

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

4

5 *Running title: Antimalarials efficacy and tolerability in DRC*

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17

18 **Abstract**

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

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

36 **Introduction**

37

38 **Background**

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

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

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

63 **Methods**

64

65 **Study area**

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

69

70 **Patient population**

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

80

81 **Trial design**

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

85

86 **Treatment**

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

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

91 mg, plus amodiaquine 67.5 mg, 135 mg or 270 mg. Tablets were administered according to the
92 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
93 tablets 100/270.

94 DP (DARTEPP, Guilin Pharmaceutical China; 40 mg dihydroartemisinin and 320 mg piperazine
95 each tablet) was administered once a day for 3 days according to body weight with the following
96 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-
97 29.9 kg, 2.0 tablets. This dosage scheme was different from the one recommended by the
98 manufacturer (5 - 10 kg, ½ tablet; 11-20 kg, 1 tablet; and 21-35 kg, 2.0 tablets). We used 5 intervals
99 of weight instead of 3 to improve the therapeutic dose of dihydroartemisinin at the upper limit of the
100 range for each interval .The mean dosage was 3.3 mg/kg/day of dihydroartemisinin and 26.6
101 mg/kg/day of piperazine.

102 AL (Coartem™, Novartis, Switzerland) was administered in 6 doses over 3 days (0, 8, 24, 36, 48,
103 and 60 hours). Each tablet contained 20 mg artemether and 120 mg lumefantrine and the mean dose
104 was 2.0 mg/kg of artemether and 12.7 mg/kg of lumefantrine for each dose. Tablets were
105 administered according to the manufacturer's instructions: 5-14.9 kg, 1 tablet; 15-24.9 kg, 2 tablets;
106 25-34.9 kg, 3 tablets; >35 kg,4 tablets.

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

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

118

119 **Sample size**

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

124

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

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

131

132 **Outcome measurements**

133 The primary outcome measure was the PCR corrected cure rate by day 42. Secondary outcome
134 measures were parasite and fever clearance and occurrence of adverse events (AE). Treatment
135 outcome was established according to standard WHO classification (8). Early treatment failure (ETF)
136 was defined as: (1) danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitemia; (2)
137 parasitemia on day 2 higher than on day 0, irrespective of axillary temperature; (3) parasitemia on day
138 3 with axillary temperature ≥ 37.5 °C; and (4) parasitemia on day 3 $\geq 25\%$ of count on day 0. Late
139 clinical failure (LCF) was defined as: (1) danger signs or severe malaria in the presence of parasitemia
140 on any day between day 4 and day 42 in patients who did not previously meet any of the criteria of
141 early treatment failure; (2) axillary temperature ≥ 37.5 °C in the presence of parasitemia on any day
142 between day 4 and day 42 in patients who did not previously meet any of the criteria of early
143 treatment failure. Late parasitological failure (LPF) was defined as presence of parasitemia between
144 day 7 and day 42 with a temperature < 37.5 °C in patients who did not previously meet any of the
145 criteria of early treatment failure or late clinical failure. Adequate clinical and parasitological response
146 (ACPR) was defined as absence of parasitemia on day 42, irrespective of axillary temperature, in

147 patients who did not previously meet any of the criteria of early treatment failure, late clinical failure
148 or late parasitological failure.

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

151

152 **Laboratory Methods**

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

158 Blood films were prepared at baseline, 6 and 12 hours and then repeated every 12 hours until 2
159 consecutive negative blood films were observed. Parasite clearance was assessed i) as the time for the
160 parasite count to reduce to 50% of its initial value (PC50) and ii) as parasite clearance rate derived
161 from the log-linear section of the log parasitemia–time curve and expressed as the parasite clearance
162 half-life ($PCT_{1/2}$; $\log_e 2$ / parasite clearance rate).

163 To compare the $PCT_{1/2}$ measured in this study with the more recent data collected in 2013 during the
164 Tracking Resistance to Artemisinin Collaboration (TRAC) project, all slides were read a second time
165 after the study was terminated by the same microscopists team using a different counting technique: if
166 more than 20 parasites were seen on the thick smear after 10 fields, parasitemia per 1,000 RBC was
167 counted on the thin smear. Below that threshold, parasites were counted on the thick smear per 500
168 WBC.

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

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

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

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

180

181 **Drug analysis**

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

185

186 **Molecular analysis**

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

194

195 **Ethical approval**

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

201 The study was monitored regularly by a qualified internal monitor (MORU Clinical Trials Support
202 Group) for adherence to GCP regulations. Serious adverse events (SAEs) were notified to all
203 Investigators and the REC of KSPH.
204 The study was registered with International Standard Randomized Controlled Trial Number Register
205 (www.isrctn.org) ISRCTN20984426.

206

207 **Statistical analysis**

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

214 For the intention-to-treat analysis, the logrank test was used to test the equality of the survivor
215 function across groups and Cox regression to estimate hazard ratio of infections post-treatment.

216 The overall fractional reduction in hematocrit was defined as the difference between the patient's
217 lowest level of hematocrit and that at baseline (i.e., pre-treatment) divided by the hematocrit at
218 baseline. The percentage of patients whose hematocrit fell >20% or 25% was compared between
219 groups. No interim analyses for efficacy or futility were done.

220

221 **Results**

222

223 **Intention to treat analysis, deviations from study protocol**

224 Between September 2011 and November 2012, 684 patients were included in the study, 228 in each
225 treatment group. Forty-two patients (6.1%) discontinued the study: 5 children were withdrawn during
226 the hospitalization because the families changed their mind and 37 were lost to follow-up between day
227 7 and 42 (DP=16; AL=10; AA=16). One patient, in the AA group, died at day 29 from causes
228 unrelated to malaria or the study drug. These cases were not included in the per protocol analysis, and

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

232

233 **Baseline Characteristics and treatment**

234 At enrollment, patients had similar demographic, clinical and parasitological characteristics (Table 1).
235 The tablets of DP contained an average of 35.6 mg dihydroartemisinin (89%) and 306 mg piperazine
236 (95.5%). This was compared to Eurartesim (Sigma Tau) product, which contained an average of 40.7
237 mg dihydroartemisinin (102%) and 300 mg piperazine (94%).

238

239 **Drug efficacy**

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

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

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

257 **Fever Clearance**

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

262
263 **Parasitemia Clearance**

264 All treatments were associated with a rapid clearance of parasitemia. The parasite positivity rate
265 (proportion of children with a positive slide at day 2) was significantly higher in the AL arm
266 ($p < 0.001$; Figure 3). Accordingly, the median PC50 was significantly longer for AL (8.4 hours, range
267 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,
268 range 0.1 to 34.4 hours, $N=212$), $p < 0.001$ (Table 5). The median $\text{PCT}_{1/2}$ was 2.2 hours (range 1.0 to 6.3
269 hours, $N=657$) with no significant differences between arms indicating a similar efficacy of the three
270 different artemisinin derivatives, $p=0.08$ (Table 6).

271
272 **Gametocyaemia**

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

280
281 **Hematology**

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

285 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
286 those not hyperparasitemic ($p<0.001$).

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

289 Ten patients developed decompensated anemia within 4 days of recruitment and required a blood
290 transfusion; 3 in the AA group, 4 in the AL group and 3 in the DP group ($p=0.62$). The risk of
291 receiving a blood transfusion was 6.5 times higher in the hyperparasitemic children (CI 95% 2.90 -
292 22.3; $p=0.005$). By day 14 the levels were comparable to admission in all patients.

293 The median WBC count was similar between the treatment groups on recruitment and at each day of
294 follow-up with an increase from day 0 to day 7 to normal values (Table 7). Neutrophil counts
295 decreased gradually from baseline values until day 14 with no differences between groups on any day.
296 Mild neutropenia (neutrophils $<1000/\mu\text{l}$) was observed in 2.5% of patients at enrollment and between
297 day 1 and 7 the neutrophils count fell below $1000/\mu\text{l}$ in 18% of patients (123/684) with no differences
298 between groups ($n=40$ in AA, 40 in AL, and 43 in DP; $p=0.92$). Fourteen of these patients developed
299 severe neutropenia (neutrophils $<500/\mu\text{l}$) (2, 4, and 8 in the AA, AL and DP groups, respectively;
300 $p=0.13$).

301

302 **Hepatotoxicity**

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

307

308 **Tolerability**

309 During the first day, children treated with DP vomited within 1 hour of the first dose significantly
310 more often ($N=21$; 9.2%) than children treated with AA ($N=10$; 4.4%) or AL ($N=5$; 2.2%), $p=0.03$.

311 The second dose of AL - administered after 8 hours - was vomited in seven cases, and taking this into
312 account the overall difference between treatments during the first day was not significant ($p=0.06$).

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

316

317 **Adverse Events**

318 At least one adverse event was reported in 37.4% of patients during the post-treatment period that was
319 not present on admission or increased in intensity and was classified as possibly or probably related to
320 the study drug. Most AEs were graded as of minor or moderate intensity. The most frequent AEs were
321 weakness, anorexia and gastrointestinal disorders (nausea, vomit, abdominal pain and diarrhea).

322 However these symptoms overlap with known malaria symptomatology. Anorexia and weakness were
323 reported more frequently in children treated with AA than those treated with DP and AL during the
324 second and third day of treatment (day 1: 15.2% (34/224) for AA, 5.3% (12/227) for AL, and 5.8%
325 (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)
326 for DP; $p=0.013$). There were otherwise no other differences in the number of AEs between treatment
327 groups. In 17 cases the adverse event was graded as severe or life-threatening. All cases were
328 classified as unlikely to be related to the study treatment. Ten patients developed decompensated
329 anemia during the hospitalization and required a blood transfusion (described above). There was one
330 case of severe skin eruption 18 days post-treatment (DP), one case of chicken-pox 12 days post-
331 treatment (AL), 1 case of abscess 11 days post-treatment (DP), 1 case of leukocytosis 2 weeks post
332 treatment (AL), and 3 cases of asthenia (2 AL and 1 AA). One child in the AA group died in a
333 different hospital at day 29. The cause of death was unknown but was considered unlikely to have
334 been caused by either malaria or the drug treatment.

335

336 **Plasma lumefantrine levels at day 7**

337 121 samples of venous blood were collected at day 7 from patients who received AL (Table 9). The
338 median concentration of lumefantrine in the blood was 377 ng/mL (range 57.1-1150 ng/mL). Drug
339 levels were positively correlated with body weight ($r=0.22$; test for trend $p=0.005$). The plasma level
340 was significantly lower in children weighing < 15 kg (median 309 ng/mL; range 57.1-1080, $N=87$)

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

347

348 **Plasma piperazine levels at day 7**

349 125 samples were collected from venous blood at day 7 from patients who received DP (Table 10).
350 The median concentration of piperazine was 31.4 ng/mL (range 10.9-189.0), and drug levels were
351 positively correlated with body weight ($r= 0.22$; test for trend $p=0.04$). In 47.2% (59/125) of patients
352 the plasma level was below 30 ng/mL, the previously published threshold associated with therapeutic
353 efficacy (12), and the 3 patients with a PCR-confirmed recrudescence had a piperazine level of 16.3,
354 31.4 and 33.1 ng/mL, respectively.

355 **Discussion**

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

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

362 In the subsample analyzed the drug level of piperaquine at day 7, reflecting the concentration of drug
363 to which residual parasites are exposed and thus predictive of outcome, was suboptimal in 47% of
364 patients. This confirms previous results (6, 14) showing that as small children have a higher body-
365 weight normalized oral clearance they need a higher dose than the one currently recommended. Of the
366 children who received artemether-lumefantrine 37% had a sub-optimal day 7 lumefantrine level, with
367 the smaller children having the lowest levels. These results are comparable to those observed in
368 Uganda (15). As there was no evidence of delayed parasitemia clearance suggestive of artemisinin
369 resistance, the PCR-confirmed treatment failures observed in the current study are likely caused by
370 either low drug exposure, as suggested by the PK results, or to parasite resistance to the non-
371 artemisinin partner drug. The former is much more likely. Moreover in high transmission areas, the
372 chances that the recurrent infection contains a parasite with the same genotype as the primary
373 infection are higher than in low transmission areas. This, along with the persistence of gametocytes in
374 the blood can lead in some cases to a misclassification of recurrent infections as recrudescences (16).
375 In this clinical trial we measured efficacy of treatments administered under supervision, with a glass
376 of milk, and retreatment was given if the first dose was vomited. Drug exposure, in a non-trial setting
377 (generally unsupervised) is expected to be lower (15). Dose optimization and schedule changes are a
378 priority for the ACT treatment especially in small children to ensure adequate drug exposure (14, 17).
379 Although the efficacy in terms of ACPR rates of the three ACTs was comparable, children treated
380 with DP were at lower risk of having a second episode of malaria during the follow-up period because
381 of the longer post-treatment prophylactic effect of DP related to the longer plasma half-life of
382 piperaquine. This chemoprophylactic effect is important in endemic areas - such as the study area –

383 and makes this drug a good candidate for replacing sulphadoxine pyrimethamine for intermittent
384 preventive treatment in pregnant women and children (18; 19).
385 This study population was characterized by hyperparasitemia and the initial high levels of parasitemia
386 affected, as expected, the recovery of hematocrit after the initial episode of malaria, but not the
387 treatment efficacy. The initial level of gametocytemia was also high (30%) and AL was significantly
388 more effective in clearing the sexual stages compared to the other ACTs. Data in literature on the
389 gametocytocidal properties of the different ACT are conflicting and the effect, if any, on malaria
390 transmission is unclear (20).
391 The median PCt_{50} was 2.2 hours (range 1.0 to 6.3 hours, N=657) and comparable to the results
392 observed in 2013 during the TRAC project: 2.2 hours (range from 1.2 to 4.6 hours, N=60) (21). The
393 difference we observed between PC50 with the three therapies (but not with the PCt_{50}) could be
394 attributed to the relatively slow conversion of artemether to dihydroartemisinin (artemether half-life
395 of 2.0 hours) compared to artesunate (artesunate half-life of 0.84 hours) and dihydroartemisinin in the
396 acute phase of malaria (22) resulting in a significantly longer lag-phase (the initial flat part of the
397 parasite clearance profile) for AL and/or the lower dosage of artemether (20). The PC50 includes the
398 lag phase of the parasite clearance curve, whereas the PCt_{50} is based on the log-linear phase alone.
399 The three combinations were well tolerated and there were no significant differences in the type and
400 number of adverse events between arms.

401

402 **Conclusions**

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

408

409 **Conflicts of interest**

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

411

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414

415 **Author contributions**

416 Study conduct and data acquisition: MO, CF. Molecular analysis: WK. Pharmacological analysis: JT.

417 Statistical analysis: CF. Preparation of the manuscript: CF, AT, AD, FN, NW, ND, JT. All co-authors

418 read the final version of the manuscript.

419

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494

Figure 1 CONSORT Flow chart

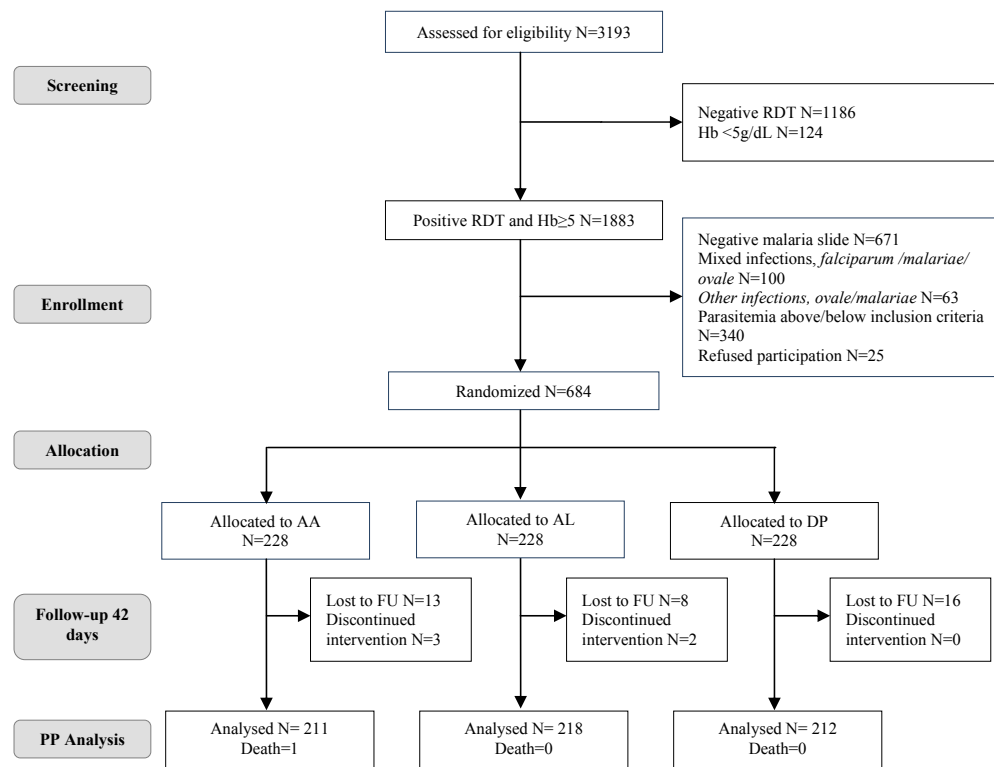


Table 1. Baseline characteristics of children at enrollment by treatment group

	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
<i>P. falciparum</i> /μL Median (range)	30,066 (2,093-199,840)	30,119 (2,040-199,720)	35,207 (2,126-199,960)
<i>P. falciparum</i> /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 (%)

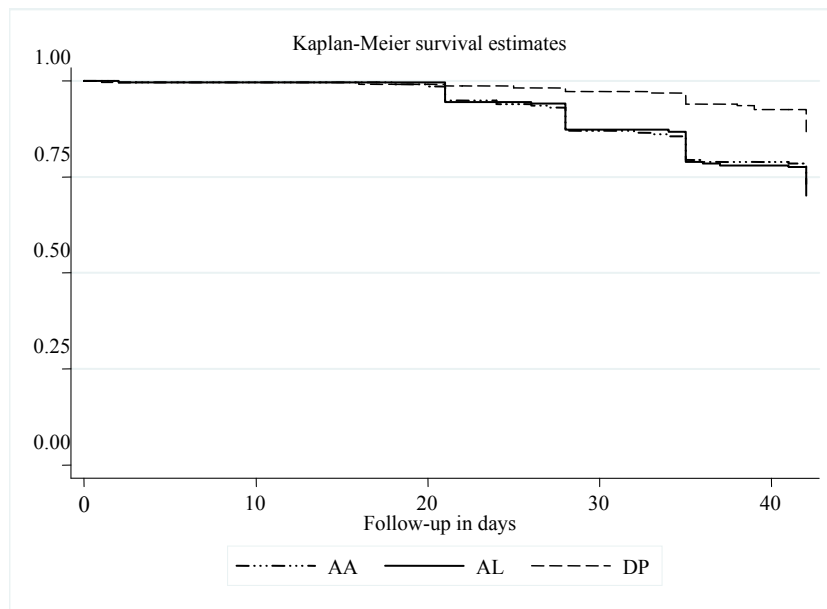
Outcome	AA	AL	DP	p-value
Total allocated to treatment	228	228	228	
Withdrawn or lost to follow-up by day 42	16	10	16	
Death	1	0	0	
Evaluable	211	218	212	
Results PCR uncorrected				
Early Treatment Failure	1 (0.47)	1 (0.46)	1 (0.47)	
Late Clinical Failure	17 (8.1)	12 (5.5)	10 (4.7)	
Late Parasitological 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 <i>P. falciparum</i>	41	39	16	
New infections <i>P. malariae</i> / <i>P. ovale</i>	2	10	1	
Recrudescences <i>P. falciparum</i>	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)

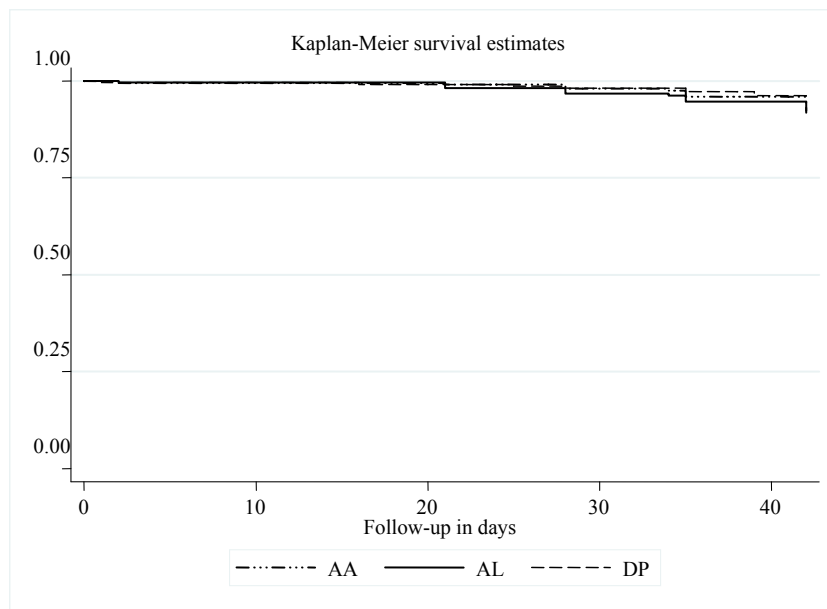
Table 3. Per Protocol Analysis: Efficacy by treatment at day 28(%)

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

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



498
499 b.



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

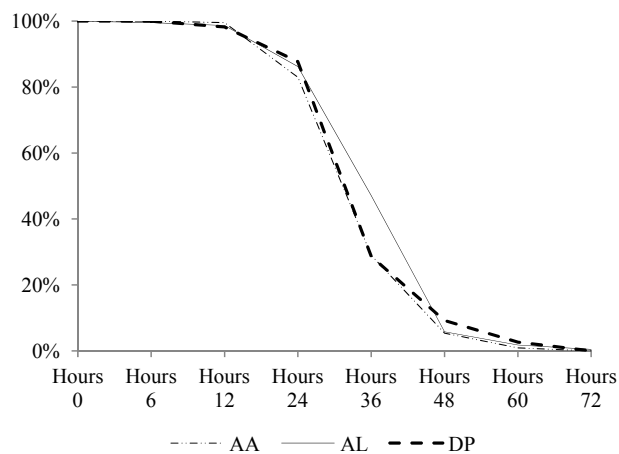
Time Days	AA	AL	DP	p-value
	n/N (%)	n/N (%)	n/N (%)	
0	63/228 (27.6)	65/228 (28.5)	55/228 (24.1)	0.53
1	3/226 (1.3)	11/226 (4.9)	8/228 (3.5)	0.1
2	1/226 (0.4)	9/226 (4.0)	1/228 (0.4)	0.003
3	2/226 (0.9)	3/226 (1.3)	5/227 (2.2)	0.50

504

505

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

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508

509

510

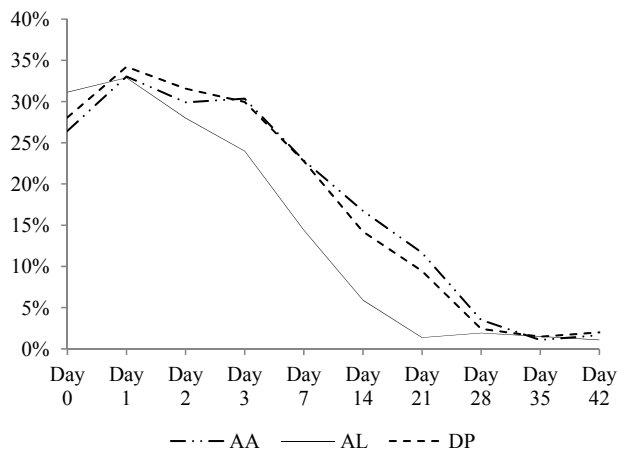
511 Table 5. Time (hours) to clear 50% of parasitemia by treatment
 512

	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

513
 514 Table 6. Slope half-life (based on the slope of the log-linear portion of the parasite clearance curve)
 515

	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

516
 517
 518 Figure 4. Gametocyte Positivity Rate by treatment days 0-42 new
 519



520
 521
 522
 523

524 Table 7. Median and interquartile range WBC μl^{-1} and differential count at days 0, 7 and 14 by
 525 treatment group
 526

Day	AA			AL			DP		
	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

527 Table 8. Mean fractional reduction (SD) in PCV and number of patients whose reduction in PCV was
 528 >20% or 25% compared to the value at admission by treatment group
 529
 530

Day		AA	AL	DP	Total	p-value
		Mean %(SD)	Mean %(SD)	Mean %(SD)	Mean %(SD)	
Day 0-3	Mean %(SD)	10.0 (8.2)	9.0 (8.4)	9.3 (7.9)	9.4 (8.1)	0.41
	>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

531

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

533

Body weight (kg)	N. analyzed	Median (range) dose received mg/kg	Median day-7 LM ng/mL (range)	Samples \leq 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 %

534

535

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

537

Body weight (kg)	N. analyzed	Median (range) dose received mg/kg	Median day-7 PQ ng/mL (range)	Samples \leq 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%

538