# Statistical Analysis Plan

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

English translation for clinical trials.gov – unofficial translation – official document in French

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# 1. Introduction

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the main analyses pre-planned for the study entitled "Clinical aspects, severity, management and outcome of febrile illnesses in the DRC" (FIKI²). The current epidemiology and outcome of febrile illnesses in the Democratic Republic of Congo (DRC) is poorly documented. A better description of the causes, clinical presentation and host biomarker profiles will enable future studies targeting optimized diagnostic and therapeutic strategies.

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

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

# 2. Study design and objectives

## 2. 1 Study design

Bi-centric (Kinshasa and Kimpese), prospective, observational cohort study of adults and children presenting to the emergency department or outpatient clinic with community-acquired febrile illness, with laboratory analyses and sample storage in a bio-bank.

A total of 1,500 people will be recruited for the study, including 1,000 children (< 18 years) and 500 adults. The study population consists of :

1. Children presenting with febrile illness in the pediatric emergency department of the Hôpital Général de Kinshasa (HGK / Mama Yemo) and in the outpatient and emergency departments of the Hôpital Général de Référence (HGR) of the Institut Médical Evangélique (IME), Kimpese, DRC.

2. Adults presenting with febrile illness at the outpatient and emergency departments of the General Reference Hospital (HGR) of the Evangelical Medical Institute (IME), Kimpese, DRC.

2.2 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) when including patients with febrile illnesses.

**Secondary objectives**

1. Estimate the proportion of symptom-free survival and death, assessed at day 21, for febrile illnesses.
2. Estimate the proportion of survival with symptom resolution, survival with symptom nonresolution, and death, assessed on days 7 and 14, of febrile illnesses.
3. Describe the epidemiology, clinical aspects, severity and management of febrile illnesses.
4. Identify factors associated with outcomes (resolution, non-resolution, death) of febrile illnesses.
5. Determine the frequency and profile of malaria (by rapid diagnostic test and thick drop), including co-infection, in patients with febrile illnesses.
6. Observe the association of CRP and WBC values with patient outcome at day 21.
7. Observe the association of CRP and WBC values with specific etiologies (malaria, bacteremia, dengue fever, etc.).

**Exploratory objectives**

1. Describe the frequency, etiology and antibiotic resistance patterns of community-acquired bacteremia detected in patients with febrile illnesses.
2. Assessing the performance and potential contribution of a rapid immunochromatographic test targeting dengue under field conditions in the DRC.
3. Evaluate metagenomic sequencing for the detection of pathogens causing febrile illness (with primary focus on RNA viruses).

## 3. Variables of interest

3.1. Final points of the study

Patients enrolled in the study will be monitored and evaluated at 4 points in time: at admission D0, at seventh follow-up day D7, at fourteenth follow-up day D14 and at twenty-first follow-up day D21. They will be categorized into 3 groups:

- **Resolution**: means (i) signs and symptoms have completely disappeared and treatment has been completed; or (ii) some signs or symptoms persist and/or treatment is still underway, but a marked improvement in these signs and symptoms suggests that the person will recover rapidly without the need for further investigation or treatment other than that already administered.
- **Non-resolution**: any combination other than those described for the "Resolution" option.
- **Deaths**

Patients with no clinical outcome (lost to follow-up, withdrawal of consent, etc.) will be excluded from analyses that include outcome. Additional sensitivity analyses will be carried out (section 4.5).

### 3.2. Primary variables

The primary variables in this study are of two types:

- Clinical: Clinical outcome on day 21 as described in section 3.1.
- Biological: CRP level, differentiated white blood cell count on admission

### 3.3. Secondary variables

- Socio-demographic characteristics: age, gender, geographical location
- Clinical outcome on days 7 and 14:
    - Resolution: means (i) signs and symptoms have completely disappeared and treatment has been completed; or (ii) some signs or symptoms persist and/or treatment is still underway, but a marked improvement in these signs and symptoms suggests that the person will recover rapidly without the need for further investigation or treatment other than that already administered.
    - Non-resolution: any combination other than those described for the "Resolution" option.
    - Deaths
- Clinical: comorbidity, signs and symptoms on admission (these are the symptoms presented by a febrile patient enrolled in the study), as described in section 3.1.
- Diagnostic hypotheses at inclusion and final diagnoses
- Therapeutic journey prior to admission (visit to health center, treatment prior to presentation, etc.).
- Disease severity on days 0, 7, 14 and 21
  *Adults:*
    - Simplified ambulatory severity index (SAHI)
    - 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, types and results of laboratory and radiology tests prescribed/actually performed
- Number and types of medications and supportive care prescribed/actually administered
- Malaria detection by rapid test (HRP II antigen, pLDH antigen) and thick drop (type and parasitaemia)
- Hemoglobin level

### 3.4. Exploratory variables

- Number of patients from whom blood cultures were taken, proportion of positive blood cultures, identification of germs from positive blood cultures and resistance

profile
- Antibiotic resistance of identified germs (per bacteremia)
- NS1 and IgM antigen results for the detection of dengue by rapid test
- Pathogens causing febrile illness by metagenomic sequencing

## 4. Statistical analysis

### 4.1. Descriptive analysis

Baseline demographic and clinical characteristics will be described in terms of medians and interquartile ranges for continuous characteristics, and in terms of numbers and percentages for categorical characteristics. Selected baseline demographic and clinical characteristics will be compared between clinical outcome groups: survival with symptom resolution vs. survival without symptom resolution, and survival with symptom resolution vs. death. In addition, other clinical outcome groupings will be explored for comparisons, such as survival vs. death, or resolution vs. non-resolution. 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 categorical characteristics for the three different strata (combination of site and age group) and for the whole cohort.

### 4.2. Primary analysis

The proportion of survival with symptom resolution, assessed at day 21, will be estimated as a proportion. The numerator will be the number of survivors who meet the definition of resolved, and the denominator will be the number who have completed the visit by day 21. The 95% confidence intervals for the proportion will be estimated using Wilson's method. A worst-case sensitivity analysis will also be carried out, defining all missing outcomes at day 21 as deaths, to examine the robustness of the results.
CRP and WBC values at recruitment will be described and compared using descriptive methods (see section 4.1). Different variables will be explored to compare CRP and WBC values, including clinical outcome, age, site, severity, diagnosis and others.

### 4.3. Secondary analysis

**Estimate the proportion of symptom-free survival and death, assessed at day 21, for febrile illnesses.**
*The frequencies and proportions of survival (with 95% CI) of febrile patients with non-resolution of symptoms and of deaths assessed at day 21 will be presented. Confidence intervals for the proportions will be estimated using Wilson's method.*

**Estimate the proportion of survival with symptom resolution, survival with symptom nonresolution, and death, assessed on days 7 and 14, of febrile illnesses.**
*The frequencies and proportions (with 95% CI) of survival of febrile patients with symptom resolution, symptom non-resolution and death assessed at day 7 and day 14 will be presented. Confidence intervals for the proportions will be estimated using Wilson's method.*

**Describe the epidemiology, clinical aspects, severity and management of febrile illnesses.**
*Standard descriptive statistics will be used to describe this objective (see section 4.1).*

**Identify factors associated with outcomes (resolution, non-resolution, death) of febrile illnesses.**
*Univariate and multivariate logistic regressions will be used to analyze the association between baseline characteristics and the outcome of febrile patients (resolution, non-resolution and death) at day 21. Two different outcomes will be used in this process: resolution vs. non-resolution (excluding death) and death vs. survival. Potential predictors will be (non-exhaustive list):*

- *Specific diagnosis (e.g. complicated malaria vs. others)*
- *Syndromic diagnosis (undifferentiated fever vs. others - neurological syndrome vs. others - respiratory syndrome vs. others)*
- *Almanach score for children under 5 years of age*
- *Modified ASAPS score and qSOFA in adults*
- *Outpatient consultation prior to admission*
- *Blood culture positive*
- *Presence of comorbidity*
- *Age*
- *Education level*
- *Hemoglobin level*

**Determine the frequency and profile of malaria (by rapid diagnostic test and thick drop), including co-infection, in patients with febrile illnesses.**
The frequency and proportion (with 95% CI) of malaria (simple and complicated; with or without hyperparasitemia) diagnosed by RDT and by thick drop and of malaria co-infections in febrile patients will be presented. Confidence intervals for the proportions will be estimated using Wilson's method.

**Observe the association of CRP and WBC values with patient outcome at day 21.**
The association between CRP values and white blood cell counts at day 21 to assess the link between these two values and individuals with outcomes of resolution, non-resolution and death will be examined with the Kruskal-Wallis test for continuous variables or Fisher's exact test for categorical variables. The outcome variables used for this comparison are *resolution vs. non-resolution (excluding death) and death vs. survival.*

**Observe the association of CRP and WBC values with specific etiologies (malaria, bacteremia, dengue fever, etc.).**
The association between CRP values and white blood cell counts with specific etiologies: malaria (simple + complicated), bacteremia, arbovirosis, other diagnoses will be examined with the Mann-Whitney test.

### 4.4. Exploratory analysis

- **Describe the frequency, etiology and antibiotic resistance patterns of community-acquired bacteremia detected in patients with febrile illnesses.**

  - *Standard descriptive statistics will be used to describe the frequency, etiology and antibiotic resistance profiles of febrile patients, as determined by bacteremia.*

- **Assessing the performance and potential contribution of a rapid immunochromatographic test targeting dengue under field conditions in the DRC.**

- *Determination of diagnostic performance with standard diagnostic measures, such as sensitivity, specificity, PPV, NPV, positive likelihood ratio and negative likelihood ratio of the dengue immunochromatographic rapid test. PCR results from metagenomic analyses and secondary research (such as the HAT-ARBO study) will be used as the gold standard.*

- **Evaluate metagenomic sequencing for the detection of pathogens causing febrile illness (with primary focus on RNA viruses).**

- *This objective is not included in the statistical analysis, but will be the subject of a specific analysis at a later date.*

### 4.5. Subgroup analysis

On the basis of retained diagnostic hypotheses and laboratory results, a subgroup analysis will be performed to assess symptom resolution according to clinical presentation, diagnostic hypotheses and demographic variables, such as age, site, severity, diagnosis and others.
As this is a stratified study, all analyses will be presented by stratum (combination of site and age group).

### 4.6. Multiplicity and missing data

An analysis of all available cases will be the primary analysis for all objectives. Missing data in all data points, except clinical status, will be flagged and, in cases 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 performed and compared to the primary analysis. Results will be adjusted for multiplicity using the Holm-Bonferroni method.