Statistical Analysis Plan
WWARN Vivax after *falciparum* study group: Recurrence of *Plasmodium vivax* parasitaemia after *P. falciparum* infection: An individual patient data pooled analysis
Version 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

Recurrent *Plasmodium vivax* parasitaemia increases morbidity and the risk of ongoing transmission of malaria. In Thailand, there is an increased risk of *P. vivax* parasitaemia after treatment of *P. falciparum* infection(1). This is hypothesized to be due to patients with falciparum malaria having occult *P. vivax* infection with the dormant liver stages (hypnozoites) being reactivated by acute malaria.

The risk of *P. vivax* after *P. falciparum* is likely to vary between regions. Dormant liver stages of *P. vivax* lead to relapse and the pattern of relapse varies with geographical region(2). The timing and frequency of relapse varies from weeks to months(3).

Chloroquine (CQ) is currently the first-line treatment for *P. vivax* infection, but in areas where CQ resistance is prevalent the World Health Organization recommends artemisinin-based combination therapy (ACT). In order to reduce the risk of *P. vivax* following *P. falciparum*, there may be a benefit in providing universal radical cure with an ACT and primaquine to patients infected with either *P. vivax* or *P. falciparum* in some co-endemic regions.

1.1. Aim of the study

Evaluate the risk of *P. vivax* parasitaemia after *P. falciparum* infection up to day 28, 42 or 63 and to identify risk factors.

1.2 The specific objectives of the study are:

1. To assess the risk of *P. vivax* parasitaemia up to day 63 following *P. falciparum* infection and compare this to the risk of *P. falciparum* recurrence

2. To quantify and compare the temporal distribution of *P. vivax* and *P. falciparum* recurrent parasitaemia

3. To identify the risk factors associated with *P. vivax* parasitaemia following the treatment of *P. falciparum* infection

4. To compare the risk of *P. vivax* parasitaemia following *P. falciparum* infection with the expected background risk of *P. vivax* infection by location

1.3. Eligibility criteria for inclusion in pooled analysis

1.3.1 Essential inclusion criteria

- Prospective therapeutic efficacy trials of uncomplicated *P. falciparum* infection (including monoinfection and mixed *P. falciparum* and *P. vivax* infection)
- Study undertaken in a location co-endemic for *P. vivax* and *P. falciparum*

- Treatment with currently recommended artemisinin combination therapy (ACT):
    - artemether-lumefantrine,
    - dihydroartemisinin-piperaquine,
    - artesunate-mefloquine, or
    - artesunate-amodiaquine.
- Record of at least one patient with *P. vivax* parasitaemia during the study
- Study observation period ≥28 days
- Available data on the patient's age and gender
- Documented day of recurrence of all species of malaria
- Study meta-data as described in the Data Management and Statistical Analysis Plan

1.3.2 Desirable criteria

- Baseline parasitaemia
- Baseline gametocytaemia
- Haemoglobin or haematocrit measured on day 0
- Genotyping to potentially distinguish recrudescence and reinfection
- mg/kg dosing
- Malnutrition as gauged by weight and age +/- height or middle upper arm circumference (MUAC)

1.3.3 Exclusion criteria

- Severe or complicated malaria
- Pregnant women
- Studies enrolling travellers, healthy volunteers, soldiers or prisoners where patients were infected with malaria in diverse regional locations

1.4. Identification of relevant studies and data acquisition

A systematic review will be carried out for all prospective clinical trials involving *P. falciparum* mono/mixed-infection at baseline that include a *P. vivax* infection during ensuing follow up. Trials which fulfil our study inclusion and exclusion criteria will be targeted through direct email to the corresponding author and/or principal investigator. Data which are not published 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 (4) for clinical data and pooled into a single database of quality-assured individual patient data.

2. Study end-points and statistical analyses

2.1 Study endpoints

Primary:
P. vivax parasitaemia between day 7 and day 28, 42 or 63

Secondary:
P. falciparum recurrence between day 7 and day 28, 42 or 63

2.2 Definitions

Primary

P. vivax parasitaemia before day X is defined as any recurrence of *P. vivax* parasitaemia between day 7 and X

Secondary
P. falciparum recurrence before day X is defined as any recurrence of *P. falciparum* parasitaemia between day 7 and X

2.3 Study and patient characteristics

The following baseline characteristics will be summarised:

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 (>37.5^{0}C axillary)

Drug: schizontocidal treatment 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 <15 years with childhood age stratified into <5 years and 5 to <15 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 the WHO (5). 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, as suggested by the WHO (5).

Treatment will be classified as supervised if all doses were directly observed, partial-planned supervision if at least the morning doses of a bi-daily regimen were observed, partial-unplanned supervision if all doses of a daily regimen were not observed and not-supervised if no doses were observed.

The doses of treatment 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. For each component, a total dose per weight will be calculated for each patient for the artemisinin components and for the partner drug components.

In studies with haematocrit measured instead of haemoglobin, haematocrit will be converted to haemoglobin using the following relationship (6):

Haematocrit (ht) = 5.62 + 2.60 * 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 (7). 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.
b) *Low (long) and high (short) periodicity of relapses* according to Battle's regions (8). 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".

In addition the entomological inoculation rate will be ascertained for each study site from updated Malaria Atlas Project estimates. These estimates will be linked to year patient enrolled in addition to location of study site.

2.4 Summary of statistical analyses

2.4.1 Description and baseline characteristics of study sample:

- A summary (study profile) of the relevant trials uploaded to the WWARN repository will be presented and compared against the available studies identified by the literature search in Section 1.4 to highlight potential selection bias.

- 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) and regional relapse periodicity. Tests of statistical significance

will not be undertaken for baseline characteristics; rather the clinical importance of any differences in the baseline distributions between studies will be noted.

2.4.2 Baseline characteristics of patients:

- A summary of relevant baseline patient characteristics will be presented including age, gender, malnutrition, treatment given, treatment supervision, haemoglobin concentration, asexual parasitaemia, gametocytaemia, temperature >37.5°C.

- 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.

2.4.3 Statistical Analyses

2.4.3.1 Quantifying the cumulative risk of PV recurrence within 28/42/63 days

- Outcomes obtained using WWARN's standardised outputs will be used to compute the **Kaplan-Meier (K-M) estimates** for the dosing groups (4). The risk of recurrence will be calculated as the complement of the derived K-M estimates (1 – KM) and this will be presented graphically together with the associated tables of number of patients in the risk-set.

- Log rank test, which compares the equality of two K-M survival curves, will be performed by stratifying by study sites to test if the K-M profiles are significantly different from each other. The estimates of cumulative incidence of Pv recurrence will be compared using the Gray's *k*-sample test. For the log-rank test, all other causes of recurrence will be censored whereas for Gray's *k*-sample test, all the causes of recurrence will be kept as competing endpoints.

2.4.3.2 Identifying the risk factors for Pv parasitaemia within 28/42/63 days

- **Cox regression analysis** for time to recurrence during follow-up (28 or 42 or 63 days) will be performed, with a random intercept for study-site through the use of shared frailty models. Risk factors of interest to be assessed include age, weight, sex, baseline parasitaemia, relapse periodicity, geographical region, drug elimination half-life, drug type, baseline haemoglobin, baseline gametocytaemia and mg/kg drug dose. Additional confounders considered for inclusion in the model are parasite prevalence, treatment supervision, vomiting, and baseline temperature. Collinearity between weight and age will be examined, as will collinearity between relapse periodicity and geographical region and parasite prevalence. Due to less data and less expected impact as confounders, some

variables such as baseline haemoglobin, baseline gametocytemia and temperature may not be included in the final multivariable model.

The assumption of proportionality will be tested using Schoenfeld's residuals and Therneau-Grambsch test (through the use of cox.zph function in R survival library) for the final multivariable model and any violation of the assumption will be reported. The functional form of the covariates will be assessed using Martingale's residuals. The overall fit of the model will be assessed using Cox-Snell's residuals.

- The median time to presentation with recurrent Pv and Pf parasitaemia will be presented and compared. Potential confounders including age, sex, weight, baseline parasitaemia, relapse periodicity, geographical region, parasite prevalence, drug elimination half-life, drug type, baseline haemoglobin, baseline gametocytemia, treatment supervision, vomiting, mg/kg drug dose and baseline temperature will be assessed.

2.4.3.3 Comparison of the risk of P. vivax parasitaemia to the expected entomological inoculation rate

- **A geospatial model** will be developed comparing the relative risk of *P. vivax* following P. falciparum to the background entomological inoculation rate. Confounders from the final multivariable model in section 2.4.3.2 will be included.

3. Sensitivity analyses

3.1 Removing data from each study site at a time

Regression models will be carried out by removing data from each site at a time to assess the influence of any given site on the derived estimates. The results of this analysis will be presented as coefficient of variation (CV) for each of the regression parameters in the final multivariable models.

4. PRISMA Statement

The analysis will adhere to the PRISMA guidelines for reporting systematic reviews and meta-analyses of individual patient data (9).

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, Publication and Ownership Policy

The Study Group comprises participating investigators who contribute relevant data sets to pooled analysis and technical experts. Datasets remain the property of the investigator. More details about sharing data with WWARN and how WWARN will use data are available on the WWARN website. Prof Ric Price (ric.price@wwarn.org) is Study Group leader, with Mohammad Sharif Hossain (sharif.hossain@wwarn.org) and Robert Commons (rob.commons@wwarn.org) as study coordinators.

The WWARN statisticians will oversee the statistical analyses. Participating investigators will be recognised in publication as contributors under the banner of the **Vivax After Falciparum Study Group**. A Writing Committee will be formed and coordinate activities including data analysis and drafting of publications and reports for complete group review and this committee will be 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 (10).

7. References

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8. Annex

8.1 List of available covariates Description

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
Haemoglobin/hematocrit at inclusion	Baseline Variable
Parasite density at Inclusion	Baseline Variable
Gametocytes (/µL) at inclusion	Baseline Variable
Max Temp Day0	Baseline Variable
Presence of mixed infection	Baseline variablV
Transmission intensity	Available Variable
Relapse periodicity region	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
Parasite density at Inclusion	Available Variable
Max falciparum asexual parasitaemia on Day1	Available Variable
Max falciparum asexual sexual parasitaemia on Day2	Available Variable
Max falciparum asexual sexual parasitaemia 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