Statistical Analysis Plan

WWARN Vivax Adherence Study Group: An individual patient data meta-analysis investigating the effect of adherence to primaquine on risk of *Plasmodium vivax* recurrence

Version 1.0

**WorldWide Antimalarial Resistance Network (WWARN)**

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A pooled analysis investigating the effect of adherence to primaquine on *Plasmodium vivax* efficacy

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WorldWide Antimalarial Resistance Network (WWARN)

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# Introduction and Rationale

Malaria is a leading cause of morbidity and mortality, with approximately 228 million clinical cases each year throughout the world. The WHO *Global Technical Strategy for Malaria 2016–2030* has set the following ambitious goals for 2030: reducing malaria case incidence and mortality rates by at least 90% and eliminating malaria in at least 35 countries(1). Efficacious treatment of malaria is one of the key factors to achieve these goals. In this regard, adherence to antimalarial drugs is an important, but the less emphasised factor, since clinical trials usually focus on the safety and efficacy of antimalarial drugs(2).

Most recommended antimalarial regimens for *P. vivax* require a three-day treatment of blood schizontocidal drugs plus a prolonged course of primaquine for hypnozoitocidal treatment. Since the primary aim of most clinical studies is to define or compare the efficacy of antimalarial regimens, treatment is usually supervised, particularly for the acute management of the blood stage infection, when the patient is clinically unwell. However, trials tend to vary in their approach to supervising the prolonged course of primaquine. Primaquine must be administered for a 14-day course or 8-weeks for malarial individuals to prevent relapses. However, adherence to these regimens is hard to follow and complete for all dosing episodes.

The conceptual and methodological concerns related to adherence measuring are essential for future research and global clinical application of antimalarial drugs. Pooled clinical trials of *P. vivax* will enable us to investigate how adherence to these regimens influences treatment efficacy. These findings will be incorporated into within-host malaria mathematical models that link drug concentration profile with drug action within a patient, to investigate the impact of missing doses on parasite clearance and recurrent episodes of malaria. Finding the most effective regimen that balances efficacy and a high level of primaquine adherence will assist in the widespread implementation of effective radical cure regimens for *P. vivax* and its elimination.

## Aim of study

This study aims to assess the effect of imperfect adherence to primaquine on *P. vivax* efficacy and define the key factors which contribute to categorising adherence levels.

## Search strategy and selection criteria in the pooled analysis

In order to collect data, we will do a comprehensive systematic review of all the prospective *P. vivax* clinical efficacy studies. We will search MEDLINE, Web of Science, Embase, and Cochrane Database of Systematic Reviews, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement from Jan 1, 1999, to Dec 31, 2019, in any language. In the analysis, we will include prospective therapeutic efficacy trials including randomised and non-randomised therapeutic trials and prospective cohort studies with active follow-up where the number of recurrent *P. vivax* episodes is recorded during follow-up.

## ****Essential inclusion criteria****

* Prospective clinical efficacy studies of uncomplicated vivax malaria:
	+ With a minimum of 28 days follow up
	+ Daily primaquine administered with schizontocidal treatments – chloroquine or artemisinin-based combination therapy
	+ Primaquine administration commenced before day 3
	+ Information on schizontocidal treatment dosing
	+ Information on supervision, timing and dose of primaquine
* Study meta-data as described in the WWARN [Clinical Data Management and Statistical Analysis Plan](http://www.wwarn.org/sites/default/files/ClinicalDMSAP.pdf)(3)
* Baseline data on patient age and gender
* Presence and/or density of parasites on day 0 and up to last day of follow-up.

## ****Desirable criteria****

* Presence of fever at baseline and during follow up
* Mg/Kg dosing
* Weight of the patient
* Number of days of symptoms before enrolment
* Haemoglobin or haematocrit measured on day 0 and during follow up
* Malnutrition as gauged by weight and age +/- height or MUAC
* Qualitative or quantitative assessment of G6PD status

## ****Exclusion Criteria****

* Pregnancy
* Not administering chloroquine or ACTs
* Adjunctive drugs
* Intermittent dosing of primaquine

## Data Pooling procedure

A systematic review of all prospective clinical efficacy studies involving *P. vivax* mono-infections will be undertaken. Studies undertaken since the year 1999 (last 20 years) 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 standardised using the WWARN Data Management and Statistical Analysis Plans(3) for clinical data and pooled into a single database of quality-assured individual patient data.

The dataset will be curated in the form of the Vivax Adherence Study Group, that is an extension of the Vivax Recurrence Study Group, which was established in April 2016(4). A systematic review was undertaken in 2017, and the relevant research groups were contacted, and the available data collated. The systematic review will be updated to includes all studies up until December 2019, and any additional datasets will be curated and added to the previous studies.

# Outline of Statistical Analysis

## **Specific objectives of the study**

How does adherence to primaquine effect the risk of recurrence following treatment of *Plasmodium vivax*?

Two specific objectives will be assessed:

* Determine the association between *P. vivax* recurrence (defined as a recurrent episode between day 7 and 42) and imperfect adherence to primaquine regimens
* Identify the key patient factors that contribute to imperfect adherence, including demographics and disease-related factors.

## **Study endpoints**

*Primary:*

*P. vivax* recurrence between days 7 and 42 of follow up.

*Secondary:*

*P. vivax* recurrence between days 7 and 28, and days 7 and 63.

##  Definitions

*2.3.1 Primary outcome*

*P. vivax* recurrence before day 42is defined as any recurrenceof *P. vivax* parasitaemia between days 7 and 42.

*2.3.2 Secondary outcome*

*P. vivax* recurrence before day 28 (63)is defined as any recurrenceof *P. vivax* parasitaemia between days 7 and 28 (63).

*2.3.3 Exposure of interest – adherence*

Adherence to treatment is an influential determinant of efficacy in clinical trials, however, specific criteria for adherence to primaquine treatment regimens have not well described. In this pooled analysis, we aim to measure adherence to primaquine based on the individual records in each study. Information on the mg dose given of hypnozonticidal treatment and treatment duration recorded for each individual, and supervision status will be gathered in the primaquine treatment arm.

*Primary measurement of adherence –supervision status*

We will use individual patient data and/or the study protocol to calculate the percentage of doses supervised. Supervision status is defined as the direct observation of the number of doses administered for each patient by a health worker at each time. The percentage of supervised doses for each individual will then be categorised into 5 groupings (see below).

*Secondary measurement of adherence –dosing information*

We will use individual patient data coming from each study to check the number of doses administered. The primaquine doses might be recorded by tablets or mg/kg of daily doses per individual. We will calculate the percentage of doses received for each individual from the number of doses administered divided by the number of doses planned according to the study protocol. As for the primary measure, this measure of adherence will be categorised into 5 groupings (see below).

We will also use individual total mg/kg dose administered as a proxy for adherence in some studies in which daily dose information was not recorded, where percentage of doses received is calculated from total mg/kg dose administered divided by total mg/kg dose according to the study protocol. For this method, we will assume that each patient was administered an equal number of daily doses over the treatment duration, where patients have only information available on total mg/kg dose.

Adherence for both measures will be categorized to five groups from 0 level of adherence to 100% full adherence, including 100, 75-99, 50-74, 25-49, and < 25 percent.

As opposed to adherence to treatment, *imperfect adherence*(5) will be defined as any deviation or failure from the clinical study protocol to follow the prescribed amount of drug in a specific treatment duration by each patient. It will be calculated according to the deviation from full adherence which is 100%.

* 1. *Study and patient characteristics*

The following baseline characteristics will be examined:

**Site**: transmission intensity, regional relapse periodicity

**Patient:** age, sex, weight, nutritional status, history of malaria in the last 28 days, history of fever in the last 24 hours, fever (>37.50C axillary), G6PD status

**Drug**: schizontocidal treatment and mg/kg dose, primaquine timing and mg/kg dose, early vomiting of the drug (within 1 hour)

**Laboratory:** parasitaemia, gametocytaemia, haemoglobin concentration

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

1. *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<0.015 will be categorized as “low” transmission areas, PvPR≥0.015 & <0.040 were classified as “moderate” transmission areas, and PvPR≥ 0.040 were classified as “high” transmission areas.
2. *Low (long) and high (short) periodicity* *of relapses* according to Battle’s regions(7), with high periodicity considered to include regions where the median periodicity was ≤42 days. Thus regions with the two highest periodicities (region 10 and 12) where the median periodicity is <47 days will be categorised as “high” and others will be categorised as “low”.

*Age* - Children will be considered as aged <15 years with childhood age-stratified into <5 years and 5 to <15 years.

*Nutritional status* – For children aged <5 years of age a weight-for-age z-score will be calculated using the igrowup package developed by WHO(8). Those with weight-for-age z-scores < -2 (i.e. below the 3rd centile) will be classified as underweight-for-age (termed underweight). Weight-for-age Z scores will be set to missing if the score is less than -6 or greater than 6.

*Drug mg/kg dose -* 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.

*Haemoglobin -* In studies with haematocrit measured instead of haemoglobin, haematocrit will be converted to haemoglobin using the following relationship(9):

Haematocrit (ht) = 5.62 + 2.60 \* Haemoglobin

*Schizontocidal treatments* – includes Artemisinin-based Combination Therapy (ACTs) and chloroquine, according to WHO guideline(10):

* Artemether+Lumefantrine
* Artesunate+Amodiaquine
* Artesunate+Mefloquine
* Dihydroartemisinin+Piperaquine
* Artesunate+Sulfadoxine-pyrimethamine (SP)

*2.5. Summary of statistical analyses*

***2.5.1 Description and baseline characteristics of the 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. Details of the relevant trials will be presented, including (but not restricted to) treatment administered, inclusion and exclusion criteria, follow up duration, study populations, parasitaemia sampling scheme and description of location by country, transmission site(s), regional relapse periodicity, chloroquine resistance, number of days of treatment supervised, and recording of dosing data. Tests of statistical significance will not be undertaken for baseline characteristics; instead, the clinical importance of any differences in the baseline distributions across study sites will be noted.
		1. ***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, temperature >37.5°C, prior antimalarial use, prior malaria history.
	4. Summary statistics will be broken down by gender and age category. The distribution of continuous variables (e.g. haemoglobin concentration) 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.
		1. ***Association between P. vivax recurrence and imperfect adherence to primaquine regimen***
* The summary distribution of adherence to primaquine as the exposure of interest will be presented by the primary and secondary measures of adherence. The distributions will be assessed separately in tables (mean (SD)) as well as visualised the distribution of adherence measures.
* Outcomes obtained using WWARN’s standardised outputs will be used to compute the Kaplan-Meier (K-M) estimates, according to the adherence groups calculated for the primary and secondary measures of adherence (3). The risk of first *P. vivax* recurrence (between days 7 and 42 (and for secondary outcomes: between days 28 and 63)), by the adherence groups, will be calculated using K-M survival analysis. The K-M curves will be presented graphically together with the associated tables.
* Cox regression analysis will be performed to estimate the association between adherence and time to the first *P. vivax* recurrence during follow-up (primary analysis is between days 7 and 42 (secondary analyses for days 28 and 63 follow-up), with a random intercept for study-site. Random intercept will be addressed with shared frailty models which are used in the Cox regression model to account for the unobserved heterogeneity in patients hazard to the rate of recurrence (11,12). The analysis will control for potential confounders including age, sex, baseline parasitemia, schizontocidal treatment (Chloroquine/ACTs), and regional relapse periodically. Body weight of the patient will not be included as a confounder due to collinearity with age. Collinearity between relapse periodicity and geographical region and parasite prevalence will be measured. Additional covariates will be examined, including vomiting, baseline temperature and haemoglobin.
	+ 1. ***Identify the key patient factors that contribute to imperfect adherence***
* The association between imperfect adherence (the primary and secondary adherence measures are ordinal variables with 5 groupings, see section 2.3.3) as an outcome and key patient factors involving age, sex, weight, history of malaria, nutritional status, different schizontocidal treatments, and G6PD status will be analysed by ordinal logistic regression, with study site included as a random effect. Other exploratory factors will be examined for potential effects including vomiting, fever, and anaemia status.
	+ 1. ***Sensitivity analysis***
* The coefficient of variation will be calculated for estimates of the primary analysis (section 2.5.3) by removal of one study site at a time to assess heterogeneity of study sites. The coefficient of variation around parameter estimates will be calculated to compare with the absolute variation of the coefficients in regression models.
* Additionally, baseline characteristics of the included studies will be compared with targeted studies that were not included.
	+ 1. ***Risk of bias***

We will consider the risk of bias which is a key component of checking the quality of included studies in systematic review using Individual Participant Data (IPD) meta-analysis methods(13). To investigate inclusion bias, baseline characteristics of targeted studies not included in the analysis will be compared with the characteristics of the studies included in the analysis.

* Data supplied for included RCTs will be checked for missing data; internal data consistency; follow up and censoring pattern.
* Summary tables will be checked with the trial protocol and latest trial report or publication. Any discrepancies or unusual patterns will be checked with the study investigator. A final copy of the form from each trial will be returned to the appropriate trial investigator for verification.
* A summary of included studies compared to potential studies will be presented to highlight potential selection bias.
* A summary of the included studies will be presented, including (but not restricted to) antimalarial treatment and description of location by country, transmission site(s), and regional relapse periodicity.
* As part of the statistical analysis a sensitivity analysis will be undertaken to identify bias related to individual studies.

# 4. PRISMA Statement

The analysis will adhere to the PRISMA guidelines for reporting systematic reviews and meta-analyses of individual patient data(14).

# 5. Tools

All statistical analyses will be carried out using R version 3.6.0.

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

The **Vivax Adherence 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. Parinaz Mehdipour, Professor Julie Simpson, Dr Saber Dini, Dr Sophie Zaloumis, Dr Robert Commons and the WWARN statistician(s) will oversee the statistical analyses. The Study Group collectively makes decisions with respect to including additional studies, and plans for publication. Participating investigators will be recognized in publication as contributors under the banner of the **Vivax Adherence Study Group**. The Study Group will assign a Writing Committee to coordinate activities including data analysis, and drafting of publications and reports for complete group review. The Writing Committee will comprise a group of interested investigators undertaking the data analysis and preparation of the manuscript. Authors will be recognized according to the ICMJE guidelines and the WWARN publication policy(15).

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