Statistical Analysis Plan
ACT Malaria and Malnutrition Study Group
Version 2.0

WorldWide Antimalarial Resistance Network (WWARN)

Suggested citation: Statistical Analysis Plan, ACT Malaria and Malnutrition Study Group: Assessing the impact of nutritional status on treatment outcome in children between 6 and 59 months with uncomplicated P. falciparum malaria treated with Artemisinin Combination Therapies (ACTs)

Version History

Version number	Revision(s) & reason for amendment	Release date
v1.0		**30.07.15**
V2.0	**More details provided** **Change in the definition of the analysis population and anthropometric indicators analysed, based on the available data** **Addition of sensitivity analyses, based on data limitations**	**20/05/2019**

WorldWide Antimalarial Resistance Network (WWARN)
www.wwarn.org

Table of Contents

1. Introduction 4
1.1 Aim of the study 4
1.2 Eligibility criteria for inclusion in pooled analysis 4
1.3 Desirable Data (not required for inclusion) 4
1.4 Study Exclusion criteria 5
1.5 Patient Exclusion 5
2. Methodologies 5
2.1 Anthropometric Indicators 5
2.2 Transmission Intensity 5
2.3 Conversion between haemoglobin and haematocrit 5
2.4 Dosing Calculation 5
3. Efficacy Endpoints 6
4. Covariates Examined 6
5. Association between nutritional status and treatment outcome adjusted for confounders 7
5.1 Analysis Population 7
5.2 Model building strategy 7
5.3 Models fitted 7
6. Sensitivity analyses 8
6.1 Multiple imputations 8
7. Risk of bias assessment 9
8. Tools 9
9. References 10

1. Introduction

Malaria and malnutrition are major causes of morbidity and mortality in low and middle income countries. Annually, approximately 3.1 million under-five mortality is attributed to malnutrition[1]. In children, the severity and extent of malnutrition is measured by various anthropometric indicators estimated from standardised growth reference curves. These metrics include wasting (low weight-for-height, a measure of acute malnutrition and the Mid-Upper Arm Circumference (MUAC) used as proxy of nutritional status in children under five years of age), stunting (low height-for-age, a measure of linear growth), and underweight (low weight-for-age, a composite of linear growth and stunting). In 2011, stunting and wasting affected at least 165 and 52 million children respectively worldwide[1]. The relationship between malnutrition and malaria is still not clearly understood. Some studies have reported malnutrition being associated with a higher risk of malaria [2,3], others have suggested a protective effect [4–6], while other studies have shown no association at all [6,7]. Similarly, the relationship between nutritional indicators and antimalarial drug efficacy has not be clearly understood with results often contrasting [8–10]. The non-generalizability of these results could be due to the heterogeneity in study population, the diversity in transmission intensity, use of different growth references and different definitions [9], but also small sample sizes of the studies. Hence, there is a knowledge gap in association between malnutrition and antimalarial drug efficacy which needs to be addressed.

1.1 Aim of the study

To assess the effect of various nutritional indicators in treatment outcome in children aged 6-59 months treated with artemisinin based combination therapies for uncomplicated *P. falciparum* malaria.

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:
Clinical trial including treatment with artemether-Lumefantrine (AL), artesunate-amodiaquine (AS-AQ), dihydroartemisinin-piperaquine (DP) and artesunate-mefloquine (AS-MQ) in children aged 6-59 months

- Data available on patients age, weight, gender and height at inclusion
- Minimum follow-up duration of 28 days
- Data available on treatments outcome and PCR genotyping
- Information on dose, study location is available

The data sets uploaded to the WWARN repository will be standardized using the WWARN Data Management and Statistical Analysis Plans [11] (DMSAP v1.11) 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) and publication will be in accordance with an agreed publication plan [12].

1.3 Desirable Data (not required for inclusion)

- Patients mid upper arm circumference (MUAC)

- Individual patient dose data (mg/kg)
- Baseline Haemoglobin level
- Daily parasite counts (days 0, 1, 2, 3)

1.4 Study Exclusion criteria

- Patients with concomitant HIV infection

1.5 Patient Exclusion

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

- No or missing plasmodium falciparum infection on enrolment
- Missing age and (or) weight and (or) gender
- Any of these other deviations, as defined by Clinical Module DMSAP:
    (a) Haemoglobin < 5 g/dL on day 0
    (b) Haematocrit < 15% on day 0

2. Methodologies

2.1 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 [13]. 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 [14].

2.2 Transmission Intensity

The study sites will be classified into 3 categories: low, moderate and high malaria transmission based on i) the observed PCR corrected re-infection rate and ii) the parasite prevalence estimates obtained from the Malaria Atlas Project [15]. Other possible factors affecting re-infection rate within study, such use of bednets and post treatment prophylactic effect of long acting antimalarials will be considered when taking re-infection rate into account.

2.3 Conversion between haemoglobin and haematocrit

Haematocrit will be converted to haemoglobin using the following relationship[16]:

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

Anaemia will defined as severe when haemoglobin concentration is below 7g/dl and as moderate if haemoglobin concentration is between 7-10g/dL

2.4 Dosing Calculation

The doses of artemisinin derivatives and the 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.

3. Efficacy Endpoints

Primary: PCR adjusted risk of *P. falciparum* recrudescence

Secondary: PCR adjusted risk of *P. falciparum* reinfection
Early Parasitological response on days 1, 2 and 3

Plasmodium falciparum recrudescence

For the analysis of Plasmodium falciparum recrudescence, failure will be defined as PCR confirmed Pf recrudescence. Patients who had a new infection (of any species) will be censored at the first day the positive parasitaemia was recorded. Patients who had a reappearance of Pf parasites but the PCR result is not available will be excluded from the analysis to avoid informative censoring. Patients with recurrent parasitaemia have higher risk of recrudescence than patients in the whole population who were lost to follow-up.

Plasmodium falciparum re-infection

For the analysis of Plasmodium falciparum re-infection, failure will be defined as PCR confirmed Pf re-infection. Patients who had a new infection other than Pf species or Pf recrudescence will be censored at the first day the positive parasitaemia was recorded. Patients who had a reappearance of Pf parasites but the PCR result is not available will be excluded from the analysis to avoid informative censoring. Patients with recurrent parasitaemia have higher risk of recrudescence than patients in the whole population who were lost to follow-up.

Early Parasitological response

A pre-defined algorithm will be used to estimate positivity status on days 2 or 3, if no observation of the blood film was recorded on that day as described in WWARN clinical Module DMSAP [11]. For studies with frequent sampling, a patient will be classified as being positive on days 1, 2 and 3 after enrolment if the measurements within a window of ±3 hours of 24, 48 and 72 hours were positive.

4. Covariates Examined

In addition to the nutritional metrics, the following variables (if available) will be examined for their association with primary and secondary treatment outcomes:

Plasmodium falciparum recrudescence: Baseline parasitaemia (after log-transformation), temperature, gender, baseline haemoglobin, anaemia, gametocytes carriage on admission, patient age, transmission intensity, treatment received, treatment supervision, mg/kg dose of partner compound.

Plasmodium falciparum re-infection: patient age, transmission intensity, gender, baseline temperature, baseline haemoglobin, anaemia, mg/kg dose of partner compound, treatment received and treatment supervision.

***Early Parasitological response*:** Baseline parasitaemia (after log-transformation), baseline temperature, gender, baseline haemoglobin, anaemia, gametocytes carriage on admission, patient age, transmission intensity, treatment received, treatment supervision, mg/kg dose of artemisinin derivatives.

5. Association between nutritional status and treatment outcome adjusted for confounders

5.1 Analysis Population

All patients with plausible estimates of WHZ will be included in the analysis of this measure. In the analysis of HAZ, only studies which recorded children age in months will be analysed.

5.2 Model building strategy

Each nutritional indicator *a* will be examined in a separate multivariate model as described below. Indicators will be modelled as continuous covariates and as categorical covariates (i.e using definitions from the WHO guidelines [14] of being stunted, underweight or wasted; and using 5 categories: $a < -3$; $-3 \leq a < -2$; $-2 \leq a < -1$; $-1 \leq a < 0$; $a \geq 0$).
The nutritional indicator variable(s), age and ACT treatment will be kept in the model regardless of their statistical significance. The remaining covariates will be investigated for inclusion in the model using a general strategy recommended by Collett[17].

i) All possible risk factors will be examined in a univariable analysis. The log-likelihood estimates $(-2 \times Log\hat{L})$ will be compared against the null model to assess if any of the variables reduce its value at 5% level of statistical significance.
ii) All the variables identified in step (i) will be fitted together in one model and variables which are not significant in the presence of other variables based on the results of the Wald test will be identified.
iii) A likelihood ratio test will be used to assess the impact of omitting variables identified in step (ii). If the omitted variable does not significantly impact the model log-likelihood, then it will be dropped. Only variables which led to significant change in log-likelihood will be retained.
iv) All variables excluded from step (i) will be added to the model identified in step (iii) one by one to check if they provided any improvement to the model.
v) A final check of the model identified in step (iv) will be carried out to ensure that none of the variables in the model could be omitted without significantly increasing the model log-likelihood, and none of the excluded variables significantly reduced the model log-likelihood.
vi) In the final model identified in v) interaction term between ACT and anthropometric measure will be added and likelihood ratio test will be used to assess the improvement to the model.

Fractional polynomials[18] will be used to explore the nonlinear relationships between outcome and continuous covariates**.**

5.3 Models fitted

PCR confirmed *Plasmodium falciparum* recrudescence/ reinfection

Risk factors for PCR confirmed recrudescence will be analysed using a Cox's proportional hazards regression with shared frailty across study sites to account for any unexplained heterogeneity[19,20].

Cox-Snell's and Martingale's residuals will be examined to assess the model fit; the underlying assumption of proportional hazards will be tested and reported when violated. The underlying assumption of proportional hazards will be tested in the final model using Schenfoeld residuals and reported when violated. For variables which violate the assumption, interaction between this variable and time intervals (0-28, 28-42 days) will be considered for inclusion in the final model. Same methodology will be used for the analysis of risk factors for PCR confirmed reinfection.

Early Parasitological response
Risk factors associated with persistence of parasitaemia on days 1, 2 and 3 will be assessed using mixed effects logistic regression with study site intercept fitted as a random effect.

6. Sensitivity analyses

In the sensitivity analysis, the final models, will be refitted

- with each study's data excluded, one at a time

Coefficient of variation around the parameter estimates will be calculated.
Exclusion of studies will identify any influential studies, that is, studies with unusual results (due to variations in methodology, patient population, or other reasons) that affect the overall pooled analysis findings

- in studies which used at least three molecular markers to distinguish recrudescence from the reinfection;
- using multiple imputations for age in studies which have not provided age in months, or in which majority of children had age given as integer (see section 6.1. for more details)

6.1 Multiple imputations

For studies with age measured in years for all or majority of children (>50%), multiple univariable imputation for such values will be conducted using an interval regression imputation method (Stata command*: mi impute intreg*[21]), assuming for a child with recorded age of x years that their true age is between x years 0 months and x years 11 months.

All variables assessed in the multivariable models: weight, height, sex, study siteanaemia, fever, $\log_{10}$ parasitaemia, ACT, transmission intensity area will be included as auxiliary variables in the imputation model, as well as parasite positivity on day 2, recrudescence by day 42, new infection by day 42, day of outcome, and cumulative hazard (Nelson-Aalen estimator) for recrudescnce and for new infection. Twenty datasets will be imputed.

For each imputed dataset, anthropometric measures will be re-estimated using the imputed age. Final models will be re-fitted on the 20 imputed datasets and final overall model coefficients and standard errors will be estimated, adjusted for the variability between imputations (stata command*: mi estimate)* according to the combination rules by Rubin[22].

7. Risk of bias assessment

Risk of bias within studies will be assessed based on:

- study design (randomization, sequence generation, blinding);
- accuracy in estimation of anthropometric indices and extent of the out-of-range values;
- the number and proportion of patients with missing outc
- the number of proportion of patients with missing baseline covariates (age, weight, parasitaemia, temperature, haemoglobin).

8. Tools

All statistical analyses will be carried out using *R* (The R Foundation for Statistical Computing) or *Stata* (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA). Using alternative statistical software does not require amendment of this SAP.

9. References

1. Black, R. E. et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 382, 427–451 (2013).

2. Nyakeriga, A. M., Troye-Blomberg, M., Chemtai, A. K., Marsh, K. & Williams, T. N. Malaria and nutritional status in children living on the coast of Kenya. Am. J. Clin. Nutr. 80, 1604–1610 (2004).

3. Ehrhardt, S. et al. Malaria, anemia, and malnutrition in african children--defining intervention priorities. J. Infect. Dis. 194, 108–114 (2006).

4. Genton, B., Al-Yaman, F., Ginny, M., Taraika, J. & Alpers, M. P. Relation of anthropometry to malaria morbidity and immunity in Papua New Guinean children. Am. J. Clin. Nutr. 68, 734–741 (1998).

5. Murray, M. J., Murray, A. B., Murray, N. J. & Murray, M. B. Diet and cerebral malaria: The effect of famine and refeeding. Am. J. Clin. Nutr. 31, 57–61 (1978).

6. Snow, R. W., Byass, P., Shenton, F. C. & Greenwood, B. M. The relationship between anthropometric measurements and measurements of iron status and susceptibility to malaria in Gambian children. Trans. R. Soc. Trop. Med. Hyg. 85, 584–589 (1991).

7. Müller, O., Garenne, M., Kouyaté, B. & Becher, H. The association between protein-energy malnutrition, malaria morbidity and all-cause mortality in West African children. Trop. Med. Int. Heal. 8, 507–511 (2003).

8. P. Mitangala Ndeba, U. D'Alessandro, P. Hennart, P. Donnen, D. Porignon, G. Bisimwa Balaluka, A. Bisimwa Nkemba, N. C. M. and M. D. W. Efficacy of Artesunate Plus Amodiaquine for Treatment of Uncomplicated Clinical Falciparum Malaria in Severely Malnourished Children Aged 6–59 Months, Democratic Republic of Congo. Clin. Exp. Pathol. (2012). doi:10.4172/2161-0681.S3-005

9. Verret, W. J. et al. Effect of nutritional status on response to treatment with artemisinin-based combination therapy in young Ugandan children with malaria. Antimicrob. Agents Chemother. 55, 2629–2635 (2011).

10. Hess, F. I. et al. Anti-malarial drug resistance, malnutrition and socio-economic status. Trop. Med. Int. Health 2, 721–728 (1997).

11. Clinical Module: Data management and Statistical Analysis Plan.Version 1.2. [http://www.wwarn.org/sites/default/files/ClinicalDMSAP.pdf]

12. WWARN. Publication policy. [http://www.wwarn.org/sites/default/files/PublicationPolicy.pdf]

13. The WHO Anthro Survey Analyser [https://www.who.int/tools/child-growth-standards/software]

14. WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva World Health Organization 2006:312.

15. Gething PW, Patil AP, Smith DL, Guerra CA, Elyazar IR, Johnston GL, Tatem AJ, Hay SI: A new world malaria map: Plasmodium falciparum endemicity in 2010. Malar J 2011, 10:378.

16. Lee SJ, Stepniewska K, Anstey N, Ashley E, Barnes K, Binh TQ, D'Alessandro U, Day NP, de Vries PJ, Dorsey G, Guthmann JP, Mayxay M, Newton P, Nosten F, Olliaro P, Osario L, Pinoges L, Price R, Rowland M, Smithuis F, Taylor R, White NJ: The relationship between the haemoglobin concentration and the haematocrit in Plasmodium falciparum malaria. Malar J 2008, 7:149.

17. Collett D. Modelling Survival Data in Medical Research. 3rd ed: CRC Press; 2015.

18. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. Applied Statistics. 1994;43:429-67.

19. Glidden, D. V & Vittinghoff, E. Modelling clustered survival data from multicentre clinical trials. Stat. Med. 23, 369–88 (2004).

20. Munda, M. & Legrand, C. Adjusting for centre heterogeneity in multicentre clinical trials with a time-to-event outcome. Pharm. Stat. 13, 145–52

21. Royston P. Multiple imputation of missing values: Further update of ice, with an emphasis on interval censoring. Stata Journal. 2007;7:445-64.

22. Rubin D. Multiple Imputations for Nonresponse in Surveys: NewYork: Wiley; 1987.