Statistical Analysis Plan
WWARN Vivax Haematology study group: A pooled analysis of haematological recovery after treatment of *Plasmodium vivax*
Version 1.2

WorldWide Antimalarial Resistance Network (WWARN)

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Version History

Version number	Revision(s) & reason for amendment	Release date
V1.1		
V1.2	Following collation and cleaning of data the analysis plan was updated. - Statistical methodology updated to include linear mixed effects modelling with non-linear terms for time. - Definitions of relapse periodicity and prevalence updated - Clinically relevant safety outcomes updated	25/05/17

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1. Contents

2. Introduction and Rationale

A significant proportion of the morbidity and mortality attributed to *P. vivax* is a consequence of severe anaemia. With each recurrence of *P. vivax* parasitaemia there is a cumulative risk of anaemia (1). Malaria-associated anaemia is a complex phenomenon, related to increased red cell destruction and haemopoeitic suppression, compounded by nutritional status, helminth carriage and drug induced haemolysis. The degree of anaemia caused by vivax malaria and its risk factors has not been evaluated widely [1].

Control and ultimate elimination of *P. vivax* requires the safe and effective prevention of recurrent infections and this requires radical cure targeting both blood and liver stages of the parasite. However, the only widely available drug that is active against the liver stages is primaquine, an 8-aminoquinoline drug that can cause drug-induced haemolysis particularly in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In order to quantify the risks and benefits of *P. vivax* radical cure,

it is crucial to determine the normal haematological response following *P. vivax* infection and treatment.

3. Aim of the study

The aim of this study is to investigate the acute haematological effect of *Plasmodium vivax* parasitaemia and its subsequent recovery following treatment.

4. Eligibility criteria

4.3.1 *Essential inclusion criteria*

- Prospective clinical efficacy studies of uncomplicated vivax monoinfection
- Asexual parasitaemia at enrolment
- Haemoglobin (hb) or haematocrit (hct) at enrolment
- Study meta-data as described in the Clinical Data Management and Statistical Analysis Plan (2)
- Baseline data on patient age and gender

4.3.2 *Desirable criteria*

- Information on the timing and dose of drug administration.
- Exact mg/kg dosing of schizontocidal and primaquine administration
- Weight of the patient
- Information on splenomegaly, hepatomegaly
- Malnutrition as gauged by weight and age or height, or Middle Upper Arm Circumference (MUAC)
- Qualitative or quantitative assessment of G6PD status
- Parasite density at days 1, 2, 3
- Outcome of malaria treatment according to standardised WHO criteria (3)
- Information on previous vivax episodes

4.3.3 *Exclusion criteria*

- Pregnancy

5. Data Pooling

A systematic review of all prospective clinical efficacy trials involving *Plasmodium vivax* mono-infection has been completed. Trials undertaken since the year 2000 that fulfil the study criteria have been 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 (2) for clinical data and pooled into a single database of quality-assured individual patient data.

6. Outline of Statistical Analysis

6.1. Specific objectives of the study

- To quantify the acute reduction in haemoglobin associated with acute vivax malaria before and after treatment
- To identify risk factors associated with anaemia at the start of treatment for acute vivax malaria.
- To identify risk factors associated with the development of anaemia during the follow-up phase of vivax malaria (up to 28 or 42 days), with and without primaquine
- To estimate the time to recovery from anaemia after administration of chloroquine and compare this with non-chloroquine treatments
- To quantify the effect of primaquine mg/kg dose on haemoglobin reduction and time to anaemia recovery.

6.2. Study endpoints

Overall haematological profile:

- Mean haemoglobin at days 0, 1, 2, 3, 7, 14, 28, 35 and 42
- Predicted normal haemoglobin level and time to predicted normal haemoglobin level

At Enrolment:

1. Mean haemoglobin
2. Presence of anaemia (Hb<10g/dL) or severe anaemia (Hb<7g/dL)
3. Fractional or absolute reduction in haemoglobin at enrolment evaluated against the patients' predicted normal haemoglobin level (NHL) in children and adults

After treatment

4. Risk of anaemia or severe anaemia within 28 days / 42 days
5. Risk of clinically relevant anaemia (fall in Hb >25% to <7 g/dL or fall >5 g/dL)
6. Time to haemoglobin nadir
7. Maximal fractional or absolute change in haemoglobin from baseline
8. Fractional change or absolute change in haemoglobin at baseline compared to the NHL (Malaria attributable fall before treatment)
9. Maximal fractional change or absolute change in haemoglobin at the nadir compared to the NHL (Total malaria attributable fall in haematocrit).
10. Time to haematological recovery

6.3 Definitions of Endpoints

Absolute reduction in haemoglobin between times t_1 and t_2 will be defined as $hb(t_2)- hb(t_1)$.

Fractional reduction in haemoglobin between times t_1 and t_2 will be defined as $(hb(t_2)- hb(t_1))/hb(t_1)$, or where $hb(t_i)$ denotes measured or estimated haemoglobin at time t_i.

Normal haemoglobin level (NHL) will be derived from data from this pooled analysis based on the shape of population haemoglobin curves over time for age, sex and region. Only data from patients who do not have a recurrence before day 42 will be included.

Duration of haematological recovery will be defined as the time from enrolment to the first time of reaching haemoglobin ≥10 g/dl after any documentation of anaemia within the first 7 days of presentation.

Time of haemoglobin nadir will be defined as the time when the minimum haemoglobin was recorded, provided measurements were available on all days 0, 3, 7, 14, 21 and 28.

6.4. Study and patient characteristics

The following baseline characteristics will be included in the analysis:

Site: transmission intensity, regional relapse periodicity, chloroquine resistance

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

Drug: schizontocidal treatment and mg/kg dose, primaquine treatment, timing and mg/kg dose, supervision of drug intake (full or partial), co-administration with fat, dose vomiting

Laboratory: baseline parasitaemia, baseline gametocytes

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 WHO (4). 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 3 morning doses were observed, and not-supervised if fewer doses were observed.

Total doses will be calculated from the recorded number of tablets administered per dose if this information is available in the individual patient data. If no individual patient dosing data was available, dose will be estimated using the protocol dosing schedule.

In studies with haematocrit measured instead of haemoglobin, haematocrit will be converted to haemoglobin using the following relationship (5):

$$Haematocrit\ (ht) = 5.62 + 2.60 * Haemoglobin\ (hb)$$

Anaemia will be defined as haemoglobin <10 g/dL with severe anaemia <7 g/dL.

WHO definitions of efficacy outcome will be used (3).

Patients early parasitological response will also be evaluated in the form of (a) estimated parasite clearance half life using the WWARN PCE tool (7); (b) positivity on Day 1; (c) positivity on Day 2.

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 < 0.015 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 (9), 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".

6.5 Summary of statistical analyses

Descriptive statistics 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, use of haematinic agents and method of haemoglobin testing.
3. The baseline characteristics of the eligible studies will be described by country, transmission site(s), regional relapse periodicity, chloroquine resistance and treatment regimens. 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.

   The distribution of continuous variables (e.g. haemoglobin, age, parasitaemia) 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.

Description of overall haematological profiles:

4. The Hb-time response will be estimated using a linear mixed effects model with non-linear terms, derived by fractional polynomial regression. Fixed effects for age, gender, baseline parasitaemia, total schizontocidal dose (mg/kg), relapse periodicity, PQ use and study site will be included. The interaction between PQ use and time will be included in order to capture the different time-course of Hb responses following the regimens with and without PQ. Day of

nadir will be determined. Differences with and without PQ will be determined for day of nadir, day 7 and day 42.

5. Separate analyses will be performed for gender as well as G6PD status.
6. The haemoglobin-time response in the subgroup of patients treated with PQ will be similarly estimated using a linear mixed effects model with non-linear terms to determine the effect of mg/kg dose of PQ on haemoglobin response. Fixed effects for age, gender, baseline parasitaemia, total schizontocidal dose (mg/kg), relapse periodicity, PQ dose and study site will be included. The interaction between PQ dose and time will be included in order to capture the different time-course of Hb responses following regimens with high and low daily dose PQ.
7. Normal haemoglobin levels

8. To determine the effect of delayed parasite clearance (cleared after day 2), the haemoglobin-time response will be similarly estimated for patients with and without delayed parasite clearance. Fixed effects for age, gender, baseline parasitaemia, total schizontocidal dose (mg/kg), relapse periodicity, PQ use, delayed parasite clearance and study site will be included. The interaction between delayed parasite clearance and time will be included.
9. To determine the effect of recurrent parasitaemia between day 7 and 42, the haemoglobin-time response will be similarly estimated for patients with and without recurrent parasitaemia. Fixed effects for age, gender, baseline parasitaemia, total schizontocidal dose (mg/kg), relapse periodicity, PQ use, recurrent parasitaemia and study site will be included. The interaction between recurrent parasitaemia and time will be included.
10. A description of safety outcomes will be presented including number and percentage of patients with reductions in haemoglobin >25%, number and percentage of patients with reductions >25% to <7 g/dL and number and percentage of patients with reductions >5 g/dL. Day of nadir, day 7 and day 28 will be examined specifically.

At Enrolment (before treatment):

11. Anaemia at enrolment

Univariable and multivariable logistic regression of risk factors for anaemia on enrolment will be performed, with random effects for study-site. Risk of anaemia will be controlled for potential confounders including age, sex, baseline parasitaemia, relapse periodicity and G6PD status. Weight will not be included due to collinearity with age. Collinearity between relapse periodicity and geographical region and parasite prevalence will be examined. Additional covariates will be examined including level of treatment supervision, vomiting, baseline temperature.

12. Fractional or absolute reduction in haemoglobin at enrolment evaluated against the patients predicted normal haemoglobin level (NHL)

Linear regression for fractional (absolute) reduction in haemoglobin on enrolment will be performed, with a random intercept for study-site. Reduction in haemoglobin will be controlled for potential confounders including age, sex, baseline parasitaemia, relapse periodicity, baseline haemoglobin, primaquine treatment and timing and G6PD status. Weight will not be included due to collinearity with age. Collinearity between relapse periodicity and geographical region and parasite prevalence

will be examined. Additional covariates will be examined including level of treatment supervision, vomiting, baseline temperature.

During Follow Up (After Treatment):

13. Risk of anaemia within 28 days / 42 days

Survival analysis for time to anaemia during follow-up (28 or 42 days) will be performed, with random effects for study-site. Potential confounders will be controlled for including age, sex, baseline parasitaemia, relapse periodicity, baseline haemoglobin, primaquine treatment and timing, recurrent infection and G6PD status. Only patients without anaemia on enrollment will be included. Weight will not be included due to collinearity with age. Collinearity between relapse periodicity and geographical region and parasite prevalence will be examined. Additional covariates will be examined including level of treatment supervision, vomiting, baseline temperature, prevalence of parasitaemia on days 1 and 2, weight, nutritional status, G6PD status, history of malaria within the past 28 days, history of fever, presence of gametocyte on enrolment, and recurrent infection.

14. Fractional or absolute reduction in haemoglobin during follow-up evaluated against the baseline haemoglobin and against the NHL

Linear regression for fractional (absolute) reduction in haemoglobin during follow-up will be performed, with a random intercept for study-site. Potential confounders will be controlled for including age, sex, baseline parasitaemia, relapse periodicity, baseline haemoglobin, primaquine treatment and timing, recurrent infection and G6PD status. Additional covariates will be examined including level of treatment supervision, vomiting, baseline temperature, prevalence of parasitaemia on days 1 and 2, weight, nutritional status, G6PD status, history of malaria within the past 28 days, history of fever, presence of gametocyte on enrolment, and recurrent infection.

15. Maximum Fractional fall in haemoglobin after treatment evaluated against the baseline haemoglobin and against the NHL

Linear regression for maximum fractional reduction in haemoglobin from enrolment and from predicted NHL during follow-up will be performed, with a random intercept for study-site. Potential confounders will be controlled for including age, sex, baseline parasitaemia, relapse periodicity, baseline haemoglobin, primaquine treatment and timing, recurrent infection and G6PD status. Additional covariates will be examined including level of treatment supervision, vomiting, baseline temperature, prevalence of parasitaemia on days 1 and 2, weight, nutritional status, G6PD status, history of malaria within the past 28 days, history of fever, presence of gametocyte on enrolment, and recurrent infection.

Figure of mean (95% confidence interval) fractional change in haemoglobin from enrolment during follow-up will be presented according to treatment group, age categories, transmission intensity, baseline parasitaemia, baseline haemoglobin, day 1 clearance and day 2 clearance.

Figure of mean (95% confidence interval) fractional change in haemoglobin from the predicted NHL at enrolment and after treatment will be presented according to treatment group, age categories, transmission intensity, baseline parasitaemia, baseline haemoglobin, day 1 clearance and day 2 clearance.

Figure of mean (95% confidence interval) fractional change in haemoglobin from the predicted NHL at enrolment and after treatment will be presented according to mg/kg dose of chloroquine, other schizontocidal agents (if numbers allow) and primaquine.

16. Time to haematological recovery

Haematological recovery will be defined in a subgroup of patients with clinically relevant anaemia (Hb<10 g/dl) at enrolment or within the first week of treatment, and the first occurrence of Hb above this level. Survival analysis for time to haematological recovery will be performed, with random effects for study-site. Potential confounders will be controlled for including age, sex, baseline parasitaemia, relapse periodicity, baseline haemoglobin, primaquine treatment and timing, recurrent infection and G6PD status. Additional covariates will be examined including level of treatment supervision, vomiting, baseline temperature, prevalence of parasitaemia on days 1 and 2, weight, nutritional status, G6PD status, history of malaria within the past 28 days, history of fever, presence of gametocyte on enrolment, and recurrent infection.

17. Sensitivity analysis

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.

7 Tools

All statistical analyses will be carried out using Stata version 13.0 and R 3.1.0 released on 2014-04-10 by The R Foundation for Statistical Computing. However, when equivalent statistical methods are applied, changing the use of statistical software does not require amendment of this SAP.

8 Study Group Governance, Management, Coordination and Publication Policy

The Vivax Anaemia 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 Anaemia 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 statistician Kasia Stepniewska 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 (10).

9 Potential Policy Outcome

The data provided by this analysis will be used to inform policy makers and research on the relative risks of anaemia, and the comparative risks and benefits of alternative treatment options including primaquine for radical cure.

10 References

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11 Annex 1

A.1 List of available covariates

Description	Type
Patent parasitaemia on day1, day2 and day3	Primary Response
Parasite Clearance on day 1 and day 2	Secondary Response
History of Fever (0/1) at inclusion within 24 hours	Baseline Variable
Haemoglobin at inclusion	Baseline Variable
Vivax density at Inclusion	Baseline Variable
Gam (/µL) at inclusion	Baseline Variable
Max Temp Day0	Available Variable
D0 Ht<20%	Baseline Variable
Age in Years	Baseline Variable
Gender	Baseline Variable
Weight	Available Variable
G6PD status	Available Variable
Antimalarial in last 28 days	Available Variable
Transmission intensity	Available Variable
Relapse periodicity region	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 Temp Day1	Available Variable
Max Temp Day2	Available Variable
Max Temp Day3	Available Variable
Dosing method (single day, broken down over days etc.)	Available Variable
Drug timing (eg primaquine)	Available variable
Total mg/kg dose at each day of dosing regimen	Available Variable
Total mg/kg dose during course	Available Variable