Statistical Analysis Plan
WWARN Vivax Recurrence Study Group: A pooled analysis investigating the effect of mg/kg drug dosage on *Plasmodium vivax* recurrence
Version 0.1

WorldWide Antimalarial Resistance Network (WWARN)

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WorldWide Antimalarial Resistance Network (WWARN)
www.wwarn.org

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1. Introduction and Rationale

Plasmodium vivax remains widespread, and is becoming the predominant cause of malaria outside of Africa. Vivax malaria is associated with recurrent symptomatic illness and anaemia with increasing recognition of an attributable morbidity and mortality. Recurrent *P. vivax* can arise from recrudescence (treatment failure), reinfection (new infections from an infected mosquito bite) and relapse (reactivation from dormant liver stages). Whilst it is currently impossible to differentiate reliably between these alternatives, early recurrence is more likely to be due to recrudescence or relapse, whereas later recurrences are more likely to be due to relapse or reinfection. The risk, frequency and timing of recurrence is dependent upon host, parasite and drug factors including: antimalarial drug resistance, the pharmacokinetic profile of the antimalarial agents administered, the use of hypnozoitocidal drugs and geographical location (relapse patterns and endemicity). The relative contributions of these risk factors have not been evaluated comprehensively. If the risks and benefits of radical cure are to be quantified, there is a need for a greater understanding of the factors that impact on recurrence, with and without radical therapy.

1.2. Aim of the study

The aim of this study is to assess the risk for early vivax recurrence before day 28 or day 42 and identify associated risk factors (including mg/kg dose of chloroquine, ACTs and primaquine).

1.3. Eligibility criteria for inclusion in pooled analysis

1.3.1 Essential inclusion criteria

- Prospective clinical efficacy studies of uncomplicated vivax monoinfection
- Asexual parasitaemia at enrolment
- A minimum of 28 days of follow up
- Data available on the exact dosage of schizontocidal treatment administered to the patients and the use, timing and dose of primaquine, according to the study protocol
- Study meta-data as described in the Clinical Data Management and Statistical Analysis Plan (1)
- Baseline data on patient age and gender

1.3.2 Desirable criteria

- Mg/Kg dosing (exact number of schizontocidal tablets administered to the patients and the timing and number of primaquine tablets; according to individual patient data)
- Weight of the patient
- Information on splenomegaly, hepatomegaly
- Malnutrition as gauged by weight and age or height, or MUAC
- Qualitative or quantitative assessment of G6PD status
- Parasite density at day 1, 2, 3

- Haemoglobin (hb) or hematocrit (hct) at enrolment
- Day 7 drug levels
- Documentation on the supervision of drug administration
- Outcome of malaria treatment according to standardised WHO criteria (2)

1.3.3 Exclusion Criteria

- Pregnancy

1.4. Data Pooling

A systematic review of all prospective clinical efficacy trials involving *Plasmodium vivax* mono-infection will be performed. Trials undertaken since the year 2000 that fulfil the study criteria will be targeted through direct email to the corresponding author and/or principal investigator. Data from unpublished and ongoing clinical studies will also be included if available. Once data are uploaded into the WWARN repository, they will be curated and standardized using the WWARN Data Management and Statistical Analysis Plans (1) for clinical data and pooled into a single database of quality-assured individual patient data.

2. Outline of Statistical Analysis

2.1 Specific objectives of the study

1. To quantify the risk of early vivax recurrence before day 28 or day 42
2. To identify key host, parasite (including parasitaemia, relapse periodicity and location) and pharmacological determinants of early vivax recurrence including:
    - The effect of schizontocidal treatments including mg/kg dose
    - The effect of different primaquine treatments including mg/kg dose
3. Determine the relationship between parasite clearance and early vivax recurrence

2.2 Study endpoints

Primary:
P. vivax recurrence between day 4 and day 28 or day 42 (PCR unadjusted)

Secondary:
Early treatment failure
Parasite clearance half-life and positivity on day 1 and 2
Gametocyte carriage at follow up days 3, 7, 14, 21 and day of recurrence

2.3 Definitions of Endpoints

Primary

P. vivax recurrence before day 28 is defined as any recurrence of *P. vivax* parasitaemia between day 4 and 28

P. vivax recurrence before day 42 is defined as any recurrence of *P. vivax* parasitaemia between day 4 and 42

Secondary
Early treatment failure (2), includes:

- Danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia
- Parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature
- Parasitaemia on day 3 with axillary temperature ≥ 37.5°C
- Parasitaemia on day 3 ≥ 25% of count on day 0

Patients early parasitological response (*parasite clearance*) will also be evaluated in the form of (a) parasite half life estimated by WWARN PCE tool (5); (b) positivity on Day 1; (c) positivity on Day 2; (d) parasite half life estimated from daily counts, depending on the available data. *Parasite positivity* is defined as the proportion of people with positive parasite counts on day x compared to the number assessable on this day.

Gametocyte presence is defined as any *P. vivax* sexual parasitaemia count/presence within 24hrs of the reading, in patients in whom this was assessed by thick film examination.

Gametocyte carriage during follow up is defined as patent *P. vivax* gametocytaemia after enrollment (>24hrs) up to study end, whilst taking account of reinfection rates, transmission levels, and concurrent asexual parasitaemia results within patients.

2.4 Study and patient characteristics

The following baseline characteristics will be examined:

Site: transmission intensity, regional relapse periodicity, chloroquine resistance

Patient: age, sex, weight, nutritional status, history of malaria in the last 28 days, history of fever in the last 24 hours, fever (>37.5^{0}C axillary), G6PD status

Drug: schizontocidal treatment and mg/kg dose, primaquine use, timing and mg/kg dose, supervision of drug intake (full or partial), early vomiting of drug (within 1 hour)

Laboratory: parasitaemia, gametocytaemia, haemoglobin concentration

Children will be considered as aged <14 years with childhood age stratified into < 1 years, 1 to 4 years, 5 to 11 years, and 12 to 14 years.

The nutritional status of children aged <5 years of age will be calculated as a weight-for-age z-score, using the igrowup package developed by WHO (3). Those with weight-for-age z-scores < -2 (i.e. below the 3rd centile) will be classified as underweight-for-age (termed underweight).

Treatment will be classified as supervised if all doses were directly observed, partially supervised if at least the morning doses of a bd regimen were observed, and not-supervised if fewer doses were observed.

The doses of treatment received i.e. primaquine, chloroquine, ACT 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. For each component, a total dose per weight will be calculated for each patient.

In studies with haematocrit measured instead of haemoglobin, haematocrit will be converted to haemoglobin using the following relationship (4):

$$\text{Haematocrit (ht)} = 5.62 + 2.60 * \text{Haemoglobin}$$

For each study, locations of study sites will be recorded. Each location will be categorised into:

a) *low, moderate and high transmission settings* based on the observed study site reinfection rate , and the malaria endemicity estimates obtained for study sites and year from the Malaria Atlas Project (6). PvPR < 0.15 will be categorized as "low" transmission areas, PfPR ≥ 0.15 & < 0.40 were classified as "moderate" transmission areas, and PfPR ≥ 0.40 were classified as "high" transmission areas.
b) *low, medium and high periodicity of relapses* according to Battle's regions (7).

2.5 Summary of statistical analyses

2.5.1 Description and baseline characteristics of study sample:

1. A summary (study profile) of the relevant trials uploaded to the WWARN repository will be presented to highlight potential selection bias.

2. A summary of the relevant studies will be presented, including (but not restricted to) treatment tested, inclusion and exclusion criteria, follow up duration, study populations, parasitaemia sampling scheme and description of location by country, transmission site(s), regional relapse periodicity and chloroquine resistance. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any differences in the baseline distributions will be noted.

2.5.2 Baseline characteristics of patients:

3. A summary of relevant baseline patient characteristics will be presented including age, gender, malnutrition, treatment given, treatment supervision, timing of primaquine, G6PD status, haemoglobin concentration, asexual parasitaemia, gametocytaemia, temperature >37.5°C, prior antimalarial use, prior malaria history.

   Summary statistics will be broken down by gender and age category. The distribution of continuous variables (e.g. mg/kg total drug dose for each dosing group) will be described using the mean and standard deviation if the data are normally distributed, geometric mean and 95% reference range if the data are normally distributed following a log transformation, or the median and interquartile range if the data are non-normally distributed.

2.5.3 Efficacy and treatment related analyses:

4. *Schizontocidal and primaquine treatment dosing*

   - A summary of the distribution of mg/kg total chloroquine and primaquine dose with inclusion of other schizontocidal agents if sufficient data available. The distributions will be calculated separately for different dosing strategies (age based and weight based) and regions (7) and presented in tables (mean(SD)) as well as visualised using box and whisker plots, histograms or scatter plots (e.g. mg/kg dosing vs age or weight).

   - Summary statistics of dosing strategies used in children (e.g. % studies using quarter/half tablets, % dissolving tablets, suspension or paediatric formulation) will be reported.

   - Multivariable linear regression analysis will be carried out to identify factors associated with a patient receiving a low mg/kg dose (as defined by section 5.5.5). These factors include age, sex, protocol type, target dose, adherence violations, location and any other available risk factors (e.g. nutritional status). The WHO weight-for-age reference database, available as *igrowup* package in Stata will be used to compute anthropometric indicators of malnutrition (Z-scores) (3).

5. *Risk of early PV recurrence within 28/42 days*

   - Outcomes obtained using WWARN's standardised outputs will be used to compute the **Kaplan-Meier (K-M) estimates** for the dosing groups (1). The K-M estimates will be presented graphically together with the associated tables. Log rank test stratified by study sites will be performed at 5% level of significance to test if the K-M profiles are significantly different from each other.

- The proportion of patients who failed before day 28 or between day 28 and day 42 will be presented. In addition, the median time to presentation with recurrent infection will be calculated for different age-groups.

- Median (IQR) mg/kg dose of chloroquine (or other schizontocidal agent such as ACTs) or primaquine in patients failing treatment (i.e. recurrence) vs successfully treated (defined as reaching the end of the study duration without failure) will be calculated.

- **Cox regression analysis** for time to recurrence during follow-up (28 or 42 days) will be performed, with a random intercept for study-site. Model building will be carried out according to Annex 2. Covariates to examine will include: age, sex, weight, nutritional status, G6PD status, history of malaria within the past 28 days, history of fever, baseline parasitaemia, presence of parasitaemia on days 1 and 2 , transmisison intensity, relapse periodicity, baseline haemoglobin, presence of gametocytes on enrolment, level of treatment supervision, schizontocidal treatment mg/kg dose, primaquine treatment mg/kg dose and timing, and vomiting within one hour of drug administration.

- **Population Attributable Risk** (PAR) of low mg/kg dose of chloroquine or ACT and primaquine on risk of recurrence will be estimated. The PAR% will be computed using formula first proposed by Levin (11) based on the prevalence of the risk factor in population and the relative risk (RR) estimate for the risk factor. The relative risks (RR) will be replaced by the adjusted hazard ratio (AHR) from the multivariable cox's regression model (12). Continuous risk factors will be categorised to compute the PAR associated with exposure to those risk factors with baseline parasitaemia categorised at >= 100,000/µL and baseline anaemia categorised as haemoglobin <10g/dL.

$$PAR = \frac{p_e(AHR - 1)}{1 + p_e(AHR - 1)}$$

Where p_e is the prevalence of the risk factor in the study population and AHR is the adjusted hazards ratio derived from the final multivariable model. An overall PAR, which is non-additive, will be calculated as 1 minus the product of 1-each of the individual PAR (13) assuming the risk factors are independent.

$$Overall\ PAR = 1 - \prod_{i=1}^{n}(1 - PAR_i)$$

- The value of the mg/kg drug dosage which maximizes the log-likelihood statistics in Cox's model will be identified as the optimal threshold based on 1,000 bootstrap samples. This will be carried out separately at day 28 and day 42.

  The use of Logrank statistics to define the breakpoint will be explored. The value of the drug dosage which maximizes the logrank statistics will be identified as the optimal threshold (14). The suitability of Receiving Operating Characteristics Curves (ROCs) for finding the optimal cutpoints for mg/kg chloroquine or ACT and primaquine dosage

which best differentiates the PCR adjusted failure will be explored. The optimal cutoff point will be defined as the maximum value of Youden's Index (*J*), which is sensitivity+ specificity-1 (15). This will be carried out by categorising each patient as Failure (1/0) on day 28 (or day 42). Patients who were lost to follow up will be excluded. The mg/kg cut offs will be defined separately for early and late *P. vivax* recurrence. Alternative methods for determining the optimal cutoff will also be explored (e.g. data driven methods such as percentiles).

Using the breakpoint for mg/kg dosing and the population attributable risk the number of patients who failed treatment due to the under dosing globally can then be estimated.

- Using the final multivariate model, the effect of increasing the drug dose of chloroquine (mg/kg) on the predicted risk of recurrence by day 28 and 42 will be calculated for every 2.5 unit increase starting from 12.5 mg/kg. The 95th percentile of this predicted risk will then be computed for each mg/kg dosage of chloroquine using each patient's individual covariates at average random effect. The dose which results in the 95^{th} percentile of the predicted risk to be <5% i.e. estimated efficacy to be >=95% will be reported.

6. *Parasite clearance following treatment*

The proportions of patients positive for parasitaemia at both day 1 and 2 will be assessed separately. Definitions are detailed on page 15 of the Clinical Module DMSAP v1.2 (1). The proportions of patients with positive parasitaemia at day 1 and 2 (Parasite Positivity Proportion, **PPPs**) will be calculated for each individual study as well as overall estimates (for the stratification of interest) with confidence intervals derived using exact methods. The effect of schizontocidal and primaquine dosage on the risk of parasite positivity will be assessed using a generalised linear mixed model with study sites fitted as a random effect and by specifying logit link function. Covariates to examine will include: age, sex, weight, nutritional status, G6PD status, history of malaria within the past 28 days, history of fever, baseline parasitaemia, transmission intensity, relapse periodicity, baseline haemoglobin, presence of gametocyte on enrolment, schizontocidal treatment mg/kg dose, primaquine treatment mg/kg dose and timing and vomiting within one hour of drug administration.

7. *Gametocyte carriage following treatment*

Gametocyte carriage will be assessed as the proportion of patients with *P. vivax* gametocytes on day 3, 7, 14, 21, and day of recurrence (GPP - Gametocyte Positivity Proportion) with confidence intervals derived using exact methods. GPP estimates will be presented separately for those with and without gametocytes on admission. The effect of schizontocidal and primaquine dosage on the risk of gametocyte carriage during the follow-up will be assessed using a generalised linear mixed model with study sites fitted as a random effect and by specifying logit link function. Covariates to examine will include: age, sex, weight, nutritional status, G6PD status, history of malaria within the past 28 days,

history of fever, baseline parasitaemia, presence of parasitaemia on days 1 and 2, transmisison intensity, relapse periodicity, baseline haemoglobin, presence of gametocyte on enrolment, schizontocidal treatment mg/kg dose, primaquine treatment mg/kg dose and timing and vomiting within one hour of drug administration.

4. PRISMA Statement

The analysis will adhere to the PRISMA guidelines for reporting systematic reviews and meta-analyses of individual patient data (16).

5. Tools

All statistical analyses will be carried out using Stata v14 (StataCorp, College Station, Texas). However, when equivalent statistical methods are applied in a different statistical software package (e.g. R statistical software), changing the use of statistical software will not require amendment of this SAP.

6. Study Group Governance, Management, Coordination and Publication Policy

The Vivax Recurrence Study Group comprises participating investigators who contribute relevant data sets to the pooled analysis. Data sets will remain the property of the investigator and will not be shared without their consent. The WWARN statistician(s) will oversee the statistical analyses. Participating investigators will be recognised in publication as contributors under the banner of the **Vivax Recurrence Study Group**. A Writing Committee will coordinate activities including data analysis, and drafting of publications and reports for complete group review. The Writing Committee will comprise Ric Price, Rob Commons, Julie Simpson, the WWARN statisticians Kasia Stepniewska and Prabin Dahal and other interested investigators. They are responsible for undertaking the data analysis and preparation of the manuscript. Authors will be recognized according to the ICMJE guidelines and the WWARN publication policy (17).

7. References

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8. Annex 1

A.1 List of available covariates Description	**Type**
WWARN Status for Pv Adj	Primary Response
ETF	Secondary Response
LCF	Secondary Response
LPF	Secondary Response
LTF	Secondary Response
Early LTF (before D14, no PCR)	Secondary Response
History of Fever (0/1) at inclusion	Baseline Variable
Severe Malaria at inclusion	Baseline Variable
Haemoglobin/hematocrit at inclusion	Baseline Variable

Vivax density at Inclusion	Baseline Variable
Gamv (/µL) at inclusion	Baseline Variable
Max Temp Day0	Baseline Variable
D0 Ht<20%	Baseline Variable
Transmission intensity	Available Variable
Relapse periodicity region	Available Variable
Chloroquine resistance	Available Variable
Age in Years	Available Variable
Gender	Available Variable
Weight	Available Variable
Height	Available Variable
Middle upper arm circumference	Available Variable
Antimalarial in last 28 days	Available Variable
Malaria in last 28 days	Available Variable
G6PD status	Available variable
Parasite density at Inclusion	Available Variable
Max Vivax Asexual parasitaemia on Day1	Available Variable
Max Vivax Asexual parasitaemia on Day2	Available Variable
Max Vivax Asexual parasitaemia on Day3	Available Variable
Max Vivax gametocytaemia on Day1	Available Variable
Max Vivax gametocytaemia on Day2	Available Variable
Max Vivax gametocytaemia on Day3	Available Variable
Max Temp Day1	Available Variable
Max Temp Day2	Available Variable
Max Temp Day3	Available Variable
Day 7 drug levels	Available Variable
Dosing method (single day, broken down over days etc.)	Available Variable
Total mg/kg dose at each day of dosing regimen	Available Variable
Total mg/kg dose during course	Available Variable

9. Annex 2 - 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 Cox's regression model. Univariable analysis of confounding factors (adjusted for the study effects) associated with the primary and secondary endpoint of interests will be conducted. The latter will include transmission intensity as a proxy of global immunity and age as a marker of host immunity. Any known confounding factors (age, parasitaemia at enrolment and chloroquine or ACT mg/kg dosing and primaquine dosing) will be forced into the multivariate model even if they are statistically non-significant. All the variables which were significant in the univariable analysis at 10% level of significance will be kept for the multivariable analysis.

A model with known confounders 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 (will be compared (for nested models) to identify the variables which results in a significant reduction in at 5% level of significance). Akaike's Information Criterion (AIC) will be used to compare competing non-nested models; models with smaller AIC will be preferred.

Inclusion of covariates in the final model will be based on their effect on model coefficients and the degree to which they improved the overall model (based on a likelihood ratio test). The final model

will then be used to estimate the hazards ratio (HR) for the under-dosed group (optimal cutoff derived from the data) relative to the normally dosed group of patients. Cox-Snell's residuals, Martingale's residuals and Schoenfeld's residuals will be examined to determine the appropriateness of model fit (10).

The assumption of proportionality will be tested for all the covariates in the final multivariable model and any departures of proportionality will be reported. Schoenfeld residuals against transformed time for the dosing group will be used to determine if the assumptions of PH across different dosing groups are reasonable. Any systematic departures from horizontal lines are indicative of non-proportional hazards (10). In addition to the Schoenfeld residuals plot, a formal chi-squared test will be used to test the assumption of proportional hazards for each of the individual covariates (and globally). Any violation of the assumption of proportionality will be reported. In case of violation of the PH assumption, interaction of the covariates and time will be fitted.

Sensitivity analysis will be carried out by removing one study site at a time and coefficient of variation (CV) around the parameter estimates will be presented. Sensitivity analysis will also be undertaken to assess the effect of chloroquine resistance using studies undertaken in areas *with and without evidence of chloroquine resistance as defined by the* WWARN Vivax Surveyor (8).