Population pharmacokinetics and clinical response of artemether-lumefantrine in pregnant and non-pregnant women with uncomplicated Plasmodium falciparum malaria in Tanzania

Dominic Mosha¹,²#, Monia Guidi³,⁴, Felista Mwingira², Salim Abdulla¹, Thomas Mercier⁴, Laurent Arthur Decosterd⁴, Chantal Csajka⁵,⁶, Blaise Genton²,⁵*

¹Ifakara Health Institute, Rufiji HDSS, Rufiji, Tanzania
²Swiss Tropical and Public Health Institute, University of Basel, Switzerland
³School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Lausanne, Switzerland
⁴Division of Clinical Pharmacology and Toxicology, Department of Laboratories, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland
⁵Department of Ambulatory Care and Community Medicine & Division of Infectious Diseases, University Hospital, Lausanne, Switzerland

Running title: PK of artemether-lumefantrine in pregnancy

#Address correspondence to Dr. Dominic Mosha, dfmosha@hotmail.com

Ifakara Health Institute, Rufiji HDSS, P.O Box 40, Rufiji, Tanzania.

* C.C and B.G contributed equally to this work.
Abstract

Background: Artemether-Lumefantrine (AL) is the first line treatment for uncomplicated malaria in second and third trimester of pregnancy. Its efficacy has recently been challenged in pregnancy due to altered pharmacokinetic (PK) properties in this vulnerable group. The aim of this study was to determine the PK profile of AL in pregnant and non-pregnant women and assess their therapeutic outcome.

Methods: Thirty-three pregnant women and 22 non-pregnant women with malaria were treated with AL (80/480mg) twice daily for 3 days. All patients provided five venous plasma samples for drug quantification at random times over 7 days. Inter- and intra-individual variability was assessed and covariates effects quantified using a nonlinear mixed-effect modeling approach (NONMEM®).

Results: A one-compartment model with first-order absorption and elimination with linear metabolism from drug to metabolite fitted the data best for both artemether (AM), lumefantrine (LF) and their metabolites. Pregnancy status and diarrhea showed a significant influence on LF PK. Lumefantrine relative bioavailability and metabolism rate into desmethyl-lumefantrine were respectively 34% lower and 78% higher in pregnant women than in non-pregnant patients. Overall PCR-uncorrected treatment failure was 18% in pregnant women and 5% in non-pregnant women (OR = 4.04; p value 0.22). A high median day 7 lumefantrine concentration was significantly associated with adequate clinical and parasitological response (p = 0.03).

Conclusion: The observed reduction in lumefantrine relative bioavailability in pregnant women may explain the higher treatment failure in this group, mostly due to lower post-treatment prophylaxis. Hence, a modified treatment regimen of malaria in pregnancy should be considered.
Background

Malaria in pregnancy is a major public health problem, which is associated with high maternal and perinatal mortality in tropical and subtropical regions (1). Pregnant women are at increased risk of clinical malaria compared to non-pregnant women because of the associated immunological and hormonal changes in pregnancy (2). Substantial direct risks to pregnant women include severe maternal anaemia, and those affecting the baby are intra-uterine growth retardation, intra-uterine death, stillbirth, premature delivery, low birth-weight, and perinatal and neonatal morbidity and mortality (3). Because of all this, malaria in pregnancy should be treated effectively.

Artemether-lumefantrine (AL) (20mg and 120mg, respectively) is one of the most popular and efficacious fixed dose artemisinin-based combination therapies (ACT) against *Plasmodium falciparum*. It is currently available at a subsidized cost in most malaria endemic countries. AL has proved to be non-inferior to quinine in East Africa for the treatment of *P. falciparum* infection in second and third trimester of pregnancy (4). ACTs are recommended by the World Health Organization (WHO) as the first line treatment for uncomplicated malaria in the second and third trimester of pregnancy (5). Unfortunately, general inter-individual variability on drug absorption, distribution to different compartments of the body and tissues, plasma binding proteins, rate of metabolism, enterohepatic recirculation, and excretion may be associated with changes in bioavailability of a drug and consequently may affect the therapeutic efficacy (6).

Pregnancy has been reported to affect the efficacy of some drugs, including antimalarials. This is due to physiological changes which lower drug absorption, speed up drug clearance and increase body fluid volume of distribution (7-9). Elevation of estrogens, progesterone, cortisol and prolactin hormones during pregnancy have been linked to altered metabolic activity of several
hepatic cytochrome P450 enzymes. For instance, catalytic activity of CYP3A4, CYP2C9 and CYP2A6 enzymes increases during pregnancy (10, 11), and these enzymes are responsible for lumefantrine and artemether metabolism (12, 13). Hence, it is expected that significant alteration of the pharmacokinetics (PK) of most antimalarial drugs during pregnancy occurs, which may be associated with lower drug concentrations and lower antimalarial cure rate, especially in advanced pregnancy (14-16). A higher treatment failure rate has indeed been observed in pregnant women when compared to non-pregnant ones living in the same area (16). Several PK studies on artemether (AM), lumefantrine (LF) and their respective metabolites, dihydroartemisinin (DHA) and debutyl-lumefantrine (DLF) have demonstrated low plasma concentration of these drugs in pregnant women compared to non-pregnant adults. However, most of these studies included healthy male adult volunteers as a comparative group rather than female malaria patients (16-19). Because of various determinants of PK and therapeutic outcome, it is essential to have a comparative population of non-pregnant women of the same study area with the same disease.

An important concern during the course of AL treatment is to achieve adequate residual LF level after complete elimination of AM and DHA so that it may clear all residual malaria parasite (9). Therefore, day 7 LF concentration level has been proposed as a good indicator of AL effectiveness (20, 21). Recent pharmacokinetics study of AL in Cambodia and Tanzania reported that the targeted day 7 LF concentration was also not achieved in a significant number of non-pregnant adult patients. In Tanzania, 35% of samples had LF concentration below the cut-off value of 175 ng/ml at day 7 (22). In pregnancy, whereby host antiparasite immunity is somehow compromised (2), a higher day 7 venous concentration of LF may be required than what has previously been proposed in studies involving non-pregnant adult patients i.e. a cut-off values of
175 ng/ml or 280 ng/ml in order to achieve effective therapeutic outcome and 600 ng/ml for maximal efficacy (23, 24). Some predictive models have suggested that a twice-daily regimen of AL for 5 days would be preferable in later pregnancy in order to achieve sufficient drug concentration in plasma (19). Increasing the duration of AL administration is indeed expected to increase the residual LF levels in the subsequent post-treatment cycle so as to reduce chances of recrudescence (22). This should be interpreted with caution because extending the duration of treatment regimen may possibly lead to lower adherence. Doubling the dose might be another option but actually it may not be appropriate because absorption of LF is dose-limited (25).

The aim of the present study was to characterize the PK profile of AL and their metabolites, to determine their variability and to identify factors that might explain variations in drugs and metabolites levels in pregnant (second and third trimester of pregnancy) and non-pregnant women of the same area, and to assess cure rate and parasitological clearance in these two groups. The model developed for lumefantrine was used to simulate day 7 concentrations under standard and alternative dosage regimens and quantify the percentages of pregnant and non-pregnant women having concentrations below different proposed cut-off thresholds.

**Material and methods**

**Study design and procedures**

This study was conducted in Rufiji district, within a Coastal region in Eastern Tanzania. The asexual parasitaemia prevalence is 14% and *Plasmodium falciparum* is the predominant species (26). The study was carried out at Kibiti health center from April to September 2012. Approval for the study was granted by two independent ethical review bodies; (i) Research Ethics Committee of Ifakara Health Institute (IHI) and (ii) National Institute for Medical Research (NIMR) Ethical Committee. All women signed an informed consent prior to enrolment.
Pregnant and non-pregnant women diagnosed with uncomplicated malaria were recruited from either out-patient department or Reproductive and Child Health (RCH) clinic. Inclusion criteria were women aged 18 years and above, resident of Rufiji study area, pregnant during their second and third trimester, and having signs or symptoms suggestive of uncomplicated malaria with fever (axillary temperature $\geq 37.5^\circ C$) or history of fever for the past 24 hours, *P falciparum* detected by microscopy, and hemoglobin level $\geq 7$ g/dl. Exclusion criteria were known allergy to AL or quinine, history of renal, liver or heart problem, hyperparasitaemia above 200,000/µL, reported intake of any antimalarial within the past 28 days, unable to take oral medication, and vomiting the medication within 1 hour of first dose intake. The same criteria applied to non-pregnant women (control group) that were recruited concurrently during the same study period after informed consent. A full medical history including concomitant illness and concomitant medication was recorded. Clinical examination on the day of enrollment was performed by an experienced physician. Patients were also seen by the clinician during follow up visits at day 1, 2, 3, 7, 14, 28 and 42 whereby axillary temperature was measured as well as evaluation of malaria related symptoms (5). Gestational age was determined from the estimated first day of the last normal menstrual period and compared to clinical examination of a fundal height. In case of any discrepancy, gestational age was recalculated from the estimated age at first RCH visit.

**Drug regimen**

Enrolled participants received four tablets of AL (Coartem® Novartis Pharma AG, Basel; 20 mg AM and 120 mg LF) over the course of 3 days at 0, 8, 24, 36, 48 and 60 hours. Each dose was administered with 200 ml of milk containing 4.5 g of fat because of the associated increase in LF bioavailability when taken with a fat rich meal (27). All patients were asked to come back to the health center for each drug administration and observed for one hour after dose intake. None of
the patients was admitted during the course of AL treatment but one pregnant woman who
developed severe malaria at day 1 was admitted and the treatment was changed to intravenous
quinine. A limited number of patients were administered drug at home by the study’s clinician or
field assistant, specifically for those who had difficulties to come to the clinic at scheduled times
for observed drug administration.

Blood samples

To determine AM, DHA, LF and DLF concentration, 2 ml of venous blood sample was drawn
from the patient at random times between 8 and 11 am on day 0, 1, 2, 3 and 7. The schedule for
sample collections agrees with WHO recommendation for lumefantrine concentration
measurement, but is suboptimal for artemether due to practical difficulties for patients to attend
all requested time points (21). Day 0 blood sample was collected before starting the medication
as a baseline so as to determine the presence of any antimalarial in patient’s plasma prior to
treatment due to intake of non-declared drugs (28, 29). The blood samples in an EDTA
vacutainer® tube were centrifuged at 2,000 x g for 5 minutes and the plasma stored in cryotubes.
Samples were kept at -25°C for at most 6 weeks before transferred to Ifakara Health Institute
(IHI) Bagamoyo clinical laboratory for temporary storage at -80°C. It is known that the storage
of plasma samples for bioassay of artemether, lumefantrine and their metabolites at -20°C for 8
months does not affect drug concentration (21). All samples were packed in dry ice and then
shipped to clinical pharmacology laboratory of the University Hospital in Lausanne, Switzerland,
to perform the drug bioassay.

To estimate the parasite density and clearance rate, capillary blood from a finger prick was taken
at day 0, 3, 7, 14, 28 and 42. Samples were collected on blood slide Giemsa stained thick and
thin blood smear were examined by two different experienced microscopists using light
microscopy. Parasite in thick film fields were counted per 200 leukocytes and the parasite count was multiplied by a factor of 40 to give parasites per µl of blood. Approximately 50µl of finger pricked blood was spotted onto Whatman® filter paper cards (3MM). DNA was extracted from Whatman® filter paper cards by Chelex method (30). In order to differentiate between recrudescence and new infection, samples were genotyped by the most polymorphic marker the merozoite surface protein 2 (MSP 2) and the amplicons were visualized in a 2% agarose gel as described elsewhere (31).

**Drug assay**

Plasma concentrations of AM, DHA, LF and DLF were determined using a validated liquid chromatography-tandem mass spectrometry method (LC-MS/MS) (32). The presence of 10 other antimalarial drugs and metabolites i.e. artesunate, amodiaquine, N-desethyl-amodiaquine, piperquine, pyronaridine, mefloquine, chloroquine, pyrimethamine and sulfadoxine were also assessed at the same time. This is a standard procedure on how LC-MS/MS operates and it helps to ensure that the malaria outcome that was observed was due to AL intake, and not to any other residual antimalarial. The assay is precise (3.1% - 12.6% for inter-day variation coefficient) and sensitive (0.15 – 3.0 ng/dl for lower limit quantification [LOQ] of basic or neutral antimalarial and 0.75 – 5 ng/dl for artemisinin derivatives).

The bioassays were carried out at the Laboratory of clinical pharmacology of the Lausanne University Hospital, which takes part in the quality control system of the worldwide antimalarial resistance network (WWARN).
AL efficacy was determined by cure rate and parasitological clearance. The definition of treatment response was according to WHO recommendations on the methods for surveillance of antimalarial drug efficacy (33). Treatment response was thus classified into early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR). Participants who developed either clinical or parasitological failure as defined above received quinine 10 mg/kg of body weight three times a day for 7 days, according to standard treatment guidelines (34).

**Pharmacokinetic analysis**

Drugs and their metabolites were modeled using the NONMEM computer program version 7.2 (NM-TRAN version II) (35) with the PsN-Toolkit version 3.5.3 (36). The program uses mixed (fixed and random) effects regression to estimate population means and variances of the pharmacokinetic parameters and to identify factors that influence them.

**Structural model.** One and two-compartment models with first-order absorption and elimination and linear metabolism to DLF and DHA were compared to describe, respectively, LF and AM pharmacokinetics with an additional compartment used to characterize metabolite data. Sequential and simultaneous parent-metabolite modeling methods were used for LF/DLF and AM/DHA, respectively. The final estimated parameters were drug and metabolite systemic clearances (CL and CL_{met}), volume of distribution of the central compartment (V_C) and metabolism rate constant from the drug to the metabolite compartment (K_{23}). Owing to identifiability problems, the volume of distributions of DLF and DHA could not be estimated and were assumed to be equal to those of LF and AM, respectively. Because of the limited number of measurements in the absorption phase, the absorption rate constants (K_a) could not be adequately estimated and were thus fixed to 0.7 and 0.54 h^{-1} to achieve AM and LF peak plasma
concentrations, respectively, 2 h and 6-8 h after drug intake (37). Finally, the known pre-
 systemic conversion of AM into DHA was modeled estimating the fraction of the AM dose
directly converted into the metabolite using $1 - F_1$, with $F_1 = 1$ representing AM relative
bioavailability. Since the drugs were given orally, these parameters represent apparent values. In
case the analysis of baseline plasma samples showed non-zero concentration of the drugs
(suggesting that AL was previously taken), a factor ($F_0$) was introduced in the model in order to
estimate the residual doses from previous treatments.

**Statistical model.** Inter-patient variability of all the PK parameters was described by exponential
ersors following a log-normal distribution, as illustrated by the equation $\theta_j = \theta \exp(\eta_j)$, where $\theta_j$ is
the pharmacokinetic parameter associated with the $j^{th}$ individual, $\theta$ is the average population
value, and $\eta_j$ is the $j^{th}$ individual component of the inter-patient random effect, an independent,
normally distributed variable with mean 0 and variance $\omega^2$. In order to constrain individual $F_1$ to
vary between 0 and 1, a logit function (logit $F_1$) was used. Correlations between PK parameters
were also investigated. Finally, proportional, additive and combined proportional-additive error
models were compared to describe the intrapatient (residual) variability for both drug and
metabolite. The correlation between drug and metabolite concentration measurements was tested
using the NONMEM® L2 item.

**Covariate model.** Available covariates were: pregnancy status, body weight, body mass index
(BMI), age, gestational age and diarrhea. The covariate analysis was performed using a stepwise
insertion/deletion approach. Visual inspection of the correlation between post hoc individual
estimates of the PK parameters and the available patients’ characteristics was conducted at first.
During the forward selection, potential covariates influencing the kinetic parameters were
sequentially incorporated in the model and retained if statistical significance was achieved in
NONMEM® (p<0.05). Backward deletion was performed once the model including all the significant factors was built. It consisted of removing the covariates one at a time, starting from the most insignificant one, until no further non-significant deterioration of the model was observed (p<0.01). The typical value of the pharmacokinetic parameters $\theta$ was modeled to depend linearly on the covariate $X$ (continuous covariates centered on the population median; dichotomous variables coded as 0 and 1) using $\theta = \theta_a \cdot (1 + \theta_b \cdot X)$, where $\theta_a$ is the mean estimate and $\theta_b$ is the relative deviation of the mean due to the $X$ covariate. Body weight (BW) effect was alternatively modeled using the allometric function $\theta = \theta_a \cdot \left(\frac{BW}{MBW}\right)^{\theta_c}$, where MBW is the median population BW and $\theta_c$ was fixed to literature values, i.e. 0.75 for CL and 1 for V. Linear and allometric functions were then compared to identify the model describing at best the relationships between BW and the pharmacokinetic parameters.

Selection of the model and parameter estimation. Drugs and metabolites were fitted by use of the first-order conditional (FOCE) method with interaction using the subroutine ADVAN5. Concentrations below the quantification limit (BQL) of the assay were treated using the M3 method described by Beal as implemented in the paper of Ahn et al (38, 39). Nevertheless, when using the L2 function, BQL data were replaced by LOQ/2 and handled with the M6 approach (39). The log likelihood ratio test, based on differences in the OFV value (\Delta OFV) provided by NONMEM®, was employed to discriminate between hierarchical models. Since a \Delta OFV between any two models approximates a $\chi^2$ distribution, a change of the objective function was considered statistically significant if it exceeded 3.84 (p < 0.05) for 1 additional parameter in model-building and covariate forward-addition steps or 6.63 (p < 0.01) in covariate backward elimination. The Akaike information criterion (AIC) was used to compare non-nested models. Shrinkage was also examined. Additional criteria for model selection were diagnostic goodness-
of-fit plots, precision of pharmacokinetic parameters estimates, and the reduction of the parameters inter-patient variability.

**Validation of the model.** The stability of the final model was assessed by means of the bootstrap method implemented in PsN, generating two-thousand datasets by re-sampling from the original dataset. Mean parameters values with their 95% confidential interval (CI_{95%}) were derived and compared with the final pharmacokinetic model estimates. Model validation was performed by visual predictive checks (VPC), simulating data for 1000 individuals based on the final model and generating 2.5th, 50th and 97.5th percentiles. The observed concentrations were plotted against the 95% prediction interval (PI_{95%}) of the simulated dataset at each time point and visually compared. Figures were generated with GraphPad Prism® (Version 6.00 for Windows, GraphPad Software, San Diego California USA, http://www.graphpad.com/).

**Model-based stimulation for LF.** The concentration-time profiles of LF in 1000 individuals receiving two different regimens of 6 doses over 3 days (at 0, 8, 24, 36, 48 and 60 h) and 5 days (at 0, 8, 24, 48, 72 and 96 h.) were derived by simulations based on the final model including inter-patient variability. Day 7 predicted median concentrations with their PI_{95%} for pregnant and non-pregnant women were derived. In addition, these simulations allowed quantifying the percentages of pregnant and non-pregnant women having a day 7 concentrations below different proposed cut-off thresholds of 175 ng/ml, 280 ng/ml and 600 ng/ml associated with treatment efficacy (24, 40, 41).

**Other statistical analyses**

The relation of the outcome variable (treatment failure) and explanatory variables were tested using a t-test for continuous variables (predicted LF day 7 concentrations, gestation age, baseline
parasite count and BMI) and Pearson chi-square for categorical variable (pregnancy status, residual antimalarial and diarrhoea). A p-value below 0.05 was considered statistically significant. All the statistical analyses were performed using STATA® 12.0 (Stata Corporation, College Station, Texas, USA).

Results

Demographic and clinical parameters. Thirty-five pregnant women and 22 non-pregnant women with acute *Plasmodium falciparum* malaria were enrolled in the study from 23rd April to 5th September 2012. Two of the enrolled pregnant women were withdrawn from the study at day 2 and 7 because they refused to continue participating in the study. Two (9.1%) non-pregnant women were lost for follow-up at day 42. None of the pregnant women were lost for follow up. Baseline characteristics of pregnant and non-pregnant women are presented in *Table 1*. Two pregnant women presented with diarrhea at the day of enrollment and throughout the course of treatment. None of the study participants vomited the drug. All participants had normal physical condition on examination with no history of any chronic disease or smoking. Twenty-six women (14 pregnant and 12 non-pregnant) reported to have taken paracetamol before enrollment. The median gestational age among pregnant women was 27 (range: 14 – 37) weeks with relatively equal numbers of women in the second and third trimester of pregnancy. No participant during the study period had miscarriage or stillbirth, or any other severe adverse drug reaction.

Residual antimalarial. Blood samples from all 57 recruited participants in the study were screened to determine the presence of any antimalarial drugs prior to initiation of malaria treatment. Fifty-five (96.5%) had at least one antimalarial in their plasma: 89.5% (29 pregnant and 22 non-pregnant) of participants had plasma LF above the LOQ but the drug concentration was generally low with the average of 37.3 ng/ml. Other antimalarial drugs which were detected...
were DLF 8 (14.0%), AM 4 (7%), DHA 0 (0.0%), sulfadoxine 14 (24.6%), pyrimethamine 11 (19.3%) and quinine 1 (1.8%). Summarized statistics are shown in Table 2. Out of 14 participants detected with sulfadoxine, 13 were pregnant with a median baseline parasitaemia of 72086 (range 3920 – 198080) counts/µL [Figure 1]. Sulfadoxine concentration persisted at relatively constant concentration throughout the first 7 days of monitoring plasma drug levels.

Population pharmacokinetic analysis

A total of 265 LF, 263 DLF, 146 AM, and 98 DHA plasma concentrations were included in the analysis. Twenty-five percent (n=37) AM, 7% (n=7) DHA and 2% (n=4) DLF concentrations were below the respective LOQs. The median (range) of samples available per study subject was 5 (4 – 5) for LF, 4 (3 – 5) for DLF, 3 (1 – 5) for AM and 2 (1 – 4) for DHA.

Artemether

AM and DHA pharmacokinetics were best described using a one-compartment model with first-order absorption from the gastrointestinal tract and linear metabolism to DHA, including pre-systemic conversion into the metabolite. Elimination of both compounds was modeled using a first-order process. The few basal AM concentrations did not allow estimating a residual dose from previous treatments. Inclusion of an inter-patient variability on \( V_c \), \( CL_{Mt} \), \( K_{23} \) or \( F_1 \) in addition to AM CL did not improve description of the data (\( \Delta OFV \geq -1.9, p \geq 0.17 \)). A mixed error model best described residual intra-patient variability for AM and a proportional one for DHA. No correlations between the drug and the metabolite concentrations could be identified. Structural model shrinkages lower than 15% were found for all the inter- and intra-individual variability. Our results show that 21% of the AM dose is converted pre-systemically into DHA. None of the available covariates significantly affected AM or DHA pharmacokinetics (\( \Delta OFV \leq 3.0, p \geq 0.08 \)). Although non-significant, an increase of 37% in drug CL in pregnant women
compared to non-pregnant ones was however observed ($\Delta$OFV = -1, $p = 0.32$). The final model parameters’ estimates and bootstrap evaluations are given in Table 3. The model was considered reliable since the obtained parameter estimates lay within the bootstrap CI$_{95\%}$. VPC graphs of AM and DHA are shown in Figure 2A.

**Lumefantrine**

A one-compartment model with first-order absorption and elimination was retained to depict LF data. A two-compartment model did not improve the model fit ($\Delta$OFV = -0.1, $p = 0.75$). Average dose from previous treatment ($F_0$) was estimated to be 3.2 mg with a large inter-individual variability ($\Delta$OFV = -40, $p = 2.5\cdot10^{-10}$). In addition to CL, an inter-patient variability on $V_c$ ($\Delta$OFV = -23, $p = 1.6\cdot10^{-6}$) and a correlation between CL and $V_c$ improved significantly the fit ($\Delta$OFV = -117, $p = 2.9\cdot10^{-27}$). The assignment of an inter-patient variability on LF bioavailability $F_1$ (fixed to 1) accounting for the correlation between CL and $V_c$ and their variability resulted in additional improvement of the model fit ($\Delta$OFV = -9.5, $p = 8.7\cdot10^{-3}$). Metabolite concentrations were included in the model using a supplementary compartment with linear metabolism from the LF central compartment. The addition of an inter-individual variability on $K_{23}$ improved significantly the description of the data ($\Delta$OFV = -41, $p = 1.5\cdot10^{-10}$), while no enhancement was observed when assigning variability on $CL_M$ ($\Delta$OFV = -0.02, $p = 0.89$). Residual intra-patient variability was best described using a proportional and mixed error model for LF and DLF, respectively. The model was further improved by including a correlation between drug and metabolite concentrations ($\Delta$OFV = -85, $p = 3.0\cdot10^{-20}$). Structural model shrinkages for the inter- and intra-individual variability were all estimated to be lower than 15%.

In univariable analyses, pregnancy and diarrhea were identified as significant covariates for both $F_1$ ($\Delta$OFV = -5.1, $p = 0.024$ and $\Delta$OFV = -15, $p = 1.1\cdot10^{-6}$) and $K_{23}$ ($\Delta$OFV = -13, $p = 3.1\cdot10^{-4}$
and $\Delta{OFV} = -4, p = 0.045$). None of the remaining covariates influenced LF and DLF pharmacokinetics ($\Delta{OFV} \geq -1.4, p \geq 0.24$). Multivariable combination of the significant covariates showed an additive influence of pregnancy and diarrhea on $F_1$ and pregnancy on $K_{23}$ ($\Delta{OFV} = -33, p = 3.2 \cdot 10^{-7}$ with respect to the model without covariates). Our results show that relative bioavailability is 34% lower and metabolism rate 78% higher in pregnant women compared to non-pregnant patients. A decrease of 83% in $F_1$ was observed in women with diarrhea as compared to those who had no diarrhea. Table 3 illustrates the final model parameters’ estimates together with their bootstrap evaluations. The model was considered reliable since the obtained parameter estimates laid within the bootstrap CI 95%. Figure 2B shows the concentration time-plots of LF and DLF for pregnant and non-pregnant women included in the analysis with average population predictions and 95% intervals.

### Concentration-time simulation of lumefantrine

The day 7 predicted median concentrations of LF after administration of a 6-dose regimen over 3 days were 908 (PI 95%: 217 – 3256) ng/ml for pregnant women and 1382 (PI 95%: 386 – 5135) ng/ml for non-pregnant women ($p = 0.10$). While considering the large inter-patient variability in the kinetics of LF, 3% of the pregnant women would have day 7 concentrations below the cut-off value of 175 ng/ml, 9% below 280 ng/ml and 31% below 600 ng/ml. For non-pregnant women, 1% would exhibit day 7 concentrations below the cut-off value of 170 ng/ml, 2% below 280 ng/ml and 15% below 600 ng/ml. Prolonging the time of drug administration over 5 days among pregnant women would provide median concentrations of 1374 (PI 95%: 367 – 5536) ng/ml, with 0.1%, 2% and 16% of patients with concentrations below the cut-off value of 175 ng/ml, 280 ng/ml and 600 ng/ml, respectively [Figure 3].

### Pharmacodynamics
There were a total of 7 therapeutic failures in the study, 6 (18.2%) pregnant women and 1 (4.5%) non-pregnant woman (OR = 4.04; p = 0.22). Among pregnant women, one developed ETF at day 1. She presented with signs and symptoms suggestive of severe malaria, was admitted and kept on full dose of intravenous quinine. One pregnant woman had LCF, presented with fever (body temperature = 38.7°C) at day 20, blood slide confirmed to have parasitaemia of 10,750 counts/µL. The remaining four pregnant women had LPF, one at day 28 and three at day 42. One non-pregnant woman had LPF at day 28. Hence, the overall PCR uncorrected efficacy of AL in the study was 87%, 82% (6/33) in pregnant women and 95% (1/22) in non-pregnant women. PCR investigation confirmed recrudescent infection in two women, one with ETF and the other with LCF, both pregnant; the remaining 5 (71%) had new infections.

Analysis of day 7 LF concentration was done irrespective of the nature of the failure (new infection or a recrudescence). The mean day 7 plasma concentration was 971 (726 – 1216) ng/ml in pregnant and 1261 (999 – 1522) ng/ml in non-pregnant women (p = 0.109) [Figure 4A]. Day 7 LF concentration was significantly lower among women with therapeutic failure than those with ACPR. The mean LF concentration among women with ACPR was 1154 (967 – 1341) ng/ml whereas, for the women with LCF and LPF it was 507 (95 – 919) ng/ml (p = 0.029) [Figure 4B]. Twenty percent of study participants had day 7 LF concentrations below 600 ng/ml. Only two patients (33%) out of six among the ones who developed LCF and LPF had day 7 LF concentrations below 600 ng/ml and all were pregnant. Potential predictors of treatment failure in addition to day 7 LF concentration were pregnancy status, gestational age, baseline parasite count, residual antimalarial and BMI and none was statistically significant.

**Discussion**
The study describes the pharmacokinetic properties of AM, LF and their active metabolites DHA and DLF in pregnant and non-pregnant women with malaria. The role of different covariates that could influence AL bioavailability, distribution and clearance in the two groups were carefully analyzed. The study differs from previous reports of population pharmacokinetics of AM and LF in pregnancy (16, 18, 19) by having a comparative group of non-pregnant women with malaria from the same population with relatively similar characteristics.

**Prior treatment.** Detectable residual antimalarial among recruited participants was unexpectedly high. This might be explained by uncontrolled prescription of AL, a first line malaria treatment, which is highly available and easily accessible from both private and public facilities (42, 43). Prevalence of residual antimalarial among participants was higher than what was reported five years ago from *in vivo* studies in Ifakara (Tanzania) and Praeh Vihear (Cambodia) which was 74.3% and 50%, respectively (28, 29). Such high prevalence of residual antimalarial levels in this population, particularly LF, is alarming because it can promote emergence and spread of drug resistance parasite. Also, the high residual prevalence of LF, irrespective of pregnancy trimester suggests a considerable AL exposure in the first trimester. There is an urgent need to monitor closely the implementation of standard malaria treatment guideline and discourage self-treatment by not acquiring antimalarial from drug venders without attended and screened for presence of malaria parasitaemia. Significant levels of detected sulfadoxine among pregnant women were probably the result of SP received from RCH clinic for Intermittent Preventive Treatment (IPTp).

**Pharmacokinetics.** LF pharmacokinetics is known to exhibit a multi-compartment disposition. The short sampling duration in the present study, however, prevented an appropriate characterization of the drug profound disposition. A simple one-compartment model was thus
employed to describe LF concentration-time profile. The important study finding was the lower
LF plasma concentration among pregnant patients compared to non-pregnant ones. This is
similar to what has been observed in a Thailand study in which the concentration of LF was
approximately half that of non-pregnant patients from historical data in the same population (16).
The reason for low LF concentration may be due to physiological changes related to pregnancy
status which accounts for reduced absorption, expanded volume of distribution, elevated drug
metabolism and clearance rate (6). The observed increase in LF metabolism rate among pregnant
women is explained by hormonal changes in pregnancy which increases catalytic activity of
hepatic enzymes such as CYP3A4, an important enzyme for LF metabolism (11). The design of
the study did not allow displaying the effect of reduced absorption on LF bioavailability.

Altered bowel condition such as having diarrhea during malaria treatment has a significant effect
on drug absorption and consequently lowers drug bioavailability. Increase of gastro-intestinal
motility due to diarrhea reduces intestinal transit time of a drug, and this time is important to
maximize drug absorption (44). The latter explains why LF concentration, a high lipophilic
compound, was 83% lower in women with diarrhea compared to the ones with no diarrhea. It is
therefore important to assess for presence of diarrhea in patients and correct dosage regimens
accordingly.

It is important to study concentration levels of a slowly eliminated partner antimalarial drug such
as LF so as to determine minimum parasiticidal concentration (MPC) and minimum inhibitory
concentration (MIC) of malaria parasite (20). The observed day 7 median concentration of LF
was lower in pregnant than in non-pregnant women. However, the concentration among pregnant
women was twofold higher compared to what had been observed in Thai pregnant patients (19).
It is also higher than the concentrations previously reported in non-pregnant adults and paediatric
patients in Ifakara-Tanzania, Thailand, Cambodia and the Lao People’s Democratic Republic (22, 24, 45, 46). Higher day 7 LF levels in the present study may be due to the administration of a standard recommended adult dose of AL with food (5) to all patients regardless of the patient’s body weight.

The observed higher AM clearance in pregnant as opposed to non-pregnant women, although not statistically significant, could explain the differences in the therapeutic outcome. Little has been done on AM bioassay in relation to its specific role on therapeutic efficacy in pregnancy as opposed to LF. AM can better explain ETF and hence, further studies are encouraged with detailed assessment on AM pharmacokinetics despite of its shorter half-life.

The simulations under the standard 6 dose of AL over 3 days schedule show that a non-negligible number of pregnant women would have LF concentrations below various proposed therapeutic threshold targets at day 7. Splitting the same recommended total dose over a 5 day regimen would greatly improve the probability of exhibiting therapeutic drug concentrations. The latter has already been shown in other pharmacokinetics studies (22, 23, 47), but the benefit might be jeopardized by poor adherence to treatment in the prolonged regimen. Hence, a formal assessment of feasibility should be performed.

**Pharmacodynamics.** The observed cure rate and parasite clearance in pregnant women was lower compared to that of non-pregnant patients despite having the same median baseline parasitaemia. The observed lower LF concentration at day 7 among the patients with therapeutic failure could be one of the reasons explaining this difference. In order to improve therapeutic efficacy, it is therefore important to consider dose increase or modifying treatment regimen to allow higher day 7 LF concentrations. Day 7 LF concentration above 600 ng/dl was associated
with 100% efficacy among pregnant patients in Thailand (40). The latter was not observed in our study; indeed 3 out of the 5 (60%) pregnant women with LCF or LTF had day 7 LF concentration above 600 ng/ml. This observation suggests that the proposed 600 ng/dl cut-off value better predicts parasite clearance of ongoing infection, rather than occurrence of new infection in the follow-up period. 600 ng/dl LF concentration at day 7 is not high enough to ensure post-treatment prophylaxis effect up to day 42. Indeed, reinfections were not all prevented with a day 7 LF concentration of 600 ng/ml. Partner drugs with longer half-life might offer better protection (20).

Baseline parasitaemia was not an important factor to determine therapeutic response among study participants. Indeed mean baseline parasite count in patients with ACPR was twofold higher compared to the ones with therapeutic failure. This is contrary to what has been reported in previous studies involving pregnant and non-pregnant patients in which patients with higher baseline parasitaemia were more likely to fail treatment (40, 47). However, baseline peripheral parasitaemia in pregnant women usually does not tell much about the actual picture of parasite level that a pregnant woman might have because of parasite sequestration in the placenta (48).

Therapeutic failure rate among pregnant women in our study was much lower than that observed in Thailand in recent AL pharmacodynamics studies whereby therapeutic failure among pregnant patients was more than 30% (19, 40). We have reason to believe that AL is more efficacious in Africa than in Southeast Asia where resistance to other antimalarial drugs such as quinine, mefloquine and artesunate has increased (14, 49).

**Conclusion**
The current AL treatment regimen in pregnancy is challenged by having low post-treatment prophylactic effect. Pregnancy is an important associated factor for low plasma concentration of LF probably due to reduced drug absorption, elevated drug metabolism and rapid clearance rate. It is therefore important to evaluate new treatment regimens of AL in this vulnerable group that would target higher day 7 LF concentration levels.

**Competing interests**

The authors declare that they have no competing interests

**Acknowledgements**

We sincerely thank the patients for their cooperation and all staff involved in the study. Special thanks are given to Sigilbert Mrema, Athumani Mzuyu, Bakari Kissa, Fadhili Mwakitete and Sajidu Ismail of Rufiji Health Demographic Surveillance System (HDSS), and Jackson Thomas and Happy Mkali of IHI Bagamoyo Clinical Laboratory. This work was supported by Ifakara Health Institute (IHI) and European and Developing Countries Trial Partnership (EDCTP) through Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) project.
References


Pharmacokinetics and pharmacodynamics of artesunate and dihydroartemisinin following oral treatment in pregnant women with asymptomatic Plasmodium falciparum infections in Kinshasa DRC. Malaria journal 10:49.


Plasmodium falciparum malaria in Luang Namtha Province, Lao People's Democratic Republic.


**Tables and figures**

**Table 1:** Characteristics of study participants with *P. falciparum* malaria on the day of enrollment

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pregnant women (n=33)</th>
<th>Median (range)</th>
<th>Non-pregnant women (n=22)</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>25 (18 - 41)</td>
<td>21.5 (18 - 35)</td>
<td></td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td></td>
<td>52 (40 – 80)</td>
<td>48.5 (41 – 79)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td>158 (147 – 169)</td>
<td>157 (150 – 174)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>21.8 (16.5 – 30.1)</td>
<td>20.3 (16.4 – 33.3)</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td></td>
<td>10.2 (7.1 – 13.3)</td>
<td>13.4 (8 – 15.5)</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>37.1 (36.0 – 39)</td>
<td>37.2 (36.0 – 39.6)</td>
<td></td>
</tr>
<tr>
<td>Parasitaemia (counts/µL)</td>
<td></td>
<td>25,280 (560 – 198,080)</td>
<td>22,280 (560 – 195,680)</td>
<td></td>
</tr>
<tr>
<td>Gestation age (weeks)</td>
<td></td>
<td>27 (14 – 37)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

* *Pregnancy – trimesters*

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women</th>
<th>Non-pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second trimester (%)</td>
<td>17 (52)</td>
<td>NA</td>
</tr>
<tr>
<td>Third trimesters (%)</td>
<td>16 (48)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Trimester presented in number (%). NA means not applicable*

**Table 2:** Plasma concentration of residual antimalarial drugs detected prior to treatment with AL in 57 recruited study patients [ng/ml]

<table>
<thead>
<tr>
<th>Antimalarial</th>
<th>Patients (%)</th>
<th>Plasma concentration [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Lumefantrine</td>
<td>51 (89.5)</td>
<td>37.3</td>
</tr>
<tr>
<td>Desbutyl-lumefantrine</td>
<td>8 (14.0)</td>
<td>2.5</td>
</tr>
<tr>
<td>Artemether</td>
<td>4 (7)</td>
<td>26.4</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td>14 (24.6)</td>
<td>1,334.3</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>11 (19.6)</td>
<td>6.9</td>
</tr>
<tr>
<td>Quinine</td>
<td>1 (1.8)</td>
<td>12.3</td>
</tr>
</tbody>
</table>

27
Table 3: Final population parameter estimates of artemether, lumefantrine and their metabolites and their bootstrap evaluations in 2000 replicates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SEa (%)</th>
<th>IIVb (%)</th>
<th>SEc (%)</th>
<th>Estimate</th>
<th>CI95%d</th>
<th>IVb (%)</th>
<th>CI95%d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Artemether</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>98</td>
<td>24</td>
<td>99</td>
<td>65</td>
<td>102</td>
<td>69-140</td>
<td>93</td>
<td>66-120</td>
</tr>
<tr>
<td>Vc (L)</td>
<td>373</td>
<td>16</td>
<td></td>
<td></td>
<td>354</td>
<td>225-492</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LogitF1</td>
<td>1.4</td>
<td>27</td>
<td></td>
<td></td>
<td>1.5</td>
<td>0.7-2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4 (h⁻¹)</td>
<td>Fixed to 0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM (L)</td>
<td>Fixed to Vc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K23 (h⁻¹)</td>
<td>0.084</td>
<td></td>
<td></td>
<td></td>
<td>0.088</td>
<td>0.05-0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL3d(L/h)</td>
<td>71</td>
<td>46</td>
<td></td>
<td></td>
<td>69</td>
<td>38-136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σprop,AM (CV%)</td>
<td>0.13</td>
<td>7</td>
<td></td>
<td></td>
<td>0.13</td>
<td>0.03-0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σadd,AM (μmol/L)</td>
<td>53</td>
<td>14</td>
<td></td>
<td></td>
<td>51</td>
<td>44-59</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lumefantrine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>2.8</td>
<td>12</td>
<td></td>
<td></td>
<td>2.8</td>
<td>2.2-3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vc (L)</td>
<td>134</td>
<td>14</td>
<td></td>
<td></td>
<td>134</td>
<td>101-174</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>Fixed to 1</td>
<td>65</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td>61</td>
<td>43-77</td>
</tr>
<tr>
<td>θPregF1</td>
<td>-0.33</td>
<td>37</td>
<td></td>
<td></td>
<td>-0.31</td>
<td>-0.52-0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>θdiarrF1</td>
<td>-0.84</td>
<td>15</td>
<td></td>
<td></td>
<td>-0.78</td>
<td>-0.95-0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4 (h⁻¹)</td>
<td>Fixed to 0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM (L)</td>
<td>Fixed to Vc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0 (mg)</td>
<td>2.7</td>
<td>18</td>
<td>87</td>
<td>46</td>
<td>2.95</td>
<td>1.9-4.4</td>
<td>116</td>
<td>70-164</td>
</tr>
<tr>
<td>K23 (h⁻¹)</td>
<td>1.6·10⁻⁴</td>
<td>46</td>
<td>54</td>
<td></td>
<td>1.6·10⁻⁴</td>
<td>(1.2-2.0)·10⁻⁴</td>
<td>44</td>
<td>31-57</td>
</tr>
<tr>
<td>θPregK23</td>
<td>0.80</td>
<td>32</td>
<td></td>
<td></td>
<td>0.80</td>
<td>0.4-1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL3d(L/h)</td>
<td>2.6</td>
<td>15</td>
<td></td>
<td></td>
<td>2.6</td>
<td>1.9-3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σprop,LF (CV%)</td>
<td>51</td>
<td>32</td>
<td></td>
<td></td>
<td>51</td>
<td>45-56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σprop,DLF (CV%)</td>
<td>39</td>
<td>40</td>
<td></td>
<td></td>
<td>38</td>
<td>32-44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation LF/RLF</td>
<td>68</td>
<td>18</td>
<td></td>
<td></td>
<td>67</td>
<td>63-69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>σadd,DLF(μmol/L)</td>
<td>4.4·10⁻³</td>
<td>17</td>
<td></td>
<td></td>
<td>4.9·10⁻³</td>
<td>(3.8-6.1)·10⁻³</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Abbreviations: CL: clearance, $V_C$: central volume of distribution, logit$F_1$: logit $F_1$ expressed as a logit function, $k_a$: first-order absorption rate constant, $V_M$: volume of distribution of the metabolite, $F_0$: residual amount from the previous treatment, $k_23$: metabolism rate constant, $CL_{met}$: metabolite clearance, $\sigma_{prop}$: exponential residual error, $\sigma_{add}$: additive residual error, $\theta_{X_{PAR}}$: effect of the $X$ covariate on the parameter $PAR$ expressed as $(1 - \theta_{X_{PAR}} X)$.

- Standard error (S.E.) of the estimate $\theta$, defined as S.E estimate/estimate, expressed as a percentage
- Inter-individual variability
- Standard error (S.E.) of the coefficient of variation or the additive component of the residual error defined as $\sqrt{S.E \text{ estimate}/\text{estimate}}$, expressed as a percentage
- 95% confidence interval (C.I.)

**Figure 1**: Relationship between parasite density at enrollment and plasma residual levels of sulfadoxine prior treatment in 14 pregnant women
**Figure 2A:** Observed AM (left panel) and DHA plasma concentrations (right panel). Filled and empty circles represent pregnant and non-pregnant women, respectively. The solid line represents the average predicted concentrations and the dashed lines the 95th prediction intervals.

**Figure 2B:** Observed LF (upper panels) and DLF plasma concentrations (lower panels) in pregnant and non-pregnant women. Triangles residual plasma concentrations of LF and DLF found prior treatment initiation. The solid lines represent the mean population prediction and the dotted lines PI95%.
Figure 3: Predicted median concentration of lumefantrine (LF) after administration of 6.480 mg regimen over 3 (continuous line) and 5 days (dotted line) in pregnant women. Day 7 (168h) median predicted concentrations (circles) with their PI95% are shown for the two dosage regimens.

Figure 4A: Day 7 plasma concentration of lumefantrine in pregnant (n = 32) and non-pregnant (n = 22) study women.
Figure 4B: Day 7 plasma concentration of lumefantrine in women with ACPR (n = 48) and those with treatment failure (n = 6) *

* Day 7 lumefantrine concentration could not be assessed in one woman since a rescue treatment with quinine was given at day 1 because of early treatment failure.