Statistical Analysis Plan

MALARIA AS A PROTECTIVE FACTOR AGAINST SEVERE VACCINATION IN THE DEMOCRATIC REPUBLIC OF CONGO

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Table of contents

1. INTRODUCTION

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the main pre-planned analyses for the study "Malaria as a protective factor against severe forms of COVID-19 in DRC". The high prevalence of malaria in sub-Saharan Africa may play a role, according to some studies, in a possible protection against the severity of COVID-19 in endemic areas. However, the scientific evidence for the physiological role of this "induced" immunity is not established. We wish to conduct this study to elucidate this hypothesis. Details on the conduct of the study are described in the protocol.

These planned analyses will be carried out by statisticians from the Clinical Research Centre (CRC/INRB) and the Clinical Trials Unit of the Institute of Tropical Medicine (CTU ITM, Antwerp). The results of the analysis will be described in a statistical analysis report, which will serve as the basis for major research publications. This document describes the statistical methods for the primary and secondary objectives of the study as defined by the protocol. Additional analyses may be performed but are not covered by the current analysis plan.

This analysis plan will be finalised and approved before the database of the first analyses is locked. Major changes in the statistical methodology used for the main and pre-planned analyses of this SAP require a detailed description and justification in the Statistical Analysis Report (SAR). The dataset, programmes and final results are archived in accordance with Good Clinical Practice guidelines (ICH E9).

2. STUDY DESIGN AND OBJECTIVES

2.1. DESIGN

An observational, multi-centre, matched case-control study. The study will compare the quality of malaria immunity in severe and non-severe cases of COVID-19.
The study will be conducted in four Treatment Centres (CTCo) to recruit severe cases (cases). Controls will be recruited at the Health Zone (HZ) level on the basis of the same characteristics as the cases, taking into account age (upper and lower 10 years), sex and HZ of origin.

2.2. OBJECTIVES

THE MAIN OBJECTIVE:

is to investigate whether the immunity conferred by Plasmodium protects against severe forms of COVID-19. This will be done by comparing Plasmodium antibody levels in severe and non-severe COVID-19 cases.

SECONDARY OBJECTIVES

They are :

- Comparing the cellular response against malaria between severe and non-severe COVID-19 cases
- To compare the proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the Thick Gauge (TG) and Rapid Diagnostic Test (RDT)
- Describe the clinical characteristics and status of severe and non-severe cases of COVID-19 with and without malaria exposure
- To assess the modulating impact of existing antimalarial immunity on the strength of humoral and cellular immune responses to SARS-COV-2 in severe and non-severe cases of COVID-19
- Compare the SARS-COV-2 specific cellular immune response between severe and non-severe cases
- Assessing the modulatory impact of antimalarial immunity on induced immunity using specific antiviral receptor stimulations of the innate immune system

3. HYPOTHESES OF THE STUDY

Anti-malarial immunity would play a protective role against severe forms of COVID-19.

4. VARIABLES OF INTEREST

4.1. PRIMARY VARIABLES OF INTEREST :

The primary variable of interest in the study is the level of anti-malarial antibodies in severe and non-severe COVID-19 cases. This level will be assessed by measuring the concentration of IgG against Circum Sporozoite Protein (CSP) using the Luminex-MAGPIX.

4.2. SECONDARY VARIABLES OF INTEREST :

- Anti-malarial IgG antibody levels against other antigens: AMAI, CSPM, GLURP, *Pf/RESA55*, as well as IgM antibodies

- Cellular response against malaria between severe and non-severe COVID-19 cases, measured by IFN-y ELISPOT
- The proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the Thick Gauge (TG) and Rapid Diagnostic Test (RDT). Indicator variables will be used for a positive RDT result, for a positive EW result and for a positive result with both tests
- Clinical features :

➔ Duration of symptoms
➔ Type of symptoms
➔ Size
➔ Weight
➔ Blood pressure
➔ Heart Rate
➔ Respiratory Rate (RR)
➔ Temperature (T°)
➔ Free Oxygen Saturation (SaO2)
➔ Splenomegaly

and clinical status of severe and non-severe cases of COVID-19 with and without malaria.

The clinical status of COVID-19 only controls is assessed by the WHO 10-point clinical progression score at 7ème days of follow-up[1] :

Patient's condition	Description	Score
Not infected	Not infected; no viral RNA detected	0
Ambulatory: illness benign	Asymptomatic; viral RNA detected	1
	Symptomatic; independent patient	2
	Symptomatic; need for assistance	3
Hospitalized: moderate illness	Hospitalized; no oxygen therapy	4
	Inpatient; oxygen therapy by mask or nasal route	5
Hospitalized: illness severe	Inpatient; high flow oxygen therapy	6
	Intubation and mechanical ventilation, pO2/FiO2 $\geq$150 or SpO2/FiO2 $\geq$200	7
	Mechanical ventilation pO2/FIO2 <150 (SpO2/FiO2 <200) or vasopressors	8

	Mechanical ventilation pO2/FiO2 <150 and vasopressors, dialysis, or ECMO	9
Deaths	Deaths	10

- SARS-COV-2 specific cellular and humoral immune response of severe and non-severe cases, measured by Luminex and ELISPOT.
- Induced immunity: Levels of antiviral cytokine release, measured by ELISA and flow cytometry, of peripheral blood monocytes (PBMC) from controls and patients with acute malaria infection (according to GE and RDT).

4.3. EXPLORATORY VARIABLES OF INTEREST :

- The proportion of patients infected with urinary and intestinal parasites.

5. POPULATION OF THE STUDY

This case-control study will be conducted in the Republic of Congo in the city of Kinshasa, the epicentre of COVID-19. Kinshasa is a malaria endemic area, the distribution of malaria prevalence is lower in urban areas compared to peri-urban areas. In fact, the city of Kinshasa is structured in four health districts and sixteen hospitals, distributed in the thirty-five health zones for the management of COVID-19.

The study consists of cases and controls: For each case, a control will be recruited.

5.1. DESCRIPTION OF CASES

The cases will be severe COVID-19 cases, which will consist of inpatients recruited from COVID-19 Treatment Centres (CTCOs).

5.2. DESCRIPTION OF

Controls will consist of asymptomatic or symptomatic cases without signs of severity. For each case, a control will be recruited after being matched in terms of age (plus or minus 10 years), gender and

Health Zone of origin. The time to recruit a control is up to 10 weeks after case recruitment.

6. POPULATION OF ANALYSIS

6.1. ANALYSIS POPULATION PRIMARY

All enrolled participants will be included in the primary analysis.

6.2. ANALYSIS POPULATION SECONDARY

Only participants who did not violate any major criteria will be included in the secondary analysis (see Table 1).

Table 1:Protocol violationsclassified as minor or major

Violation of the	Minor/major violation	Comments
Protocol Inclusion		
For CAS		
Criteria		
1. At least 18 years of age	Major	
2. Having an SARS-CoV infection	Major	
2 confirmed by RT-PCR or rapid antigen test within 72 hours of inclusion.	Major	
3. Have given their informed consent or that of their guardian/ representative to participate in the study.		
4. Clinical signs of pneumonia: fever, cough, dyspnea or crepitations and (Respiratory rate > 30 cycles/min or severe respiratory distress or SpO2 < 90% on room air)	Major	
5. Being admitted to a care unit for COVID-19	Major	
6. Reside in the health zone for at least 6 months	Minor	

For WITNESSES	
1. Be at least 18 years old	Major
2. Have a PCR or antigenic test confirmed SARS-CoV- 2 infection within 72 hours prior to inclusion.	Major
3. Have given informed consent to participate in the study	Major
4. Be asymptomatic or symptomatic, but without any signs of severe pneumonia	Major
5. Not to be admitted to a care unit for COVID-19.	Major
6. Reside in the study health area for at least 6 months	Minor

Exclusion Criteria (CASES AND WITNESSES)	
1. The subject has a contraindication at venipuncture, determined by clinical judgment	Major
2. The subject is vaccinated against SARS- CoV-2	Major
3. The subject has been infected with SARS- CoV-2 in the past and now presents with a reinfection	Major

7. ANALYSIS

A conditional logistic regression model will be fitted with the logarithm of malaria antibody levels to Circum Sporozoite Protein (CSP) as the independent variable. The p-value for the test of no association between malaria antibody levels and case/control will be presented.

8. ANALYSIS SECONDARY

- Compare malaria antibody levels to other antigens between severe and non-severe COVID-19 cases:
    - A similar model to the one for the primary objective is fitted with the respective malaria antibody levels as the independent variable.

- Compare the cellular response against malaria between severe and non-severe COVID-19 cases:

    - A conditional logistic regression model will be fitted with the cellular response to malaria as the independent variable.

- Compare the proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the Thick Gauge (TG) and Rapid Diagnostic Test (RDT):

    - A conditional logistic regression model will be fitted with acute malaria infection as the independent variable.

    Note: Acute infection will be assessed in 3 ways: first with the EWG alone, then the RDT alone and finally the EWG or RDT taken together.

- Describe the clinical characteristics and clinical status of severe and non-severe cases of COVID-19 with and without malaria (former exposure or active infection):

    - Medians with quartiles or frequencies and proportions will be presented in 3 types of tables:
        - Cases versus controls
        - Malaria present versus malaria absent
        - Malaria cases present/absent versus Controls-Malaria present/absent

Statistical comparisons between groups (cases versus controls and malaria present versus absent) will be performed. The Fisher's exact statistical test or Chi-square test will be used to compare categorical clinical characteristics and the non-parametric Man-Whitney test for numerical characteristics.

- To assess the modulating impact of existing antimalarial immunity on the strength of humoral and cellular immune responses to SARS-COV-2 in severe and non-severe cases of COVID-19

    - The Spearman correlation between malaria immunity and immune responses to SARS-COV-2 will be calculated with a 95% confidence interval. separately for cases and controls

- Compare the SARS-COV-2 specific cellular immune response between severe and non-severe cases

    - A conditional logistic regression model will be fitted with the SARS-COV-2 specific cellular immune response as the independent variable

- Assessing the modulatory impact of antimalarial immunity on induced immunity using specific antiviral receptor stimulations of the innate immune system

    - The Spearman correlation between malaria immunity and induced immunity will be calculated with a 95% confidence interval separately for cases and controls

9. ANALYSIS EXPLORATORY

- Compare the proportion of patients infected with urinary and intestinal parasites between severe and non-severe cases of COVID-19 by microscopic examination of stool and urine

    - A conditional logistic regression model will be fitted with intestinal/urinary parasitosis as an independent variable.

10. ANALYSIS OF SUB GROUPS

No subgroup analysis will be performed.

11. MULTIPLICITY AND MISSING DATA

As this is a single-primary objective study, no multiplicity adjustment is required. Participants with missing data will be excluded from the missing data analysis.

12. BIBLIOGRAPHY

1. Marshall JC, Murthy S, Diaz J, et al. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis*. 2020;20(8):e192-e197. doi:10.1016/S1473-3099(20)30483-7