Statistical Analysis Plan

Low-Dose Primaquine Efficacy Study Group

Version 1.0

WorldWide Antimalarial Resistance Network (WWARN)

Suggested citation: Statistical Analysis Plan, Low-dose Primaquine Efficacy Study Group: Pooled analyses of the efficacy of single low-dose primaquine to interrupt P. falciparum malaria transmission.

Version History

Version number	Revision(s) & reason for amendment	Release date
1.0	**Final version approved by SG members**	**25.08.2016**

WorldWide Antimalarial Resistance Network (WWARN)

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Table of Contents

1. Introduction 4
2. Specific objectives of the study 5
3. Efficacy Endpoints 6
4. Covariates Examined 7
5. Methodologies 7
6. Outline of Statistical Analysis 8
7. Statistical Methodology 10
8. Tools 12
9. References 15

1. Introduction

Primaquine is the only commercially available drug that can clear mature P. falciparum gametocytes, the parasite lifecycle stage responsible for the transmission of malaria from the human to the mosquito (White 2013). Primaquine can cause hemolysis in some individuals with an inherited enzyme deficiency, glucose-6-phosphate dehydrogenase (G6PD) deficiency (von Seidlein et al. 2013). The level of hemolysis experienced by an individual depends primarily on the dose of primaquine used and G6PD enzyme level in that individual (Eziefula et al. 2014). Other safety and tolerability issues with primaquine include gastrointestinal upset and methaemoglobinaemia (Ashley et al, 2014).

In 2012, in areas facing artemisinin resistance or approaching malaria elimination, the WHO recommended the addition of a single low (0.25 mg/kg) dose of primaquine to an artemisinin-based combination treatment (ACT), without G6PD testing (WHO 2012). This recommendation was based on pooled, unstandardized efficacy studies of primaquine and plasmoquin (its more toxic predecessor) and a variety of different blood schizonticide partner drugs and was further supported by a comprehensive analysis of published literature (Ashley et al, 2014). As no formal dose-finding studies have been conducted, this recommendation, did not come with the statement “strong recommendation, high quality evidence” typical of WHO recommendations for antimalarial use (WHO 2012).

Adoption of the WHO recommendation to add a 0.25 mg/kg dose of primaquine to ACT therapy has been slow (Chen & Gosling 2014). To support the WHO recommendation, stakeholders collaborated to identify the critical gaps and barriers that are preventing the widespread implementation of this approach, and to develop a low-dose primaquine roadmap (link is external). A key priority identified by the roadmap is establishment of the safe, effective therapeutic range of single-dose primaquine, values that would define the lowest efficacious dose and the highest safe dose in G6PD-normal and deficient individuals. Determination of this safe therapeutic range could avoid hemolysis in G6PD-deficient individuals, and guide the establishment of appropriate dosing recommendations in field settings (Chen et al. 2015).

1.1 Aim of the study

This analysis will provide evidence to inform clinical and policy decisions on use of low-dose primaquine as an agent to block the transmission of malaria infection. In particular, the study provide information on efficacy of primaquine as measured by gametocyte carriage and investigate the dose-reponse relationship for primaquine.

1.2 Eligibility criteria for inclusion in pooled analysis

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

- Individual patient data from a clinical efficacy trial of patients with uncomplicated P. falciparum infection containing at least one study arm with a combination of an ACT + single low-dose primaquine
- Patient demographics (including age, gender, weight)
- Weekly follow-up during at least 14 days
- Transmisison potential assessed by weekly gametocyte carriage (i.e. prevalence) and density (microscopy and/or molecular methods) and/or membrane feeding assay. At least data on day 0 and any day of follow-up is required.
- Information on dosing (mg/kg) of the ACTs and primaquine

Desirable Data (not required for inclusion)

- Known G6PD status (qualitative and/or quantitative) of patients treated
- CYP 2D6 phenotype
- Gametocyte density estimates
- Any data on the performance of assays in the membrane feeding experiment

Study Exclusion criteria

- Studies that do not include the use of single-dose primaquine

1.3 Patient Exclusion

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

i. No or missing plasmodium falciparum infection on enrolment
(mixed infections with pf will be included in the analysis)
ii. Missing age and (or) weight and (or) gender

In this analysis only patients who presented with gametocytes on enrolment will be included.

2. Specific objectives of the study

- To quantify the change in gametocyte prevalence and density with time following administration of a single dose of primaquine, given in conjunction with a blood schizonticide partner drug

- To assess the infectivity of gametocytes with time as measured by membrane feeding assay, following administration of a single dose of primaquine, given in conjunction with a blood schizonticide partner drug
- To assess whether age, sex, G6PD status, of CYP 2D6 metabolism phenotype impact the efficacy of primaquine given at single doses ≤0.75 mg/kg

3. Efficacy Endpoints

Primary:

The carriage of gametocytes on follow-up as measured by:

Presence of gametocytes at any time after enrollment

Presence of gametocytes on Day 7

Time to gametocytaemia in patients with no gametocytes on enrollment

Time to gametocyte clearance in patients with gametocytes on enrollment

Secondary:

Infectivity of patients to mosquitoes on Day 0, Day 7 and any other day of follow-up (if data exists) as measured by a proportion of infected mosquitoes in the membrane feeding experiment

Proportion of patients infectious to mosquitoes on Day 0, Day 7 and any other day of follow-up (if data exists) as measured by a propotion of patients with at least one mosquito with at least one oocyst in the membrane feeding experiment

Area under the infectivity time curve within study follow-up

Area under the gametocyte density time curve

Gametocyte sex-ratio. Proportion of gametocytes that is male, determined at all time-points during follow-up.

4. Coariates Examined

- Dose of primaquine administered (mg/kg)
- Gametocyte density on enrolment
- Method of gametocyte measurement
    - Microscopy
    - QT-NASBA
    - RT-PCR
    - Target for gametocyte detection (Pfs25, Pfg377, Pfs230, P230p, etc)
- Age
- Sex
- Baseline parasitaemia
- Fever on enrolment
- G6PD status
- CYP 2D6 metabolism phenotype
- Type and dose of blood schizonticide drug administered (mg/kg)
- Transmission intensity
- Hemoglobin/haematocrit on enrolment
- Anthropometric measures for children (WAZ score)
- Asexual parasite prevalence on day 2 and day 3

5. Methodologies

5.1 Data Pooling

The data sets uploaded to the WWARN repository will be standardized using the WWARN Data Management and Statistical Analysis Plans (DMSAP v1.11) (WWARN, 2012) 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) as per WWARN's Terms of Submission (WWARN, 2013) and publication will be in accordance with an agreed publication plan (WWARN, 2015).

5.2 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 (for methodology see Gething et al. 2011) for specific location and year of study.

5.3 Anthropometric Indicators

Nutritional status would be assessed by using standardised age, weight, height and gender specific growth reference according to the WHO 2006 recommendations using *igrowup* Stata package (WHO 2006). Anthropometric indicators include weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ). The nutritional status of a child will be given as a Z-score and classified as stunted, underweight or wasted as defined in the WHO guidelines.

5.4 Conversion between haemoglobin and haematocrit

Haematocrit will be converted to haemoglobin using the following relationship (Lee etal. 2008), :

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

Anaemia will be defined according to WHO guidelines (WHO 2011)

5.5 Dosing Calculation

The doses of primaquine, artemisinin derivatives and 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. For each component, a total dose per weight will be calculated for each patient.

6. Outline of Statistical Analysis

1. A summary of study profile of all studies uploaded to WWARN repository will be presented, and studies in which a membrane feeding assay is used to assess for infectivity will be ascertained

2. A summary of key study characteristics
3. A summary of patient baseline characteristics
4. Gametocyte carriage at baseline and followup days (0, 7, 14) by ACT and primaquine treatment, subgrouped by type of assessment used for gametocyte carriage (microscopy, RT-PCR, QT-NASBA). These prevalences will be subgrouped for patients with (100% carriage on day 0) and without gametocytes on enrollment (0% carriage on day 0)
5. Survival curves for time to gametocytaemia and time to gametocyte clearance
6. AUC for gametocyte density presented by ACT, primaquine treatment and method of measurement, separately for patients with and without gametocytes on enrolment.

7. A summary graph for efficacy based on gametocytes:
   X axis = dose of primaquine used (regardless of partner drug choice)
   Y axis = gametocyte prevalence on day 7 in gametocyte-positive individuals at enrolment

   And, if feasible:
   X axis = dose of primaquine used (regardless of partner drug choice)
   Y axis = time to reach zero infectivity (= no gametocytes present in any of the patients)

8. For membrane feeding studies: infectivity of gametocytes (% of gametocytemic individuals that infected mosquitoes) versus time (day 0, day 7, and any other days data is available for), presented by primaquine dose for each type of ACT treatment.

9. For membrane feeding studies: prevalence of infectious individuals (infecting >= infected mosquito) versus time (day 0, day 7, and any other data is available for), presented by primaquine dose for each type of ACT treatment

10. For membrane feeding studies: proportion of infected mosquitoes on any day during follow-up relative to baseline (prior to treatment, day 0, day 1), presented by primaquine dose for each type of ACT treatment

11. For membrane feeding studies: graph box of % infected mosquitoes versus time (day 0, day 7, and any other days data is available for), presented by primaquine dose for each type of ACT treatment.

12. Data permitting, a summary graph for efficacy based on membrane feeding:

    X axis = dose of primaquine used (regardless of partner drug choice)

    Y axis = time to reach zero infectivity (= no oocysts present on followup, based on time of membrane feeding)

13. For membrane feeding data: univariate and multivariate logistic regression model for (a) proportion of infected mosquitoes in the membrane feeding experiment and (b) proportion of

patients with at least one mosquito with at least one oocyst in the membrane feeding experiment. Possible risk factors: time since treatment, primaquine dose, ACT, age, initial gametocyte count, gametocyte count at the time of sampling, gametocytes measurement method and sensitivity.

14. Data permiting, univariate and multivariate survival regression models for time to gametocyte clearance will be fitted with random effects for study site, seperately for patients with gametocytes on enrolment and patients who developed gametocytaemia after starting treatment. Possible risk factors: patient baseline characteristics, parasitaemia measures, transmission intensity, primaquine dose, dose and type of ACT, gametocytes measurement method and sensitivity, recrudescence, new infection.

7. Statistical Methodology

7.1 Definitions

Gametocytes on enrollment is be defined as any sexual parasitaemia count/presence within 24hrs of the reading, in patients in whom this was assessed by microscopy (thick film examination), QT-NASBA or RT-PCR.

Gametocyte carriage during follow-up is defined as patent gametocytaemia after enrollment (>24hrs) up to day 14 of follow-up, whilst taking account of reinfection rates, transmission levels, and concurrent asexual parasitaemia results within patients.

The appearance of gametocytes will be defined as gametocyte carriage during study follow-up in patients with no detectable gametocytes present at enrollment (within first 24hrs).

Prevalence of gametocytes during follow-up will be determined on days 7, 14 according to patent gametocytaemia by microscopy QT-NASBA or RT-PCR on each day of observation. Patients with missing counts on that day will be excluded from the analysis, unless a missing count is between two positive counts (it will be assumed to be positive).

The duration of gametocyte carriage during follow-up is defined as the time between the first positive gametocytaemia record and half-way between the last positive record and the subsequent negative record (e.g. last positive record +3.5days). For patients with missing records after a positive record, the duartion of gametocytes carriage is censored at the day of the last record +3.5days. Some examples are given below.

Day	**0**	**7**	**14**	**21**	**28**	**Classification**
Pt1	+	null	+	-	-	Gct carriage 17.5 days
Pt2	+	+	null	null	null	Gct carriage 10.5 days then censored
Pt3	+	-	+	-	-	Gct carriage 17.5 days
Pt4	*+*	-	-	-	*+*	*Will be excluded from the analysis*

Maximum gametocytaemia is defined as the maximum density of gametocytaemia recorded over the whole follow-up period. Only patients with all available counts will be included.

The area under the gametocyte density time curve (AUC) will be calculated using trapezoid rule and the actual gametocyte counts at days 0,7,14 and any later follow-up data. Patients with all counts available and patients with a missing count between two positive counts (the missing count, level of gamtocytaemia will be interpolated from the two neighbouring values using simple regression) will be be included in this analysis.

Infectioussness prevalence is defined as proportion of individuals infecting at least one mosquito.

As a measure reflecting **overall infectivity of an individual**, a range of methods will be explored including the area under the infectivity curve (AUIC) of the probability of infecting a biting female

anopheleine mosquito and time estimated (Stepniewska et al. 2008, Bousema et al. 2012, Churcher et al. 2013).

7.2. Descriptive statistics

Descriptive statistics will use the mean and SD if data re normally distrubited, geometric mean and range if data are log-normally distributed (as assessed by Shapiro-Wilk test), and as median and range otherwise.

7.3. Survival regression models

Survival models will be used to investigate two outcomes: duration of gametocyte carriage and duration of infectivity.

Random effects in the form of frailty parameters will be used to adjust for study-site effect (Glidden and Vittinghoff 2004). A Cox 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 (Collett 2003). In the Cox regression model, the proportional hazard assumption will be tested based on Schoenfeld residuals(Schoenfeld 1982). Except for key variables included in all models as specified in section 6, 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.

7.4. Model selection for risk factors

For any regression model, the following strategy will be employed to determine independent risk factors. Initially all possible risk factors will be examined in the univariate model, and will be included in model building in the the multivariate analysis. Model with known confounders will be fitted first (asexual parasite density, age in categories, transmission intensity baseline model). Variables and covariates will then be added to the baseline model in a stepwise forward fashion 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 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.

The relationship between outcome and gametocyte density will be examined using factional polynomials.

8. 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 13.1* (StataCorp, 4905 Lakeway Drive, College Station, Texas

77845 USA). Nutritional status will be computed using *Stata 13.1.* Using alternative statistical software does not require amendment of this SAP.

Table 1. Gametocyte-related variables extracted from study data

Variable	Description
Gametocytes count	
sid	study id
pid	patient id
date	date of measurement
time	time of measurement
days since treatment	
method of measurement	microscopy / QT-NASBA/ PCR
detection limit	
count (per microlitre)	
species	
Memberane feeding	
sid	study id
pid	patient id
date blood sample taken	
time blood sample taken	
days since treatment	
duration of feeding (min)	
number of mosquitoes used	
number of mosquitoes fed	
are unfed mosquitoes discarded	
time to dissection (days)	
number of mosquitoes surviving until dissection	
number of mosquitoes examined	
number of infected msoquitoes	
number of oocysts per infected mosquito	

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