RESEARCH STUDY PROTOCOL

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MALARIA AS A PROTECTIVE FACTOR AGAINST SEVERE COVID-19 IN THE DEMOCRATIC REPUBLIC OF CONGO

Version 1.3 on 07/12/2021

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PROTOCOL HISTORY

Protocol / Amendment #	Date	Reason for amendment
Protocol v1.0	30/05/2021	/
Protocol v1.2	24/06/2021	Minor amendment as suggested by the ITM IRB
Protocol v1.3	12/07/2021	Minor amendment to replace "final outcome" with "clinical condition" as secondary objective

DECLARATION OF CONFORMITY

This protocol contains the information required to carry out the present research study. By signing this document, the Investigator agrees to conduct the study in compliance with the protocol, applicable ethical guidelines such as the Declaration of Helsinki, the European General Data Protection Regulation (GDPR), the ESF/ALLEA Code of Conduct for Research Integrity, as well as in compliance with international scientific standards and all applicable regulatory requirements. The Investigator will also make every reasonable effort to complete the study within the designated calendar deadlines.

Once the protocol has been published and signed by the Investigator(s) and authorized signatories, it can no longer be modified informally. Amendments to the protocol have the same legal status, and must go through the mandatory review and approval stages before being implemented.

Principal Investigator INRB

Prof Mumba Dieudonné		Date :	07/13/2021
Signed	:		

Principal Investigator ITM

Dr Liesenborghs Laurens		Date :	07/13/2021
Signed	:		

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SUMMARY

Justification	Covid-19 has enormous socio-economic consequences worldwide. Africa remains the continent least affected, despite the poverty level of its population and the fragility of its health systems. In inter-tropical African countries with high malaria prevalence, low prevalence of COVID-19 is reported. According to some studies, the high prevalence of malaria in sub-Saharan Africa could play a role in protecting against the severity of COVID-19 in endemic areas. However, scientific evidence for the physiological role of this "trained" immunity has not been established. We wish to undertake this study to elucidate this hypothesis.
Objectives	The main objective is to assess whether the immunity conferred by Plasmodium protects against severe forms of COVID-19. This will be achieved by comparing anti-plasmodium antibody levels in severe and non-severe COVID-19 cases. The secondary objectives are : ▪ Comparing the cellular response to malaria between severe and non-severe COVID-19 cases ▪ Compare the proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the Thick Gout (TG) and Rapid Diagnostic Test (RDT). ▪ Describe the clinical features and status of severe and non-severe cases of COVID-19 with and without malaria exposure ▪ Evaluate the modulatory 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 ▪ Evaluate the modulatory impact of antimalarial immunity on trained immunity using specific stimulations of antiviral receptors in the innate immune system.
Methodology	Observational, multicenter, matched case-control study. The study will compare anti-plasmodium antibody levels between severe and non-severe cases of COVID-19.
Study population	The study will be carried out in four COVID-19 treatment centers for the recruitment of severe cases (cases). For each case, two controls will be recruited from the community based on the following concordance criteria: ▪ Place of origin: same Health zone ▪ Gender ▪ Maximum 5-year age difference
Inclusion criteria	<u>For cases :</u> ▪ Must be at least 18 years old ▪ PCR-confirmed SARS-CoV-2 infection within 72 hours of inclusion. ▪ Have given informed consent to participate in the study. ▪ Diagnosed as <u>severe</u> COVID-19 according to the following criteria: o Clinical signs of pneumonia: fever, cough, dyspnea or crepitations **AND** o With at least one of the following : ▪ Respiratory rate > 30 cycles/min **OR** ▪ Severe respiratory distress or SpO2 < 90% on room air ▪ Admission to a care unit for COVID-19

	▪ Have lived in the health zone for at least 6 months <u>For witnesses :</u> ▪ Must be at least 18 years old ▪ PCR-confirmed SARS-CoV-2 infection within 72 hours of inclusion. ▪ Have given informed consent to participate in the study ▪ Diagnosed as a <u>non-severe</u> COVID-19 <u>case</u> according to the following criteria: o Be asymptomatic **OR** o Be symptomatic, but without any signs of severe pneumonia: ▪ Respiratory rate > 30 cycles/min **OR** ▪ Severe respiratory distress or SpO2 < 90% on room air ▪ Not to be admitted to a care unit for COVID-19. ▪ Have lived in the study area for at least 6 months
Exclusion criteria	▪ The subject has a contraindication to venipuncture, determined by clinical judgment ▪ The subject is vaccinated against SARS-CoV-2 ▪ The subject has already been infected with SARS-CoV-2 in the past and now presents with reinfection.
Sample size	300, including 100 cases and 200 controls: Power is calculated by simulation. Power is calculated for different effect sizes (i.e. difference in anti-plasmodium antibody levels between cases and controls).
Calendar	▪ Protocol drafting: February-May 2021 ▪ Ethics committee submission and approval: June 2021 ▪ Formation of study team: July 2021

	▪ Data collection : July 2021 - December 2021 ▪ Data analysis: as soon as samples are received - March 2022 ▪ Presentation of results: May 2022

LIST OF ABBREVIATIONS AND ACRONYMS

AMA-1	Apical Membrane Antigen 1
COVID-19	Corona virus disease 2019
CSP	Circum Sporozoite Protein
CRF	Case Report Form' (data collection form, observation notebook)
CRC	Clinical Research Center
CTCo	COVID-19 processing center
ELISA	Enzyme-Linked Immunosorbent Assay
FM	Thin smear
GE	Thick drop
GLURP	Glutamat Rich Protein
IC	Investigator Coordinator
IC(F)	Informed Consent (Form)' - Formulaire de Consentement Eclairé
IC	Confidence interval
IFN-ɣ	Interferon gamma
IgG	Immunoglobulin G
INRB	National Institute for Biomedical Research
HbA1	glycated haemoglobin
MSP-1	Merozoite Surface Protein 1
WHO	World Health Organization
Pf	Plasmodium falciparum
PBMC	peripheral blood mononuclear cell
GROUND FLOOR	Democratic Republic of Congo

RT-PCR	Reverse transcription polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TDR	Rapid diagnostic test
ZS	Health Zone

1 INTRODUCTION

Corona virus disease 2019 (COVID-19) is caused by a new virus, severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), which was first reported in a group of pneumonia patients in the Chinese city of Wuhan on December 31, 2019 **[1]**. The epidemic that began in Wuhan then spread very rapidly worldwide and became a pandemic declared by the World Health Organization (WHO) on March 11, 2020 **[2].** COVID-19 in its first wave and the current second wave is having enormous socio-economic consequences worldwide. The disease is therefore a major public health problem. According to data compiled by the American Johns-Hopkins University, up to May 2, 2021, more than 150 million people have tested positive for Covid-19 worldwide, and more than 3 million have died from the disease **[3].**

No continent is spared from COVID-19, although Africa remains the least affected despite its population's level of poverty and the fragility of its health systems, which predispose it to high mortality. However, the realities on the ground are proving to be contrary to all predictions **[4].** Although there are many factors that can influence the transmission of COVID-19, including demographics, socio-cultural aspects, the young age of the population, the warm climate and genetics, this does not fully explain why the mortality rate is lower than those observed in Europe and the USA **[5-7].**

According to data compiled by the American Johns-Hopkins University on March 2, 2021, Africa has around thirty million confirmed cases of COVID-19 and 104,384 deaths, with proportions varying from region to region. There is a high prevalence of COVID-19 in regions with low malaria prevalence, such as the Maghreb region, notably Morocco with 513,314 confirmed cases and Tunisia with 318,236 cases, and South Africa with 159,2626 cases. Conversely, in inter-tropical African countries with high malaria prevalence, low prevalence of COVID-19 has been reported, perhaps due in part to under-diagnosis. This is notably the case in Nigeria, with 9,7478 confirmed cases, and the Democratic Republic of Congo (DRC), with over 29,000 cases **[8].**

The DRC is the second malaria-endemic country in Africa after Nigeria, and malaria remains the main cause of morbidity and mortality. At the time of writing on May 2, there are over 18 million cases and more than 18,000 deaths per year [**9**].

The prevalence of malaria in the DRC varies according to geographical location and depends on individual and community diversity; incidence is generally high in rural areas **[10].** The DRC has a population of over 90,000,000, with a precarious health system and lax protective measures. However, according to the COVID-19 DRC epidemiological report of March 5, 2021, there are around 712 deaths due to COVID-19, despite effective circulation of the virus in the population**.** 75% of cases are concentrated in the capital Kinshasa, which is the

epicenter of COVID-19, where malaria is meso-endemic, with a lower prevalence in urban areas compared with peri-urban areas **[11]**.

Several recent studies have demonstrated that the "early" innate immune response can be shaped differently by prior exposure to immune stimuli. However, little is known about how endemic micro-organisms such as *Plasmodiums* and helminths shape the innate immune response and direct the reactivity and efficacy of the immune response as a whole **[12, 13].**

The high prevalence of malaria in sub-Saharan Africa has been assumed to confer possible protection against the severity of COVID-19 in endemic areas **[14].** Our preliminary *in* vitro data indicate that prior and repetitive stimulation of anti-parasitic innate immune receptors induces an immune entrainment mechanism, stimulating the type I IFN response while attenuating the release of pro-inflammatory cytokines during SARS-CoV-2 infection (prof. Eva Bartok et al., unpublished results). According to several studies, high IFN type I release in conjunction with low induction of pro-inflammatory cytokines correlates with a less severe clinical course of COVID-19. This suggests a potential protective effect against COVID-19 virus in malaria-endemic areas **[15, 16].**

However, scientific evidence for the physiological role of this "trained" immunity is not established. This is why we are undertaking this study to shed light on a possible protective role of malaria in patients testing positive for COVID-19. The aim of this study is to show whether the immunity conferred by repeated *Plasmodium* infections protects against severe forms of COVID-19.

2 STUDY OBJECTIVES

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

The secondary objectives are :

- Comparing the cellular response to malaria between severe and non-severe COVID-19 cases
- Compare the proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the thick drop (GE) and rapid diagnostic test (RDT).
- Describe the clinical features and status of severe and non-severe cases of COVID-19 with and without malaria exposure

- Evaluate the modulatory 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
- Evaluate the modulatory impact of antimalarial immunity on trained immunity using specific stimulations of antiviral receptors in the innate immune system.

3 VARIABLES OF INTEREST

3.1 PRIMARY VARIABLES OF INTEREST :

The study's primary variable of interest 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.

3.2 SECONDARY VARIABLES OF INTEREST :

- Anti-malarial IgG antibody levels against other antigens: AMAI, CSPM, GLURP, Pf/RESA55, as well as IgM
- The cellular response against malaria between severe and non-severe COVID-19 cases, measured by IFN-y ELISPOT
- Proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the Thick Gout (TG) and Rapid Diagnostic Test (RDT).
- Clinical characteristics and clinical status of severe and non-severe cases of COVID-19 with and without malaria
- SARS-COV-2 specific cellular and humoral immune response of severe and non-severe cases
- Antiviral cytokine release levels, measured by ELISA and flow cytometry, of peripheral blood monocyte cells (PBMC) from controls and patients with acute malaria infection (according to GE and TDR).

4 DESIGN OF THE STUDY

An observational, multicenter, 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 carried out in four Treatment Centers (CTCo) to recruit severe cases (cases). Controls will be recruited from the Health Zone (ZS) on the basis of the same characteristics as the cases, taking into account age (upper and lower 5-year intervals), sex and ZS of origin.

5 METHODS

5.1 STUDY ENVIRONMENT AND POPULATION.

This case-control study will be carried out in the Republic of Congo, in the city of Kinshasa, the epicenter of COVID-19. Kinshasa is a malaria-endemic zone, with a lower prevalence of malaria in urban areas than in peri-urban areas. In fact, the city of Kinshasa is structured into four health districts and sixteen hospital centers, spread across the thirty-five Health Zones for the management of COVID-19.

The study will consist of cases and controls:

- The cases will be severe COVID-19 cases, and will comprise hospitalized patients recruited from COVID-19 Treatment Centers (CTCOs).
- Controls will consist of asymptomatic or symptomatic cases without signs of severity. For each case, two controls will be recruited after being matched in terms of age, sex and Health Zone of origin.

5.1.1 INCLUSION CRITERIA

To be eligible, study patients must meet the following criteria:

For cases :

- Must be at least 18 years old
- RT-PCR-confirmed SARS-CoV-2 infection no more than 72 hours prior to inclusion.
- Have given their informed consent or that of their guardian/representative to participate in the study.
- Diagnosed as severe COVID-19 according to the following criteria:
    - Clinical signs of pneumonia: fever, cough, dyspnea or crepitations
      AND
    - Presenting at least one of the following signs:
        - Respiratory rate > 30 cycles/min
          OR
        - Severe respiratory distress or SpO2 < 90% on room air

- Being admitted to a care unit for COVID-19
- Have lived in the health zone for at least 6 months

For witnesses :

- Must be at least 18 years old
- PCR-confirmed SARS-CoV-2 infection within 72 hours of inclusion.
- Have given informed consent to participate in the study
- Diagnosed as a non-severe COVID-19 case according to the following criteria:
    - Be asymptomatic

      OR
    - Symptomatic, but no signs of severe pneumonia
        - Respiratory rate > 30 cycles/min

          OR
        - Severe respiratory distress or SpO2 < 90% on room air
- Not to be admitted to a care unit for COVID-19.
- Have lived in the study area for at least 6 months

5.1.2 Exclusion criteria

To be eligible, study patients must not meet the following criteria for cases and controls:

- The subject has a contraindication to venipuncture, determined by clinical judgment
- The subject is vaccinated against SARS-CoV-2
- The subject has already been infected with SARS-CoV-2 in the past and now presents with reinfection.

5.1.3 RECRUITING SEVERE CASES

Severe cases will be recruited from four state CTCOs. The choice of CTCOs takes into account the frequency of severe cases and the geographical distribution of malaria in the city of Kinshasa. The centers are as follows:

- Saint Joseph Hospital
- Kinkole General Hospital
- Cliniques Universitaires de Kinshasa
- Ngaliema Clinics

After political and administrative authorizations have been obtained by a qualified member of the research team, all severe cases in the care units of the selected centers will be investigated for their eligibility for the study, and then invited to participate and give informed consent.

5.1.4 RECRUITING WITNESSES

For each case, two controls will be recruited in the Health Zone from which the severe cases originated. The Health Zone nurse assigned to the COVID-19 response and the investigator assigned to the site will record the witness information in the register of RT-PCR-positive cases for COVID-19.

The first two controls on the linear list with a COVID-19 RT-PCR test result of 72 hours or less and with the same characteristics will be contacted and invited to take part in the study. If participation is refused, the next person on the list will be taken. Controls must be matched to the case on the basis of the following concordance criteria:

- Age: +/- 5 years relative to case age
- Gender: same as case
- Place of origin: same health zone as the case

During the follow-up home visit planned by the ZS for the non-severe COVID19 patient, the investigator will accompany the ZS nurse supervisor, and the study will thus be introduced to the patient. Once informed consent has been obtained, the questionnaire and blood sample will be taken during the same visit. In compensation for their time, the controls will receive masks and soap to limit the spread of the virus in their environment.

5.1.5 SAMPLE SIZE AND STATISTICAL POWER

The sample size is 300 participants, made up of 100 cases and 200 controls. Sample power is calculated according to the following assumptions:

- Median antibody titers in controls are estimated at 898 mfi (*mean fluorescence intensity*). This estimate is based on a previous study in which antibody titres against CSP were determined by the same Luminex technology in a malaria-endemic context **[17]**.
- The logarithm of the anti-malarial anti-CSP antibody levels of the controls is normally distributed, with a mean of 6.8 and a standard deviation of 1.
- The logarithm of malaria antibody levels in cases is normally distributed with a standard deviation of 1.
- For each case, two matched controls are recruited.

- The correlation between the logarithm of anti-malarial antibody levels in cases and matched controls is 0.7.

The data collected will be analyzed using conditional logistic regression with a two-tailed test at the 5% significance level.

Power is calculated for different effect sizes (i.e. difference in malaria antibody levels between cases and controls). The table below shows the power for different effect sizes, or rather design effects. With a sample size of 300, the study is able to detect a 17% difference in median antibody titres with 80% power, which is considered clinically relevant.

Mean of logarithm of anti-malarial antibody levels in controls	Average of the logarithm of anti-malarial antibody levels in cases	Median malaria antibody levels of controls	Median malaria antibody levels of cases	Power
6.8	6.7	898	812	0.3
6.8	6.69	898	804	0.35
6.8	6.68	898	796	0.4
6.8	6.67	898	788	0.48
6.8	6.66	898	781	0.53
6.8	6.65	898	773	0.6
6.8	6.64	898	765	0.65
6.8	6.63	898	757	0.7
6.8	6.62	898	750	0.76
6.8	6.61	898	742	0.81

6.8	6.6	898	735	0.83
6.8	6.59	898	728	0.87
6.8	6.58	898	721	0.9
6.8	6.57	898	713	0.93
6.8	6.56	898	706	0.94
6.8	6.55	898	699	0.96

In conclusion, with a sample size of 300, the study is able to detect a 17% difference between median antibody titers with 80% power.

5.2 PROCEDURES

5.2.1 OVERVIEW OF STUDY PROCEDURES

Using the CRF, the investigator will interview both conscious cases and witnesses. For cases who are unconscious or unable to communicate, the medical file will serve as a source of information. Blood samples for cases and controls will be taken on D1 of the study.

5.2.2 STUDY/VISIT SCHEDULE

5.2.2.1 Day 1: main study visit

Participants will be informed of the study's objectives and design; if they agree, an informed consent form will be signed. After inclusion, the main study procedures will take place (questionnaire, sampling and follow-up).

The following variables will be collected:

- **Socio-demographic variables** :
  - Age
  - Gender
  - Profession
  - Civil status
  - Study level
- **Medical history**

- Comorbidities: diabetes, tuberculosis, cancer, HIV, renal failure, chronic liver disease, chronic lung disease, heart disease
- Medicines
- Smoking
- Pregnancy

- **Clinical variables** :
  - Clinical status according to WHO scale
  - Duration of symptoms
  - Type of symptoms
  - Size
  - Weight
  - Blood Pressure
  - Heart Rate
  - Respiratory Rate (RR)
  - Temperature (T°)
  - Free Oxygen Saturation (SaO_2)
  - Spleen size

<u>The following biological samples will be collected:</u>

- 30 ml of venous blood will be collected using Lithium-Heparin vaccuitenaire tubes for the various immunological analyses (described below).
- 5 µl of capillary blood will be taken for the thick smear, two µl of blood for the thin smear (GE/FM) and one drop of blood for the TDR.

The results of the thick drop and RDT will be communicated to the attending physicians for hospitalized patients (cases). For outpatients (controls), the results will be communicated to the ZS team in charge of malaria management, in accordance with national guidelines.

<u>Day 7 +- 2: follow-up</u>

For controls: a telephone contact on Day 7 with the treating clinician or the patient will be considered in order to obtain information on their clinical evolution. If, in the meantime, they have developed severe COVID-19, they will be excluded from the study and will no longer be considered as controls.

During participation in the study, the telephone number of the control as well as that of a close relative and the attending physician will be recorded in order to monitor the participant's health status. The patient's telephone number will be recorded separately in the patient identification register, which will be accessible only to local investigators. This

number will not be included in the CRF or patient recruitment register. If no telephone contact is possible, a home visit by the control will take place at D7.

5.2.3 LABORATORY PROCEDURES.

All analyses will be carried out by the INRB laboratory in Kinshasa.

30 ml of venous blood will be collected using Lithium-Heparin vaccuitenaire tubes. After centrifugation, the plasma fraction will be stored at -80°C and subsequently used to determine antibodies against malaria and COVID-19 using Luminex. A Luminex test will be developed specifically to determine the following *Plasmodium* antigens*:* CSP, AMAI, CSPM, GLURP, Pf/RESA55 and SARS-CoV-2 antigens. In the unlikely event of it not being possible to install the Luminex test in Kinshasa, a commercial ELISA test will be used as an alternative.

The PBMC fraction will be isolated for ELISPOT assays with malaria and SARS-CoV-2 antigens and *in vitro* experiments on entrained immunity, and the remainder cryopreserved afterwards. The potency of cellular immunity against Plasmodium and SARS-CoV-2 will be measured by IFN-γ ELISPOT assays. In a subgroup of individuals, flow cytometry measurements may be performed to validate the functionality and phenotype of responding cells.

For experiments on trained immunity, the PBMC fraction will be stimulated with ligands specific to antiviral receptors of the innate immune system (TLR3, TLR7-9, RIG-I, MDA-5, cGAS) **[18].** The release of antiviral cytokines (e.g. type I IFN, CXCL10) will be measured by commercial ELISAs and multiplexed flow cytometry assays.

In addition, 5 µl of capillary blood will be taken for the thick smear, two µl of blood for the thin smear (GE/FM) and one drop of blood for the TDR.

5.2.4 WITHDRAWAL AND TERMINATION OF THE STUDY

Participants may withdraw from the study at any time. This is the case if the participant or legal representative withdraws consent, or if the investigator considers that continuation of the study would have a negative effect on the patient's health.

Data and biological samples collected up to the time of the participant's withdrawal may be used for analysis purposes. However, if the participant expressly requests that they not be used, the data and samples will be destroyed.

6 DATA MANAGEMENT

6.1 DATA MANAGEMENT RESPONSIBILITIES

Although the principal investigator assumes ultimate responsibility for data management, practical implementation and monitoring will be carried out by the INRB data manager in collaboration with study staff at the sites. This will be done in accordance with the study's data management plan, which broadly describes the aspects below. The ITM Data Manager and other ITM colleagues will help support the implementation of this DMP.

6.2 DATA COLLECTION AND MANAGEMENT

6.2.1 DATA TYPES

Study data includes individual participant-level data at baseline and follow-up study visits. These include variables such as study participant identifiers, socio-demographic variables, medical history, symptoms, vital signs, laboratory results and final results status). See also 5.2.2.1 for further details.

6.2.2 COLLECTION AND MANAGEMENT METHODS

Study data will be collected on paper and/or directly via electronic questionnaires in the study database using REDCap. Only data defined by the study protocol will be collected.

REDCap (Research Electronic Data Capture) is a software application widely used in the academic research community to create and manage surveys, databases and research studies. It is a secure web application as well as a mobile application that can be used to collect, cleanse and manage subject information in compliance with various applicable standards and regulations (GCP, CFR 21 part 11, FISMA, HIPAA). Its mobile application enables offline data collection with smartphones or tablets. In addition, the software includes a query management system and capabilities for importing and exporting data in a variety of formats.

Paper questionnaires will be used where possible and appropriate. In addition, electronic questionnaires on tablets or laptops will be used to collect data from participants at remote sites. Subsequently, when connected to the Internet or cellular networks, data from the mobile device can be synchronized with the study server. Edit controls and branching logic, programmed on the electronic forms, will validate at the point of data entry and support data quality. To ensure better data quality, appropriate data management training will be organized before the actual data collection (and processing) begins. In addition, data will be

reviewed and monitored during the course of the study by various study collaborators to achieve timely database lock.

Because of the biological risk of COVID-19 contamination, any original written source document created at the patient's bedside (i.e. in the "red zone") should be treated as potentially infectious unless an effective means of decontamination can be instituted. Wherever possible, photographs, digital scans or other electronic data capture methods can be used.

6.3 DATA SECURITY AND CONFIDENTIALITY

The IT/data management system to be used incorporates a robust security architecture including encryption, firewalls, anti-virus software and controlled user access (tablet/computer authentication, REDCap username, personal password and authorized user role). Data backup will be provided at server, computer, mobile device and/or database level, where applicable.

ITM and INRB will also ensure that the necessary measures are taken to ensure the safekeeping of all data management documents (Data Management Plan, procedures, completed questionnaires) and that IT equipment (tablets, computers, server) is secured (locked cupboards, locked offices and/or rooms and/or controlled by badge).

Study participants' private data will be treated confidentially. Study participants' names and contact data will be replaced by an identification code specific to the study subject (= pseudonym). Only pseudonymized data will be used and distributed within the wider study team. See also 7.4 for further details.

6.4 DATA SHARING

The study will make its research data available for secondary research or to support the Congolese response to COVID-19 and considering the following key aspects:

- ITM's data sharing policy will be followed, with the following key aspects mentioned below
- The European FAIR data principles ("Findable"; "Accessible"; "Interoperable"; "Reusable") will also be respected ("as open as possible, as closed as necessary").
- Ensure confidentiality by anonymizing data (e.g. by replacing the study subject's identification code with a new code that can no longer be used to identify the patient; by generalizing and randomizing specific variables).
- Ensure that ethical approvals have been obtained by ethics committees and study participants.

- Making data available, mainly medical, personal and sensitive data, through a regulated data access mechanism and along a transparent decision-making process, with :

  (i) Filling in a data request form,

  (ii) Evaluation through access to committee data,

  (iii) agreement to share data through this committee,

  (iv) secure transfer of anonymized data,

  (v) additional metadata that clarify the research data. Metadata will include study protocol, questionnaire(s), data dictionary.

6.5 RECORDING RETENTION

The principal investigator is responsible for the retention of all essential documents listed in the Good Clinical Practice guidelines: 'Informed Consent (Form)' or IC(F) or Formulaire de Consentement Eclairé. All essential documentation for all study subjects must be retained by the principal investigator in a secure storage facility for at least twenty years in accordance with INRB policies or local country regulatory requirements (whichever is longer). The DRC requires that study files be kept for up to 2 years after publication. These records must also be retained in accordance with the ethics committee's medical record retention requirements, whichever is longer. All stored records must remain confidential to the extent required by national, state and local laws of the jurisdiction in which they are stored.

7 DATA ANALYSIS

A detailed statistical analysis plan will be developed prior to completion of the statistical analysis. The analysis plan will provide a full description of the variables of interest, statistical methods (including management of missing data, statistical adjustment, management of confounding factors and interactions), subgroup definitions and sensitivity analyses.

7.1 PRIMARY ANALYSIS

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

7.2 SECONDARY ANALYSIS

- Compare anti-malarial antibody levels against other antigens between severe and non-severe COVID-19 cases:
  - A model similar to that for the primary objective is fitted with the respective anti-malarial antibody levels as the independent variable.
- Comparing the cellular response against malaria between severe and non-severe COVID-19 cases:
  - A conditional logistic regression model will be fitted with cellular response against malaria as the independent variable.
- Compare the proportions of acute malaria infection between severe and non-severe cases of COVID-19 using the Thick Gout (TG) and Rapid Diagnostic Test (RDT).
  - A conditional logistic regression model will be fitted with the proportions of acute malaria infection as the independent variable.
- 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 numbers and proportions will be presented.
- Evaluate the modulatory 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
  - A conditional logistic regression model will be fitted with the SARS-COV-2 specific cellular immune response as the independent variable.

7.3 SUBGROUP ANALYSIS

No subgroup analysis will be performed.

7.4 MULTIPLICITY AND MISSING DATA

As this is a study with a single primary objective, no multiplicity adjustment is necessary. The management of missing data will be specified in the statistical analysis plan.

8 ETHICAL CONSIDERATIONS

8.1 ETHICAL (AND REGULATORY) REVIEW

The investigators would be committed to conducting the study in accordance with the principles of the Declaration of Helsinki. Prior to carrying out the study, official approval would be obtained from the ethics committee; the study protocol would be submitted for approval to the ITM's institutional review, to the ethics committee of Belgium's Antwerp University Hospital, and to the national ethics committee of the DRC's Ministry of Public Health. Authorization will also be sought from the relevant health zones before the study begins.

8.2 PROTOCOL CHANGES

Once the final study protocol has been issued and signed by the authorized signatories, it cannot be modified informally. Amendments to the protocol have the same legal status and must go through the appropriate steps before being implemented. Any substantial modification must be approved by all the bodies and the Ethics Committee (EC) that approved the original protocol, before being implemented, unless it is due to participant safety concerns (in which case immediate implementation may be necessary for participant protection). If changes to the protocol or an amendment are requested by a local EC during the review process, they must be discussed and agreed with the sponsor prior to any resubmission incorporating these changes.

8.3 OBTAINING INFORMED CONSENT

Before starting the study, the investigator assigned to the site (CTCO or ZS) will obtain informed consent from the participants; this will consist of asking for their opinion and explaining the purpose, objectives, rights of the participants and responsibilities of the investigation team. Consent will ensure that each participant understands all procedures. Participants will receive clear explanations of all possible techniques that will be used by their samples.

The informed consent form will be translated into the local language (Lingala) at the language service of the University of Kinshasa. The informed consent procedure will be conducted in the patients' mother tongue or preferred language by a qualified person, indicated by the principal investigator.

Patients will have sufficient time to ask questions and make a free decision. If the person is willing to participate, he or she will be asked to sign the consent form. If the participant cannot read and/or write, an impartial witness should be present during the informed

consent discussion. After the written informed consent form has been read and explained to the participant, and after he/she has orally consented to participate in the study and provided his/her fingerprint, the witness should complete the participant's name and add the date of the fingerprint, then personally sign and date the consent form. By signing the consent form, the witness attests that the information contained in the consent form and all other written information has been accurately explained to the participant, that the participant has apparently understood it, and that the participant has freely given consent.

The person obtaining informed consent will be asked to sign and date a written confirmation that he/she has been sufficiently informed about all relevant aspects of this study and confirms that he/she has freely and voluntarily consented to participate in the study.

For patients who are unable to give consent due to their clinical condition, consent will be obtained from a legally acceptable representative. As soon as the patient is able to give consent, the consent procedure will be repeated and the patient will be given the opportunity to confirm/refuse participation.

8.4 PRIVACY

Individual medical information collected in this study will remain confidential. Members of the study team are bound by professional secrecy. Individual data will be made available to physicians in charge of patients upon request to the investigators. Disclosure of this information to third parties is strictly forbidden.

Electronic files will not contain any data that could identify the participant (e.g. surname, first name, full date of birth, telephone number). Each study participant will be assigned a unique identification code (ID). On all study documents or files, study participants will be identified by the participant's ID code.

A correspondence list will establish the link between the participant ID and the participant's personal data so that examination results can be used for patient care. This list containing personal data and other documents containing participants' names or signatures (e.g. informed consent) will be kept separate from other study documents. All study documents will be kept in a secure, locked location, with access limited to the site principal investigator and authorized personnel.

Access to all paper documents and electronic files required for data management and study follow-up will be restricted to authorized personnel at international, regional and local levels. Study computers and CRF files will only be accessible via a login with a personal username

and password. A list of authorized CRF and database users will be kept at ITM and updated throughout the study.

8.5 RISKS AND BENEFITS

The direct benefit of the study is that each participant will receive a free malaria test with an additional examination, namely a blood sample. This will be the only additional examination compared to basic care**.** In addition, as compensation for their time, the controls will receive masks and soap to limit the spread of the virus in their environment.

Participation in the study will also benefit the community; it will contribute to the acquisition of knowledge about COVID-19, and the information it will generate will enable researchers or the response team to improve management. The only additional procedure specific to the study will be follow-up visits for controls: telephone contacts, or for patients for whom this is not possible: a physical visit either at the study site or at home.

The greatest risk for participants is a potential breach of confidentiality, for which all measures will be in place to limit this as much as possible (by de-identification). The sting may also cause a little pain and swelling at the sampling site, but this is temporary. In the event of significant risk, as assessed by the clinician, the sample will not be taken and the patient will be excluded (see inclusion/exclusion criteria).

8.6 SAMPLE STORAGE

The samples collected can be stored for a maximum of 20 years, so that they can be used for further research into COVID-19 immunization in line with future scientific discoveries. These samples will be stored and registered at INRB's bio-banking facilities in Kinshasa. Subjects will be asked to indicate on the informed consent form whether or not they agree to long-term storage.

The decision whether or not to use the samples for future research will be made by the study investigators in consultation with, and only after receiving approval from, the Institutional Review Board (IRB) and ethics committees.

8.7 INSURANCE

The coordinator of this study, ITM, has obtained study (no-fault) insurance to cover any injury, damage or loss to study participants caused directly or indirectly by their participation in the study. Information about the insurance is not specified in the Informed Consent Form for this study due to the expressed concern about unreasonable claims for compensation. Furthermore, we do not anticipate any specific physical injuries or mental health problems as a result of participation in this observational study.

8.8 MONITORING AND QUALITY CONTROL

Detailed SOPs will be developed to cover all laboratory processes, from sample collection to shipping, analysis and storage. Immunological and parasitological tests will be carried out by qualified laboratory technicians. The INRB and ITM PIs will be responsible for ensuring that all study-specific analyses are carried out in accordance with Good Clinical Laboratory Practice (GC(L)P) guidelines, study-specific Standard Operating Procedures (SOPs), and the internal/external quality control systems of the respective tests. During visits by the ITM research team, compliance with the protocol and GC(L)P will be monitored, including data management and documentation.

9 DISSEMINATION OF RESULTS AND INTELLECTUAL PROPERTY

All study documents are provided by the principal investigators to the investigators and their designated personnel in strict confidence. None of these documents may be disclosed to any party not directly involved in the study, without the written authorization of the principal investigators.

Once the results are considered final, arrangements will be made to communicate them to the Kinshasa community in the most appropriate way, with the active participation of the health authorities. Oral communications and written publications will mention the name of the sponsor and the names of all partners and researchers involved.

10 CALENDAR

N°	Tasks	Managers	Mars 21	April 21	May 21	June 21	July 21	August 21	Sept 21	Oct 21	Nov 21	Dec 21	Janv 22	Feb 22	March 22
1	Drafting the protocol	- Principal investigator - Scientific Committee													
2	Submission and approval Ethics Committee	- Investigator - Developer													
3	Preliminary steps : - Contact with political and administrative authorities	- Investigator - Supervisor - Scientific Committee													

	- Survey tool finalized - Interviewer training														
4	Collecting data	Investigators													
5	Laboratory analysis of samples	Survey team													
6	Data capture and analysis	- Project management/Monitoring - Data management													
7	Summary of results	Scientific Committee													

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