IMT RESEARCH STUDY

Clinical aspects, severity, management and outcome febrile illnesses in the DRC FIKI² ("Febrile Illness in Kinshasa and Kimpese")

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DECLARATION OF CONFORMITY

By signing this protocol, the researcher(s) acknowledge(s) and agree(s) to

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

Once the final protocol has been published and signed by the researcher(s) and authorized signatories, it cannot be modified informally. Amendments to the protocol have the same status
and must go through the obligatory review and approval stages before being implemented.
implementation.

COORDINATING INVESTIGATOR :

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Title, Name: Prof. Jean Pierre Elongi Date:

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PRINCIPAL INVESTIGATOR :

Title, Name: Prof. Charles Mbala Date:

Signed:

By signing this document, I undertake to carry out the trial in accordance with the protocol
clinical practices and applicable ethical and regulatory requirements. I also acknowledge the
paragraph concerning the confidentiality of the study and I authorize the Institute of Tropical Medicine, Antwerp,
Belgium, to record my data on a computerized system containing all data
relevant to the study.

TABLE OF CONTENTS

DECLARATION OF CONFORMITY **4**
SYNOPSIS **6**
1. INTRODUCTION **12**
1.1 BACKGROUND 12
1.2 JUSTIFICATION 13
2. STUDY OBJECTIVES **15**
2.1 PRIMARY ENDPOINT 16
2.2 SECONDARY ENDPOINTS 16
3. STUDY DESIGN **16**
3.1 GENERAL STUDY DESIGN 17
4. PARTICIPANTS, POPULATION AND ELECTIONS **17**
4.1 RECRUITMENT SITES 17
4.2 SELECTION AND RECRUITMENT 17
4.3 INCLUSION AND EXCLUSION CRITERIA 18
4.4 SAMPLE SIZE 18
4.5 WITHDRAWAL AND TERMINATION OF 18
5. STUDY PROCEDURES **19**
5.1 STUDY/VISIT SCHEDULE 19
5.2 OBTAINING INFORMED CONSENT 20
5.3 LABORATORY PROCEDURES 23
6. STATISTICAL METHODS **23**
6.1 VARIABLES OF INTEREST 24
6.2 STATISTICAL METHODS 24
6.3 SAMPLE SIZE AND POWER 25
7. QUALITY CONTROL AND ASSURANCE **25**
8. DATA MANAGEMENT **26**
9. ETHICAL ISSUES **28**
9.1 ETHICAL AND REGULATORY REVIEW 28
9.2 PROTOCOL MODIFICATIONS 28
9.3 INFORMED CONSENT 29
9.4 CONFIDENTIALITY 29
9.5 RISKS AND BENEFITS 29
9.6 ACCIDENTAL DISCOVERIES 30
9.7 COMPENSATION FOR PARTICIPATION 30
9.8 INSURANCE 30
10. DISSEMINATION OF RESULTS, INTELLECTUAL PROPERTY **30**
11. ARCHIVING **31**
12. REFERENCES **31**
13. LIST OF ABBREVIATIONS **32**
14. APPENDICES **34**

SYNOPSIS

BACKGROUND AND RATIONALE	The current epidemiology and outcome of febrile illnesses in The Democratic Republic of Congo (DRC) is poorly documented. A better description of the causes, presentation and host biomarker profiles will make it possible to future studies targeting diagnostic and optimized therapies. The FIKI² clinical trial initiated (see protocol version 1.0), which prospectively describes febrile illness in 2 sites of DRC, has integrated a prospective sample collection of enrolled patients; it will enable analysis of the host responses (biomarker profiles, in particular the 'C-Reactive Protein' - CRP) and explore the pathogens involved (blood cultures; metagenomic sequencing for the detection of pathogens, with a primary focus on RNA viruses and arboviroses). The storage of these samples, well documented with clinical data, will enable the development and evaluation diagnostic and monitoring platforms, as well as secondary etiological or immunological investigations.
DESIGN	Bi-centric prospective observational cohort study of adults and children presenting to emergency departments or outpatient clinic with community-acquired febrile illness, with laboratory analysis and sample storage in a biobank.
STUDY SITES AND POPULATION	1. Pediatric Emergency Department, Hôpital Général de Kinshasa (HGK / Mama Yemo), DRC : urban site, children < 15-year-old with febrile illness. 2. Hospital outpatient and emergency services Reference General (HGR) of Institut Médical Evangélique (IME); health services outpatient consultation at one or more health centers in the IME reference area, Kimpese, DRC: rural site, adults and children with febrile illnesses.
OBJECTIVES AND CRITERIA JUDGEMENTS	**Primary objectives** 1. Estimate the proportion of survival with resolution of symptoms, assessed on day 21, of febrile illnesses. 2. Describe the biomarker profile (CRP, blood cell count with differentiation) on inclusion of the patients with febrile illnesses.

	Secondary objectives 1. Estimate the proportion of survival with non-resolving symptoms and death, assessed on day 21, of the febrile illnesses. 2. Estimate the proportion of survival with resolution of symptoms, survival with non-resolution of symptoms, and death, assessed on days 7 and 14, of the diseases feverish. 3. Describe epidemiology, clinical aspects and severity and management of febrile illnesses. 4. Identify factors associated with outcomes (resolution, unresolved, death) of febrile illnesses. 5. Determine the frequency and profile of malaria (by rapid diagnostic test and thick drop), including in co-infection, in patients with severe feverish. 6. Observe the association between CRP values and with patient outcome at day 21. 7. Observe the association between CRP values and the rate of white blood cells with specific etiologies (malaria), bacteremia, dengue fever, etc.). **Exploratory objectives** 1. Describe the frequency, etiology and profiles of antibiotic resistance in bacteremia detected in patients with febrile illnesses. Evaluating the performance and potential contribution of a test rapid immunochromatographic test for dengue fever under field conditions in the DRC. 2. Evaluate metagenomic sequencing for the detection of fever-causing pathogens (with a primary focus on RNA viruses). **Strategic objectives** 1. Create at ITM and in research institutions a biobank of clinical samples with a strong to enable the development and the future evaluation of diagnostic platforms and monitoring, as well as other secondary research. 2. Strengthen clinical research capacity and laboratory training at participating sites and direct support. 3. Establish etiologies, epidemiology and inflammatory/immunological characteristics and the basic outcome of febrile illnesses in preparation

	subsequent studies focused on optimizing diagnostics (biomarkers, platforms, etc.) or therapeutic interventions. **Primary endpoints** ● Proportion of survival with symptom resolution evaluated on day 21, febrile illnesses. ● CRP and white blood cell values with differentiation on day 0 (inclusion). **Secondary endpoints** ● Clinical status on a 3-category ordinal scale (resolution, non-resolution, death) evaluated on **days 7, 14 and 21.** ● Diagnostic hypotheses at inclusion and diagnoses final. ● Disease severity on **days 0, 7, 14 and 21**. - *in adults :* o Simplified ambulatory severity index (SAHI) o Quick Sepsis Related Organ Failure Assessment (qSOFA) - *in children under 5 years of age*: o criteria of the algorithm for managing childhood illnesses (ALMANACH) ● Initial and secondary hospitalization (assessed on **day 21**). ● Length of hospital stay (initial hospitalization and/or secondary) (assessed on **day 21**). ● Number of secondary visits (assessed on **day 21**). ● Number and types of laboratory and radiology tests prescribed / actually carried out. ● Number and types of medications and supportive care prescribed / actually administered. ● Detection of malaria by rapid test (HRP II antigen), pLDH antigen) and by thick drop (type and parasitaemia). **Exploratory judgment criteria** ● Frequency of bacteremia, with etiological distribution and antibiotic resistance profiles of germs identified. ● Detection of dengue by rapid test (NS1 antigen), IgG and IgM antibodies). ● Detection and profiling of disease-causing pathogens by metagenomic sequencing.
INCLUSION CRITERIA AND EXCLUSION	**Inclusion criteria:** ● Ongoing fever detected on presentation, or documented at home or in another health center within 24 hours of presentation, defined by: temperature

	axillary or tympanic > 37.5°C, or oral temperature or rectal > 38°C. ● Possibility of contact between the patient (or a relative) and and the study team on days 7, 14 and 21. ● Informed consent to participate signed by patient (adult) or a legally acceptable representative (child) or patients whose condition does not allow them to sign the informed consent), with the children's assent to From the age of 12, wherever possible. **Exclusion criteria** ● Children under two months old. ● Hospitalization > 48 hours in the last 14 days.
SCREENING AND RECRUITMENT	Sample size (participants recruited in the study laboratory, following amendment 1 of the protocol) - 500 children at HGK / Mama Yemo, Kinshasa - 500 children at and 500 adults at HGR-IME/centres de health, Kimpese Patients will be enrolled in the study at the time of hospital presentation. The 1,500 participants will include uniformly throughout the study period.
FOLLOW -UP	Each patient will be monitored for 21 days. Patients will be evaluated at inclusion, and during fixed time points (D7, J14 and J21) for ambulatory patients, and daily for patients hospitalized at study sites. A blood sample (venous +/- capillary) is taken at the time of inclusion. Venous blood volume of maximum 14.5 ml (in children aged <14 years) or 30.5 ml (≥14 years) is taken. to meet the clinical and monitoring needs of for additional laboratory analyses related to and for sample storage. For children < 14 years of age, the maximum volume to ensure safety during blood sampling will be calculated on the basis of age, weight and capillary haemoglobin level. If the total quantity required is not possible, priority will be established as follows: 1. clinical necessity and monitoring 2. primary study analyses 3. secondary and exploratory study analyses 4. sample storage
LABORATORY ANALYSES	The following samples will be collected (according to consent and site capacity) 1. Fingertip capillary blood test (participants) of < 14 years)

	a. Hemoglobin (quantity determination in children under 14 years of age): QuikRead Go®, bowls CRP/hemoglobin or HemoCue® Hb 301 b. CRP (only on capillary blood if simultaneously with hemoglobin by Quikread Go®) c. Preparation of up to 5 bloodstains dried: for storage + secondary research For participants ≥ 14 years of age, blood sampling The capillary blood sample is taken after the venous blood sample. 2. Venous blood sampling: volume-dependent that can be collected, the samples in order of priority a. 4 ml (2 ml if < 14.5 ml can be taken from children < 14 years) EDTA tube ▪ According to clinical need: blood glucose ▪ Malaria TDR: SD Bioline pan-LDH & pf-. HRP2 ▪ Thick drop ▪ Dengue TDR: SD Bioline Dengue Duo ▪ White blood cells and differentiation : HemoCue® WBC/DIFF ▪ CRP (if not yet performed on blood capillary) : QuikRead Go ®, cuvettes CRP/Hemoglobin (with hemoglobin); CRP TDR Centrifuge the remaining volume to aliquoting (in 2-5 aliquots of 330 µL plasma) and stored in the biobank at -80°. The aliquots will be used in part for metagenomic analyses, the remaining volume (the will be stored for research purposes secondary. b. Blood culture bottle (1-4 ml in the case of children < 14 years, 20 ml in patients ≥ 14 years): according to routine c. 2 ml serum tube: biochemical analysis according to clinical necessity d. 2 ml additional EDTA: storage and secondary research

	e. 2.5 ml PAXgene: storage in the biobank at -. 80°C, for secondary research Blood cultures are taken and analyzed in accordance with the monitoring protocol (protocol approved in DRC and Belgium; IRB IMT 613/08, UZA CE 8/20/96). Study analyses (except metagenomic analyses) will be carried out at the IME laboratory and the study laboratory of HGRK. Samples for metagenomic analysis will be transported to the ITM laboratory in Antwerp. Samples for secondary research will be stored in the biobank on research sites, or at ITM, Antwerp.
ANALYSIS METHODS	Description of the proportion of patients meeting the criteria for 3 categories (resolution, non-resolution, death) on days 7, 14 and 21, overall and by basic characteristics (age, gender...), geographical location, syndromic category, characteristics biomarker results) in terms of medians and interquartile ranges for continuous characteristics, and in terms of numbers and percentages for categorical characteristics. Estimates will be given with a Wilson 95% confidence interval. Models of univariate and multivariate logistic regression will be used to analyze the association between basic characteristics and condition on day 21. Biomarker data will be described mainly using descriptive statistics of the as described above. The prevalence of malaria in the study population will be calculated as proportion with a Wilson 95% confidence interval. The association of CRP and white blood cell values with patient results at day 21, or with specific etiologies, will be examined using the Mann-Whitney test. The evaluation diagnostic performance will be calculated by calculating the standard diagnostic performance measures, such as the sensitivity and specificity.

1. INTRODUCTION

1.1 Background

Of the 54.8 million deaths worldwide in 2013, 8.3 million (15.2%) were caused by
products in sub-Saharan Africa. Over 60% of deaths in Africa are linked to disease.
infectious diseases, and more than 50% of all infectious disease-related deaths worldwide occur in the workplace.
Africa. Compared with global mortality data, the incidence of death from disease
is 3.5 times higher in sub-Saharan Africa (1). This burden of disease
is all the more intolerable given that many of the infectious diseases that kill
are both preventable and curable. This also means that the main causes of death in
Africa are avoidable, and any effort to prevent and cure them can lead to a
significant reduction in mortality.
The aetiology and burden of syndromes such as diarrhoea and pneumonia have been the subject of much debate.
particularly in children (2,3). However, many pathogens can
cause fever with focal signs in various organ systems, leading to
different syndromic presentations, or cause an "undifferentiated fever" without signs of
focalization (4,5). Febrile syndromes are generally divided into localized infections
(respiratory, intestinal, cutaneous, genitourinary, etc.) and undifferentiated infections (without associated symptoms or focal signs). Fever (undifferentiated or not) is one of the most common main reasons for seeking healthcare in Africa
sub-Saharan Africa, but it represents a huge challenge, given the wide range of differential and
the wide etiological spectrum (6,7). Among hospitalized patients with a febrile illness
undifferentiated, for example, case-fatality rates reach 12% (8).
The burden of specific infections, such as malaria (9) and certain bacterial infections
septicemia, is relatively well quantified (1), but viral infections, infections
fungal diseases, bacterial zoonoses and parasitic diseases that have not been adequately
may be underestimated. New emerging diseases such as fevers
hemorrhagic viruses, or COVID-19 risk developing into major health threats.
and pose important and dynamic challenges for disease diagnostic capacity.
feverish.

With the decline in malaria transmission and increased use of diagnostic tests
(RDT), appropriate management of febrile patients with a malaria RDT
is a growing clinical challenge for which there is no clear recommendation (9).
In some contexts, the deployment of RDTs for malaria has ultimately contributed to
transform the problem of antimalarial drug abuse into that of antibiotic abuse (10, 11). The
antibiotic resistance has become a major issue for global health, and
the rational use of antibiotics in the care of sick people is seen as an important
key strategy for combating this problem (alongside veterinary control).

Clinical research is a key element in the development of evidence-based strategies for
to improve the prevention, diagnosis and management of febrile illnesses. The WHO has therefore
stated that "multicenter studies on the causes of fever are needed to identify
the main conditions that can be treated" and "provide evidence for the adaptation of
clinical algorithms for fever management by countries" (11); and that studies on "the importance

of febrile illnesses for populations and patients are necessary" to "produce evidence-based recommendations for the management of febrile patients, improve health status, reduce the prevalence of serious illness and death, and maintain the effectiveness of drugs" (11).

However, research capacity in Africa is severely limited, as illustrated by the the fact that only 5003 (2.4%) of the 208491 clinical trials registered on clinicaltrials.gov in 2019 are on the African continent (www.clinicaltrials.gov), which contrasts sharply with the needs in research linked to the burden of morbidity.

1.2 Justification

As in most sub-Saharan African countries, very little data is available in this area. Democratic Republic of Congo (DRC) on the epidemiology, clinical presentation, causes and the outcome of febrile illnesses; research capacity is generally very low.

The CREDO Project, an extension of the agreement between Antwerp's Institute of Tropical Medicine (ITM) and the Directorate General for Development Cooperation and Humanitarian Aid (DGD) aims to improve research capacity on (re)emerging epidemics in the DRC. The study is part of this general objective and is planned for 2 clinical sites (see below). below), who have little experience of clinical research. In collaboration with 2 partners local: Centre de Recherche Clinique (CRC) of the Institut National de Recherche Biomédicale (INRB), and the Kimpese Health Research Centre (CRSK). In this way, the first foundations to build these hospitals into research centers will be established, and, even more important Congolese research teams will be trained through ongoing supervision in the field. In addition, the study provides the epidemiological and clinical basis for future research into optimizing diagnostic and therapeutic strategies.

The initial protocol (and its title) were inspired by experience gained during the FISSA study. ("Febrile Illness in sub-Saharan Africa"). This is a multi-center cohort study designed to document the clinical aspects, severity, management and outcomes of febrile illnesses in health centers in West, Central and East Africa. This study was developed by African Coalition for Epidemic Research, Response and Training (ALERRT) : a consortium of 21 African and European institutions. The consortium has secured funding of the European Union's 5-year EDCTP program. The study protocol has been approved by the ITM IRB.

The use of a fairly similar protocol in the DRC will have the advantage of enabling studies with common inclusion and evaluation criteria, and thus complete the picture. with scientific information from a Central African country, which is relatively under-represented in the the FISSA study.

The purely descriptive and observational clinical component of the initial study is in this This phase is complemented by laboratory procedures, with the additional aim of providing better understanding of the causes of febrile illness and optimization of strategies diagnosis and monitoring :

1. Analysis of inflammatory biomarkers: C-reactive protein (CRP), white blood cells and their differentiation by different types of point-of-care (POC) equipment: analyzers and RDTs. An evaluation of the performance of a few TDRs for CRP will be carried out. as an additional comparative analysis. The objectives and methodology of this laboratory-only analysis is not described in a specific sub-protocol (Appendix 1). This new component will enable the future evaluation of strategies Diagnostics based on the use of RDTs for these inflammatory biomarkers, integrated into clinical routine.
2. Etiological research into febrile illnesses
    a. Blood culture monitoring. Although pilot projects exist on both sites already, as part of a project to improve monitoring of resistance to antibiotics and clinical management (protocol approved in the DRC and the Belgium; IRB IMT 613/08, UZA CE 8/20/96), the systematic application on FIKI² study populations will make it possible to evaluate bacteremia as an etiology of febrile illnesses, and to evaluate the biomarkers analyzed as factors of prediction of blood culture positivity.
    b. Systematization of febrile illnesses using RDTs targeting specific specific pathogens: malaria and dengue fever. Unlike malaria, RDTs for dengue are poorly validated under field conditions in sub-Saharan Africa. A systematic use of dengue RDTs, and reference diagnoses (in metagenomic analyses, or other secondary research) will enable us to assess their respective roles in the diagnostic approach to diseases feverish in this context.
    c. Evaluation of a new platform for the detection of pathogens causing febrile diseases (primary focus on RNA viruses such as arboviruses) by metagenomic sequencing (performed at ITM in Belgium or on site after implementation).
    To identify the etiology of an infection, clinicians rely on tests that specific laboratory diagnostics that focus on one or a small number of pathogens (by PCR, antigen detection, etc.). The narrow scope of these targeted tests by their very nature neglects untested pathogens or agents previously unknown emerging pathogens. Metagenomic sequencing, which is proposed here will provide an interesting alternative to targeted testing with a potential for the diagnosis and monitoring of febrile illnesses (12). This technique enables the sequencing of all the genomic material present in a given patient sample without prior knowledge of the target. The sequences recovered can be compared with databases from sequences of well-coded infectious pathogens and new pathogens viruses can also be identified in this way. Given that RNA have a marked epidemic potential (13), we will first focus on detection of RNA viruses. Our aim here is to demonstrate that sequencing metagenomics can be a useful tool for epidemiological and environmental mapping. regional surveillance of pathogens linked to acute fevers (observational).
3. Well-documented sample storage with clinical data will enable the development and evaluation of future diagnostic and monitoring platforms, and

other secondary post-hoc research. Future analyses may include profiling of peripheral blood to search for early biomarkers to distinguish bacterial from viral infections.
Separate ethical approvals will be requested from all ethics committees with the submission of a detailed study protocol.

These objectives will make it possible to integrate a laboratory component consisting in the development and
strengthening the clinical research capacity of the 2 sites, with a focus on the ITM biobank and in partner research institutions, and the evaluation of POC analyses.

2. STUDY OBJECTIVES

Primary objectives

1. Estimate the proportion of survival with symptom resolution, assessed at day 21, of febrile illnesses.
2. Describe the biomarker profile (CRP, white blood cell count with differentiation) in the case of
   inclusion of patients with febrile illnesses

Secondary objectives

1. Estimate the proportion of survival with non-resolution of symptoms and death, assessed on day 21, febrile illnesses
2. Estimate the proportion of survival with symptom resolution, survival with non resolution of symptoms, and death, assessed on days 7 and 14, of febrile illnesses
3. Describe the epidemiology, clinical aspects, severity and management of diseases feverish.
4. Identify factors associated with disease outcomes (resolution, non-resolution, death) feverish.
5. Determine the frequency and profile of malaria (by rapid diagnostic test and gout) including co-infection, in patients with febrile illnesses.
6. Observe the association of CRP values and white blood cell counts with the outcome of the patients on day 21
7. Observe the association of CRP and white blood cell values with etiologies specific (malaria, bacteremia, dengue fever, etc.)

Exploratory objectives

1. Describe the frequency, etiology and antibiotic resistance profiles of bacteremias detected according to the surveillance criteria in patients with febrile illnesses
2. Assessing t h e performance and potential contribution of a rapid immunochromatographic test targeting dengue under field conditions in the DRC
3. Evaluating metagenomic sequencing for the detection of pathogens causing febrile diseases (with a primary focus on RNA viruses)

Strategic objectives

1. Set up a biobank at the ITM and partner research institutions of well-documented clinical samples to enable the development and

future evaluation of diagnostic and monitoring platforms, as well as other secondary research.

2. Strengthen the clinical and laboratory research capacity of participating sites by formal training and direct support
3. Establish etiologies, epidemiology, inflammatory/immunological characteristics and basic outcome of febrile illnesses in preparation for subsequent focused studies on diagnostic optimizations (biomarkers, platforms, etc.) or interventions therapeutics

2.1 Primary judging criteria

- Proportion of survival with symptom resolution assessed at day 21, for febrile illnesses.
- CRP and WBC values with differentiation on day 0 (inclusion)

The word " **resolution"** refers to the following situations: (i) the signs and symptoms have completely disappeared and treatment has been completed; or (ii) certain signs or symptoms and / or treatment is still underway, but there has been a marked improvement in these signs and symptoms suggest that the person will recover quickly without needing exploration or treatment other than those already administered.
Any other combination suggests that the situation is "**unresolved**".

2.2 Secondary endpoints

- Clinical status according to a 3-category ordinal scale (resolution, non-resolution, death) evaluated on **days 7, 14 and 21.**
- Diagnostic hypotheses at inclusion and final diagnoses.
- Disease severity on **days 0, 7, 14 and 21**.
  - *in adults :*
    - Simplified ambulatory severity index (SAI)
    - Quick Sepsis Related Organ Failure Assessment (qSOFA)
  - *in children under 5 years of age*:
    - criteria of the algorithm for the management of childhood illnesses (ALMANACH)
- Initial and secondary hospitalization (assessed on **day 21**).
- Length of hospital stay (initial and/or secondary hospitalization) assessed on **day 21**).
- Number of secondary visits (assessed on **day 21**).
- Number and types of laboratory and radiology tests prescribed/actually performed.
- Number and types of medications and supportive care prescribed/actually administered.
- Detection of malaria by rapid test (HRP II antigen, pLDH antigen) and thick drop test (type and parasitemia)

2.3 Exploratory judgment criteria

- Frequency of bacteremia, with etiological distribution and drug resistance profiles antibiotics for identified germs
- Detection of dengue by rapid test (NS1 antigen, IgM)
- Detection and profiling of pathogens causing febrile illnesses by sequencing metagenomics

3. STUDY DESIGN

3.1 General study design

This is a bi-centric, prospective, observational cohort study of adults and children in the
presenting to the emergency department or outpatient clinic with a community-acquired febrile illness (with
or without focal signs) at 2 clinical sites in the DRC. The study included blood sampling
at inclusion for study analysis and storage.
The study will describe the epidemiology, causes, clinical aspects and biomarker profile of the disease.
the severity, management and outcome of febrile illnesses, using the data from
collected during routine diagnostic and therapeutic procedures, and study analyses.
Each patient will be monitored for 21 days. Follow-up will include:

- Daily visits for hospitalized patients,
- Telephone calls (or a visit to the study center or home visit) on days 7, 14 and 21 for outpatients and discharged patients.

4. PARTICIPANTS, POPULATION AND ELECTIONS

4.1 Recruitment sites

Kinshasa General Hospital (also known as Mama Yemo Hospital) is a 2,000-bed hospital,
centrally located in the Commune de Gombe, serving an urban population. The
pediatrics has 170 beds. Participants in the FIKI² study will be recruited from the following departments
pediatric emergencies, which currently see around 5 febrile children a day.

The Institut Médical Evangélique (IME) de Kimpese is the General Reference Hospital (HGR) of
Kimpese, a 400-bed hospital in the province of Kongo Central, west of DRC. The department
The pediatrics department has 64 beds, while the internal medicine department has 78 beds.
Some of the participants in the FIKI² study will be recruited there from emergency departments and the outpatient consultations. The monthly number of outpatient consultations and admissions to the HGR is of around 2257 and 167 respectively. Approximately 10% of patients seen at the outpatient clinic were present with fever.
The population served by this health facility is estimated at 187,796 (Zone de Santé de
Kimpese). There are several health centers in this area. Recruitment will take place
also, if necessary, in one or more of these health centers (selected on the basis of their
proximity, attendance, clinical standards), by the study team based at the IME and following
exactly the same study protocol.

4.2 Selection and recruitment

The frequency of febrile illnesses can vary from season to season. In order to balance the
seasonal recruitment, the FIKI² study will be carried out in two phases
staggering inclusions evenly over the duration of the study, to take account of these
seasonal variations. Recruitment will be carried out in such a way as to avoid, as far as possible, any influence from members of
the study team on the choice of participants to be included. Recruitment will be stratified by age and site, with the number of patients (adults and children) to be recruited defined for the 2 sites.
This number will be evenly distributed over the duration of the study, with a recruitment target of

weekly. Participants will be recruited consecutively from the beginning of the week.
until the target is reached. If the planned number of patients has not been reached by the end of a week, the supplement will be paid the following week.

4.3 Inclusion and exclusion criteria

Inclusion criteria

- Fever in progress and objectified at presentation, or documented at home or in another location
  health center within 24 hours of presentation, defined by:
  - Axillary or tympanic temperature > 37.5°C
    OR
  - Oral or rectal temperature > 38°C

  AND
- Possibility of contact between the patient (or his or her designated relative) and the team responsible for
- study on days 7, 14 and 21.
  Informed consent signed by patient (adults) or legal representative
  acceptable (children or patients whose condition does not allow them to sign informed consent),
  with the consent of children aged 12 and over, wherever possible

Exclusion criteria

- Children under two months old.
- Hospitalization > 48 h in the last 14 days (to exclude nosocomial fevers).

4.4 Sample size

The sample size is of convenience and was adapted to the number of febrile patients.
and feasibility forecasts. The sample size refers to the
participants recruited in the laboratory phase of the study (following amendment 1 of the protocol). We plans to include 500 children in the HGK / Mama Yemo site, and 500 children + 500 adults in the HGR-IME and health centers in Kimpese. Sample size calculations can be found in
section 6.3.

4.5 Withdrawal and termination of the study

Participants may be withdrawn from the study if:

- The participant or legal representative withdraws consent
- The investigator believes that continued participation would have a negative effect on the health of the
  participant

Data from participants withdrawn from the study will be used until the time of the last visit.
study.

In the absence of any news from a participant, the interviewer will do everything within his or her power to contact the participant.
(limited to a maximum of 3 calls to the available telephone numbers and/or visits spread over a number of days)
within the visit period (window of ± 2 days) to try to re-establish a connection with the participant for follow-up. A patient included in the study who, however, is no longer available for the follow-up visits, will be considered as a loss of follow-up/abandonment.

If the study is prematurely interrupted or suspended due to force majeure, the sponsor will promptly inform the investigators, institutions and the regulatory authority of any interruption or

of the suspension and the reason(s) for the interruption or suspension. CEs will also be informed without delay and will receive from the sponsor or investigator/institution the reason(s) for interruption or suspension, as specified in the applicable regulatory requirements.

5. STUDY PROCEDURES

5.1 Study/visit schedule

Inclusion visit

Participants will be included in the study as soon as the consent form has been signed. The study investigator or his or her representative will fill in the data collection form (box report form (CRF), the patient's demographic information, history, symptoms and signs (type and severity), co-morbidities, initial diagnosis, medications and examinations and his immediate decision (hospitalization or discharge).

The study team will work with patients to arrange the following contacts on days 7, 14 and 21.

- For patients with whom telephone contact is considered possible, the study team records the patient's telephone number and the number of the telephone number of one or more relatives authorized by the patient to provide information on health status up to day 21. Patients will also receive the member of staff responsible for the study.
- Patients for whom telephone contact is not an option will receive offer to return to the study center on days 7, 14 and 21. Home visits by community health workers participating in the study will also be considered if necessary and feasible.

- If the participant gives permission for the laboratory part of the study, a blood sample will be taken.
  (venous +/- capillary blood) is taken during the inclusion visit. This blood test is integrated with the routine sampling required for patient management. Immediate test results (CRP, white blood cell count with differentiation), blood cultures, thick drop, malaria RDT, dengue RDT) will be collected in the and some of them (hemocultures) communicated directly to the clinicians at study. Samples stored for further analysis (metagenomic evaluation) or of secondary research will be registered in the ITM biobank and the institutions research partners, depending on the logistical capacities being developed and well-established memorandums of understanding (based on existing collaborations).

Follow-up visits

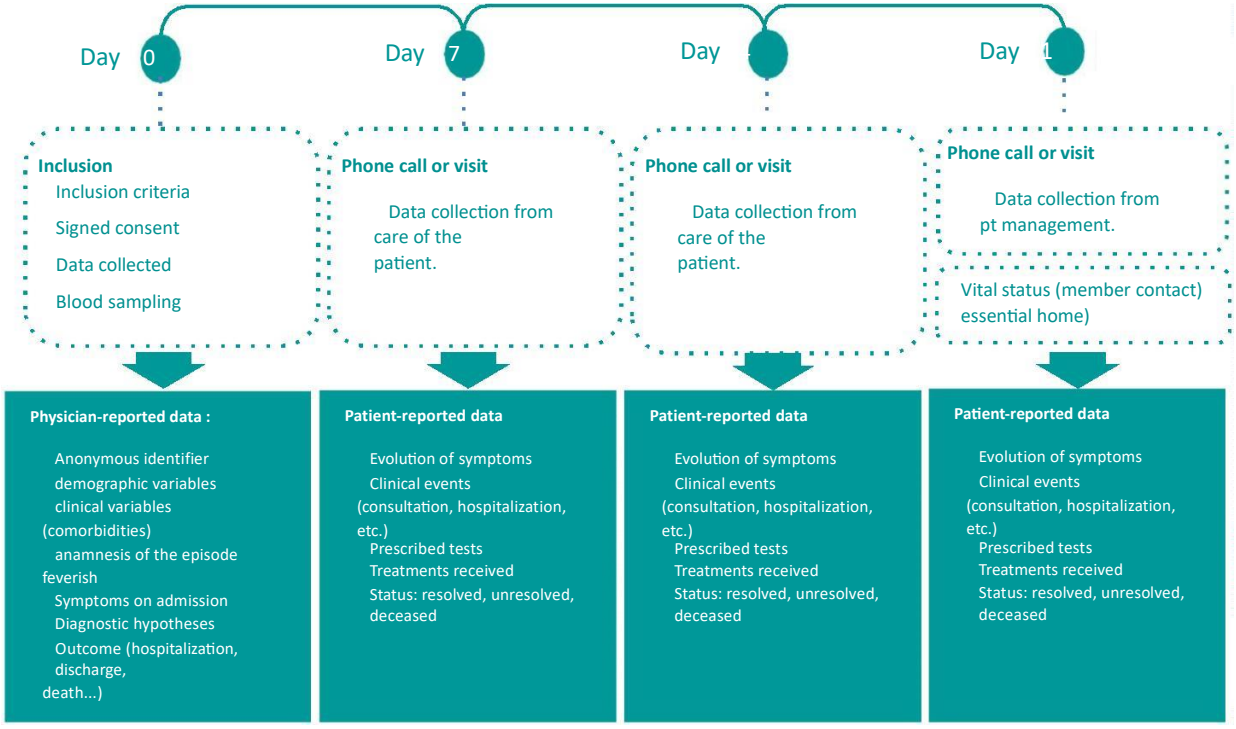
After inclusion, each patient will be followed up for 21 days. Follow-up will include:

- Telephone calls (or visits to the study center or home visits by health agents) community) on days 7 (D5-9), 14 (D12-16) and 21 (D19-23) for patients followed in outpatients, discharged patients, or patients hospitalized in facilities other than the hospital. inclusion site.
- Daily bedside visits for hospitalized patients

Every daily hospital visit (inpatients) and every visit or phone call whether planned or not, the study staff will collect the patient's symptoms and signs in the CRF. (type and severity), comorbidities, drugs and tests prescribed, medications received,

examinations performed, results of examinations, secondary visits and/or hospitalizations and the status (resolved, unresolved, dead).
After day 21, the investigator will establish a "final diagnosis", based on examination of the entire file. This final diagnosis will be syndromic and/or specific, according to standard definitions.
There are no laboratory procedures for follow-up visits.

Figure 1: Schedule of visits and clinical data collected*.



* For hospitalized patients, the same variables will be collected daily

5.2 Obtaining informed consent

Adults who agree to participate in the study, children's representatives who agree to the their child's participation and the representatives of adults whose condition does not allow them to sign the form.
informed consent (impaired consciousness, insufficient level of comprehension) is impaired and who agree to the patient's participation will be invited to sign the consent form after a oral explanation by a qualified professional. If a participant (or parent or representative) is unable to read or write, the signature of a witness to the informed consent discussion will be obtained.
For the witness, preference will be given to persons indicated by the patient or representative, or if not possible, it will be carefully checked that the proposed independent witness is worthy of and freely accepted by the patient.
In the case of children aged 12 and over, the age at which the child's consent to participate is sought, to give informed consent, will be asked to sign a consent form.
consent (in addition to parental consent).

After the informed consent process, the form will be given to the patient or his/her representative. A patient with a disturbance of consciousness whose legal representative has given consent, will receive information about the study and will be asked to give formal consent when his or her condition is diagnosed.
will allow.
Informed consent and assent forms will be available in French, Lingala and English.
(for Kinshasa) and Kikongo (for Kimpese).
The informed consent procedure will describe the (legitimate) purpose of the study, the procedures to be followed, the
risks and benefits of participation, and data management. Participants (or parents
or representatives) will be informed that participation in the study is entirely voluntary and that the participant may withdraw from the study at any time without any negative consequences.
It will be explained to participants that the study and its laboratory procedures include a
sample storage for secondary research (e.g. transcriptomics):
transcriptional profiling of peripheral blood to identify early biomarkers for
distinguish bacteria from viral infections), and that these investigations can also include
genetic analysis *. Specific consent will be required for these analyses.

*Note for the metagenomics protocol: unlike procedures with the intention
host sequences, we do not consider the metagenomic protocol to be
a "genetic analysis", as it aims to sequence, analyze and report RNA sequences
rather than human sequences. However, since the technique is based on
on the sequencing of all the RNA material present in the patient's sample, a small amount of material
RNA of human origin will inevitably (incidentally) be sequenced in addition to the material
pathogenic RNA. We will use laboratory and bioinformatics procedures to
depleting human RNA sequences and enriching pathogenic sequences. Since this
subtraction is not 100% efficient, some human sequences may remain in the
the final data set used for pathogen identification. Some
publishers can request free access to the raw data set.

For participants whose samples will be used for secondary research, which may include
influence clinical management (e.g. important endemic infections such as
trypanosomiasis, which is a severe disease that can remain asymptomatic for a long time), he will be explained that the medical team would be informed in the event of detection, so as to warn them. They will
will then be advised to go to a nearby referral center, where a diagnostic evaluation will be carried out.
(and possibly treatment) will be offered free of charge.
Similarly, patients will be informed that during metagenomic analyses and the
secondary research, certain chronic infection diagnoses could be made in the same way.
examples of HIV, or hepatitis B or C will be provided in the information form.
so that the participant fully understands what it's all about and what the implications are.
The consent process and documents will enable participants to give their
consent only for the clinical and purely observational part, or to add a
consent for (i) study laboratory analyses, (ii) sample storage for
minimum of 10 years in the biobank for secondary research (iii) genetic analyses in
secondary research. If participants give their permission for the storage of

samples, it will be explained that this consent for storage can be withdrawn at any time.
without having to provide any particular justification, and that at that time the patient's samples will be
destroyed.

5.3 Laboratory procedures

A blood sample (venous +/- capillary) is taken at inclusion. A volume of blood of a maximum of 14.5 ml (in children < 14 years) or 30.5 ml (≥14 years) is taken for to meet routine clinical and monitoring needs, study laboratory analyses and sample storage.

For children < 14 years, the maximum volume to ensure safe sampling will be calculated on the basis of age, weight and capillary haemoglobin. If less than the required is possible, priority will be given as follows:

1. clinical necessity and monitoring
2. primary study analyses
3. secondary and exploratory study analyses
4. sample storage

The following samples will be collected (subject to consent and site capacity)

1. Fingertip capillary blood test (participants < 14 years of age)
   a. Hemoglobin (determination of maximum blood volume in children < 14 years): QuikRead Go®, CRP/hemoglobin cells or HemoCue® Hb 301
   b. CRP (only on capillary blood if simultaneously with hemoglobin by QuikRead Go ®)
   c. Preparation of a maximum of 5 dried bloodstains: for storage + for secondary research

   For participants ≥ 14 years of age, capillary blood is taken after the blood test. venous.

2. Venous blood sampling: depending on the maximum volume that can be withdrawn, the The following samples will be taken in order of priority
   a. 4 ml (2 ml if < 14.5 ml can be collected from children < 14 years) EDTA tube
      - According to clinical need: blood glucose
      - Malaria TDR: SD Bioline pan-LDH & pf-HRP2
      - Thick drop
      - Dengue TDR: SD Bioline Dengue Duo
      - White blood cells and differentiation: HemoCue ® WBC/DIFF
      - CRP (if not yet performed on capillary blood) : QuickRead Go ®, cuvettes CRP/Hemoglobin (with Hemoglobin) or RDT

      Centrifuge the remaining volume for aliquoting (into 2-5 aliquots of 350 µL of plasma) and stored in the biobank at -80°. The aliquots will be partly used for metagenomic analyses, the remaining volume (if any) will be stored for secondary searches.
   b. Blood culture bottle (1-4 ml in children < 14 years, 20 ml in adults) patients ≥ 14 years of age): according to routine monitoring criteria

c. 2 ml serum tube: biochemical analysis according to clinical need
d. 2 ml additional EDTA: storage and secondary research
e. 2.5 ml PAXgene tube: storage in the biobank at -80°C, for research purposes. secondary (e.g. transcriptional profiling)

Blood cultures will be taken and analyzed in accordance with the monitoring protocol. (protocol approved in DRC and Belgium; IRB IMT 613/08, UZA CE 8/20/96).

Study analyses (except metagenomic analyses) will be performed at the IME and the HGK study laboratory. Samples for metagenomic analysis will be transported to the ITM laboratory in Antwerp (as agreed in a 'Material Transfer Agreement' - MTA). Local empowerment of partner institutions is planned as part of a second time.
Samples for secondary research will be stored in the biobank at the following sites or at the ITM, Antwerp. All relevant sample data will be recorded in the biobank database. Samples obtained and stored in the biobank belong to the research site.

The sponsor guarantees that an MTA will be drawn up and signed for each sample transfer in outside the research site. The intellectual property of the results belongs to the institution which receives samples An agreement will be reached between the study sponsor and the institutions partners (INRB, CRSK or IME) prior to any secondary research, as well as the approval of the relevant ethics committees. No data will be shared before signature a data-sharing agreement.

The study will ensure that all laboratory activities, including transport, processing and storage, are carried out in compliance with the applicable standards, analysis, reporting of results and storage of samples, will be carried out in accordance with to GCLP. The laboratory will perform diagnostic tests in accordance with the following procedures and EN-ISO 15189 standards. A laboratory analytical plan will be created before the start of the study, describing all aspects in accordance with the WHO GCLP.

6. STATISTICAL METHODS

A detailed statistical analysis plan will be drawn up before recruitment begins. The analysis plan will provide a full description of the variables of interest, statistical methods (including the management of missing data, statistical adjustment, management of confounding factors and interactions), subgroup definitions and sensitivity analyses.

6.1 Variables of interest

- Demographic characteristics
- Basic clinical data (previous treatment, history, symptoms, clinical examination)
- Diagnostic hypothesis
- Patient outcome
- Examinations and treatments prescribed and carried out
- Outcome (survival with symptom resolution, survival without symptom resolution, death)

- Results of inflammatory biomarker analyses: CRP, white blood cells, and differentiation
- Results of etiological analyses: malaria, blood cultures, and germ antibiograms identified, metagenomic analysis, dengue TDR

6.2 Statistical methods

Baseline demographic and clinical characteristics will be described in terms of median and mean values.
of interquartile ranges for continuous characteristics, and in terms of numbers and
percentages for categorical characteristics. Certain demographic and
will be compared between clinical outcome groups: survival with resolution of clinical
symptoms vs. survival with no symptom resolution vs. survival with symptom resolution
vs. death. Comparisons between groups will be made using the Mann-Whitney test.
for continuous characteristics and the Chi-square test or Fisher's exact test for continuous characteristics.
categorical characteristics for the three different strata (combination of site and group)
age)
We describe the proportion of patients meeting the criteria of resolution, non-resolution and death,
overall and by basic characteristics (age, gender, geographical location, category, etc.).
syndromic, patient characteristics) using percentages and intervals of
Wilson's 95% confidence level. Univariate and multivariate logistic regression models
will be used to analyze the association between baseline characteristics and clinical status on the
21
following days.

We will use Kaplan-Meier estimates to describe the probability of survival and resolution.
over time, globally and by category. In addition, death will be used as a risk
in this model. Univariate Cox proportional hazard models and
will be used to analyze the association between baseline and follow-up characteristics.
and mortality. Biomarker data will be described mainly using
descriptive statistics in the same way as described above. Prevalence of malaria
in the study population will be calculated as a proportion with a confidence interval of
Wilson at 95%. The association of CRP and white blood cell values with patient outcome
at day 21, or with specific etiologies, will be examined using the Mann-Whitney test.
Diagnostic performance will be assessed by calculating the following performance measures
standard diagnostic parameters, such as sensitivity and specificity.

6.2.1 Subgroup analyses

As this is a stratified study, all analyses will be presented by stratum (combination of strata).
site and age group).

6.2.2 Multiplicity and missing data

An analysis of all available cases will be the main analysis for all objectives. The data
data points, with the exception of clinical status, will be reported, and in
In the case of limited missing data, multiple imputation will be used to recover the data.
A subsequent analysis with the full set of imputed data will be carried out and compared with
primary analysis. No adjustment for multiplicity is required in the analyses.

6.3 Sample size and power

Taking into account the main and strategic aims of this study (to describe the presentations and issues on the 2 sites, initiate the research capacity of the teams, provide the bases epidemiological studies), the expected sample size is based on the budget, reality and logistical feasibility. Size refers to the number of participants recruited in the laboratory phase of the study (following amendment 1 of the protocol). We plan to to include 500 children at HGK / Mama Yemo, and 500 at HGR-IME + health centers in Kimpese. children + 500 adults for a one-year study period. This number is sufficient to provide data relevant to the study's objectives, and will also enable teams to manage study requirements. With an expected drop-out rate of 20%, we hope to have an accuracy of at least less ± 4.9% in each stratum (combination of site and age group) in the estimation of the proportion of the primary result.

7. QUALITY CONTROL AND ASSURANCE

The study will be conducted and monitored in accordance with this protocol and the applicable regulations.
for clinical research, in particular ICH-GCP and WHO-GCLP, and the procedures manual (MOP).

The investigator nator and the local principal investigators will coordinate all study follow-up activities, including :

- Develop and monitor workflows, schedules, milestones and performance measurement tools. progress.
- Hold regular team meetings to discuss project progress, activities and to resolve any problems that may arise. These can be face-to-face meetings or remotely, depending on distance.
- Ensure data quality and query resolution.
- Visit each clinical site regularly to :
    - check compliance with study protocol and procedures
    - verify that informed consents are valid
    - verify the source data by comparing it with data stored in the database
    - verify that confidentiality is fully respected. Monitoring standards require full verification of the presence of informed consent and the compliance with eligibility criteria.
    - Verify/monitor the quality of laboratory tests carried out according to GCLP practices and SOPs

The PI and the research staff at the site concerned will devote the necessary time and resources to this project.
to these monitoring activities. The investigator will also ensure that the insurance monitors of the sponsor (ITM CTU), the CRC (INRB) and the CRSK have access to all the documents and facilities related to the above-mentioned study and has the necessary space and resources to monitor and verify source data. The promoter will contact investigators concerned as soon as it is informed of an ongoing study center inspection by a regulatory authority or funder. Similarly, the investigator will inform the sponsor of any ongoing inspections. Monitoring of the study will be carried out by the CTU monitor and will be done by
according to the monitoring plan.

8. DATA MANAGEMENT

Data management procedures will comply with the
ITM.

At each participating clinical site and each coordinating center, the investigator will keep a record of all the data collected.

hard copy of the main study file (IF: Investigator File), containing all the elements of the study (protocol, CRF, MOP) and regulatory requirements (ethics committee approvals), task delegation descriptions), CVs, memorandums of understanding).

Data management will be organized and carried out by the ITM/CTU data manager in collaboration with the INRB/CRC team and the CRSK. Full details of the treatment of study data will be described in the "data management plan", which will comply with SOPs and regulatory requirements. The study data will take into account the following essential elements.

Data collection

Only data defined by the study protocol will be collected. Data collected will be registered via electronic forms (eCRF) in the study database at using a REDCap platform.

REDCap is a data capture and management software for clinical studies. REDCap complies with ICH quality standards and good clinical practice, as well as the recommendations and requirements such as CFR 21, Part 11 (e.g. electronic signature, audit trail, etc.). electronic). The software includes, among other things, an integrated system for tracking and managing queries, as well as data import and export functions in a variety of formats. shapes.

The eCRF and database have been designed in accordance with the specifications drawn up in the FIKI² study. The specifications include the forms, the list of included variables in forms, programmed checks on eCRF to validate data at the time of data entry and automated queries to verify data and quickly identify or inconsistent. A complete validation of the eCRF system will be carried out, by in particular the input controls. It is only after final approval of the test phase and the validation report that data entry can begin.

Data capture and further processing will be carried out at all sites by our own staff. under the responsibility of the site's principal investigator. Participants will be identified by study-specific participant code on the CRF and in the database (pseudonymization). The name and any other identifying details will be kept only on the recruitment site and will not be disclosed to third parties.

will NOT be included in any electronic study data file.

An ITM server hosts the REDCap platform and eCRF data. ITM will have access at all times to access to a database export for follow-up activities.

Confidentiality and security of participant data

Study participants' personal information will be treated confidentially.

Information such as the participant's name or any other data likely to make it possible to identification will not be recorded in the study database, nor in the database of the no other paper documents or electronic files used for the study. Each patient will receive a unique study code that will be used for registration in the study database. The

the name and contact details of each participant and the link to the unique study code will be retained and access to them will be restricted to clinical site personnel.
Access to paper documents and electronic files required for data management will be provided by strictly limited to ITM-authorized study personnel. Study computers and files eCRF will only be accessible with a personal username and password. A list of of authorized users of eCRFs (and the database) will be kept at ITM and updated as required. study.
A data backup will be performed at server, computer and/or database level. data.

Data quality and verification
Best practices in CRF design, such as the use of codes and checkboxes, and drop-down menus, will improve data quality. A system will be set up for data entry, verification, query resolution, backup and, last but not least, database locking. In addition to automatic verification checks will also be carried out to identify out-of-spec data, data with a high and inconsistent data. The central data manager, the coordinating investigator, the INRB team and the sites, will hold regular teleconferences on data management throughout the study.

Record-keeping
Electronic documents for data management purposes, including txt and pdf formats, the Excel files, REDCap databases and their extractions, eCRF-generated files (in study descriptions), programs and e-mails will be stored in a secure and organized in a specific access-controlled data management subfolder on the server of ITM. Paper documents used for data management, such as mail printouts, data lists and data management documentation will be stored at the ITM in special specific files. ITM will ensure that access to these paper and electronic files is controlled. (e.g. in closed cabinets or rooms). Details on shelf life paper documents and electronic files are available in the section Archiving (see below).

Data sharing
Study data relating to participants will be available for sharing within a given timeframe. reasonable after the study and in accordance with the research data sharing principles of ITM.

The notion of "data sharing for secondary research" will also be indicated in the informed consent form.
The study's anonymized data will be supplemented by metadata and the documentation, such as the study protocol, annotated CRFs and other information such as codes that together form a dataset, but will not include any direct identifiers. The researchers can ask ITM to access anonymized data for specific research purposes. or secondary analyses via a controlled access procedure.

Training

For the study to be optimally implemented, all the teams involved must have a good understanding of the subject.
knowledge of the study documents and tools, as well as good clinical practice, and
principles of research ethics. Specific training will be given to all members
of the study team involved in patient care, data collection and data entry
data verification and data monitoring. All those involved in the study will be
trained before inclusion begins. They will also receive ongoing training throughout the year.
throughout the study.

9. ETHICAL ISSUES

9.1 Ethical and regulatory review

This clinical trial will be submitted to the Institutional Review Board for formal review and approval.
of the ITM, the CE of the University Hospital of Antwerp and the relevant ethics committees in the DRC. No
specific intervention will not take place until written approval has been obtained from the study committees.
ethics, compliance with local regulatory requirements and signature of the clinical study protocol.
of each contractual party involved. Ethics committees to be kept informed of progress
and then informed of the study's conclusions.
The study will be carried out in compliance with current national and international regulations, including
including CIOMS international ethical guidelines for biomedical research
involving human subjects (16), the Declaration of Helsinki (17), the harmonized tripartite directive
for ICH Good Clinical Practice E6 (ICH-E6) (14), the WHO GCLP (15) and the
Nagoya (18).
The study will also be included in the public registry [Clinicaltrials.gov] before recruitment begins.
of participants.

9.2 Protocol changes

Once the final clinical study protocol has been issued and signed by the authorized signatories, it cannot be used for any other purpose.
can be amended informally. Amendments to the protocol have the same legal status
and must pass through the appropriate stages before being implemented. Any modification
substantial must be approved by all the organizations and CEs that have approved the protocol
before it is implemented, unless it is due to concerns about the safety of
participants (in which case immediate implementation may be necessary to protect
participants). If changes to the protocol or an amendment are requested by an EC/CA
during the review process, they must be discussed and agreed with the promoter
before any new submission incorporating these changes.

9.3 Informed consent

No participant may be admitted to the study until the investigator or designated person
has obtained the written informed consent form.

9.4 Privacy policy

Individual medical information collected in this study will remain confidential. Visit
members of the study team are bound by professional secrecy. The data

will be made available to doctors in charge of patients on request.
to the investigators. Disclosure to third parties is strictly forbidden.
The electronic files will not contain any data that could allow the identification of the customer.
(e.g. surname, first name, full date of birth, telephone number). Each
participating in the study will be assigned a unique identification code (ID). On all documents or
study files, study participants will be identified by the participant identification code.
At each clinical site, a correspondence list will establish the link between the participant ID and the
personal data of the participant so that the results of the examinations can be used for the
patient care. This list containing personal data and other documents containing the
names or signatures of participants (e.g., informed consent) will be retained.
separately from other study documents. All study documents will be stored in a
a secure, locked area, with access limited to the site's principal investigator and staff
authorized.
Access to all paper documents and electronic files required for data management
and monitoring of the study will be limited to authorized personnel, at international, regional and local
levels.
The study computers and the eCRF files will only be accessible via a connection with a
user name and a personal password. A list of authorized users of eCRF and
the database will be kept at ITM and updated throughout the study.

9.5 Risks and benefits

A venous +/- capillary blood sample is part of the study procedures, but is in any case
also necessary in the clinical management of most patients. The 2
samples will be integrated. Sampling can be uncomfortable, even painful. Visit
risk of complications from blood sampling is very low (may include haemorrhage in elderly patients).
people with coagulation disorders, puncture site infection or thrombosis
superficial veins). The amount of blood to be collected for this study, both for routine
and additional analyses, in no case exceeds the maximum of what is considered acceptable.
as safe for the patient. In any case, routine analyses for management will always be
priority, if sufficient volume cannot be achieved.
The greatest risk for participants is a potential breach of confidentiality of
data, for which all measures will be put in place to limit them as much as possible.
Apart from access to laboratory analyses that can improve management (some research
biomarkers), there are no advantages to participating in the study. Compensation
is still provided for a requested trip, this compensation does not exceed the
usual burden of effort.

9.6 Incidental discoveries

All results of immediate laboratory analyses in the FIKI² study (with the exception of TDR
for dengue fever, currently being evaluated in the African context) will be communicated to the team.
the patient. In general, these are analyses that should already be part of the
routine management of febrile illnesses.

As explained above, however, participation in the study can lead to chance discoveries.
pathogens that can give rise to chronic infections (asymptomatic for long periods),

requiring further action at the level of the individual patient. These discoveries can occur in metagenomic sequencing and secondary research.

If an endemic infection with immediate importance for management such as trypanosomiasis is detected during secondary research, the patient and medical team will be informed, and the patient will be offered a free diagnostic evaluation (and possible treatment) at a nearby referral center.
If a chronic infection (HIV, HBV, HCV, etc.) is discovered incidentally during the analyses metagenomics or subsequent secondary research), only the results considered to be will be communicated. The results of this technique are not considered conclusive for the time being (the metagenomic analysis currently being development is not yet a validated clinical laboratory test) and would require a additional confirmation through a standard clinical workup. The study teams will then be responsible for informing the participant in question and directing him or her to the care structure appropriate for the health problem detected.

9.7 Compensation for participation

There is no compensation for participation in the study. Patients who cannot be contacted by telephone and who return to the study center on days 7, 14 and 21 will receive a amount sufficient for a round trip using standard means of transport in addition to any daily allowance, in line with that given by similar studies on this site, and approved by local ethics committees.

9.8 Insurance

The coordinator of this study, the Institute of Tropical Medicine, obtained study insurance (without liability) to cover any injury, damage or loss suffered by study participants and which is caused directly or indirectly by their participation in the study.
Information on insurance is not specified in the GSI of this study, due to the fear that of unreasonable claims for compensation. In addition, we do not anticipate any physical injury or mental health problems as a result of participating in this event. observational (non-interventional) study.

10. DISSEMINATION OF RESULTS, INTELLECTUAL PROPERTY

All study documents are provided by the sponsor to the investigators and their staff. confidentiality. None of these documents may be disclosed to any party who is not a not directly involved in the study, without the written authorization of the sponsor.

Once the results are considered final, arrangements will be made for the communicate to the community, in the most appropriate way, with the active participation of health authorities.
Oral communications and written publications must mention the name of the sponsor and the name of the author.
All the partners and researchers involved
declaration and publication will be made in accordance with the CONSORT declaration.
(http://www.consort-statement.org/consort-statement)

11. ARCHIVING

The sponsor and the investigator must keep adequate and accurate records to allow for fully document the course of t h e study and verify study data. Visit relevant (essential) documents are those documents that individually and to assess the progress of the trial, the quality of the data produced and compliance with PCM standards and applicable regulatory requirements. The investigator's file must contain at least all the (essential) documents listed in the "Setting up and maintaining the investigator's file". A copy of all source data and report forms from must always be kept on site.

Once the study has been completed, the study master file (IF) on the site and in the coordination center remain available for internal audits and/or inspections by regulatory authorities for a period of 20 years, unless otherwise requested by the national authorities. The sponsor must be informed before the files are destroyed.

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13. LIST OF ABBREVIATIONS

ACVPU	alert, confusion, voice, pain, unresponsive'
ALMANACH	'algorithm for the management of childhood illness'.
ALERRT	the African Coalition for Research, Intervention and Training in the epidemic
AQ	Quality assurance
CA	Competent authorities
CIOMS	Council for international organizations of medical sciences
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRC	Clinical Research Center
(e-)CRF	'(electronic -) Case Report Form' - the data collection form
CRP	C-reactive protein
CRSK	Kimpese Health Research Center
CQ	Quality control
DGD	Swiss Agency for Development and Humanitarian Aid
DSMB	Data and Security Control Board
EDCTP	The European & Developing Countries Clinical Trials Partnership
FISSA	'Febrile Illness in sub-Saharan Africa'.
GCP	Good Clinical practice

GCLP	Good clinical laboratory practice
GDPR	General Data Protection Regulation
HGK	Kinshasa General Hospital
HGR	General Reference Hospital (Kimpese)
IC	Investigator Coordinator
IC(F)	Informed Consent (Form)' - Formulaire de Consentement Eclairé
ICH	The International Council for Harmonisation - 'Conférence internationale sur harmonization
(I)CE	Ethics Committee (independent)
IGSA	Simplified ambulatory severity index
IME	Evangelical Medical Institute
ITM	Institute of Tropical Medicine
INRB	French National Institute for Biomedical Research
IRB	Institutional Review Board
MOP	Operating procedures manual
MTA	Material Transfer Agreement
IF	Investigator's File' - main study file
IP	Principal Investigator
WHO/WHO	World Health Organization/Organisation mondiale de la Santé
POC	Point of care
qSOFA	Quick Sepsis Related Organ Failure Assessment
GROUND	Democratic Republic of Congo
FLOOR	Statistical analysis plan
SDV	Checking source data
SOP	Standard Operational Procedure
TDR	Rapid diagnostic tests
TMG	Trial Management Group
EU	European Union
UZA	Universitair Ziekenhuis Antwerpen (~ University Hospital Antwerp)

14. Appendices

Appendix 1: Protocol for the evaluation of rapid diagnostic tests for CRP

Background and justification	Acute febrile illnesses are a major cause of death and disability. morbidity worldwide. In countries with limited resources, they are often treated empirically with antibiotics due to a lack of the absence of reliable diagnostic methods, whereas in most In some cases, this is not necessary. It is well established that inappropriate use of antibiotics, including in the first line of care, contributes to the the spread of antibiotic resistance. C-reactive protein (CRP) is a biomarker of inflammation, which can help distinguish between bacterial and non-bacterial causes fever. Currently, rapid diagnostic tests (RDTs) for CRP determination, are becoming increasingly available and can be used to could be very useful in the management of febrile illnesses in primary health care centers. Before evaluating the use of these tests in the routine practice of primary health centers in the DRC, we want to evaluate a selection of 2 immunochromatographic tests (lateral flow) measuring CRP (a qualitative and semi-quantitative) in a controlled laboratory environment.
Design	Evaluation study of 2 RDTs targeting CRP in the clinical laboratory, as part of the FIKI² study
Site and population study	The study uses existing samples (EDTA blood) from a subpopulation of participants in the FIKI² study, and will be carried out in the site laboratory correspondents : 1. Laboratory of the pediatric emergency department of the Hôpital Général de Kinshasa (HGK) 2. Institut Médical Référence (IMR) of Evangelical Medical Reference (HMR) Laboratory Kinmpese
Objectives and criteria judgment	1. Evaluation of the performance of 2 CRP RDTs in comparison with a reference test (QuikRead go®) on patient samples of fever obtained in the FIKI² study. **Judging criteria** ● degree of correlation between RDTs and reference test (with reading at minimum and maximum time depending on mode and late reading) ● proportion of invalid tests ● proportion of migration errors ● low background lightening ratio ● proportion of test line intensities by scale ● degree of inter-observer agreement ● degree of repeatability of TDRs ● presence of the hook effect for very high CRP values high (> 150 mg/l)

	2. Assessment of operational features and ease of use 2 CRP RDTs in the laboratory **Judging criteria** ● systematic analysis of the characteristics of TDRs and their packaging, labelling and instructions for use as per checklist
Criteria inclusion and exclusion	**Inclusion criteria**: ● samples from patients who have given consent for the laboratory part of the FIKI² study ● CRP value (Quikread Go®) according to selection and planned distribution (as described in the 'Selection' section) and sample distribution') **Exclusion criteria :** ● samples with a maximum sampling volume of blood (depending on age, weight and Hb, as described in the FIKI² study procedures) could not be obtained
Selection and distribution of samples	1. Assessment of correlation between RDTs and reference test, degree of concordance between 2 readers, proportion of invalid tests, errors in migration, low background brightening, and line intensity ● Number of samples planned: 140 (70 per site, at adapt to recruitment speed) ● Distribution ■ 2x14 samples with CRP < 5 mg/L ■ 2x14 samples with CRP ≥ 5 - < 10 mg/L ■ 2x14 samples with CRP ≥ 10 - < 40 mg/L ■ 2x14 samples with CRP ≥ 40 - < 80 mg/L ■ 2x14 samples with CRP ≥ 80 mg/L ● 2 TDR 1x on each sample, with 2 readings by TDR 2. Evaluation of the croquet effect (HGK only): ● Number of samples: 5 ● Distribution: 5 samples with CRP > 150 mg/L ● 2 TDR 1x on each sample, with 1 reading by TDR 3. Repeatability assessment (HGK only) ● Number of samples: 5 ● Distribution ■ 1 sample with CRP < 5 mg/L ■ 1 sample with CRP ≥ 5 - < 10 mg/L ■ 1 sample with CRP ≥ 10 - < 40 mg/L ■ 1 sample with CRP ≥ 40 - < 80 mg/L ■ 1 sample with CRP ≥ 80 mg/L ● 2 TDR 5 x on each sample, with 1 reading by TDR
Procedures laboratories	2 RDTs for CRP will be analyzed: - Qualitative Biopanda® RDT (threshold 10 mg/l) - Actim® semi-quantitative RDT (thresholds 10 mg/l, 40 mg/l and 80 mg/l)

	Reference test for performance evaluation: PoC QuikRead go®. (CRP+Hb kit; range 5-200mg/l ~ hematocrit) Assessment of correlation between RDT and reference test, degree of concordance between 2 readers, proportion of invalid tests, test errors, etc. migration, low background brightening, and line intensity : TDRs will be performed on the EDTA samples of FIKI² participants. according to the planned distribution and the producer's recommendations, and read out minimum and maximum incubation times, and also beyond the set time by the manufacturer TDR. Readings will be taken and recorded by two independent readers at the 2 sites and result by Quikread Go. A test result will be considered as invalid when the control line is missing, mottled or irregular. A scale will be used to note the intensity of the line of test for each reading. A photo will be taken for each reading. In case of discrepancy for a test invalid, migration error or poor background illumination, the result of a third reading of the photo by the principal investigator will be taken as a reference. Evaluation of the hook effect, evaluation of repeatability : TDRs will be performed on the EDTA samples of FIKI² participants. according to the manufacturer's recommendations. These analyses will only be performed at HGK by the principal investigator of this assessment. Systematic analysis of TDR characteristics and packaging, labeling and instructions for use (based on a checklist of features) will be made and evaluated for ease of use.
Methods analysis	The kappa index (Cohen's coefficient of agreement) will be used to evaluate the degree of correlation between TDR (reading at minimum, maximum, and and reference test for the 2 different readings, and for the inter-observer agreement. With sample size, the distribution and an expected kappa value of 0.6 - 0.7, the interval size of confidence would be 0.184-0.22 (Actim® test) and 0.26-0.30 (Actim® test). Biopanda®) for correlation with the reference test. The proportion of invalid tests, migration errors, clarification of the background At a low level, the intensities of the test lines according to scale will be calculated with the 140 TDRs performed and read at maximum time as the denominator. Evaluation of the hook effect and repeatability will be carried out in the following way descriptive.