase of malaria (22) resulting in a significantly longer lag-phase (the initial flat part of the parasite clearance profile) for AL and/or the lower dosage of artemether (20). The PC50 includes the lag phase of the parasite clearance curve, whereas the $PCt_{½}$ is based on the log-linear phase alone.

The three combinations were well tolerated and there were no significant differences in the type and number of adverse events between arms.

Conclusions

The three combinations tested were equally efficacious and well tolerated for the treatment of children with acute uncomplicated *falciparum* malaria. Dihydroartemisinin-piperaquine had the longest lasting chemo-prophylactic effect which prevented repeated clinical attacks in the treated children. The recommended dosage of DP provides suboptimal piperaquine plasma concentrations in particular in small children.

Conflicts of interest

The authors have no conflicts of interest concerning the work reported in this paper.

Financial support

The study was financed by the Wellcome Trust, United Kingdom.

Author contributions

Study conduct and data acquisition: MO, CF. Molecular analysis: WK. Pharmacological analysis: JT. Statistical analysis: CF. Preparation of the manuscript: CF, AT, AD, FN, NW, ND, JT. All co-authors read the final version of the manuscript.

Acknowledgments

We thank our young patients and their families, the study staff at Kingasani Research Centre and the hospital staff and management at Kingasani hospital.

We thank Prof. Kevin Marsh, KEMRI, for donating amodiaquine-artesunate and Guilin Pharmaceutical, China, for donating dihydroartemisinin-piperaquine.

References

1. **WHO.** 2013. World Malaria Report: 2013, Geneva.
2. **Alker AP, Kazadi WM, Kutelemeni AK, Bloland PB, Tshefu AK, Meshnick SR.** 2008. Dhfr and dhps genotype and sulfadoxine-pyrimethamine treatment failure in children with falciparum malaria in the Democratic Republic of Congo. Trop Med Int Health **13:**1384-1391.
3. **Bonnet M, Broek I, van Herp M, Urrutia PP, van Overmeir C, Kyomuhendo J, Ndosimao CN, Ashley E, Guthmann JP.** 2009. Varying efficacy of artesunate+amodiaquine and artesunate+sulphadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria in the Democratic Republic of Congo: a report of two in-vivo studies. Malar J **8:**192.
4. **Mobula L, Lilley B, Tshefu A, Rosenthal P.** 2009. Short Report: Resistance-mediating Polymorphisms in Plasmodium falciparum Infections in Kinshasa, Democratic Republic of the Congo. American Journal of Tropical Medicine and Hygiene **80:**555-558.
5. **Naing C, Mak JW, Aung K, Wong JY.** 2013. Efficacy and safety of dihydroartemisinin-piperaquine for treatment of uncomplicated Plasmodium falciparum malaria in endemic countries: meta-analysis of randomised controlled studies. Trans R Soc Trop Med Hyg **107:**65-73.
6. **Tarning J, Zongo I, Some FA, Rouamba N, Parikh S, Rosenthal PJ, Hanpithakpong W, Jongrak N, Day NP, White NJ, Nosten F, Ouedraogo JB, Lindegardh N.** 2012. Population pharmacokinetics and pharmacodynamics of piperaquine in children with uncomplicated falciparum malaria. Clin Pharmacol Ther **91:**497-505.
7. **WHO.** 2013. Management of Severe Malaria A Practical Handbook, 3rd Edition ed, Geneva.
8. **WHO.** 2009. Methods for surveillance of antimalarial drug efficacy., Geneva.
9. **ICH-GCP.** 1996. ICH Harmonised Tripartite Guideline for Good Clinical Practice E6(R1) ICH Expert Working Group.
10. **WHO.** 2010. Basic Malaria Microscopy, 2nd Edition, Geneva.

11. **Ezzet F, Mull R, Karbwang J.** 1998. Population pharmacokinetics and therapeutic response of CGP 56697 (artemether + benflumetol) in malaria patients. Br J Clin Pharmacol **46:**553-561.

12. **Price RN, Hasugian AR, Ratcliff A, Siswantoro H, Purba HL, Kenangalem E, Lindegardh N, Penttinen P, Laihad F, Ebsworth EP, Anstey NM, Tjitra E.** 2007. Clinical and pharmacological determinants of the therapeutic response to dihydroartemisinin-piperaquine for drug-resistant malaria. Antimicrob Agents Chemother **51:**4090-4097.

13. **Tshefu AK, Gaye O, Kayentao K, Thompson R, Bhatt KM, Sesay SS, Bustos DG, Tjitra E, Bedu-Addo G, Borghini-Fuhrer I, Duparc S, Shin CS, Fleckenstein L.** 2010. Efficacy and safety of a fixed-dose oral combination of pyronaridine-artesunate compared with artemether-lumefantrine in children and adults with uncomplicated Plasmodium falciparum malaria: a randomised non-inferiority trial. Lancet **375:**1457-1467.

14. **WWARN.** 2013. The effect of dosing regimens on the antimalarial efficacy of dihydroartemisinin-piperaquine: a pooled analysis of individual patient data. PLoS Med **10:**e1001564; discussion e1001564.

15. **Checchi F, Piola P, Fogg C, Bajunirwe F, Biraro S, Grandesso F, Ruzagira E, Babigumira J, Kigozi I, Kiguli J, Kyomuhendo J, Ferradini L, Taylor WR, Guthmann JP.** 2006. Supervised versus unsupervised antimalarial treatment with six-dose artemether-lumefantrine: pharmacokinetic and dosage-related findings from a clinical trial in Uganda. Malar J **5:**59.

16. **Greenhouse B, Dokomajilar C, Hubbard A, Rosenthal PJ, Dorsey G.** 2007. Impact of transmission intensity on the accuracy of genotyping to distinguish recrudescence from new infection in antimalarial clinical trials. Antimicrob Agents Chemother **51:**3096-3103.

17. **Staehli Hodel EM, Guidi M, Zanolari B, Mercier T, Duong S, Kabanywanyi AM, Ariey F, Buclin T, Beck HP, Decosterd LA, Olliaro P, Genton B, Csajka C.** 2013. Population pharmacokinetics of mefloquine, piperaquine and artemether-lumefantrine in Cambodian and Tanzanian malaria patients. Malar J **12:**235.

18. **Nankabirwa JI, Wandera B, Amuge P, Kiwanuka N, Dorsey G, Rosenthal PJ, Brooker SJ, Staedke SG, Kamya MR.** 2014. Impact of intermittent preventive treatment with dihydroartemisinin-piperaquine on malaria in Ugandan schoolchildren: a randomized, placebo-controlled trial. Clin Infect Dis **58:**1404-1412.

19. **Lwin KM, Phyo AP, Tarning J, Hanpithakpong W, Ashley EA, Lee SJ, Cheah P, Singhasivanon P, White NJ, Lindegardh N, Nosten F.** 2012. Randomized, double-blind, placebo-controlled trial of monthly versus bimonthly dihydroartemisinin-piperaquine chemoprevention in adults at high risk of malaria. Antimicrob Agents Chemother **56:**1571-1577.

20. **Price RN.** 2013. Potential of artemisinin-based combination therapies to block malaria transmission. J Infect Dis **207:**1627-1629.

21. **Ashley E.** Accepted. **The spread of artemisinin resistance in falciparum malaria** New England Medical Journal.

22. **Suputtamongkol Y, Newton PN, Angus B, Teja-Isavadharm P, Keeratithakul D, Rasameesoraj M, Pukrittayakamee S, White NJ.** 2001. A comparison of oral artesunate and artemether antimalarial bioactivities in acute falciparum malaria. Br J Clin Pharmacol **52:**655-661.

Figure 1 CONSORT Flow chart

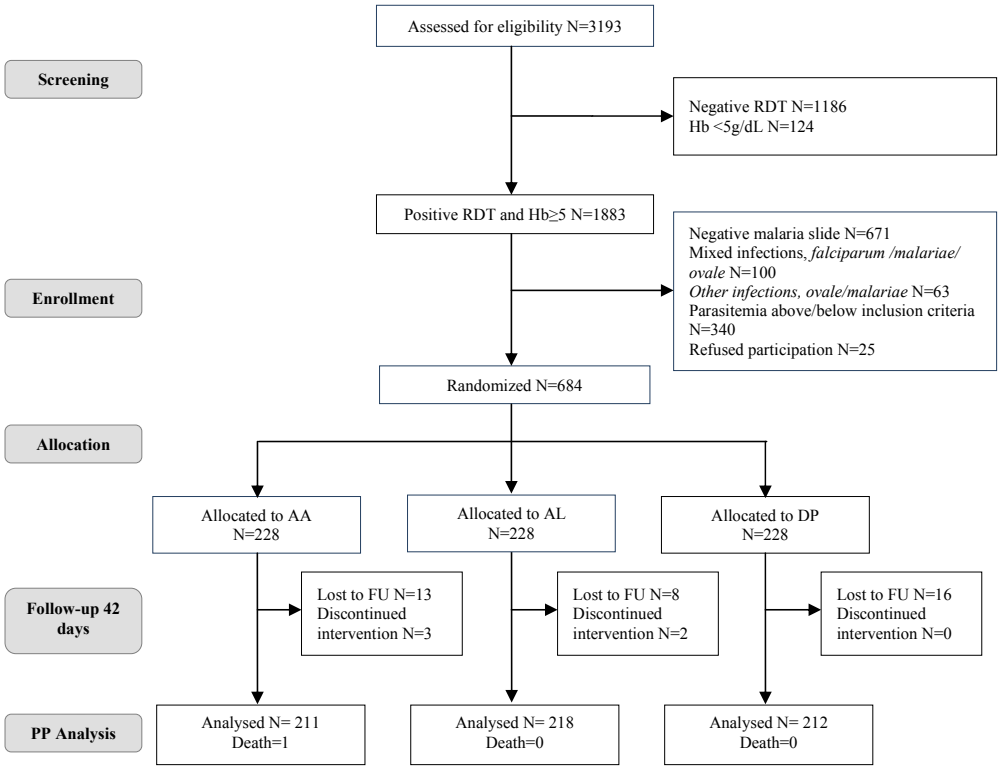


Table 1. Baseline characteristics of children at enrollment by treatment group

	AA	AL	DP
Number of patients at admission	228	228	228
Female: Male ratio	115:113	105:123	105:123
Mean age in months (range)	35.3 (3 - 59)	33.5 (5 - 59)	33.7 (5 - 59)
Mean weight in kg (95%CI)	12.5 (12.1 - 12.8)	12.5 (12.1 - 12.9)	12.3 (11.9 - 12.7)
Axillary temperature °C (median, range)	37.5 (36.0 - 40.5)	37.2 (36.0 - 40.8)	37.1 (36.0 - 40.2)
Splenomegaly %	72/226 (31.9)	84/228(36.8)	88/228(38.6)
Hepatomegaly %	3/228 (1.32)	1/228 (0.44)	0/228
P. falciparum/µL Median (range)	30,066 (2,093-199,840)	30,119 (2,040-199,720)	35,207 (2,126-199,960)
P. falciparum/µL Geometric mean (95% CI)	25,179 (21,188-29,921)	25,681 (21,828-30,216)	30,403 (25,657-36,026)
Patients with > 150,000/µL (%)	21/228 (9.2)	14/228 (6.1)	29/228 (12.7)
Mean hemoglobin g/dL (95%CI)	9.7 (9.4 - 9.9)	9.7 (9.5 - 10.0)	9.6 (9.4 - 9.8)

Table 2. Per Protocol Analysis: Efficacy by treatment at day 42 (%)

Outcome	AA	AL	DP	p-value
Total allocated to treatment	228	228	228	
Withdrawn or lost to follow-up by day 42	16	10	16	
Death	1	0	0	
Evaluable	211	218	212	
Results PCR uncorrected				
Early Treatment Failure	1 (0.47)	1 (0.46)	1 (0.47)	
Late Clinical Failure	17 (8.1)	12 (5.5)	10 (4.7)	
Late Parasitological Failure	39 (18.5)	52 (23.9)	18 (8.5)	
Adequate Clinical and Parasitological Response	154 (73.0)	153 (70.2)	183 (86.3)	0.001
PCR results on recurrent episodes				
New infections *P. falciparum*	41	39	16	
New infections *P. malariae* /*P. ovale*	2	10	1	
Recrudescences *P. falciparum*	10	11	9	
Undetermined PCR result*	3	4	2	
Results PCR corrected				
Adequate Clinical and Parasitological Response	197 (93.4)	202 (92.7)	200 (94.3)	0.78

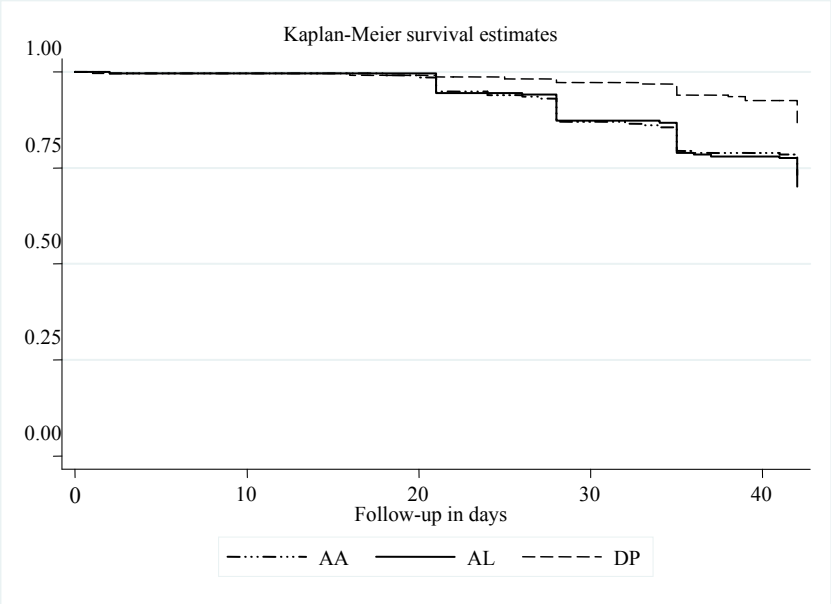
(* the samples were collected, but the PCR results were undetermined: cases were excluded from the PCR-corrected analysis)

Table 3. Per Protocol Analysis: Efficacy by treatment at day 28(%)

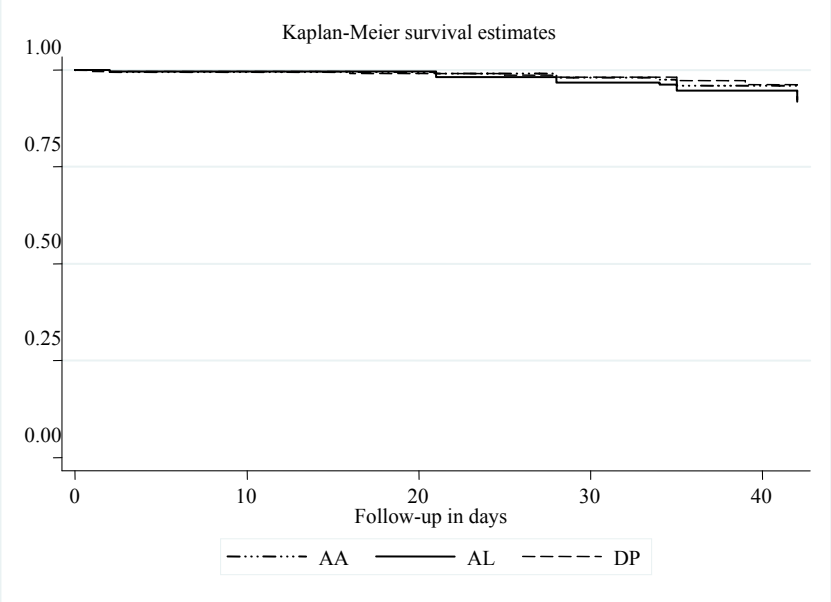
Outcome	AA	AL	DP	p-value
Follow-up not completed by day 28	12	5	10	
Results PCR uncorrected				
Adequate Clinical and Parasitological Response	183 (86.7)	190 (87.1)	206 (97.2)	0.001
Results PCR corrected				
Adequate Clinical and Parasitological Response	207 (98.1)	211 (96.8)	208(98.1)	0.578

Figure 2. Intention to Treat Analysis: Kaplan Meier plots of failure rates a) without and b) with PCR correction

a.



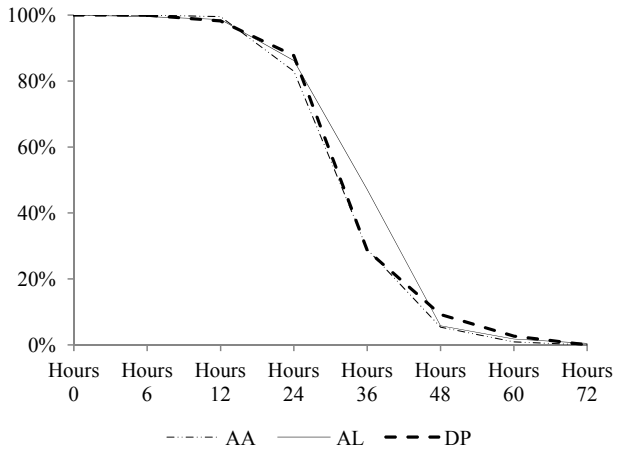
b.



503 Table 4. Fever clearance: percentage still febrile on day 0 to day 3 by treatment group

Time Days	AA	AL	DP	
	n/N (%)	n/N (%)	n/N (%)	p-value
0	63/228 (27.6)	65/228 (28.5)	55/228 (24.1)	0.53
1	3/226 (1.3)	11/226 (4.9)	8/228 (3.5)	0.1
2	1/226 (0.4)	9/226 (4.0)	1/228 (0.4)	0.003
3	2/226 (0.9)	3/226 (1.3)	5/227 (2.2)	0.50

504
505
506 Figure 3. Parasite positivity rate by day and treatment group
507



508
509
510

Table 5. Time (hours) to clear 50% of parasitemia by treatment

	AA	AL	DP	total
N. Observations used for the estimation	204	214	212	630
Median	5.71	8.44	6.54	7.31
Range	0.09-24.26	0.18-23.85	0.08-34.40	0.08-34.40
IQR	5.39	5.64	5.12	5.63

Table 6. Slope half-life (based on the slope of the log-linear portion of the parasite clearance curve)

	AA	AL	DP	total
N. Observations used for the estimation	214	223	220	657
Median	2.15	2.23	2.13	2.18
Range	1.05 -4.35	1.05-6.32	0.97-4.85	0.97-6.32
IQR	0.87	0.73	0.76	0.82

Figure 4. Gametocyte Positivity Rate by treatment days 0-42 new

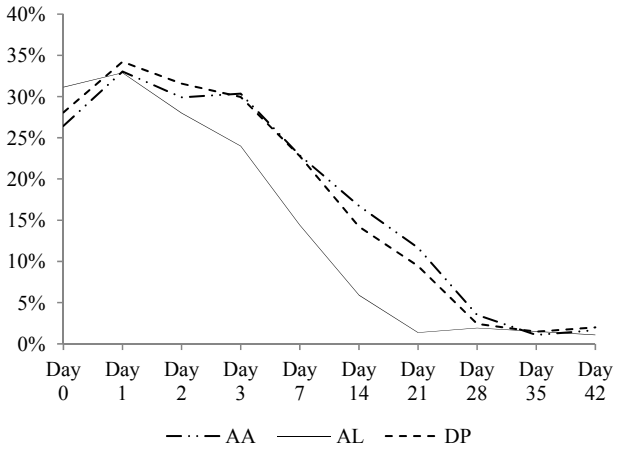


Table 7. Median and interquartile range WBC µl -1 and differential count at days 0, 7 and 14 by treatment group

	AA			AL			DP		
Day	0	7	14	0	7	14	0	7	14
N	227	216	212	228	221	217	228	219	215
WBC	6800	8200	7000	6650	7600	6900	6875	7600	7700
IQR	4000	4475	3075	4050	4000	2900	5050	3750	3700
Neutrophils	3096	2677	2014	2829	2496	2118	3201	2520	2352
IQR	2311	1846	1288	2414	1706	1306	3254	1745	1476
Lymphocytes	2919	4651	4489	2840.8	4623	4300	3075	4550	4590
IQR	2170	2884	2234	2388	2817	2240	2730	2394	2792

Table 8. Mean fractional reduction (SD) in PCV and number of patients whose reduction in PCV was >20% or 25% compared to the value at admission by treatment group

		AA	AL	DP	Total	p-value
	Mean %(SD)	10.0 (8.2)	9.0 (8.4)	9.3 (7.9)	9.4 (8.1)	0.41
Day 0-3	>20%	12.4 (28/226)	11.8 (27/228)	9.7 (22/228)	11.3 (77/682)	0.62
	>25%	6.2 (14/226)	4.4 (10/228)	5.7 (13/228)	5.4 (37/682)	0.68
Day 0-7	Mean %(SD)	10.3 (8.2)	9.4 (8.4)	10.0 (8.2)	9.9 (8.3)	0.51
	>20%	12.8 (29/226)	11.8 (27/228)	12.7 (29/199)	12.5 (85/682)	0.94
	>25%	6.6 (15/226)	4.4 (10/228)	6.1 (14/228)	5.7 (39/682)	0.55
Day 0-14	Mean %(SD)	10.4 (8.2)	9.7 (8.7)	10.2 (8.2)	10.1 (8.3)	0.65
	>20%	12.8 (29/226)	12.7 (29/228)	13.2 (30/228)	12.9 (88/682)	0.99
	>25%	6.6 (15/226)	4.8 (11/228)	6.6 (15/228)	6.0 (41/682)	0.65

Table 9. Lumefantrine (LM) plasma level at day 7

Body weight (kg)	N. analyzed	Median (range) dose received mg/kg	Median day-7 LM ng/mL (range)	Samples ≤ 280 ng/mL
5-9.9	22	27.9 (24.5-45.3)	294.5 (63.4-1050)	45.5 %
10–14.9	65	20.0 (17.1-24.0)	364.0 (57.1-1080)	43.1 %
15–20.9	34	30.0 (24.0-32.0)	473 (108-1150)	20.6 %
Total	121	24.0 (17.1-45.3)	421.9 (57.1-1150)	37.2 %

Table 10. Piperaquine (PQ) plasma levels at day 7

Body weight (kg)	N. analyzed	Median (range) dose received mg/kg	Median day-7 PQ ng/mL (range)	Samples ≤ 30 ng/mL
5-7.9	8	20.8 (20.3-33.3)	23.6 (10.9-65.2)	75 %
8-9.9	17	28.2 (17.2-30.0)	31.8 (16-70.8)	41.2%
10-14.9	65	26.7(22.1-32.0)	28.1 (12.6-135)	52.3 %
15-20.9	35	30.0(26.7.0-32.0)	44.3 (15.8-189)	34.3 %
Total	125	28.2 (17.2-33.3)	31.4 (10.9-189)	47.2%