Statistical Analysis Plan
WWARN Haematology study group: A pooled analysis of haematological recovery after treatment with an ACT for *Plasmodium falciparum*
Version 19.07.2018

WorldWide Antimalarial Resistance Network (WWARN)

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Version History

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1. Introduction and Rationale

Artemisinin-based combination therapies (ACTs) have been adopted as first-line antimalarial treatment in most malaria endemic countries, and have now become the mainstay for uncomplicated malaria treatment [1]. ACTs have demonstrated high cure rates and because of their fast mode of action, achieve rapid parasite clearance [2]. Malaria-associated anaemia is a complex phenomenon, related to increased red cell destruction and haemopoeitic suppression[3], compounded by nutritional status and helminth carriage [4]. Different ACTs may produce a different response to anemia but quantification of antimalarial-drug attributable fall in haemoglobin level following treatment of *Plasmodium falciparum* infections with different ACTs has not been evaluated widely. Recent reports have described variable reductions in haemoglobin during recovery after treatment with different ACTs [5]. There have also been reports of rebound anaemia following the use of artesunate monotherapy for severe malaria which have been attributed to the effect of artesunate on bone marrow. An understanding of the normal haematological response and recovery following the treatment of uncomplicated malaria is crucial if one is quantifying the risks and benefits of different treatment options of both ACTs and other antimalarials such as single dose primaquine.

2. Aim of the study

The aim of this study is to investigate the reduction in heamoglobin and hematological recovery in *Plasmodium falciparum* infection.

3. Eligibility criteria for inclusion in the pooled analysis

- Clinical efficacy studies of uncomplicated falciparum malaria:
    - patients treated with either an ACT or non-ACT Regimen
    - haemoglobin (hb) or hematocrit (hct) measured on day 0
- Study meta-data as described in the Clinical Data Management and Statistical Analysis Plan
- Baseline data on patient age and gender

3.1 Desirable criteria –but not required for inclusion

- Hb or hct during follow up
- PCR genotyping results
- Mg/kg dosing
- Weight of the patient
- Information on splenomegaly and hepatomegaly
- Malnutrition as gauged by weight and age +/- height
- Information on measurement of Hb or Hct

3.2 Exclusion criteria

- Studies in pregnant population

4. Outline of Statistical Analysis

4.1. Specific objectives of the study

- To quantify the reduction in haemoglobin associated with acute uncomplicated malaria before and after treatment
- To assess independent risk factors associated with anaemia at presentation
- To assess risk factors associated with the development of anaemia during follow up (including day 3 and 7 and within 28 and 42 days)
- To assess the time to anaemia recovery after administration of different ACTs
- To assess the additional effect of single dose primaquine in patients receiving early primaquine treatment.

4.2. Study endpoints

At Enrollment

- Mean haemoglobin at enrollment
- Anaemia at enrollment

After treatment

- Risk of anaemia at day 3 and 7 and within 28 days / 42 days
- Risk of large fractional fall in haemoglobin (>25% from baseline) on day 3 or day 7 after treatment
- Absolute reduction in haemoglobin during follow-up evaluated against the enrollment haemoglobin Day of nadir in haemoglobin
- Time to hematological recovery Mean haemoglobin level during treatment and follow-up time

4.3. Definitions of Endpoints

Anemia will be classified as moderately severe if haemoglobin level is <7 g/dL and as moderate if haemoglobin level is ≥7 g/dL and <10 g/dL.

Absolute change in hemoglobin between times t_1 and t_2 will be defined as $hb(t_2)- hb(t_1)$;
fractional change in haemoglobin between times t_1 and t_2 will be defined as $(hb(t_2)- hb(t_1))/hb(t_1)$, where $hb(t_i)$ denotes measured or estimated haemoglobin at time t_i.

A large reduction in haemoglobin at time t will be defined as fractional reduction in heamoglobin, compared to baseline value, greater than 25%, i.e. $(hb(t)- hb(t_0))/hb(t_0) < -0.25$

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

Time of nadir of haemoglobin will be defined as the time when the minimum haemoglobin was recorded.

4.4. Study and patient characteristics

The following baseline characteristics will be included in the analysis:

Site: transmission intensity
Patient: age, sex, weight, nutritional status, past history of malaria, history of fever
Drug: artemisisin derivative and its dose, partner drug and its dose, supervision of drug intake (full or partial), co-administration with fat, dose vomiting, and date of admission, primaquine treatment and dose at the start of the study (days 0-3).
Laboratory: baseline parasitaemia, species (Pf versus Pf mixed infection)

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 [6]. 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 artemisinin component and partner drug 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 [7]:

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

Anaemia will be defined according to WHO guidelines [8], based on measured or estimated haemoglobin values.

WHO definitions of efficacy outcome will be used [9]
For each patient, early parasitological response will also be evaluated in the form of (a) parasite half life estimated by WWARN PCE tool [10]; (b) positivity on Day 2; (c) positivity on Day 3; delayed parasite clearance (positivity after day 2); (e) parasite half life estimated from daily counts, depending on the available data (WWARN Parasite Clearance Study Group).

For each study, study locations/sites will be recorded. Each location will be categorised into (a) low, moderate and high transmission settings based on the observed study site PCR confirmed reinfection rate, and the malaria endemicity estimates obtained for study sites and year from the Malaria Atlas Project [11] and (b) according to geographical region (Africa, Asia, and S. America).

4.5. Summary of statistical analyses

Descriptives and baseline characteristics:

- A summary (study profile) of the relevant trials uploaded to the WWARN repository will be presented 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, and parasitemia sampling scheme.
- The baseline characteristics of the eligible studies will be described by country, transmission site(s) and treatment regimens. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

Description of overall haematological profiles:

- Figure of mean (+/- 2 sd) haemoglobin concentrations at each observation will be presented by: region, treatment group, age categories and transmission intensity.

- Population haemoglobin levels will be examined over time using fractional polynomial models with random coefficients. The effect of the following covariates on the coefficients will be examined: region, transmission intensity, age category, treatment group. This model will be used to estimate the day of nadir and the mean reduction in haemoglobin from day 0 to day of nadir and day 7.

At Enrollment (before treatment):

- Moderately severe anaemia at enrollment

Uni- and multivariable logistic regression of risk factors for moderately severely anemia on enrollment will be performed, with random effects for study-site. Covariates to examine will include: age, sex, weight, nutritional status, , history of fever, baseline parasitaemia, species, presence of gametocytes on enrolment, transmisison intensity. In addition, fractional polynomials will be used to define the nonlinear relationships between outcome and continuous covariates.

During Follow Up (After Treatment):

- Risk of moderately severely anemia on day 3 or day 7

Uni- and multivariable logistic regression for moderately severely anemia on Day 3 or Day 7 (two separate outcomes) will be performed, with random effects for study-site. Covariates to examine will include: age, sex, weight, nutritional status, , history of fever, baseline parasitaemia, species (pure Pf or mixed species), presence of gametocytes on enrolment, transmisison intensity, delayed parasite clearance. In addition, fractional polynomials will be used to define the nonlinear relationships between outcome and continuous covariates.

- Risk of large large fractional fall in haemoglobin on day 3 or day 7

Uni- and multivariable logistic regression for anemia on Day 3 or Day 7 (two separate outcomes) will be performed, with random effects for study-site. Covariates to examine will include: age, sex, weight, nutritional status, past history of malaria, history of fever, baseline parasitaemia, species (pure Pf or mixed species), presence of gametocytes on enrolment, transmisison intensity and delayed parasite clearance. In addition, fractional polynomials will be used to define the nonlinear relationships between outcome and continuous covariates.

- Risk of anaemia within 28 days / 42 days

Survival analysis for time to anemia during follow-up (28 or 42 days) will be performed, with random effects for study-site. Covariates to examine will include: age, sex, weight, nutritional status, past history of malaria, history of fever, baseline parasitaemia, prevalence of parasitaemia on days 1,2 and 3, species (pure Pf or mixed species), transmisison intensity, baseline haemoglobin, presence of gametocyte on enrolment, ACT treatment, primaquine treatment, AS dose, recrudescent infection. Only patients without aneamia on enrollment will be included.

- Figure of median (+95% centile) fractional change in haemoglobin from enrolment during follow-up with be presented according to treatment group, age categories and transmission intensity.

- Time to hematological recovery

Survival regression analysis of time to hematological recovery will be perfomed. 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. Covariates to examine will include: age, sex, weight, nutritional status, past history of malaria, history of fever, baseline parasitaemia, species, transmisison intensity, baseline haemoglobin, presence of gametocyte on enrolment, ACT treatment, primaquine treatment, AS dose.

5. Statistical Methodology

5.1. Descriptive statistics

Descriptive statistics will use mean and standard deviation if data are normally distributed, geometric mean and range if data are log-normally distributed (as assessed by Shapiro-Wilk test) , or median and range otherwise.

5.2. Survival regression models

Survival models will be used to investigate two outcomes: time to haematological recovery and time to anaemia.
Random effects in the form of shared frailty parameters will be used to adjust for study-site effect [12]. 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 [13]. In the Cox regression model, the proportional hazard assumption will be tested based on Schoenfeld residuals [14]. 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 and based on the residuals, as described below. Robustnes of the coefficients in the final model will be explored using 1000 boostrap samples. Coefficient of Variation (CV, %) of the estimates derived from bootstrap analysis will be reported.

5.3. 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 multivariable analysis. Known confounders will be fitted first (eg for the baseline anaemia model : asexual parasite density, age in categories, transmission intensity). Variables and covariates will then be added in a stepwise forward fashion using model deviance/Likelihood Ratio Test (LRT) i.e. changes in log likelihood *(*$-2\ Log\hat{L}$) will be compared (for nested models) to identify the variables which results in a significant reduction in $-2\ Log\hat{L}$ 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 continuous variables (baseline Hb, age and parasitaemia) will be examined using factional polynomials

6. 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.

7. Study Group Governance, Management and Coordination

The Study Group comprises participating investigators who contribute relevant data sets to the pooled analysis. Data sets will remain the property of the investigator. The Study Group collectively makes decisions with respect to including additional studies, data analysis and plans for publication, in line with the WWARN Publication Policy. The Study Group will identify one or two people to coordinate activities including data analysis, and drafting of publications and reports for group review. The WWARN statistician(s) will be responsible for statistical analyses
Once data are uploaded into the WWARN repository, they will be curated and standardized into one format using the WWARN Data Management and Statistical Analysis Plans (DMSAP v1.2 http://www.wwarn.org/sites/default/files/ClinicalDMSAP.pdf) for clinical data and pooled into a single database of quality-assured individual patient data. The WWARN statistician will be responsible for statistical analyses.

8. Publication Policy

The policy regarding authorship will be widely discussed in the group. However, in view of the large number of eligible studies we propose publication in 'group' name. The manuscript preparation and writing committee will be composed of Ric Price, Philippe Guerin, Rob Commons and the WWARN statisticians Rashid Mansoor and Kasia Stepniewska. They will be responsible for circulating drafts for comments to the study group members.

The data sets uploaded to the WWARN repository will remain the property of the individual donor(s) and publication will be in accordance with an agreed publication plan (see publication policy document https://www.wwarn.org/tools-resources/publication-policy).

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. Definition of the normal haematology recovery profile following malaria will inform ongoing studies which are quantifying the haematological consequencs of single dose primaquine for transmission blocking.

10. References

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