Statistical Analysis Plan
Malaria in Pregnancy Treatment Efficacy Study Group

Project 1 Evaluation of the treatment efficacy of artemisinin-based and quinine-based treatments and optimal dose of artemisin–based treatments for a single malaria episode during pregnancy
Version 0.1

WorldWide Antimalarial Resistance Network (WWARN)

Suggested citation: Statistical Analysis Plan: Project 1 **Evaluation of the treatment efficacy of artemisinin-based and quinine-based treatments and optimal dose of artemisin–based treatments for a single malaria episode during pregnancy**, Malaria in Pregnancy Treatment Efficacy Study Group, WorldWide Antimalarial Resistance Network, 2018.

Version History

Version number	Revision(s) & reason for amendment	Release date
1		21/June/2018

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Introduction

About 60% of all pregnancies take place in malaria endemic areas [1]. In addition, pregnant women and children are the most vulnerable group for malaria infection leading to higher morbidity and mortality. Although studies on the efficacy of antimalarials in malaria treatment have been conducted extensively, pregnant women have been excluded from the majority of clinical trials in the past, mainly because of safety concerns for the fetus. Due to the lack of evidence for both efficacy and safety, quinine, rather than artemisinin combination therapy (ACT), has been recommended as the first-line treatment for pregnant women in the first trimester by the World Health Organization (WHO) [2]. However, recent studies measuring the safety of artemisinin derivatives during pregnancy, including in the first trimester, have shown reassuring results [3][4][5][6] and consequently ACT may be recommended as a first-line treatment option for pregnant women regardless of the trimester in the next WHO guideline [7]. As the current treatment recommendation for pregnant women is based on limited available data, vigorous and prompt assessment of treatment efficacy in pregnancy is warranted. The WorldWide Antimalarial Resistance Network (WWARN) has established a unique individual participant data (IPD) sharing platform facilitating large scale pooled analysis. We plan to expand the platform to include both published and unpublished studies exploring the efficacy of the treatment of malaria and the impact of malaria infection during pregnancy.

Assessment of antimalarial efficacy in non-pregnant patients is standardised by the WHO guidelines. However, there is no consensus on how long pregnant women should be followed up to assess the treatment efficacy. The duration of follow-up in pregnancy can be longer than 28–42 days [8] particularly because of placenta sequestration [9]; altered pharmacokinetics and pharmacodynamics [10]; and altered immunity balance in pregnancy [11]. We will conduct a one stage IPD meta-analyses for the majority of antimalarial drugs used for the treatment of *P. falciparum* malaria in pregnancy and explore techniques to derive the efficacy of antimalarials. We will describe correlation of treatment efficacy and long-term outcomes (i.e. placentala malaria and maternal anaemia) after single and repetitive episodes of *P. falciparum* malaria infection and treatment in pregnancy.

Aims and objectives of the study

The aim of this study is to estimate treatment efficacy and explore the methodology for assessing treatment efficacy of artemisinin-based and quinine-based treatment for *P. falciparum* during pregnancy. Our hypotheses are: for all trimesters, artemisinin-based treatment is better than

quinine-based therapy in the clearance of parasites; optimal duration of follow-up of pregnant women is longer than the current recommendation; the dosing of antimalarials needs to be modified for pregnant women; higher treatment efficacy leads to better long-term outcomes.

To test these hypotheses, the study is devided into three projects (as listed below) and each project is addressed in a separate analysis plan.

- Project 1 Evaluation of the treatment efficacy of artemisinin-based and quinine-based treatments and optimal dose of artemisin–based treatments for a single malaria episode during pregnancy
- Project 2 Analysis of the optimal duration of follow-up for artemisinin-based and quinine-based treatment for a single malaria episode during pregnancy in pregnancy
- Project 3 Analyses of the haematological change following malaria treatment in pregnancy

Project 1 Evaluation of the treatment efficacy of artemisinin-based and quinine-based treatments and optimal dose of artemisin–based treatments for a single malaria episode during pregnancy

Primary objectives are:

- To compare antimalarial efficacy between artemisinin-based and quinine-based treatments
- To identify risk factors associated with treatment failure
- To assess the relationship between the dosing (dose per body weight) of artemisinin-based treatments and treatment efficacy

Secondary objectives are

- To evaluate the risk of gametocyte carriage following antimalarial treatment
- To evaluate the risk of *Plasmodium vivax* infection following antimalarial treatment
- To evaluate the risk of developing adverse symptoms following antimalarial treatment

1. Eligibility criteria for inclusion in pooled analysis

We will include both asymptomatic and symptomatic women because even asymptomatic parasitaemia is reported to be related to adverse pregnancy outcomes. We will include all pregnant women regardless of the trimester, parity, and gravidity to assess the impact of these factors on treatment outcomes.

A study will be deemed eligible for the purpose of this analysis if they meet the following criteria:

- Prospective clinical efficacy studies of parasitologically confirmed uncomplicated *falciparum* malaria with a minimum 28-day active follow-up in patients treated with quinine- or artemisinin-based regimens
- Information on name, date and dose of antimalarial drugs: artemisinin-based and quinine-based treatments will be included
- Baseline data on patient age, estimated gestational age (ega) or trimester of pregnancy (all trimesters will be included)
- Parasite density on day 0 and during the follow-up
- Information on body temperature (or history of fever) on day 0 (both symptomatic or asymptomatic will be included)
- Date of the last day of follow-up or length of follow-up
- Genotyping to distinguish recrudescence and reinfection by polymerase chain reaction (PCR)

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1.1 Desirable Data (not required for inclusion)

- Information on symptoms including number of days of symptoms before enrollment
- Body weight of the patient at treatment
- Body weight before pregnancy (or body weight in the first trimester)
- Parasitae clearance time
- Fever clearance time
- Gametocyte carriage on day 0 and during the follow-up
- Parity
- Gravidity
- Information on the supervision of drug administration
- Information on intermittent presumptive treatment (IPTp)
- Information on ega estimation method used
- Information on adverse symptoms

1.2 Study Exclusion criteria

The following studies will be excluded

- No active follow-up within 28 days after treatment (e.g. studies which followed up only at delivery will be excluded)

1.3 Patient Exclusion

The following patients (from the studies which are in the analysis) will be excluded from all analyses:

i. No or missing parasitologically confirmed *Plasmodium falciparum* infection on enrolment
ii. Any of deviations, as defined by Clinical Module DMSAP [12]

2. Methodologies

2.1 Data Pooling

The data sets uploaded to the WWARN repository will be standardized using the WWARN Clinical Data Management and Statistical Analysis Plans [12] for clinical data and pooled into a single database of quality-assured individual patient data. Data will remain the property of the individual donor(s) and publication will be in accordance with an agreed publication plan [13].

2.2 Transmission Intensity

The study sites will be classified into 3 categories: low, medium and high malaria transmission based on the parasite prevalence estimates obtained from the Malaria Atlas Project [14][15] for specific location and year of study.

2.3 Dosing Calculation

The doses of quinine, artemisinin derivatives and the partner compounds received will be calculated from the number of daily tablets administered to each patient. If the daily tablet counts are not available, doses will then be back-calculated using the dosing scheme available from study protocols. Two body weights, namely body weight before pregnancy (if available) and body weight at treatment, will be used for the assessment.

3. Efficacy Endpoints

Primary: PCR adjusted *P. falciparum* recrudescence

Secondary: PCR adjusted *P. falciparum* reinfection

PCR unadjusted *P. falciparum* recurrence

Parasite clearance time

Tertiary: Gametocyte carriage during follow up

P.vivax infection during follow-up

Adverse symptoms

All the primary and secondary endpoints on interest will go through the same analysis as outlined below. Analysis of tertiary endpoint will be carried out provided enough data is present; else, only summary statistics will be reported.

4. Covariates Examined

In the analysis the following baseline characteristics of patients will be included, as appropriate: age, estimated gestational age (or trimester), parity/gravidity, weight (weight before pregnancy and

weight at treatment), body mass index (BMI), baseline parasitaemia, presence of fever (body temperature >37.5 degrees), haemoglobin (or haematocrit), anemia (Hb<11g/dL or Hct<30% for anaemia and Hb<7g/dL or Hct<20% for severe anaemia) [16], gametocytes on presentation, past history of malaria (time from the last treatment or the number of past infection in the same pregnancy), description of infection (mixed species infections), total mg/kg dose for each drug component, and supervision of drug administration. *P.vivax* intercalated infection will be regarded as censored if the original study did not test PCR for falciparum recurrences after vivax intercalated infection. If falciparum recurrences were tested with PCR regardless of vivax intercalated infection, vivax infection will be regarded as a time-dependent covariate (i.e. vivax infection status will be 0 until the first appearance of vivax and the status will be regarded as 1 after the day of vivax infection). For each study, also details of study locations and transmission intensity will be considered.

5. Outline of Statistical Analysis

5.1 An overall summary of studies

Overall study profile as per PRISMA-IPD guidelines of all the studies identified, studies shared at the WWARN repository will be presented.

5.2 Baseline characteristics of the patients included in the analysis

Summary of the studies and baseline characteristics of patients included in the analysis will be presented. The number of available patients will be summarised for all variables, proportion will be used for categorical or binary variables, and mean and standard deviation (or median and range) will be used for continuous variales.

5.3 Diversity in dosing strategies of artemisinin-based treatment

Descriptive statistics for different methods of dosing strategies (fixed dose, weight-based, loose of fixed dose combination) will be calculated for artemisinin-based treatments. Summary statistics of dosing strategies used in pregnancy (e.g. proportion of studies using quarter/half tablets or dissolving tablets) will be reported. The following figures will be presented for artemisinin derivates and partner drugs:
  (a) Box plot or histogram of the mg/kg distribution together with their descriptive statistics.

(b) Scatter plot of mg/kg dosing (Y axis) and age (X axis).

(c) Scatter plot of mg/kg dosing (Y axis) and weight (X axis).

5.4 Efficacy on day 28, day 42 or day 63 and risk factors for treatment failure

In studies peripheral malaria smears were examined every week or every two weeks. Time of parasite recurrence will be defined as a time of the first positive parasite smear after parasite clearance after treatment. For patients with no recurrent parasitaemia recorded, day of their last negative smear will be treated as their last visit and censoring time.

In case of intermittent follow-up, the following rules will be applied:

(i)   blood smears will be assumed to be negative between the two negative observations.

(ii)   if patient came back to be followed up with a positive smear, the date of positive parasitaemia will be assumed to be the date of observation if this date is within 31 days from the last observation.

(iii)   if parasite clearance is not recorded after treatment but the positive parasite count is recorded at least 7 days after starting the treatment, day of the first positive count will be treated as day of recurrence

Definitions of status and other censorship are detailed on page 14 of the Clinical Module DMSAP [12] except the above modification. Early early late treatment failure are not applicable for quinine-based treatment.

All episodes of malaria will be included for each patient.

Univariable and multivariable Cox analysis of possible risk factors (as define in section 4) (adjusted for the study-site effects) for the primary and secondary endpoints of interests (Section 3) will be conducted for pooled data including all treatments and also for each treatment. In order to account for within study clustering and repeated episodes, study sites and patients (if data permits and there are any longitudinal studies) will be fitted as random effects.

Time to recrudescence (median, range) for each treatment will be calculated for different trimester for the patients who failed. The effect of the level of supervision on the risk of recurrence will be assessed. The efficacy outcomes for the partially, fully and not supervised groups will be computed. Analysis of mg/kg dose of artemisinin-based treatments as a risk factor for the three recurrence outcomes, after controlling for known confounders will be carried out. Median (IQR) mg/kg dose of artemisinin derivatives or partner drugs in patients failing treatment (i.e. recurrence) vs successfully treated (defined as reaching the end of the study duration without failure) will be calculated.

5.5 Parasite clearance

The proportions of patients cleared at day 1, 2 and 3 will be assessed. Definitions are detailed on page 15 of the Clinical Module DMSAP [12]. All episodes of malaria will be included for each patient. Univariable and multivariable analyses of risk factors (as define in section 4) associated with parasite positivity status on different days will be conducted using generalised linear mixed model (logit link), in a one-stage analysis by combining all of the individual patient data. In order to account for within study clustering and multiple episodes in the same person, study site and patient will be fitted as random effects.

5.6 Gametocyte carriage

Gametocyte carriage will be assessed as the proportion of patients with *P. falciparum* gametocytes on day 0, 3, 7, 14 or 21. Proportions after day 0 will be stratified by whether or not gametocytes were present on admission. If enough data is available, generalised linear mixed model with logit link will be used to assess the risk factors (as define in section 4) for gametocytes carriage on day 0 and after treatment stratified by the gametocyte carriage at baseline. In order to account for within study clustering and repeated episodes, study sites and patient will be fitted as random effects.

5.7 Adverse symptoms

Adverse symptoms will be assessed as the proportion of patients who developed adverse symptoms after treatment. Proportions of patients who developed symptoms after day 0 will be stratified by whether or not that symptom was present on admission. If enough data are available, logistic regression will be used to assess the risk factors (define in section 4) for adverse symptoms after treatment. Symptoms on day 0 (before treatment) will be adjusted as a covariate. In order to account for within study clustering and repeated episodes, study sites and patient will be fitted as random effects. Adverse symptoms will include abdominal pain, dizziness, headache, body pain/myalgia, weakness/fatigue, vomiting, nausea, anorexia and tinnitus if data permit. Primarily the symptoms developed in the first week will be included, symptoms developed at any time during study period may be added as supplement.

6. Statistical Methodology

6.1 Descriptive statistics

Descriptive statistics will use mean and variance/standard deviation if the data are normally distributed, geometric mean and range if data are log-normally distributed (as assessed by Shapiro-Wilk test [17]) or median and range/ Interquartile range otherwise. Statistical tests or plots will be used to check the assumption of normality. Patients will be categorised into four age groups (≤20 years, 20-25 years, 25-30 years and >30 years). The summary statistics will be further broken down by treatment group, trimester and parity/gravidity.

6.2 Survival analysis

PCR-adjusted and unadjusted outcomes (see [12] for definitions) obtained using WWARN standardised outputs will be used to compute the Kaplan-Meier (K-M) estimates. The K-M estimates will be presented graphically together with the associated tables. To obtain a K-M estimate for each treatment across studies and to test if the overall K-M profiles are significantly different between treatment, cloglog transformation with inverse variance weighting will be used for comparing different groups [18]. Different mg/kg dosing groups of the same drug will be also compared. Also, the efficacy between treatments will be compared at fixed time points (i.e. on day 28, 42, and 63) by constructing a chi-squared test statistics using the stratified (by study sites) approach recommended by Klein et al (2007) [18].

Two-level random effects will be used to adjust for study-site and patient effect [19]. A semi-parametric Cox's regression model and models with parametric hazard functions such as: Gompertz, Weibull, lognormal and log-logistic will be examined and the best regression model will be selected based on Cox-Snell residuals [20]. In the Cox's regression model, the proportional hazard assumption will be tested based on Schoenfeld residuals [21] and Therneau and Grambsh test [22]. Inclusion of covariates in the final model will be determined based on how they improve the overall model (likelihood ratio test) and if they change the coefficient estimates for other factors and based on the residuals, as described below.

6.3 Model selection for determinants

Model building will be carried out first by investigating if any of the available variables (Annex A.1) are related to the treatment outcome using regression model. Any known confounding factors will be forced into the model even if they are statistically non-significant. Interaction between gravidity

(parity) and endemicity will be assesed as the impact of gravidity (i.e. pregnancy-specific immunity) is known to be different depending on the endemicity. All the available variables will be used for the multivariable analysis. If data permit, linear and non-linear relationship will be examined for continuous variables. Model with known confounders (baseline parasitaemia) will be fitted first (baseline model). Variables and covariates will then be added to the baseline model and the Likelihood Ratio Test (LRT) i.e. changes in log likelihood ($-2\,Log\hat{L}$) will be compared (for nested models) to identify the variables which results in a significant reduction in $-2\,Log\hat{L}$ at 10% level of significance. Akaike's Information Criterion (AIC) [23] will be used to compare competing non-nested models; models with smaller AIC will be preferred. In the multivariable analysis, the treatment group will be added to the model containing the significant covariates. A LRT will be used to test if there is a significant effect of treatment group. The final model will then be used to estimate the hazards ratio (HR). Cox-Snell residuals and Martingale residuals will be examined to determine the appropriateness of model fit.

6.4 Sensitivity Analysis

Three types of sensitivity analysis will be performed. Firstly, a model will be refitted with each study's data excluded, one at a time, and a coefficient of variation around the parameter estimates will be calculated. This would identify any influential studies, that is, studies with unusual results (due to variations in methodology, patient population, and so on) that affect the overall pooled analysis findings. Secondly, to assess the impact of missing data, sensitivity analysis will be performed to see if our main conclusion is affected or not by the exclusion of patients with missing data. Multiple imputation approach assuming that the data are missing at random will be used to handle missing data using one of the several commonly used statistical packages i.e. R and Stata. The number of imputed dataset will be decided on the basis of the fraction of missing information. Finally, sensitivity analysis will be conducted to test whether assumption about timing of recurrence in patients with intermittent follow-up affect the results. Final models will be rerun after excluding patients with missing visits more than 18 days before the positive parasitaemia.

In addition, a bootstrap analysis of the final model will be performed. A random sample of size n (where n= sample size of the final model) will be drawn with replacement 1000 times from the final dataset (used for fitting the final model) and the final model with be refitted. The summary distribution of the parameter estimates derived from bootstrapping will be presented as a supplementary file.

6.5 Finding the optimal dosing cut-off points

The suitability of Receiver Operating Characteristics Curves (ROCs) for finding the optimal cut-off points for mg/kg drug dosage which best predicts the PCR-adjusted failure will be explored. The optimal cut-off point will be defined as the maximum value of Youden's Index (J), which is sensitivity+ specificity-1 [24]. The use of Logrank statistics to define the cut-off point will be explored. The value of the drug dosage which maximizes the logrank statistics will be identified as the optimal threshold [25].

Alternatively, model based approach will be used to derive the optimal mg/kg dose of the partner components; the optimal dose is defined as one which will be predictive of 95% drug efficacy [26].The cumulative baseline hazard function derived from the final multivariable model of putative risk factors for each of the treatment regimen separately will be used to compute the predicted risk of treatment failure. Relationship between the predicted risk and mg/kg dose will be explored in order to define a cutoff corresponding to 95% efficacy. If data permit, Linear and nonlinear relationship will be explored.

7. Tools

All statistical analyses will be carried out using *R 3.1.2* (released on 2014-10-31 by The R Foundation for Statistical Computing) or *Stata 14.1* (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA). Using alternative statistical software will not require amendment of this SAP.

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