TB-SPEED (STRENGTHENING PAEDIATRIC TB SERVICES FOR ENHANCED EARLY DETECTION)

DEVELOPMENT OF A DIAGNOSTIC PREDICTION SCORE FOR TUBERCULOSIS IN HOSPITALIZED CHILDREN WITH SEVERE ACUTE MALNUTRITION

TB-Speed SAM

International protocol version No. 7.0 - 13/10/2021

CONFIDENTIAL

RESEARCH PROTOCOL INVOLVING HUMAN PERSON

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Corresponding country protocol versions and approvals:

	Uganda	Zambia
Country protocol version		
Approved by		
Date		
Approved by		
Date		

Coordinating Investigators		
Olivier Marcy (IDLIC), Bordeaux Population Health – Inserm U1219 Université de Bordeaux, 146 Rue Léo Saignat, 33076 BORDEAUX Cedex **Tel:** +33 (0)5 57 57 47 23 **Email:** olivier.marcy@u-bordeaux.fr	Maryline Bonnet IRD UMI233 / Inserm U1175 Université de Montpellier, 911 Avenue Agropolis 34394 Montpellier Cedex 05 **Tel**: +33 (0)4 67 41 64 91 **Email***:* maryline.bonnet@ird.fr	Eric Wobudeya MU-JHU Care Ltd Upper Mulago Hill Road P.O. Box 23491 Kampala, Uganda **Tel**: +256 712 846163 **Email**: ewobudeya@mujhu.org

☐ **Monocentric study**
☐ **French multicentre study**
☐ **European multicentre study**
☒ **International multicentre study**

Sponsor:	**Contact:**
Inserm - Pôle Recherche Clinique / Clinical Research Department Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris (FRANCE)	Benjamin Hamzé, Inserm Project Manager benjamin.hamze@inserm.fr Tel: +33 (0)1 82 53 34 72

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SIGNATURES PAGE

Sponsor's signature:

French National Institute for Health and Medical Research (Inserm)

Director: Hélène Espérou 101, rue de Tolbiac 75013 Paris, France **Tel:** +33 153 94 80 90 / 60 46 / 60 00 **Email:**	Paris, Date: Name: Hélène Espérou Signature:

Coordinating Investigators' signatures:

Olivier Marcy Bordeaux Population Health - Inserm U1219 Université de Bordeaux 146 Rue Léo Saignat 33076 Bordeaux Cedex, France **Tel:** +33 (0)5 57 57 47 23 **Email:** olivier.marcy@u-bordeaux.fr	Bordeaux, Date: Name: Olivier Marcy Signature:

Maryline Bonnet Institut de Recherche pour le Développement (IRD) UMI233 / Inserm U1175 Université de Montpellier 911 Avenue Agropolis 34394 Montpellier Cedex 05 **Tel**: +33 (0)4 67 41 64 91 **Email**: maryline.bonnet@ird.fr	Montpellier, Date: Name: Maryline Bonnet Signature:

Eric Wobudeya Makerere University - Johns Hopkins University Research Collaboration (MU-JHU Care Ltd) Upper Mulago Hill Road P.O. Box 23491 Kampala, Uganda **Tel**: +256 712 846 163 **Email**: ewobudeya@mujhu.org	Kampala, Date: Name: Eric Wobudeya Signature:

PRINCIPAL CORRESPONDENTS

Coordinating investigators:

Olivier MARCY
Infectious Diseases in Resource-Limited Countries (IDLIC)
Bordeaux Population Health - Inserm U1219
Université de Bordeaux
146 Rue Léo Saignat
33076 Bordeaux Cedex, France
Tel: +33 (0)5 57 57 47 23
Email: olivier.marcy@u-bordeaux.fr

Maryline BONNET
IRD UMI233 / Inserm U1175
Université de Montpellier, France
911 Avenue Agropolis, BP 64501
34394 Montpellier Cedex 05, France
Tel: +33 (0)4 67 41 64 91
Email: maryline.bonnet@ird.fr

Eric WOBUDEYA
Makerere University - Johns Hopkins University Research Collaboration (MUJHU Care Ltd)
Upper Mulago Hill Road
P.O. Box 23491
Kampala, Uganda
Tel: +256 712 846 163
Email: ewobudeya@mujhu.org

Sponsor:

French National Institute for Health and Medical Research (Inserm)
101 rue de Tolbiac
75013 Paris, France
Tel: +33 (0)1 53 94 60 00

Project Manager:
Benjamin Hamzé
Clinical Research Department
BIOPARK Bâtiment A
8 Rue de la Croix-Jarry
75013 Paris, France
Tel: +33 (0)1 82 53 34 72
E-mail: benjamin.hamze@inserm.fr

Pharmacovigilance:
Noémie MERCIER
Clinical trial safety office
101 rue de Tolbiac
75013 Paris, France
Tel: +33 (0)1 44 23 67 67
E-mail: noemie.mercier@anrs.fr

International Coordinating Clinical Trials Unit (CTU):

IDLIC/Mereva Inserm U1219 Université de Bordeaux 146 rue Léo Saignat 33076 Bordeaux Cedex, France Phone: +33 557 57 17 67 *CTU Coordinator:* Olivier MARCY E-mail: olivier.marcy@u-bordeaux.fr *International trial manager:* Aurélia VESSIERE E-mail: aurelia.vessiere@u-bordeaux.fr	*Biostatistics:* Hélène FONT E-mail: helene.font@u-bordeaux.fr *Monitoring:* Aurélie BEUSCART E-mail: aurelie.beuscart@ u-bordeaux.fr Aurélie CHARPIN E-mail: aurelie.charpin@u-bordeaux.fr *System development and data management:* Sophie KARCHER E-mail: sophie.karcher@ u-bordeaux.fr Eric BALESTRE Email: eric.balestre@u-bordeaux.fr

Reference microbiologist:

Sylvain GODREUIL IRD-Mivegec / CNRS U5290 911 Avenue Agropolis, BP 64501 34394 Montpellier Cedex 05, France Tel: +33 (0)4 67 41 63 57 Email: s-godreuil@chu-montpellier.fr

Laboratory coordinator:

Manon LOUNAS IRD-Mivegec / CNRS U5290 911 Avenue Agropolis, BP 64501 34394 Montpellier Cedex 05, France Tel: +33 (0)7 86 85 32 59 Email: manon.lounnas@ird.fr

Country principal investigators: (countries appear in alphabetical order)

UGANDA Eric WOBUDEYA MUJHU Care Ltd Upper Mulago Hill Road P.O. Box 23491, Kampala Tel.: +256 712 846 163 Email: ewobudeya@mujhu.org	***ZAMBIA*** Chishala CHABALA University of Zambia School of Medicine, University Teaching Hospital Nationalist Rd P.O Box 50110, Ridgeway, Lusaka Tel: +260 977 849 537 Email: cchabala@gmail.com Veronica MULENGA Children's Hospital University Teaching Hospital 1st Nationalist Road Private Bag RW IX, Ridgeway, Lusaka Tel: +260 966 953 792 Email: veromulenga@gmail.com

Associated investigators:

Paul PEREZ
Clinical Epidemiology Unit (USMR)
University Teaching Hospital
Université de Bordeaux
146 rue Léo Saignat
33076 Bordeaux Cedex, France
Tél. +33 (0)5 57 57 14 42
Email: Paul.PEREZ@u-bordeaux.fr

Cécile CAMES
IRD UMI233 / Inserm U1175 TransVIHmi
911 avenue Agropolis, BP 64501
34394 Montpellier Cedex, France
Tél: +33 (0)4 67 41 61 10
Email : cecile.cames@ird.fr

Ezekiel MUPERE
Department of Pediatrics and Child Health
College of Health Science
Makerere University, P.O. Box 7062
Kampala, Uganda
Tel: +256 776 161 327
Email: mupez@yahoo.com

Country Clinical Trials Units:

UGANDA	***ZAMBIA***
MUJHU Care Ltd Upper Mulago Hill Road P.O. Box 23491 Kampala	University of Zambia University Teaching Hospital School of Medicine Nationalist Rd P.O Box 50110, Ridgeway Lusaka

PROTOCOL WRITING COMMITTEE

- Olivier MARCY, Coordinating investigator (Inserm U1219, Bordeaux University, France)
- Eric WODUBEYA, Coordinating investigator (MUJHU, Kampala, Uganda)
- Maryline BONNET, Coordinating investigator (IRD UMI233 / Inserm U1175 Montpellier, France & Epicentre, Mbarara, Uganda)
- Aurélia VESSIERE, International Trial Manager (Inserm U1219, Bordeaux University, France)
- Delphine GABILLARD, Statistician (Inserm U1219, Bordeaux University, France)
- Paul PEREZ, Methodologist (University Teaching Hospital/Bordeaux University, France)
- Julien ASSELINEAU, Statistician (University Teaching Hospital/Bordeaux University, France)
- Chishala CHABALA, Principal Investigator (University of Zambia, Lusaka)
- Cécile CAMES, Nutritionist/Epidemiologist (IRD UMI233 / Inserm U1175, Montpellier, France)
- Ezekiel MUPERE, Paediatrician (College of Health Sciences, Makerere University, Kampala, Uganda)
- Ayeshatu MUSTAPHA, Paediatrician (Ola During Children Hospital, Free Town, Sierra Leone)

HISTORY OF PROTOCOL VERSIONS

TB-Speed SAM

Version No.	Version Date	Amendment Summary
1.0		
2.0	19/02/0219	- Addition of a secondary objective: "To develop a screening score without use of microbiological samples and Ultra to identify children with presumptive TB among hospitalized children with SAM." - Methodology and diagnostic strategy: MLR, CRP, QFT as well as abdominal ultrasound findings will be made available to the clinicians - Patient schedule and informed consent modified to better differentiate research activities from routine care. - Description of biobank governance and patients' rights
3.0	16/04/2019	- Modification of the study title - Clarification on the methodology (precision about the internal validation step) - Clarification and reformulation of the primary objective and associated endpoint - Clarification and reformulation of secondary objectives objective and associated endpoints - Addition of contra-indications for GA and NPA collection - Clarification on the roles of study nurses, site clinicians and attending clinicians - Addition of a section to describe capacity building activities
4.0	29/05/2019	- Role of site clinicians in the algorithm development added - Training of clinicians on the developed algorithm added in the capacity building section - Participants amenities: addition of a time compensation for time spent during study visit
5.0	04/12/2019	- Implementing sites: replacement of the Sierra Leone study site by a second site in Zambia - Inclusion criteria: precisions added on the minimum age of inclusion (i.e., 2 months), and that the MUAC criterion only applies for children over 6 months
6.0	09/04/2021	- Inclusion of the TB-Speed Covid sub-study - Details added on the cost-effectiveness ancillary study - Update of the study schedule - Switching to a competitive recruitment - Clarification made that recruitment in the Covid sub-study is proposed prospectively to newly enrolled children
6.1	14/06/2021	- Update of the study schedule - Suppression of blood samples at D15
7.0	13/10/2021	- Update of the study schedule

LIST OF ABBREVIATIONS

ANRS	French National Agency for Research on HIV/AIDS and Hepatitis
AE	Adverse Event
ART	Antiretroviral therapy
AUROC	Area under the receiver-operating-characteristic curve
CPC	Country Project Committees
Covid-19	Coronavirus disease 2019
CRA	Clinical Research Assistant
CREDIM	Centre de Recherche et Développement en Informatique Médicale
CRF	Case Report Form
CRP	C reactive protein
CXR	Chest radiography or chest X-ray
CTU	Clinical Trials Unit
DALYs	Disability Adjusted Life Years
DBS	Dried Blood Spot
DMP	Data Management Plan
DR	Digital radiography
DST	Drug susceptibility testing
eCRF	Electronic Case Report Form
ERC	Ethical Review Committee
FDC	Fixed-dose combinations
GA	Gastric aspirate
GCP	Good Clinical Practices
HIV	Human Immunodeficiency Virus
ICER	Incremental Cost-Effectiveness Ratio
IDLIC	Infection Diseases in Low Income Countries
IDMC	Independent Data Monitoring Committee
IRD	Institut de Recherche pour le Développement
MLR	Monocyte-lymphocyte ratio
MTB	Mycobacterium tuberculosis
MUAC	Mid-Upper Arm Circumference
NPA	Nasopharyngeal aspirate
NTP	National Tuberculosis Program
OSC	Output Steering Committee
PI	Principal Investigator
PPE	Personal Protective Equipment
QC	Quality control
RIF	Rifampicin
SAB	Scientific Advisory Board
SAE	Serious Adverse Event
SAM	Severe Acute Malnutrition
SCD	Sickle cell disease
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedures
TB	Tuberculosis
TeAM/SPI	Technical Assistance for Management/Soutien Pneumologique International
TMF	Trial Master File
UBx	University of Bordeaux
WHO	World Health Organization

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1. INTRODUCTION: BACKGROUND, RATIONALE AND HYPOTHESIS

Child undernutrition rates remain alarming in resource-limited countries

There are four broad sub-forms of undernutrition: wasting (low weight-for-height), stunting (low height-for-age), underweight (low weight-for-age) and deficiencies in vitamins and minerals. Joint effect of undernutrition conditions is a cause of 3.1 million child deaths annually, or 45% of all child deaths in 2011 [1]. These mostly occur in low- and middle-income countries.

Moderate wasting (-3 ≤ weight-for-height Z score [WHZ] < –2 standard deviation (SD)) and **severe wasting** (WHZ < –3)[1] **or severe acute malnutrition (SAM)** usually indicates recent and severe weight loss resulting from hunger and/or disease. According to joint analyses by UNICEF, the World Health Organization (WHO), and the World Bank, in 2016 nearly 52 million (7.7%) children under the age of 5 were wasted, and 17 million (2.5%) severely wasted [3]. More than one quarter of all wasted children live in Africa, where 14.0 million (7.4%) children under 5 are wasted including 4.1 million severely wasted.

Young children moderately or severely wasted face an increased risk of death. Although treatment and care is possible, severe wasting accounted for 7.4% of all child deaths in 2011 [1].

In 2016, the United Nations General Assembly proclaimed 2016–2025 the *United Nations Decade of Action on Nutrition*. The 2017 joint estimates however reveal insufficient progress to reach these global nutrition targets as well as the Sustainable Development Goals set for 2030.

Africa in particular has experienced slow progress in reducing malnutrition over the past 20 years compared to Asia and Latin America, being the only region where the absolute number of stunted children has risen. Western Africa in particular accounts for half of the stunting increase of the continent [3].

Malnutrition and infection: a synergistic association

All degrees of anthropometric deficits (stunting, underweight and wasting) are associated with increased risk of under-five mortality, especially from infectious diseases. Conversely, severe infectious diseases in early childhood can cause acute wasting. A pooled analysis of ten longitudinal studies involving more than 55,000 children revealed that undernutrition was associated with increased hazards of death from diarrhoea, pneumonia and measles; the association was also noted for other infectious diseases including tuberculosis (TB) [4].

The bidirectional causal link between malnutrition and TB

TB is a significant source of morbidity and mortality among children in resource-limited settings, with approximately 1.04 million new cases and 253,000 deaths in 2016 [5]. Malnutrition is indeed highly prevalent in children living in TB endemic countries. Socio-economic risk factors of malnutrition like poverty, poor sanitation, overcrowding, food insecurity, and human immunodeficiency virus (HIV) play also a significant role in continued TB transmission.

Based on 2008 figures from 22 high TB burden countries, a study estimated that 27% of overall TB cases were attributable to undernutrition [6]. However, the mechanisms underlying the association between malnutrition and childhood TB remain unclear [7], knowing also that TB is responsible of wasting, and studies are limited. This may be due to the challenges in diagnosing paediatric TB, difficulty in establishing a causal role of malnutrition on TB, and an overall low research priority.

On one hand, severe malnutrition and younger age individually increase the risk of household contacts of TB patients of developing TB disease [8]. Malnutrition is thought to both predispose to respiratory infections through deficits in innate immunity and contribute to progression to TB disease through reduced proliferation of T-cells and impaired cell-mediated immunity which in

[1] on the basis of the WHO Child Growth Standards [2]

turn leads to increased susceptibility to infection [9]. The resulting inflammatory response further worsens nutritional status.

On the other hand, it is well known that TB can cause or worsen undernutrition. TB leads to a decrease in body mass index (BMI), resulting from a loss of fat-free and fat mass [10]. Weight loss (or failure to thrive) is one of the main symptoms for the clinical diagnosis of TB in children, and weight variation is commonly used as a marker of treatment response follow-up [11].

SAM as a predictor of TB outcome

In adults, the presence of moderate-to-severe malnutrition (wasting) has been identified as a risk factor for mortality during the first 4 weeks of TB treatment [12]. A study conducted in South African children with drug-resistant TB showed that malnutrition was the most important predictor of both treatment outcome and mortality, even among those HIV co-infected [13]. In another recently published study from rural Uganda, severe malnutrition was independently associated with mortality of children with presumptive TB [14].

However, there is a paucity of data on TB prevalence in malnourished African children. Most studies exploring morbidity and mortality associated with TB in hospitalized children with SAM under 5 years were conducted in Asia, since the region bears the highest burden of child wasting. In a review of literature published up to 2014, TB prevalence was found particularly high in children with SAM hospitalized in tertiary hospitals in India and Pakistan (22% and 28% of cases respectively) and in high HIV prevalence settings such as South Africa, Zambia or Swaziland were it reached 23%, 30%, and 31% of cases respectively [15]. Recent publications confirm the wide range of TB prevalence observed among hospitalized children with SAM. Studies in India, mainly retrospective, reported a TB prevalence ranging from 2.0% to 22.0% [16]–[18]. In African countries, TB prevalence appeared lower, spanning 1.6% to 6.7% [19]–[21]. Programmatic data from MSF in Mali and Niger showed TB prevalence rates significantly higher in poor responders to nutritional therapy compared to responders, reaching up to 50.0% based on clinical signs and chest X-rays [15].

This wide range in the reported TB prevalence in hospitalized children with SAM could be partly explained by the heterogeneity in TB screening and diagnosis strategies across settings, worsened by the absence of a clear, standardized case definition.

In Dhaka hospital, Bangladesh, Chisti et al. reported no inpatient mortality but high post-discharge mortality (11%) in SAM children receiving TB treatment [22]. In a retrospective cohort study in Malawi, the case fatality rate among children with SAM and TB was 56%, with significant annual fluctuations [20]. On multivariate analysis, children with SAM and TB were 40% more likely to die compared to those without TB. Similarly, children with SAM and HIV infection were twice times more likely to have TB compared to HIV uninfected children. TB is probably a non-negligible cause of mortality in SAM cohorts with a high prevalence of HIV.

Challenges of TB diagnosis in malnourished children

Recent modelling showed that the vast majority of children dying from TB are young children below the age of five not accessing treatment, most likely because they are not diagnosed [23]. Indeed, in 2016 less than 45% of childhood TB cases were reported to the World Health Organization (WHO). This low notification rate is likely due to poor diagnostic performances of existing tests, as well as the result of the paucibacillary nature of the disease and difficulty to produce suitable bacteriological samples in children.

Besides usual challenges in microbiological confirmation of childhood TB cases, TB diagnosis in children with SAM poses an even greater problem due to the poor specificity of clinical and radiological features of the disease. Apart from severe wasting, these children are often asymptomatic yet with TB disease. They are however usually not considered as presumptive TB cases, and do not benefit from systematic TB assessment. Systematic TB screening in children with SAM seems a reasonable option but there is no clear WHO recommendation in favour of this strategy. The current approach is to consider TB in children not responding to nutritional rehabilitation and supportive treatment [11], which may delay TB treatment initiation and worsen the prognosis.

Political and institutional barriers

Malnutrition is an important target for future interventions in children at risk of TB. In its guidelines, WHO recognizes severe malnutrition as a key risk factor for TB in children and underlines the need for novel diagnostics in this population [11]. Despite increased awareness within the TB community and the launch of the Roadmap for Childhood Tuberculosis in 2013 [24], there is a significant gap in decentralization and integration of TB services into broader child health programs such as those addressing malnutrition.

Due to unspecific presentation of symptoms in this population, routine systematic TB screening of all children with SAM may offer clinical benefits. A study conducted in a rural hospital in Uganda however showed no statistical difference in TB case-detection yield between routine and targeted screening among 172 newly admitted children with SAM, despite a few more TB cases identified through routine screening [25]. Other authors recommend continued clinical TB screening with a low treatment threshold in severely malnourished hospitalized children suspected of TB [19].

In a recent article reviewing guidelines for the management of acute and severe malnutrition from 17 high TB burden countries, only seven (41%) countries recommend routine TB screening among children with acute malnutrition, and six (35%) recommend obtaining a TB exposure history [26]. Systematic TB risk assessment among children with SAM combined with improved linkage with TB services should help increase TB case detection. Training and operational research are needed to generate evidence on the impact that these efforts may have on case finding, treatment initiation and child survival.

Weaknesses of current TB diagnostic approaches

WHO criteria for the diagnosis of TB in children employs simple clinical features and CXR. These include prolonged duration of cough and/or fever, failure to thrive, result of tuberculin skin tests (TST), suggestive clinical signs, and a suggestive CXR [11]. However no definite cut-offs, such as duration of symptoms, have been validated and accuracy of screening and diagnostic approaches will depend on context. Strict symptom criteria have lower sensitivity and specificity in very young children, children living with HIV, or severely malnourished children. Cough, fever and poor weight gain are not specific to TB in a malnourished child. Due to the wasting and general weakness, these children may not be able to cough even when they have significant parenchymal disease. Lethargy and reduced playfulness, which are part of the symptoms used for TB screening in children are common in severely malnourished children without any other co-disease.

Chisti et al. evaluated WHO criteria in children with SAM aged <5 years admitted in Dhaka Hospital with radiological pneumonia. For the detection of culture-confirmed TB, sensitivity of WHO criteria was only 40% [27].

Radiography and TST, although recommended by WHO, have limitations in young children and particularly in those immunosuppressed. Though malnutrition is recognized as a cause of false-negative TST, it may be used in conjunction with other diagnostic tests [11]. Limited data are available from children in TB-endemic settings regarding interferon-gamma release assays (IGRAs), especially from malnourished children.

Performance of the WHO-endorsed molecular assay, Xpert MTB/RIF, in children with SAM is largely unknown. Studies in hospitalized children with SAM in Malawi and Uganda showed high prevalence of unconfirmed TB using the National Institutes of Health standard case definitions of intrathoracic TB, and high proportion of TB contact history but very low rates of Xpert and culture-confirmed TB on various samples including induced sputum (0.7% and 1.7% respectively) [21], [28]. These data do not support routine screening of hospitalized children with SAM using Xpert or culture. Conversely, in another study from Bangladesh, Xpert provided higher case detection rate than microscopy and culture on sputum in children with SAM with pneumonia. When using culture as the gold standard, the sensitivity and specificity of Xpert was 67% (95% CI: 24–94) and 92% (95% CI: 87–95) respectively [22].

Xpert may have increased utility on alternative specimens such as nasopharyngeal aspirates (NPA) or stool as demonstrated by our team in HIV-infected children [29]. The increased

detection yield of the recently released Xpert MTB/RIF Ultra (Ultra) will potentially benefit most patients with paucibacillary TB disease as seen in severely malnourished children [5]. However, use of Xpert for paediatric TB diagnosis is still scarce in resource-limited settings, particularly at low level of health care. In practice, even when laboratory and radiological diagnostic resources for TB are available, treatment is initiated empirically based on clinical features for most young children.

Research gaps in childhood TB biomarkers

Recent efforts in the field of paediatric TB diagnostics are calling for non-sputum based point-of-care diagnostic tools which are more efficient, affordable, and adapted to high-burden and limited resource settings. The WHO-endorsed Priority Target Product profiles (TPP) for TB includes a biomarker-based non-sputum-based rapid test for detecting active TB with the purpose of initiating treatment [30]. In young children with SAM in particular, who more likely to present few TB symptoms, have a paucibacillary disease and are usually unable to expectorate, detection of host markers in accessible non-sputum samples would be of great value. However, to date no single biomarker has proven to reliably discriminate childhood TB from other diseases, as well as TB disease from latent TB infection.

Findings from metabolomics studies have provided useful information on the host metabolic response to MTB infection, but their potential as TB diagnostics has yet to be confirmed. Measurement of immune response molecule concentrations, such as interferon gamma and C-reactive protein (CRP), is a complementary strategy to the direct detection of MTB. However, IGRAs are unable to discriminate between latent and active TB, and results are contradictory regarding the diagnostic value of CRP in children with SAM [31], [32]. IP10 has also been identified as a potential interesting inflammatory biomarker for diagnosis of TB in children in previous study and would deserve further investigation [33]. The blood Monocyte-to-Lymphocyte ratio (MLR) correlates with TB disease in HIV-infected adults, but very few data are available on its validity in children. Findings from a cohort of 183 hospitalized HIV-infected children, presented at the 2017 Union Conference, showed that the MLR distinguished HIV-infected children with microbiologically-confirmed TB from those with unconfirmed or unlikely TB, possibly reflecting bacterial burden [34], [35].

Lipoarabinomannan (LAM), a mycobacterial antigen and virulence factor which is found in the urine of many TB patients, has been the most studied TB biomarker for the development of a low-cost point-of-care assay. However, the adoption of LAM tests has been limited due to their relatively poor clinical sensitivity across the spectrum of incident TB cases. The observation that LAM levels in urine tend to be higher in HIV-positive TB patients than in HIV-negative TB patients led to the WHO recommendation to use the Alere Determine™ LAM assay (only commercially available test) to diagnose TB only in HIV-positive individuals with CD4 counts <100 cells/µl who have TB symptoms [36]. Urinary LAM assays have an interest as a complement to Xpert for people living with HIV, given the relatively low sensitivity of Xpert in this population. A novel urine-based assay co-developed by FIND and Fujifilm, The Fujifilm SILVAMP TB LAM, has shown a higher sensitivity and specificity compared to the Alere LAM test based on initial results on frozen urine samples of almost 1000 HIV-infected patients ([37]; non-peer-reviewed manuscript). Further prospective and operational studies are nevertheless needed in paediatric populations.

Emerging research using genome-wide transcriptional biosignatures in whole blood has been the most promising (accuracy >80%). Studies in cohorts of children from South-Eastern Africa, South America and India have identified mRNA signatures and gene sets distinguishing active disease from latent TB infection as well as TB from non-TB pneumonia and other diseases [38]–[42]. However, these candidate transcript signatures now require further exploration as well as cross-validation in prospective cohorts of patients from multiple settings and genetic backgrounds [39].

TB diagnostic algorithms are urgently needed for children with SAM

Systematic reviews on clinical scoring systems for the diagnosis of TB in children reveal that a minority of systems are relevant to developing countries with a high burden of malnutrition and

HIV/AIDS. No studies have focused on children with SAM, or compared validity of systems between SAM and well-nourished children [43], [44]. This is partly because acute malnutrition itself (especially if unresponsive to nutritional rehabilitation) is a criterion in many of the scoring systems.

Although these scoring systems and diagnostic criteria are commonly used, they are currently not recommended by WHO. Their reliability and validity remain unclear due to lack of an established and practicable gold standard.

Different diagnostic criteria are used in different settings, and they may or may not have been validated for those locations [43].

One major limitation for the development and evaluation of new diagnostic tests or algorithms in children is the absence of reference standard of TB disease. Indeed, as previously mentioned the microbiological based reference standard using sputum mycobacterial culture that is commonly used in adults is not adapted for children and would miss a proportion of true TB cases. Therefore, group of experts have proposed robust Clinical Case Definition for Classification of Intrathoracic Tuberculosis for the evaluation of TB diagnostic tests in children [45]. Although this alternative approach is probably the best we have today, there are several methodological issues associated with the use of composite reference standard and with the use of these definitions to evaluate the accuracy of diagnostic algorithms that may include signs that are also used in the standard case definitions [46].

A study conducted in India reviewing the application of a new national algorithm for TB diagnosis in malnourished children showed that the yield of TB was higher among children evaluated as per algorithm (4%) as compared to those who were not (0.3%) [16]. It also identified several operational challenges, including non-availability of full-time paediatricians, non-functioning X-ray machines, difficulties in demonstrating a positive TST and difficulties in adhering to a complex diagnostic algorithm increasing the chances of loss to follow-up.

There is now strong evidence that undiagnosed and untreated TB increases the risk of death in children, especially those severely malnourished who are highly vulnerable. Specific decision-making tools are therefore urgently needed to guide clinicians from high-TB burden and low-income countries to initiate treatment quickly in children with SAM with suspected TB.

The TB-Speed strategy: development of a diagnostic score and algorithm in children with SAM

A diagnostic prediction score and algorithm was recently proposed by our team for TB treatment decision in HIV-infected children with presumptive TB (developed in the ANRS 12229 PAANTHER 01 study). Based on easily collected clinical features, chest X-Ray, Xpert MTB/RIF, and abdominal ultrasonography, the score aims to help clinicians make a same-day treatment decision [47]. Such a prediction score improving TB diagnosis and shortening time to treatment initiation would be a key benefit in children with SAM. Based on this experience, we are therefore proposing a diagnostic cohort study enrolling hospitalized severely malnourished children. The study will include the evaluation of several diagnostic tests that could be integrated in the development of a prediction model and subsequent score for the diagnosis of TB in hospitalized children with SAM. This will include ultrasonography which has shown its interest for diagnosis of TB in both HIV-infected adults and children [48]–[50]. In the PAANTHER study, it detected abdominal lymphadenopathy in 50% of culture confirmed TB cases and 35% of all confirmed and unconfirmed cases, with a specificity of 85%. In order to reduce costs and sample collection in all hospitalized children with SAM, if possible, we will develop a first-step screening prediction score to identify children with presumptive TB, requiring further investigations including sample collection and Ultra testing.

The score(s) will be used to propose a stepwise diagnostic algorithm, targeting microbiologically confirmed and unconfirmed TB cases.

Participating countries and added value of the multi-country aspect

The impact of this innovative diagnostic strategy may vary with TB incidence, prevalence of malnutrition and aggravating co-morbidities like HIV as well as seasonal variations that can affect nutritional status in young children. To provide a better basis for the generalisation of results, the project will take place in two countries with different epidemiological and environmental backgrounds, in Eastern Africa (Uganda), and Southern Africa (Zambia) (Table 1).

Zambia is among the 30 high TB burden countries according to the WHO classification. For the purpose of this study, we will differentiate between 'high' and 'very high' TB incidence countries using a cut-off annual incidence rate of 300 cases/100,000 population.

Table 1: TB incidence and child acute malnutrition in participating countries

Region	Country	TB incidence rate /100,000 pop[a]	Wasting[b,*] % in children 0-59 months (year)	Paediatric HIV prevalence[c]
Eastern Africa	**Uganda**	201	4.3 (2012)	17.1%
Southern Africa	**Zambia**	376	6.3 (2013-2014)	32.6%

*Sources: [a]WHO Global TB report, 2017; [b]UNICEF/WHO/World Bank Group Joint Child Malnutrition Estimates, 2017; [c]Dodd et al., 2017. * Moderate and severe wasting: Weight for Height < -2 SD*

National TB Programs (NTPs) from these countries support the TB-Speed project and are members of Country Project Committees (see Chapter 13.3). NTPs will play an instrumental role in the scale up of the TB-Speed strategy, taking the opportunity of the 2020 country dialogue for funding request to the Global Fund. This will be further supported by the WHO-Unitaid TB enabler grant, through which the project will receive support from WHO to ensure country preparedness for accelerated uptake and integration of the TB-Speed approach into national and international guidance for the management of childhood TB.

2. OBJECTIVES

2.1. Primary Objective

- To develop a diagnostic prediction score for TB in hospitalized children with SAM

2.2. Secondary Objectives

1. To assess the prevalence of TB among hospitalized children with SAM
2. To describe the symptoms and clinical characteristics of TB disease in hospitalized children with SAM
3. To develop a first-step screening prediction score to identify children with presumptive TB among hospitalized children with SAM
4. To propose a stepwise diagnostic algorithm based on the score(s) developed
5. To assess the diagnostic performance and the added value in a diagnostic prediction score for TB in hospitalized children with SAM of the following tests:
    a. Ultra performed on one NPA and one stool sample
    b. Chest radiography features as assessed by the simplified TB-Speed CXR reading tool
    c. Abdominal ultrasound
    d. QuantiFERON®-TB Gold In-Tube (QFT) IGRA

e. Monocyte-to-lymphocyte ratio (MLR)

f. CRP

6. To assess the feasibility of collecting NPA and stool in children with SAM
7. To assess the safety and tolerability of NPA collection in children with SAM
8. To assess mortality and weight gain at 6 months in children with SAM, with or without anti-TB treatment
9. To assess the effect of bacteriological features (Xpert and/or culture-confirmed TB) and other key patient characteristics (age, HIV status, initial severity markers, percentage weight gain, CXR features) on TB treatment outcome
10. To evaluate the cost effectiveness of implementing the new diagnostic prediction score for TB treatment compared to the estimated effect of the standard of care in children with SAM.

3. STUDY ENDPOINTS

3.1. Endpoints for the primary objective

i) Sensitivity of the score obtained using predicted probability cut-off with the prediction model for the diagnosis of TB, defined as either confirmed or unconfirmed using the updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis (see section 3.3)

ii) Specificity of the score obtained using predicted probability cut-off with the prediction model for the diagnosis of TB

3.2. Endpoints for the secondary objectives

1. Proportion of confirmed and unconfirmed TB in the study population
2. Clinical (symptoms, anthropometric measures, physical signs), laboratory (bacteriological, haematological, immunological signs) and radiological features (chest X-ray and abdominal US) of children with tuberculosis (confirmed and unconfirmed)
3. Sensitivity and specificity of the score obtained using predicted probability cut-off with the screening prediction model for the identification of children with presumptive TB requiring further diagnostic evaluation
4. Estimated time to TB treatment decision in hospitalized children with SAM, with and without presumptive TB based on the first-step screening prediction score
5. Diagnostic accuracy measures (Sensitivity, specificity, negative and positive predictive value) of the different tests evaluated for the diagnosis of TB (Ultra performed on NPA and stools, CXR, abdominal ultrasound, QFT, MLR, CRP) and AUROC of diagnostic prediction models with and without the different tests results

   Diagnostic accuracy of Ultra performed on NPA and stool samples will also be estimated against a specific microbiological reference standard including Ultra and mycobacterial culture from gastric aspirates (GAs)
6. Feasibility of NPA and stool specimen collection: proportion of children with samples collected as per study protocol
7. i) Safety of NPA collection procedure: adverse events (AEs) collected by study nurses (vomiting, nose bleeding, low oxygen saturation, respiratory distress)

   ii) Tolerability of NPA collection procedure: discomfort/pain/distress assessed from the child (Wong-Baker face scale), by the parents (visual analog scale), by the nurses (FLACC behavioural scale) (quantitative assessment) measured in a subset of children
8. i) Mortality at 6 months, with or without TB treatment

ii) Percentage weight gain, WHZ at 6 months

9. TB treatment outcomes as defined per WHO recommendations [51]
10. Incremental cost-effectiveness ratio (ICER)

3.3. Reference diagnosis/case definition for the study and validation by the Expert Committee

Children will be classified into 3 categories, according to the updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children [45], detailed in Table 2: *confirmed*, *unconfirmed*, or *unlikely* tuberculosis or any subsequent published update at the time of final case review if feasible and approved by the Scientific Advisory Board (SAB).

Table 2. Updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children (adapted from Graham et al, 2015 [45]***)***

Case definition	Refined criteria
Confirmed tuberculosis	Bacteriological confirmation obtained (Mycobacterium tuberculosis confirmed by culture or Xpert MTB/RIF assay from at least 1 respiratory specimen)
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: • Symptoms/signs suggestive of tuberculosis[1] • CXR consistent with tuberculosis[2] • Close tuberculosis exposure or immunologic evidence of *M. tuberculosis* infection • Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment — no time duration specified) AND no spontaneous improvement of symptoms in the absence of antituberculosis treatment
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND criteria for "unconfirmed tuberculosis" NOT met (including spontaneous improvement of symptoms in the absence of antituberculosis treatment)

[1] Clinical signs/symptoms suggestive of tuberculosis include: (a) Persistent cough: persistent (>2 wk), unremitting cough. (b) Weight loss/failure to thrive: (b1) Unexplained weight loss: >5% reduction in weight compared with the highest weight recorded in last 3 months OR (b2) Failure to thrive defined as (i) Clear deviation from a previous growth trajectory, and/or (ii) Documented crossing of percentile lines in the preceding 3 months, and/or (iii) Weight-for-age z score of ≤−2 in the absence of information on previous/recent growth trajectory, and/or (iv) Weight-for-height z score of ≤−2 in the absence of information on previous/recent growth trajectory AND (b3) Not responding to nutritional rehabilitation (or antiretroviral therapy if HIV infected). (c) Persistent unexplained fever: Persistent (>1 week) and unexplained fever (>38°C) reported by a guardian or objectively recorded at least once. (d) Persistent, unexplained lethargy or reduced playfulness: persistent, unexplained lethargy or decrease in playfulness/activity reported by the parent/caregiver. (e) Infants 0–60 day (or neonate): additional signs and symptoms suggestive of tuberculosis include: (e1) neonatal pneumonia or (e2) unexplained hepatosplenomegaly or (e3) sepsis-like illness.

[2] CXR will be considered consistent with tuberculosis if reviewers agree on the presence and location (right/left) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, tracheal compression (as suggested by Graham et al. 2012 [52]*).*

CXRs will be read by 2 independent experts experienced in reviewing CXRs in children, blinded to all of the clinical information and to each other's interpretation. In case of disagreement, advice from a third expert will be sought.

An Expert Committee will be set up at national level for the purpose of case review and validation of TB diagnosis including causes of death in children who would die after inclusion in the study. The Expert Committee will not review all cases systematically. We will use an

algorithm to select children who will be reviewed. The Expert Committee will not review cases which are Ultra (bacteriologically)-confirmed TB cases, or true negative TB (Ultra-negative untreated children alive at 6 months with normal M6 follow-up CXR). The Expert Committee will review other cases considering the following parameters: initial and follow-up clinical data, microbiological data and radiological features.

The Expert Committee will classify children using the Revised Classification of Intrathoracic Tuberculosis Case Definitions for Diagnostic Evaluation Studies in Children (see Table 2 above), following Standard Operating Procedures (SOPs) specifically developed for the study. A centralized Expert Committee will review part of all cases reviewed at country level to ensure homogeneity of classification across countries.

4. STUDY DESIGN

4.1. Study type

TB-Speed SAM is a multicentric, prospective diagnostic cohort study conducted in two countries with high and very high TB incidence (Uganda, and Zambia). It aims at assessing several diagnostic tests that could result in the development of a score and algorithm for TB treatment decision in hospitalised children with SAM.

4.2. Methodology

This will be a prospective cohort study enrolling 720 children with SAM (see 4.3. Sample size).

The diagnostic strategy will include an initial clinical, radiographic and bacteriological evaluation of all enrolled children. For the purpose of the study, additional diagnostic methods will be added and evaluated including: abdominal ultrasonography, QFT IGRA, MLR, CRP as well as alternative sample collection methods (NPA, stools samples). MLR, CRP, QFT, and abdominal ultrasound findings will be made available to the clinicians. Although not currently recommended by the WHO and NTPs, these tests may contribute to patient care. Training on how to interpret tests results will be provided during the study preparation phase (see Chapter 6.2), together with specific SOPs. In particular, caution will be recommended to avoid using these tests to rule out TB.

Clinicians will make a diagnosis using national TB algorithms (referred to as “the clinician’s diagnosis”). Results will be provided to the attending clinicians, i.e. not members of the study, who will make the final decision to initiate TB treatment according to current National Guidelines.

At the end of the study, children will be retrospectively classified as confirmed, unconfirmed, or unlikely TB, using the updated version of the Clinical Case Definition for Classification of Intrathoracic Tuberculosis [45] (Table 2). For model development, the reference diagnosis will be *confirmed* and *unconfirmed* TB.

Using logistic regression, we will develop a score for TB diagnosis, considering confirmed and unconfirmed TB as reference diagnosis, in hospitalized children with SAM. As a secondary objective, and in order to reduce costs, sample collection, and complexity of the diagnostic process, we will aim at developing a first-step screening score to identify children with presumptive TB who would benefit from further diagnostic testing. This will be done similarly using logistic regression with the individual predicted probability of TB dichotomized to maximise sensitivity as predicted variable. We will exclude the following tests from model development: Ultra results on microbiological samples, abdominal ultrasound, as well as CXR if possible. Both scores will be internally validated using resampling and will be incorporated in a stepwise algorithm to guide practical implementation of the screening and diagnosis process.

The stepwise algorithm will be discussed with local clinicians involved in the study to better adapt it for future use in their routine practice.

- **Study design adaptation to the Covid-19 outbreak**

All enrolments in the TB-Speed SAM study have been stopped as of April 1st, 2020 due to the Covid-19 outbreak. This was justified by the complete or partial lock down in project countries, the lack of protective equipment to ensure safety of participants and study staff, and the difficulty to conduct proper study monitoring. The decision was approved by the TB-Speed Scientific Advisory Board. All study sites were instructed: 1) to suspend all enrolments until further notice; 2) to conduct phone-based follow-up when physical visits are not possible, provided the participant's safety is not compromised, based on ad hoc Standard Operating Procedures for remote follow-up; 3) to limit face-to-face contact to that strictly necessary to provide care that cannot be suspended, provided the safety of staff is protected, based on ad hoc Standard Operating Procedures for infection prevention and control during health care, samples collection and samples processing at the laboratory.

Enrolments in the main TB-Speed SAM study will resume progressively at sites, on condition that the respective country situations allow for it, in particular the availability of personal protective equipment (PPE). The Covid-19 ancillary study will be implemented to document SARS-CoV-2 prevalence and its impact on outcome in children, and enable access to specific Covid-19 treatment if needed, as soon as approved by Ethics Review Committees.

4.3. Sample size

Data on reported prevalence of TB in children with SAM from African settings is scarce and heterogeneous, largely depending on diagnostic strategies, case definitions as well as bias inherent to retrospective studies. Prevalence data gathered from the published literature varies from 1.6% to more than 30% in HIV-prevalent, urban tertiary health care hospitals, including Zambia [36], [37]. Hypothesizing an intermediate TB prevalence among children with SAM in participating sites of 15%, we will need a sample size of approximately 720 children, including children with either confirmed or unconfirmed TB, to develop a diagnostic algorithm or score with an expected sensitivity of 80%, an unacceptable sensitivity of 65% (minimal acceptable lower confidence interval limit), and 10% missing data on key predictors entering the model.

4.4. Provisional study schedule

- First inclusion: 04/11/2019
- Last inclusion : 31/12/2021
- Total inclusion period: 26 months
- Duration of follow-up for each participant once enrolled: 6 months
- Enrolment stop due to the Covid-19 pandemic: April 1st, 2020
- Enrolment restarting: August-October 2020
- Expected last visit of the last patient: 30/06/2022
- Overall duration of the study (from the first inclusion to the last visit): 32 months

4.5. Cost-effectiveness ancillary study

A cost-effectiveness study will be performed as part of the TB-Speed Project Output 5 ("Evaluation of cost-effectiveness of the proposed diagnostic approaches"). Cost-effectiveness and budget impact analyses will evaluate the incremental cost-effectiveness ratio (ICER) and the long-term impact of improving TB diagnostics in children with SAM, guide health authorities' decisions and support the implementation of the TB-Speed approach in resource-limited settings.

A mathematical model will be developed to project health-economic outcomes including TB cases and mortality in children with SAM. The model will be developed in collaboration with ScHARR of the University of Sheffield (UK) and the CaP-TB project (see Chapter 12.2.3).

The cost-effectiveness analysis will be from the health payer perspective and only direct health care costs will be included. A budget impact analysis will be conducted to evaluate the actual impact of implementing the TB-Speed approach on healthcare budgets at 2- and 5-year horizons in the countries participating in TB-Speed.

A separate analysis plan will be written for the cost-effectiveness analysis. Data collection methods for cost data are outlined in Chapter 10.1.2.

5. STUDY ENROLLMENT

5.1. Study population

5.1.1. Inclusion criteria

- Children aged 2 to 59 months
- Severe acute malnutrition defined as weight-for-height Z score (WHZ) < -3 standard deviation (SD) or mid-upper arm circumference (MUAC) < 115 mm (in children over 6 months) or clinical signs of bilateral pitting oedema [2]
- Hospitalized per hospital clinician's decision*
- Parent/guardian informed consent

**Usual criteria for hospitalisation of children with SAM recommended by WHO include: medical complications including sepsis and dehydration, severe oedema, poor appetite, and presentation of one or more Integrated Management of Childhood Illness danger signs (unable to drink or breastfeed; vomiting everything; more than one or prolonged convulsions (>15 min); lethargic or unconscious; convulsing now)* [53].

5.1.2. Non-inclusion criteria

- Ongoing TB treatment or history of intake of anti-TB drugs in the last 3 months

5.2. Recruitment sites

The study will be implemented at inpatient nutrition centres from three selected tertiary hospitals in Uganda and Zambia (see Table 1). Participating hospitals are University Teaching Hospitals or equivalent in terms of level of care, located in the country capital city or major city. They are also implementing sites for the TB-Speed Pneumonia and TB-Speed HIV studies.

Table 3 shows indicative recruitment capacities of participating hospitals, based on a capacity assessment questionnaire including the number of children admitted annually for SAM. It should be noted the high prevalence of HIV infection among these children.

Table 3: Implementing sites and number of admissions for severe malnutrition

Country	Hospitals, City	No. children admitted for SAM (year)	Proportion of HIV infected
Uganda	Mulago National Referral Hospital, Kampala	1,200* (2016)	30%
Zambia	University Teaching Hospital, Lusaka	2,000* (2016)	50%
	Arthur Davidson Children's Hospital, Ndola	541 (2017)	

** Approximate number*

6. STUDY INTERVENTIONS

6.1. Description of the diagnostic strategy

The diagnostic strategy will include:

- Assessment of household TB contact history
- Medical history and duration of symptoms in the previous 4 weeks
- Physical examination including vital signs
- Clinical, anthropometric and biochemical assessment of malnutrition
- Clinical assessment for other non-dietary causes of malnutrition, including

identification of important comorbidities like HIV
- A digitalized CXR (standard anteroposterior and lateral view) performed and interpreted using standardised approach
- Ultra performed on NPA and stool samples, as well as on the first of the two GAs collected
- Mycobacterial culture performed on two GAs
- Abdominal ultrasonography to assess the presence of intra-abdominal lymphadenopathies or ascites
- MLR
- QFT
- CRP

TB diagnosis will be made according to national TB guidelines (i.e. clinician's diagnosis).

6.2. Capacity building

Implementation of the TB-Speed SAM study will include a preparation and capacity building phase conducted at participating hospitals, which are tertiary level reference university teaching hospitals also involved in the TB-Speed Pneumonia and HIV studies in Zambia and Uganda. Sites which are not already equipped will be provided with digital radiography (DR) plates for digital CXR, ultrasound machine, sample collection material (including battery-operated suction machines for NPA collection) and IGRA kits. Laboratories will be equipped with GeneXpert devices if not available through National Programs as well as -80°C freezers for biobanking.

Specific training will be provided to site investigators and hospital staff in nutrition wards (doctors and nurses) on TB diagnosis, study procedures, and Good Clinical Practice (GCP). Initial training will be provided onsite by the study central coordinating team. Based on didactic and practical presentation, trainings will include the following topics:

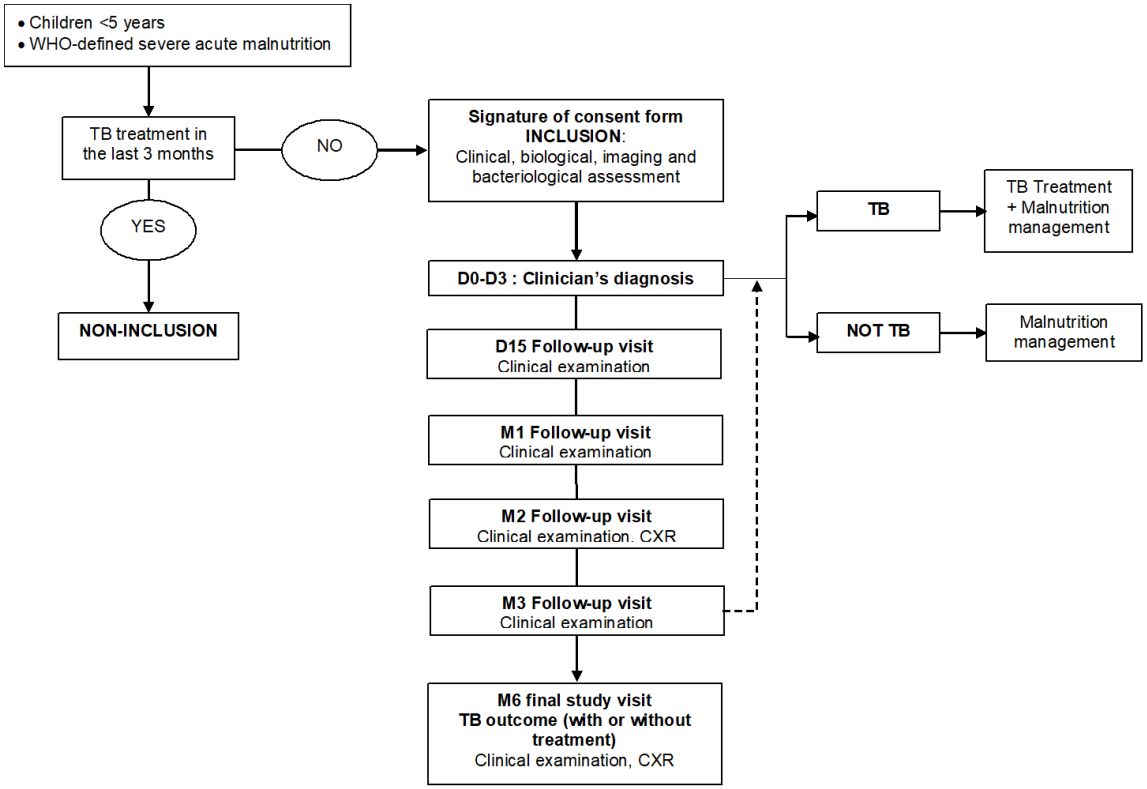
- Childhood TB diagnosis and management
- Clinical follow-up (eligibility criteria, informed consent process, patient schedule, TB assessment, management in the case of AE/SAEs)
- Biological samples collection (NPA) and laboratory procedures including biobanking
- Non-routine TB assessment procedures and their interpretation (QuantiFERON®-TB Gold, MLR, CRP, abdominal echography)
- Data collection and use of electronic CRF
- Data management and monitoring
- Use of DR plates

At the end of the study, site clinicians will be trained on the use of the developed algorithm and related interpretation of the different diagnostic tests.

6.3. Implementation at site level

The TB-Speed SAM study will be implemented in 3 tertiary healthcare level hospitals in Uganda and Zambia (see Table 1 and Table 3). A total of 720 children <5 years old with WHO-defined severe acute malnutrition will be enrolled, that is approximately 240 participants per hospital, with a competitive recruitment. The inclusion period will last until the expected number of children enrolled is reached, which could be achieved in about 32 months based on hospital admission numbers (see Chapter 5.2) and the overall delay incurred by the study, in particular due to the Covid-19 pandemic.

6.4. Study flow chart



7. STUDY PROCEDURES

7.1. Selection process

Any child younger than 5 years old, hospitalized and presenting with SAM at inpatient nutrition centres of selected hospitals, will be consecutively screened for eligibility by study nurses and offered to participate to the study.

Assessment for eligibility will be detailed in specific clinical SOPs.

7.2. Obtaining informed consent

The consent process will be conducted only once the child is stabilized, i.e. by 24 to 48 hours following admission. In any case, the clinical management of the child will be prioritized over research.

Parent(s)/guardian(s) of eligible children will be explained the purpose, the nature of constraints and the foreseeable risks and benefits of the study. Parent(s)/guardian(s) will be informed that participation is voluntary and that they will be free, without justification, to withdraw from the study at any moment without consequence on the quality of care and follow-up provided to their child. In addition to oral explanations, a written information sheet will be systematically provided (see Appendix 6). Documents with pictures will be available to help the parents to better understand the exams proposed in the study.

The informed consent process will be implemented by the study nurses. They will ensure that parent(s)/guardian(s) have read and understood the content of the information sheet, and that they have received answers to all their questions before signing the informed consent. If one of the parent(s)/guardian(s) does not agree on the child's participation, the child will not be enrolled. If the participation agreement is given by the parent(s)/guardian(s), the consent form will be completed, signed and dated by the parent(s)/guardian(s) and the study nurses and/or site clinicians/investigators. Oral consent in the presence of a literate witness (not from the medical team) is acceptable in the case of illiteracy. The consent form will include separate consent for frozen samples conservation.

A copy of the signed consent will be given to parent(s)/guardian(s). The original consent form will be retained by the site Principal Investigator (PI) in a safe place inaccessible to others, even when moving, throughout the study period and for 15 years after its end.

In the absence of national regulation, will be considered as a guardian a person who usually assumes responsibility for the child's custody, care, and maintenance even though no court order exists formally appointing the person as the guardian, custodian, or adoptive parent of the child. Should any of the child's parents be alive but not living with the child, the usual caregiver will be considered as a guardian.

7.3. Patient schedule

Table 4: Patient schedule including specimen collection in children

	Study visits						
	Inclusion (D0)	**D3** *(+/-2 days)*	**D15** *(+/-2 days)*	**Month 1**	**Month 2**	**Month 3**	**Month 6**
				(+/-7 days)			
Eligibility criteria	X						
Informed consent	X						
Clinical evaluation[1]	X	X	X	X	X	X	X
Medical and treatment history	X		X	X	X	X	X
Initial TB assessment	X						
Chest X-Ray	X(○)[2]	Result			X		X
Abdominal ultrasound	X	Result					
Lab assessment:							
- HIV diagnosis[3]	○	Result					
- Malaria test	X(○)[4]						
- Complete Blood Count (CBC)	X(○)[5]	Result		X			
- Transaminases + CRP	X	Results		X			
- QFT	X	Result		(X)[6]			
Maximal number of tubes collected[6,7]	10 (9)			6 (2)			
Maximal volume of blood collected[6,7,8]	13 mL (12.5 mL)			4 mL (6 mL)			
NPA	X			(X)	(X)		
Stool sample	X	(X)		(X)	(X)		
2 GAs		X(○)[2]		(X)	(X)		
Xpert MTB/RIF Ultra	X	Result		(X)	(X)		
Mycobacterial culture (on 2 GAs)		X		(X)	(X)		
Safety/feasibility/tolerability/ acceptability assessment	X						
Biobank (frozen samples): - Plasma - Whole blood - NPA, stool, GA leftovers - Urine	(X)[6] X X X						
TB treatment if needed[9]		○	○	○	○	○	○
TB drug adherence assessment			○	○	○	○	○
Treatment of SAM[9]	○	○	○	○	○	○	
Treatment and care of co-morbidities if needed[9] (HIV infection, other)	○	○	○	○	○	○	○
Nutritional status assessment	○	○	○	○	○	○	○
TB treatment response							○

○ *Routine care*
X Research

(1) Content of clinical evaluation varies with the visit
(2) Performed as part of routine care in case of TB suspicion
(3) Performed if not available in the patient medical chart
(4) Possibly performed as part of routine care in case of clinical suspicion of malaria
(5) Possibly performed as part of routine care for anaemia assessment
*(6) **In children <18 months weighing <5 kg, or children presenting with signs of severe anaemia (conjunctival or palmar pallor):** QFT test will be performed at M1 and plasma sample for biobank will not be*

collected, except in children with signs of severe anaemia if they will benefit from blood transfusion; QFT test will be preferably done just before transfusion. Plasma sample for biobank will not be collected

*(7) **In children >18 months:** 2 mL of whole blood collected on plain tube for HIV serology; 3 x 2 mL collected on EDTA tube for CBC, malaria test and plasma biobank; 2 mL collected on heparin tube for transaminases and CRP; 4 x 1 mL collected on QFT tubes; 2.5 mL collected on Paxgene Blood RNA tube for biobank. **In children <18 months**: HIV testing will be performed by PCR on 500 µL (maximum) dried blood spot.*

(8) Volume of blood draw must not exceed 3 ml/kg/visit and 7 ml/kg/6 weeks.

(9) According to national treatment guidelines based on WHO recommendations

7.4. Inclusion visit

After obtaining informed consent, the following procedures will be performed during the inclusion visit:

- **Complete clinical evaluation**

The following data will be abstracted form existing file; additional data will be collected by site investigators or study nurses

- Reason for hospitalization
- Demographic information (sex, month and year of birth)
- Physical examination including vital signs (respiratory rate, heart rate, temperature, measurement of oxygen saturation), severe dehydration, features of shock
- Clinical and anthropometric assessment of malnutrition: WHZ, MUAC, presence and severity of oedema, medical complications[2], danger signs[3], anaemia
- Clinical assessment for other non-dietary causes of malnutrition, including identification of important comorbidities like HIV infection, intestinal infections, malaria
- Interview of parent/guardian on medical history, past and current medication and immunization (including BCG)
- HIV status (if unknown) and history of antiretroviral therapy (ART)
- TB assessment: family exposure, history and duration of symptoms in the previous 4 weeks, including cough, signs of pneumonia, fever, diarrhoea, neurological signs

- **Medical imaging**

  - A digitalized CXR (standard anteroposterior and lateral view) will be realized and interpreted using standardised approach
  - Abdominal ultrasonography will be systematically performed to assess the presence of intra-abdominal lymphadenopathies, splenic micro-abscesses, hepatic abscesses or ascites

Medical imaging will be performed as soon as possible and within 24 hours of inclusion to enable quick TB diagnosis. In very sick children, CXR and abdominal ultrasonography could be postponed by 2 to 3 days.

- **Blood samples** (see section 8.2.5)

  - For HIV testing (in children with unknown HIV status, using national guidelines)
  - For malaria testing (rapid test and/or thick smear)
  - For complete blood count (anaemia assessment), transaminases, and CRP
  - For the QFT assay
  - For biobanking (storage of frozen samples)

- **Bacteriological specimen collection** (see section 8.1)

Initial bacteriological specimen collection will be done as soon as possible and within 24 hours to 48 hours of stabilization by trained nurses, including:

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[2] hypoglycemia, hypothermia, dehydration, sepsis, lethargy; skin, respiratory or urinary tract infections [53].

[3] unable to drink or breastfeed; vomits everything; has had convulsions (more than one or prolonged >15 min); lethargic or unconscious; convulsing now [53].

- 1 NPA, collected by the nurse on the day of admission without prior nasal instillation (see Appendix 3);
- 1 stool sample, collected as soon as the child is able to produce stool (see Appendix 4);
- 2 GAs.

NPA and GAs will be done under SpO2 monitoring. They will not be attempted in children presenting severe respiratory distress or SpO2 <90% under appropriate oxygen therapy, severe dehydration or features of shock. Advice will be sought from a clinician not directly involved in the study to make the determination on a case by case basis for very sick children.

The safety of bacteriological specimen collection (NPA and GA) will be assessed by study nurses. Contraindications, implementation of sample collection procedures, and safety monitoring during collection will be detailed in study procedures.

The feasibility and tolerability of NPA will be assessed using qualitative and quantitative tools (Cf Chapter 7.6) in a sub-group of children.

Bacteriological tests performed on collected specimen are detailed in Chapter 8.2.

Additionally, a urine sample will be collected for biobanking.

Extra tests not planned by the protocol will be requested by the clinician in accordance with his/her practice and the national recommendations.

7.5. Follow-up

Children will be followed-up by site clinicians for 6 months upon enrolment, regardless of their TB diagnosis, with the following schedule (Table 4):

- Day 0 to Day 3: TB diagnosis (clinical, radiological, bacteriological and biological evaluation)
- Day 15, Month 1, Month 2, Month 3, and Month 6: assessment of nutritional recovery and TB disease evolution with or without TB treatment depending on the clinician's diagnosis, and "catch-up" TB diagnosis

The dates of each visit must comply with the provisional patient schedule generated from the date of inclusion of the child. In case of delayed visit at a specific date, dates of subsequent visits must comply with the initial visit schedule. If a child fails to attend a study follow-up visit, the clinical team on site will confidentially contact the parent(s)/guardian(s) and encourage/assist them to bring back the child for follow-up. For children receiving longer TB treatment duration, an additional visit will be scheduled after 6 months to assess treatment outcome.

Each follow-up visit will comprise:

- Clinical evaluation: complete physical examination, vital signs
- Medical history since the last visit: any new clinical and/or AE, with special attention to severe AEs resulting from NPA collection
- Chest radiography (M2 and M6 visits)
- TB drug prescription and dispensation to cover time to the next follow-up visit if indication of treatment
- Evaluation of adherence to TB treatment, if initiated
- Clinical, anthropometric and biochemical assessment of nutritional status
- Dietary and food security assessment

As part of routine care and per WHO recommendations, hospitalized children with SAM will be given nutritional support, parenteral antibiotics to treat possible sepsis and appropriate treatment for other medical complications such HIV, surgical conditions or disability [53]. In HIV-infected children, ART should be initiated as soon as possible after stabilization of metabolic complications and sepsis, i.e. return of appetite and resolution of severe oedema.

TB treatment (new dispersible paediatric fixed dose combinations, FDCs) will be initiated at the discretion of the site clinician, and will be procured by each country NTP. ART will be provided by national HIV programs. The study will facilitate communication with the NTP to ensure that adult index case tracing is done according to national recommendations.

Assessment of nutritional status evolution and response to treatment will be made at M1, M2, M3, and M6 (stabilization, transfer to outpatient, recovery, treatment failure, death).

In children not initially diagnosed with TB who show no clinical improvement or present additional signs of presumptive TB at M1 or M2, bacteriological specimen collection and Ultra testing will be repeated.

Assessment of TB treatment response (if initiated) will be made by the site clinician at the M6 final study visit (symptoms improvement, treatment completion, treatment failure, death, lost to follow-up).

- **TB treatment initiation**

TB treatment using 2HRZ(E)/4RH with new paediatric formulations according to National Guidelines will be initiated immediately in children with positive Ultra results, without waiting for the next early morning and overnight fasting. Longer treatment will be prescribed for extra-pulmonary TB (like meningitis) according to guidelines. To facilitate immediate treatment initiation, the study will provide to implementing sites a drug buffer stock if needed. Children with rifampicin resistance detected by Ultra will have culture and phenotypic drug susceptibility testing (DST) performed on leftovers from NPA and stool (and additional samples if needed), and will be started on empirical MDR-TB treatment.

- **Evaluation of adherence**

Adherence to the TB treatment will be evaluated by an adherence questionnaire administered to parent/guardian of the child.

7.6. Feasibility and tolerability assessment of NPA and stool collection procedures

- **Feasibility**

Feasibility will be defined using a series of indicators including the proportion of children with NPA and stool samples performed when the test is expected per study protocol, the proportion of samples collected tested by Ultra, and the turnaround time.

- **Tolerability**

Tolerability of NPA collection will be measured according to the level of discomfort/distress/pain felt as assessed by the child, the parents/guardians and the nurses, using a set of validated tools. This evaluation will include: at child level, the Wong-Baker Face scale; at parent(s)/guardian(s) level, the Visual Analog Scale (evaluating child's tolerability); at nurse level, the FLACC (Face Legs Activity Cry Consolability) behavioural scale. These assessments will be conducted in a randomly selected subset of children in all participating countries. Study nurses will coordinate and perform data collection on site.

7.7. Unscheduled visits and care in case of clinical adverse events

Whenever enrolled children become ill, they will have access to medical personnel during the business hours of their respective study site. Children will receive care in the form of consultations, inpatient day care and inpatient hospitalizations, depending on the severity of disease. Care will be provided in accordance with national guidelines. Data will be collected similarly to routine protocol visits.

7.8. Management in the case of selected adverse events

7.8.1. Management in the case of sample collection adverse events

Expected AEs occurring from NPA collection procedure include, by decreasing order of frequency: cough (this induced cough reflex is expected as it is the mechanism by which sample is obtained), nausea, local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnea/low O2 saturation and heart rate deceleration <60/mm [54].

NPA will be performed by study nurses under SpO2 monitoring. In case of acute O2 desaturation or respiratory distress occurring during sample collection, the procedure will be immediately interrupted and the child will be started on O2 therapy. If a sample could not be obtained, a new attempt will be performed as soon as the child respiratory status allows for it.

Management in case of AEs will be detailed in specific SOPs.

7.8.2. Management in the case of treatment-limiting adverse events

Treatments will not be provided by the study. Management of drug toxicity will be performed according to National guidelines and will be detailed in the clinical SOPs.

7.9. Final study visit

For each enrolled child, the last protocol visit will occur 6 months after inclusion. Once completed the 6-month follow-up period, children will continue to benefit from regular treatment and care provided by National Programs.

7.10. End of the research

7.10.1. Definition

Each child will be followed up for 6 months after inclusion. The official end of the research, except in case of premature termination, is defined by the last visit of the last patient included in the study. At the end of the study, children will benefit from regular treatment and care provided by National Programs.

The sponsor or its representative will notify the end of the study to the ethical and regulatory authorities of each participating countries within 90 days.

A premature end may be decided by the sponsor, following the advice of the Scientific Advisory Board (SAB), the Independent Data Monitoring Committee (IDMC) or the ethical and/or regulatory authorities issuing an unfavourable opinion to the continuation of the research. In case the study is ended prematurely, the sponsor or its representative will notify the ethical and regulatory authorities within 15 days, and clarify the reasons for such a premature termination. The sponsor and the investigators, in close collaboration with the country health authorities, will take appropriate decision to ensure that patients have access to the best available care and treatment according to each country conditions.

7.10.2. Withdrawal from the study

Withdrawal of the participant from the study may be at the initiative of the investigator or the participant himself (withdrawal of consent or premature exit).

An investigator may withdraw a subject from some or all study components at any time, at his/her discretion. Circumstances can include: when the child's safety may be compromised such as when experiencing AEs, when the child's participation to the study prevents him/her to participate in another research which would be more beneficial, when the study is being closed by the sponsor related to increased risk to participants, or when the subject is non-compliant with required study regimens or procedures. In such a case, the investigator will inform parent(s)/guardian(s) that the child has been withdrawn from participating in the study, and the reasons therefor.

The parent(s)/guardian(s) may decide to withdraw the child from the study at any time if they wish to, without any consequence on the quality of subsequent follow-up and care. When parent(s)/guardian(s) withdraw their consent for the child's participation in the study as they have the right to do at any time, no new information must be collected and recorded in the database after the date of withdrawal. Similarly, no samples must be collected after that date in the context of the study.

When parent(s)/guardian(s) who withdraw consent explicitly express the will that the child's data be removed from the database and the laboratory samples be destroyed, the study team will carry out such will. When parent(s)/guardian(s) who withdraw consent do not express such will, data and samples collected prior to the date of the withdrawal will be used for the analysis.

Withdrawals of consent to participate in the study must be reported to the country Clinical Trial Unit (CTU) as soon as possible. The investigator must document the date of withdrawal in the CRF. The reason for withdrawal either decided by the investigator or the parent(s)/guardian(s) (if possible and if parent(s)/guardian(s) are willing to), and the parent(s)/guardian(s)' decision regarding conservation or destruction of their child's data will be documented in a specific study document.

7.10.3. Loss to follow-up

When a child for whom parent(s)/guardian(s) have not explicitly withdrawn consent does not show up for routine clinic visits, the study team must make every effort to contact him/her. With their prior agreement, the study team will contact the parent(s)/guardian(s) via telephone or any other means available and acceptable locally (home visits, home base care team). The investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient.

A child who has not withdrawn consent or transferred out, and who does not show up at Month 6 visit is considered definitely lost to follow-up, unless he/she is known to be deceased. The date of lost to follow-up will be the date of his/her last contact with the study team (either at the hospital, via telephone, or at home).

A particular attention will be given to the descriptive analysis of patients lost to follow-up and protocol withdrawals during the study: numbers, characteristics and reasons for refusal/lost to follow-up.

7.11. Post-study care conditions

At the end of the study, children will receive treatment and care if still required, according to the conditions defined by their country authorities. Children with ongoing TB treatment at M6 will be followed-up and treated under the responsibility of NTPs. Until treatment completion of the ongoing TB episode, the study investigators, in close collaboration with NTPs, will actively contribute to facilitate their access to the best available TB disease management.

8. LABORATORY AND IMAGING ASSESSMENT

8.1. Biological specimen collection

NPA, stool, GA, urine, and blood samples are collected at inclusion (see Table 4) for laboratory tests performed on-site or at the country reference laboratory, as well as for biobanking (see Chapter 8.3).

All specimen collection methods and biological exams procedures will be detailed in specific SOPs.

- **Nasopharyngeal aspirate**

The collection of the contents of the oropharynx is done by mechanical suction through a graduated suction tube inserted into the nostril while the child is seated (See appendix 3). NPA will be performed under SpO2 monitoring.

- **Stool sample**

Stools cannot be tested with the GeneXpert device without prior processing to avoid invalid results due to the presence of PCR inhibitors. In this study, stool processing will be performed using the flotation method based on Sheather's sucrose solution previously used in the PAANTHER 01 study (Appendix 4). Stool processing optimization will be largely developed as a separate work-package of the TB-Speed project (Output 4). It will assess centrifugation-free methods for stool processing hence generating evidence on the diagnostic value of stools as alternative specimen for TB diagnosis in resource-limited settings.

- **Gastric aspirate**

The collection of gastric contents is done in the morning through a nasogastric tube in a child fasting since midnight the previous day, in supine position, before any rise in case of hospitalization and after 1 hour of supine for children seen in consultation. If it is impossible to collect at least 5 ml of gastric fluid, 10 to 20 ml of sterile water is injected through the catheter and aspirated.

- **Blood samples**

Table 5 a: summary of blood samples collected in children <18 months

	Tube/sample	**Minimal volume required**	**Inclusion D0**	**Month 1**
HIV serology (rapid test)	(finger/heel/toe prick)	50 µL	X	
HIV DNA PCR	EDTA/Dried Blood Spot (DBS)	500 µL		
Malaria test (thick smear)	EDTA [a]	2 mL [a]	X [a]	
CBC	EDTA [a]	2 mL [a]	X [a]	X
Transaminases + CRP	Heparin	2 mL	X	X
QFT	4 QFT tubes or 1 heparin	2 mL	(X)[b]	(X)[b]
Biobank:				
- Plasma	EDTA	2 mL	(X)[c]	
- Whole blood	PAXgene	2.5 mL	X	
Maximal number of tubes collected:				
weight >5kg			10 (9)[a]	2
weight <5 kg or anaemia			5 (4)[a]	6
Total Minimal volume of blood required:				
weight >5kg			13 mL (11 mL)[a]	4 mL
weight <5 kg or anaemia			8.5 mL (6.5 mL)[a]	6 mL

[a] *Wherever possible, malaria test and CBC should be performed on the same EDTA tube to avoid additional sampling.*

[b] ***In children weighing <5 kg or children presenting signs suggestive of severe anaemia (conjunctival or palmar pallor)****: QFT test will be performed at M1, except in children with signs of severe anaemia if they will benefit from blood transfusion. In this case, QFT test will be preferably done just before transfusion, as the result may be falsely positive due to the adult blood transfusion donor.*

[c] ***In children weighing <5 kg or children presenting signs suggestive of severe anaemia (conjunctival or palmar pallor)****: plasma sample for biobank will not be not collected.*

Volume of blood draw must not exceed 3 mL/kg/visit and 7 mL/kg/6 weeks.

Table 5 b: summary of blood samples collected in children >18 months

	Tube/sample	Minimal volume required	Inclusion D0	Month 1
HIV serology (rapid test)	(finger/heel/toe prick)	50 µL	X	
Malaria test (thick smear)	EDTA [a]	2 mL [a]	X [a]	
CBC	EDTA [a]	2 mL [a]	X [a]	X
Transaminases + CRP	Heparin	2 mL	X	X
QFT	4 QFT tubes or 1 heparin	2 mL	X	
Biobank:				
- Plasma	EDTA	2 mL	X	
- Whole blood	PAXgene	2,5 mL	X	
Maximal number of tubes collected			9 (8) [a]	2
Total minimal volume of blood required			12,5 mL (10.5 mL) [a]	4 mL

[a] *Wherever possible, malaria test and CBC should be performed on the same EDTA tube to avoid additional sampling.*
Volume of blood draw must not exceed 3 ml/kg/visit and 7 ml/kg/6 weeks

Blood collection will be performed following specific SOPs guiding nurses on highest priority tests in case sub-optimal volumes of blood are obtained from a child. Tests planned for clinical management of the child will be prioritised over samples for biobank.

8.2. Laboratory assessment

8.2.1. HIV testing

HIV serology will be performed at inclusion if not available in the patient medical chart, according to national routine practices.

Definitive exclusion or confirmation of HIV infection in children aged less than 18 months should be based on an HIV RNA or DNA nucleic acid tests.

8.2.2. Xpert MTB/RIF Ultra

The Ultra assay will be performed on the following samples:

- one untreated NPA (or sputum)
- one stool sample, with prior processing
- first GA out of two collected

Xpert testing will be done at the hospital laboratory on standard G4 platforms.

The Ultra assay will be carried out according to the manufacturers' guidelines and will be defined as positive, negative or invalid based on the manufacturers' recommended criteria.

Results will be interpreted as follows:

- In case of positive result for the presence of MTB (including "trace call" positive result) on any sample, the global result will be given as "MTB detected".
- In case of negativity of Ultra performed on the two samples, the global result will be given as "MTB not detected".
- In case of an invalid or error result for MTB detection, the test will be repeated if sample volumes allow for it.
- In case of rifampicin resistance detected on one or more sample, the result will be given as "MTB detected, rifampicin resistance detected". Otherwise the result will be given as "MTB detected, rifampicin resistance not detected (or indeterminate)". Children with

rifampicin resistance detected by Ultra will have culture and phenotypic DST performed on leftovers from NPA and stool (with additional samples taken if needed), and will be started on empirical MDR-TB treatment according to national guidelines.

Ultra results will be communicated by the laboratory to the nurse or clinician as soon as the result is available.

In case of TB treatment failure or poor treatment response, appropriate samples will be performed and resistance to TB drugs will be assessed using methods available on sites (phenotypic DST or line probe assays).

Leftovers of NPA, sputum, GA and stool samples will be kept at -80°C for future ancillary/sub-studies.

8.2.3. Mycobacterial culture and Drug susceptibility testing (DST)

Culture and DST will be done on two GAs at the country Central Laboratory level.

Mycobacterial culture will be done in liquid medium (MGIT) by an automated method (BACTEC 960), and on solid media (Lowenstein Jensen) if available.

Identification of mycobacteria will be done by the molecular method Gen-Probe®, or Niacin test, or MPT64 antigen test depending on availability at the laboratory level. Detection of TB drug resistance will be performed using first-line DST on liquid media (MGIT) or solid media or line probe assays (LPA).

8.2.4. Interferon Gamma Release Assay

The whole blood assay QuantiFERON®-TB Gold In-Tube (Qiagen) will be carried out according to the manufacturers' guidelines and will be defined as positive, negative or indeterminate based on the manufacturers' recommended criteria. IGRA will be performed at the country Central Laboratory level by laboratory staff blinded to the clinical status of children. Blood samples will be carried to the central laboratory following study SOPs.

8.2.5. Blood tests

Blood analyses including leucocyte differential counts, red blood cells values, CRP, and transaminases measurements will be performed using standard procedures usually implemented on sites and local reference ranges.

MLR will be calculated as the quotient of absolute monocyte and lymphocyte counts.

CRP will be tested using both ELISA testing and the point-of-care NycoCard™ CRP assay (Alere).

8.2.6. Laboratory quality control

Internal QC will be routinely performed for Ultra testing, including calibration tests and procedures provided by the manufacturer. Internal QC results and logs will be available for monitoring.

Procedures for laboratory quality assurance will be detailed in study-specific SOPs.

8.3. Frozen samples and biobank

Leftovers from NPA, stool, gastric aspirates or sputum, as well as whole blood, plasma, and urine samples will be frozen and stored at the Central Laboratory level.

Biobank samples are planned in case further bacteriological analyses are retrospectively performed, as well as host immunologic, metabolic, and genomic studies.

Procedures for preparation of frozen samples and biobank conservation will be detailed in study-specific SOPs.

8.3.1. Type of samples and purpose

- **NPA, sputum, stool, gastric aspirate**

Leftover samples from NPA, sputum, stool and GAs could be used for retrospective bacteriological analyses such as culture and mycobacterial antigens assays.

- **Cultured mycobacterial strains**

In the same way, cultured strains will be stored to establish a mycobacteria repository for future sub-studies.

- **Whole blood**

2.5 mL of whole blood will be collected on PAXgene® Blood RNA tube.

Whole blood could be used for potential genomics or transcriptomics analyses to discriminate between TB and other diseases, including mRNA transcripts and micro RNAs, based on state-of-the-art subsequent to the study.

- **Plasma**

1 mL of plasma will be aliquoted from a 2 mL ETDA tube collected at inclusion.

Plasma samples could be used to further characterize the proteomic, metabolic and immunologic profile of children presenting with signs of SAM, with or without TB infection. Moreover, measurement of prealbumin level could be done retrospectively, as it is a sensitive serum marker for the early diagnosis of malnutrition and response to nutritional therapy in malnourished patients [55]. Though it is a better indicator than albumin, due to its shorter half-life, it is not done routinely by laboratories and will have to be transferred to a specialized laboratory out of the countries.

- **Urine**

Fresh urine samples (20mL) will be collected in sterile urine containers for storage.

Urine could be used for evaluation of biomarkers such as LAM, including the novel Fujifilm SILVAMP TB LAM assay.

8.3.2. Storage

During the study, biological samples will be stored at -80°C at the country reference laboratory. Transfer of samples will be done according to the internal procedures of implementing sites, which are checked by the international coordinating CTU before the beginning of the study. A centralized biobank database will be constituted by the international CTU.

Subject to approval by the SAB and relevant Ethics Committees, frozen samples may be sent to external laboratories (inside or outside the country) for additional analyses performed as part of post-study ancillary studies. A Material Transfer Agreement will be submitted to appropriate ethics committees and regulatory authorities.

8.4. Radiological assessment

CXR is part of the inclusion, M2 and M6 final study visit (see Table 4).

A 2-view chest radiography (anteroposterior and lateral) will be performed using standard analogue X-ray machines with digital plates, or digitalized radiography machines where possible. Clinicians will be invited to use the simplified TB-Speed CXR reading tool to interpret CXR results. This tool focus on 6 CXR features including enlarged lymph nodes, alveolar opacity of the lung tissue, airway compression, miliary, cavitation, and pleural or pericardial effusion.

Digitalized CXRs will be archived on a centralized database accessible through a secured website (the Mereva tool, as described in Chapter 10).

For the purpose of TB reference diagnosis classification, CXRs will be reviewed independently by two readers blinded to clinical and biological data to identify CXR lesions consistent with TB, as proposed in the Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children [45]. Discordant opinions will be resolved by a third reader.

8.5. Abdominal ultrasonography

Abdominal ultrasonography will be performed by the hospital radiology department by a trained radiologist or paediatrician who is not the physician in charge of the TB diagnosis or treatment decision. Data will be collected using standardized forms. Presence of abdominal lymphadenopathy and splenic micro-abscesses, previously described as associated with childhood TB will be assessed [48], [49]. Additional signs such as hepatic abscesses, and peritoneal effusion will also be sought.

9. STUDY VIGILANCE

9.1. Definitions

- **Adverse events**

An “adverse event” (AE) is defined as any unfavourable, expected or unexpected sign (clinical or biological) occurring during the study in a human subject participating in the research, whether or not considered related to treatment or procedures or to participation in the study.

- **Serious adverse events**

A “serious adverse event” (SAE) (ICH-E6 step 4. 1996) refers to any untoward medical occurrence that:

- Results in death;
- Is life-threatening (means that the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect.
- Is an "important medical event" (medical events, based upon appropriate medical judgment, which may jeopardize the subject or may require medical or surgical intervention to prevent one of the above characteristics/consequences). Examples: allergic bronchospasm requiring intensive treatment at an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization.

- **New fact**

A new fact is defined as any safety data that could modify significantly the evaluation of the benefit/risk ratio of the research or the study product, likely to affect the safety of participants or that could modify the study product administration, the study documentation or the conduct of the study, or to suspend or interrupt or modify the protocol or similar studies.

- Examples: a SAE which could be associated with the study procedures and which could modify the conduct of the study, recommendations of the IDMC, if any, where relevant for the safety of subjects.

- **Severity**

The severity of an AE caused by NPA collection will be graded using the “Division of AIDS table for grading the severity of adult and paediatric adverse events” (Version 2.1 – July 2017) as included in the SOPs [56].

- **Causality**

“Causality” refers to causal relationship between a specific AE, the study intervention and any other concomitant intervention/medication

9.2. Expected adverse events related to the study intervention

Expected AEs occurring from NPA collection procedure include, by decreasing order of frequency: cough (this induced cough reflex is expected as it is the mechanism by which sample is obtained), nausea, local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnea/low O2 saturation and heart rate deceleration <60/mm [54].

No AEs are expected from stool sample collection.

Overall, children tolerate anti-TB drugs very well when using currently recommended dosages. SAEs are rare and even mild symptoms such as nausea or vomiting are uncommon. There are occasional case reports of severe hepatotoxicity [57].

Occurrence of AEs will be monitored by study nurses and reported in the Case Report Form (CRF). Management in case of AEs will be detailed in clinical SOPs (see Chapter 7.7.1).

9.3. Reporting of adverse events

AEs occurring as a consequence of NPA collection, primary and secondary diagnosis of illness causing initial hospitalisation, death and cause of death, primary and secondary diagnosis of illness causing any hospital readmission will be reported in the CRF.

There will be no systematic reporting of other AEs in the study.

9.4. Notification of serious adverse events

In this diagnostic study without investigational medicinal product, and with very low expected risk of AEs linked to the intervention, there will be no systematic notification of SAEs to the sponsor at the exception of:

- Death;
- Life-threatening AEs, excluding asymptomatic biological AEs of grade 4
- SAEs related to NPA collection.

9.5. Responsibilities of the investigators

The investigators are responsible for:

- Grading the severity of AEs occurring from NPA collection reported by study nurses as severe or potentially life-threatening.
- Reporting SAEs, as defined above, to the sponsor and to the appropriate country authorities, according to the procedures described in section 9.4.
- Assessing the causality of all SAE in relation to the study intervention and to concomitant intervention/medication.

The assessment on expectedness will be done by the sponsor.

SAEs, as defined above, should be reported as soon as they are known to the country CTU according to the last updated SOPs. A specific “SAE report form” will be used. SAEs will be reported immediately by the country CTU to the Inserm Pharmacovigilance Department according to appropriate SOPs. If needed, queries on SAEs will be sent to the investigators by the Inserm Pharmacovigilance Department representative.

All SAE must be reported if it occurs in a participant:

- from the date of signature of the informed consent to the study;
- during the follow-up of the participant scheduled by the study;
- until 4 weeks after the end of follow-up when it is related to the study intervention.

9.6. Responsibilities of the sponsor

9.6.1. SAE Recording and assessment

The sponsor shall keep detailed records of all SAEs which are reported to him by investigators.

The sponsor is responsible for the assessment of the causality of the SAE in relation to the NPA collection. In the absence of information on causality from the reporting investigator, the sponsor should consult the reporting investigator and encourage him to express an opinion on this aspect. The causality assessment given by the investigator should not be downgraded by the sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided in the report to the National Competent Authority.

All SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as suspected Serious Adverse Reaction (SAR).

The expectedness of the SAR shall be determined by the sponsor. The sponsor assesses if the SAE is expected or not using information described in the protocol (section 9.2), especially concerning, acts and methods performed for the research. An unexpected adverse reaction is an adverse reaction, the nature, the outcome or severity of which is not consistent with this information.

9.6.2. New fact reporting

When a new event is likely to affect the safety of participants, the sponsor and the investigator take appropriate urgent safety measures to protect participants against any immediate hazard.

The sponsor inform without delay the Competent Regulatory Authorities of safety data that may be relevant in terms of subject safety, or safety issues which might alter the current benefit-risk assessment of the study.

The Inserm Pharmacovigilance department shall transmit a written report to the Competent Regulatory Authorities and concerned Ethic Committee.

10. DATA COLLECTION AND PROCESSING

10.1. Description of data collected

10.1.1. Individual patient data

Once enrolled in the study, the following data will be collected for each patient by study nurses or clinician, on remote data capture devices:

- Individual identifiers: month and year of birth, sex
- Anthropometric and clinical data: weight, height, vital signs, symptoms, treatments and adherence, AEs
- Data on the tolerability of NPA collection
- Radiological and ultrasonography data: digital images and interpretation
- Laboratory data: mycobacterial culture and Ultra assay, HIV testing, QFT, blood tests
- Samples collected for biobanking
- Management of SAM: antibiotics, nutritional support, other
- TB treatment if initiated
- Comorbidity management: antiretroviral treatment, other
- Outcome data: end of study status or early study termination (death, lost to follow-up, withdrawal)

For each new patient included in the study, an anonymised individual identification code will be attributed and used as the only patient identifier in the REDCap database.

10.1.2. Cost data

We will collect data to estimate the costs of the TB diagnostic approaches being compared. This will include the costs of TB diagnostics, including collection and testing of samples, and the other direct health costs required to treat patients, including treatment for SAM, TB and other comorbidities, use of equipment and staffing costs.

We will measure and record, or where necessary estimate, resource utilization for both use of the new diagnostic prediction score and standard of care in children hospitalized with SAM. Unit costs will be collected from relevant sources including clinic and hospital site visits, accounts and invoices from the TB-Speed project and individual facilities, pharmaceutical and medical equipment manufacturers, Ministries of Health and NTPs.

To estimate human labour costs, we will conduct a time and motion study survey to estimate quantities of staff time involved in different health care tasks. To do so, we will ask nurses, doctors and other health workers participating in this study to self-complete timesheets recording the length of time they spend conducting each task.

Costs will be expressed in U.S. dollars, converted using purchasing power parity exchange rates, i.e. market exchange rates adjusted for differences in purchasing power between countries.

10.2. Definition of source data

Source data must be available to document the existence of patients enrolled in the study and should substantiate integrity of the data collected. It must include the original documents relating to the study, the medical treatments and medical history of the patient.

The following information should be collected from source medical records filled by site physicians:

- Patient's demographic data (month and year of birth, sex)
- Study name(s) and protocol number(s) in which the patient participates
- Details related to the inclusion criteria
- Date of signing informed consent form
- Dates of follow-up visits
- Medical history and physical examination details
- Laboratory print-outs
- AEs and concomitant treatments

For the purpose of the study, specific forms may be developed for source data collection to be inserted into medical records.

In addition to the source medical records, radiological data will be collected as digital imaging and communications files (.dicom files). CXRs interpretation will be directly reported in the patient's CRF using standardized forms developed as part of the study.

In the same way, Ultra test result files (.gxx files) will be extracted directly from the GeneXpert software.

Data transfer for dicom and gxx files is detailed in Chapter 10.3.6.

10.3. Electronic data entry

The eCRF system, the methods to ensure restricted access to the database, and the data management procedures, including the procedures to check completeness, accuracy, quality and validity of the data, will be described in specific trial SOPs in accordance with good clinical, scientific and data management principles.

10.3.1. eCRF

A real time data collection is needed for optimised monitoring of data entry. No paper CRFs will be used; patient data collected at inclusion and follow-up visits will be recorded directly in an electronic CRF (eCRF) by study nurses, mostly through single data entry.

The TB-Speed data management system will be based on the electronic data capture application REDCap (Research Electronic Data Capture; https://www.project-redcap.org/). REDCap is an online tool for eCRF development, allowing data input from anywhere in the world over a secure connection with authentication and data logging.

Design and conception of the eCRF will be done by the international trial manager in close collaboration with the international data manager and international Clinical Research Assistant (CRA).

10.3.2. Data hosting

The REDCap MySQL database server will be hosted by the international CTU at the University of Bordeaux (UBx), France. REDCap is a free, secure web application for building and managing online surveys and databases, it's geared to support online and offline data capture.

Developed by Vanderbilt University, the REDcap software complies with internationally recognized standards including the Health Insurance Portability and Accountability Act (HIPAA, 1996), the United States legislation providing data privacy and security provisions for safeguarding medical information, as well as part 11 of Title 21 of the Code of Federal Regulations (CFR) that establishes the United States Food and Drug Administration (FDA) regulations on electronic records and electronic signatures.

10.3.3. Data security

The eCRF will be accessible 24/24h by secure authentication to a restricted users group. The connection will be authenticated by a user ID, password and digital certificate enabling data encryption during transfer and storage to the central server. Access and rights levels will be granted and managed by the international data manager (international CTU).

The server hosted at UBx will be saved every weekend on a hard drive, and send to bands (rotation frequency of the bands will be every five weeks). The database will be saved incrementally on a hard drive every working day.

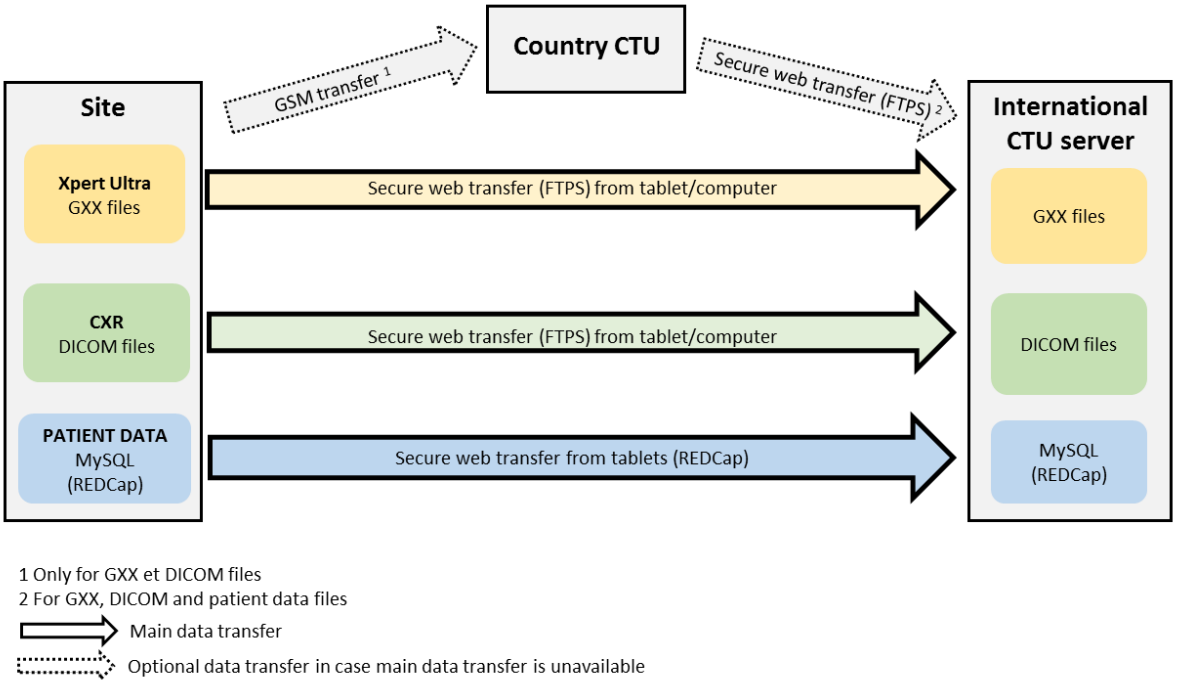
10.3.4. Data entry

Field-based users will be able to access REDCap either through a classical Internet-connected tablet or computer, or through the REDCap mobile App application.

The mobile App also enables offline data entry through a tablet or an Android mobile phone. In such a case, the tablet or mobile phone will be brought by the CRA to the country CTU and further synchronized with the central database once connected to the Internet (Figure 2).

Tablets will be purchased locally by country CTUs. Configuration of tablets will be managed by the international data manager.

Figure 2: Secure data flow



Project Managers from country CTUs will be in charge of training the relevant study staff for data collection and issuing of electronic data queries for quality control. The investigator is responsible for ensuring that all sections in the eCRF are completed correctly and that entries can be verified against source data. If the investigator authorizes other persons in their staff to make entries on the eCRF, the names, positions, and signatures must be documented in writing. eCRF must be completed during/after each study visit. Any person entering data in the eCRF must be trained beforehand and appointed to do this task.

10.3.5. Data coding

The Anatomical Therapeutic Chemical (ATC) system will be used for drug classification and coding. As part of safety monitoring, AEs will be coded using the Medical Dictionary for Drug Regulatory Affairs (MedDRA, version 17.1). Coding will be performed by country CTUs based at TB-Speed consortium members institutions.

10.3.6. Data transfer

Individual patient data will be transferred from tablets to the server located at UBx using a secure file transfer protocol (ftps) with individual authentication and data logging.

CXRs (.dicom files) and Ultra test result files exported from GeneXpert (.gxx files) will be transferred to the international CTU central server using a secure web transfer (ftps). In case implementing sites experience web access issues, transfer of CXRs and Ultra result files will be done 1/ by GSM with a tablet (equipped with a SIM card) from the implementing sites to the country CTU, and 2/ from the country CTU to the international CTU central server via a secure web transfer (ftps).

In the context of the Covid pandemic, due to travel restrictions, remote site monitoring visits might be conducted by the international CTU. Where needed, pseudonymized source documents will be forwarded by the country CRA through a ftps for monitoring purpose only; source documents will be destroyed afterwards.

10.4. Description of the data verification, validation and processing (data management)

A data management plan (DMP) will be established and validated by the study coordination team at UBx. Verification of the completeness and consistency of the data is performed for all key data as well as a list of additional data defined in the DMP.

A data management system (DMS) will be developed at UBx to enable generation of standardized lists of data management queries at country level. Queries will be programmed by the international data manager for data completeness, integrity and consistency as defined in the DMP. They will be run on an at least monthly basis at the country level. Data management checks will be implemented at central level on a monthly basis. If needed, centralized correction queries will be sent by the international coordinating CTU to the country CTU, and by the country CTU to study sites. At country level, queries will be managed by the country data manager or CRA. Queries will be solved by data managers along with the country CRA.

The investigator, co-investigators, head of laboratory must allow access to relevant hospital, laboratory or clinical records, to confirm their consistency with the CRF entries. All research staff working in the study, including study nurses, national CTU team (PMs, CRAs), PIs, international coordination team (CRA, Trial Manager, Coordinating Investigators) will sign a confidentiality agreement with regards to access to individual patient data and medical records.

Central statistical monitoring will also be implemented by the international coordinating CTU to look at variables for which distributions differ from the rest of the observed data at the country, site or patient level. Its purpose is to highlight systematic (non-random) faults in filling the CRF data, protocol compliance, AEs, and to guide targeted monitoring. Variables subject to statistical monitoring are specified in the monitoring plan. Comparison of distributions is made by statistical tests or models.

Before final database freezing, a final data review will be conducted and remaining issues will be adjudicated. Closure of the database will be performed by the international data manager.

10.5. Length of data retention, archiving conditions and management

All data will be stored in a server hosted by the CREDIM (*Centre de Recherche et Développement en Informatique Médicale*) at UBx and will be declared to the French data protection authority CNIL (*Commission Nationale de l'Informatique et des Libertés*) in accordance with French regulations on personal data protection.

The server is located in a secure computer room. The network is protected by uninterrupted power supply firewalls and up-to-date viruses and malwares scanning softwares. Data backups are performed regularly. Reading, entry, modification or deletion of data will be granted via the standard authentication and access-control features.

Medical records will be stored in the clinical sites as per standard practices. Electronic data and files will be maintained on password-protected computers. Essential study documents will be retained at the coordinating centre for 15 years.

No displacement or destruction of data will be done without the agreement of the sponsor. At the end of the regulatory archiving period, the sponsor will be consulted for destruction.

10.6. Study documents archiving conditions and management

Essential documents and study records will be kept secured for 15 years after study completion, under the responsibility of each country investigator, the international CTU, and the sponsor.

Study documents constituting the Trial Master File (TMF) will be made available online to investigators on a secured website. The international coordinating CTU will be responsible for routinely updating global documentation on the study website. Country CTUs will be responsible for routinely updating national documentation on the study website.

Investigators will ensure that study records are not disposed of or removed from the study sites or the country CTU without prior notification and approval from the sponsor or his representative.

Each investigator will keep a hard copy of original documents whenever those are manually signed or generated. This includes, at site level, medical records (source documents) and study ID assignment log which are subjected to professional secrecy and confidentiality, and task delegation lists.

Data, documents, reports and SOPs should be available to be audited or inspected at any time.

11. TB-SPEED COVID SUB-STUDY

11.1. Background and rationale

Since December 2019, a novel coronavirus emerged in Wuhan, Hubei province, China and has rapidly spread to the rest of the world, becoming a pandemic [58], [59]. The WHO has named it "2019 novel coronavirus" (2019-nCoV or SARS-CoV-2) and has declared Covid-19 a pandemic and public health emergency of international concern. This is the 3rd coronavirus epidemic of large public health threat after the severe acute respiratory syndrome (SARS-CoV-1) and the Middle East respiratory syndrome (MERS-CoV) [60], [61]. Community transmission of SARS-Cov-2 virus has been reported in many African countries, with figures on cases and deaths likely to be underestimated due to the limited access to tests. As of June 23, 2020, there were 797 and 1430 cases reported in Uganda and Zambia, respectively[4].

Children are largely untouched by Covid-19. Data from China, the United States (US) and Italy showed that only about 2% of all cases were children [62]–[66]. First data from Africa show than only 2.5% of cases were young children below the age of 5 years [66], [67]. No racial predilection has been observed in children, although emerging US data in adults suggest that minority communities may be affected disproportionately [68]. Despite the worldwide spread, the epidemiological, clinical, therapeutic and evolutionary patterns of Covid-19 remain largely unclear among children. In the largest Chinese paediatric case series to date, of 2 143 subjects, children younger than 5 years old represented 40% of all paediatric cases and 60% of the severe cases [69]. In this study, there were more boys (56.6%) than girls. Slight male predominance was also seen in the US data [70]. Few neonate cases have been reported and no cases of intra-uterine transmission was notified [71]. The median incubation period in children is usually 3 ~ 7 days (range of 1-14 days) [72].

Covid-19 has been diagnosed using real-time RT-PCR assays for 2019-nCoV nucleic acids [73], [74] on nasal swabs, sputum, lower respiratory tract secretions, stool, and blood. Few paediatric cases with negative respiratory samples were diagnosed using stool samples, on which RT-PCR is usually less sensitive [75]. Two study in French children reported similar SARS-CoV-2 viral loads in children than in adults that SARS-CoV-2-positive children exhibited viral loads that do not differ significantly compared to those of adults [76] [77]. Clinical diagnosis has also been proposed using a combination of at least two symptoms, laboratory tests and chest X-ray findings [78], [79]. Chest radiography and computed chest tomography show in the early stage of disease, multiple small plaques and interstitial changes, which are obvious in the lung periphery, that further deteriorate to bilateral multiple ground-glass opacity and/or infiltrating shadows. Lung consolidation may also occur in severe cases.

Immunological assays for SARS-Cov-2 infection will be useful to assess seroprevalence and seroconversion of infected people. The Institut de Recherche pour le Développement (IRD) has developed a high throughput serological screening assay using the Multiple Analyte Profiling (xMAP) technology (Luminex Corp. Austin, TX, USA). The assay was evaluated on a panel of well documented positive and negative samples (IgG and/or IgM antibodies) obtained from reference laboratories in Europe and collaborators [80]. It is also developed for IgM

[4] Source: COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University.

antibodies to SARS-CoV-2 to allow detect recent infections on plasma, whole blood, DBS, saliva and faecal samples.

Although epidemiological and clinical data from China, Europe, and the US show that children, and notably those aged less than 5 years, are less affected by the Covid-19 than adults, we do not know what happens when SARS-Cov-2 infection occurs in children with comorbidities affecting their immune response. This includes malnutrition that is common in low/moderate income countries in Sub-Sahara Africa, and that can be associated with other frequent co-morbidities like HIV infection. Additionally, children with sickle cell disease (SCD) who present viral respiratory infection may have an increased risk of bacterial sur-infection and therefore worsening of the clinical presentation as shown in a small study of children with sickle cell disease and H1N1 influenza viral infection [81].

This sub-study will assess the prevalence and impact of the Covid-19 in young children hospitalized with severe acute malnutrition. The sub-study findings are expected to guide policy makers and clinicians on potential specific screening and management measures for this vulnerable group of children. In addition, the evaluation of the primary objective of the TB-Speed SAM study relies on clinical case definitions for the retrospective classification of children (as confirmed, unconfirmed or unlikely TB), which are based on several parameters including clinical presentation and outcome. In the context of the Covid-19 pandemic, it is therefore very important to identify SARS-CoV-2 infections at enrolment in the study and during follow-up based on clinical evolution.

11.2. Sub-study objectives

- To assess the prevalence of Covid-19 (confirmed and probable cases) in children below 5 years hospitalized with SAM
- To assess the impact of SARS-CoV-2 infection on the clinical outcomes of children with SAM
- To describe the clinical, laboratory, and radiological characteristics of Covid-19 cases
- To assess the yield of stool as compared to naso/oropharyngeal swab for the detection of the SARS-CoV-2 by real time RT-PCR
- To assess seroprevalence and seroconversion (IgM and IgG to SARS-CoV-2) at Day 0 and Month 6

11.3. Case definition

Confirmed and probable cases are defined according to WHO Covid-19 technical guidance [82].

- **Confirmed case**: A person with laboratory confirmation of Covid-19 infection, irrespective of clinical signs and symptoms (using nucleic acid amplification tests such as RT-PCR).
- **Probable case**:
  - A suspect case for whom testing for the Covid-19 virus is inconclusive OR
  - A suspect case for whom testing could not be performed for any reason

See detailed definitions of ***suspect case*** per country in Appendix 6.

11.4. Sub-study population

- All children enrolled in the TB-Speed SAM study accepting enrolment in the Covid sub-study

11.5. Sub-Study design

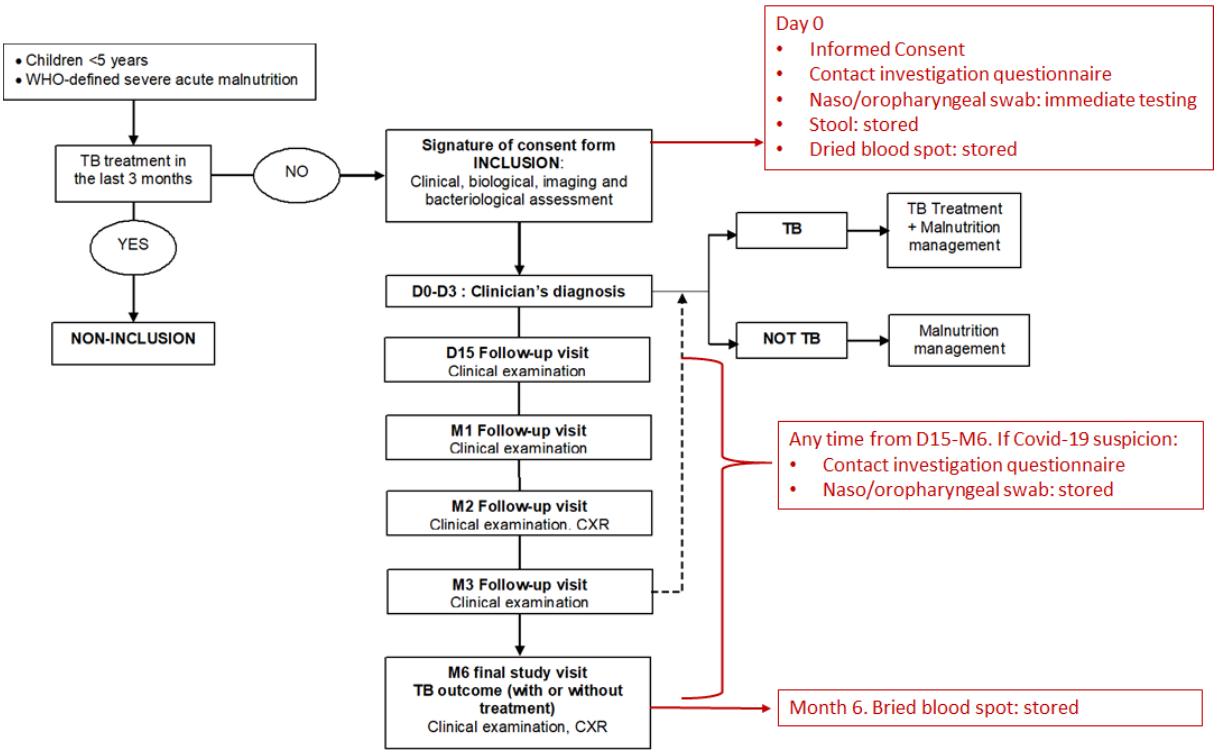
This will be an observational sub-study nested in the TB-Speed SAM study. At the time of enrolment in the main TB-Speed SAM study, patients will be also offered to participate to the Covid sub-study. Children will be tested for SARS-Cov-2 on the day of enrolment. The duration

of enrolment in the sub-study will be of 6 months in total. Duration of follow-up for a particular participant will be 6 months, as in the main SAM study.

For hospitalised children with SAM presenting respiratory symptoms or signs, the detection of SARS-Cov-2 is routinely done in both countries and used for case management. For others, it will be done for the purpose of the study only.

The study scheme of the nested Covid-19 study is presented in Figure 3 below.

Figure 3: TB-Speed Covid sub-study flow chart



- **Sample size**

There is no sample size calculated. All children consecutive enrolled in the TB-Speed SAM study will be invited to participate to the Covid Sub-study.

- **Statistical analysis**

Prevalence (and 95% CI) of SARS-CoV-2 infection at enrolment will be calculated. Summary statistics of patient characteristics will be described. Differences in patients' characteristics between SARS-Cov-2 infected and non-infected patients will be assessed using Chi-square, Student's t-test or Wilcoxon rank-sum test as appropriate. Bi- and multivariate analysis will be used to identify effect of the Covid-19 disease on the children's outcomes. Factors associated with children's outcomes will be also analysed.

A preliminary analysis of prevalence and outcomes will be performed at 3 months (mid-study) and discussed with the SAB in order to support case detection and management decision.

11.6. Management of Covid-19 cases

At each study site, management of Covid-19 cases will follow national recommendations (see Appendix 6). This will require isolation of cases and potential antiviral treatment in addition to the standard care for SAM, as well as TB diagnosis on NPA, stool and GA as part as study procedures. Prescription of specific treatment against Covid-19 will be decided by site clinicians in accordance with country guidelines, subject to modification during the study implementation period.

As soon as a child will be identified as a Covid-19 case, the disease control team at site level will be informed in order to ensure that contacts are traced and additional suspect cases tested and isolated according to the national policy.

In the event that a child is transferred to a Covid isolation centre, study staff will ensure compliance and continuity of the main TB-Speed SAM study procedures and follow-up visits, including appropriate TB diagnosis and timely access to TB treatment if needed.

11.7. Sub-study procedures

As part of the overall informed consent process of the TB-Speed SAM study, parent(s)/guardian(s) will be informed about the Covid sub-study (see Appendix 6). Participation in the Covid sub-study will be proposed on a prospective basis to children newly enrolled in the TB-Speed SAM main study. Patient participation in this ancillary study is voluntary; parents will be free to opt out from the Covid sub-study without any consequence on the participation of the child to the main SAM study. Only children whom parents have approved participation to main study will be able to participate in the sub-study.

- **Inclusion visit (D0-D3)**

The following procedures will be performed during the TB-Speed SAM inclusion visit:

- Questionnaire on Covid-19 contact investigation: parent/guardian will be asked questions regarding the child's exposure to the SARS-Cov-2
- Unless national recommendation for SARS-Cov-2 sample collection is different (Appendix 5), one nasopharyngeal (or oropharyngeal) swab will be collected for immediate SARS-Cov-2 RT-PCR
- A blood sample collected for diagnosis of SCD if not previously documented
- A DBS collected and stored for further serological testing of the SARS-Cov-2 specific antibodies (IgM and IgG)
- A stool sample stored for further SARS-Cov-2 RT-PCR (see Table 6).

- **Follow-up visits (D15 to M6)**

A second DBS will be collected and stored for further serological testing of SARS-Cov-2 at M6.

In children with a negative SARS-Cov-2 RT-PCR at inclusion but showing new symptoms suggesting of Covid during follow-up, another naso/oropharyngeal swab will be collected (see Table 6).

There will be no additional specific follow-up visits for Covid-19 on top of those planned for children enrolled from the main TB-Speed SAM study (see 7.3. Patient schedule).

11.8. Laboratory evaluations

- **Biological samples collection**

Procedures for sample collection, transportation, and processing at the laboratory will be detailed in specific SOPs. To minimize the risk for participants, collection of naso/oropharyngeal swabs for SARS-CoV-2 detection will be performed either by the study nurses collecting NPA, who will receive specific training, or by specialized staff from the Covid surveillance team according to country policy.

Table 6: Biological samples collected for the TB-Speed Covid sub-study

Sample type	Minimal amount required	Laboratory test	D0-D3	M6	D15-M6
1st naso/oropharyngeal swab[a]	minor (swabbing of the nasal/pharyngeal mucosa surface)	SARS-CoV-2 RT-PCR	X		
Stool sample[b]	1 g	SARS-CoV-2 RT-PCR	X		
2nd naso/oropharyngeal swab[c]	minor (swabbing of the nasal/pharyngeal mucosa surface)	SARS-CoV-2 RT-PCR			X
Blood (EDTA or dry tube)[d]	2 mL[d]	Sickle cell disease	X		
DBS	approximately 50 µL (0.05 mL) collected by finger/heel/toe prick	SARS-CoV-2 serology	X	X	

[a] *For young infants (< 6 months) an oropharyngeal swab is preferred due to the small diameter of nasal passage and increased risk of trauma.*
[b] *If the volume of the stool sample collected for the TB-Speed SAM study does not allow for 1) Ultra testing, 2) biobanking, and 3) SARS-CoV-2 testing, an additional stool sample will be collected.*
[c] *The 2nd swab will be collected in children negative for SARS-CoV-2 at inclusion showing new symptoms suggestive of Covid during follow-up.*
[d] *Possibly performed on the same tube than CBC for the SAM study to avoid additional sampling. Volume of blood draw must not exceed 3 ml/kg/visit and 7ml/kg/6 weeks.*

The swabs will be referred for immediate testing whenever possible. However, depending on laboratory testing capacities and national policies, it may be possible only for children fitting indication of Covid-19 testing (i.e. meeting suspect case definition, see Appendix 6). If the testing delay is above 72h, the swabs will be stored at -80°C until further testing. The stool sample and the DBS will be stored at the country Central Laboratory to be tested later on.

- **RT-PCR for SARS-Cov-2**

SARS-CoV-2 virus infection confirmation will be performed by real-time RT-PCR either by in-house or commercial assays at national Covid-19 Reference Centres, namely (subject to modification depending on laboratory capacities and new accreditations at the time of the sub-study implementation): Uganda Virus Research Institute/MRC Center in Entebbe, or Medical and Molecular Laboratories Limited, Makerere University, in Uganda; and Virology Laboratory, UTH, Lusaka, or TDRC Laboratory, Ndola, in Zambia.

- **Immunological assays**

DBS collected at Day 0 and Month 6 will be sent to the Virology Laboratory, TransVIHMI Research Unit, IRD (Montpellier, France) for testing with the Multiple Analyte Profiling (xMAP) serological screening assay (Luminex Corp. Austin, TX, USA). Recombinant Nucleocapsid (NC) and Spike (S) proteins from human coronaviruses (SARS-CoV1, SARS-CoV2, MERS-CoV) will be used for a large spectrum screening and to evaluate the immune imprints of past infections with the two SARS coronaviruses. In addition, synthetic peptides derived from immunodominant regions of the NC, S and membrane proteins (designed by bioinformatics and through literature searches) will be used to discriminate among known coronavirus infections.

- **Diagnosis of sickle cell disease**

Sickle cell disease will be diagnosed by haemoglobin electrophoresis according to usual procedures at site level.

11.9. Safety measures

Risk assessment by study activities and recommended infection prevention and control measures will be detailed in specific SOPs.

Study nurses will be equipped with the appropriate PPE following WHO recommendation [84], including:

- Droplets and contact precautions (clinical examination, blood, stool, and DBS collection): gowns, gloves, googles, surgical masks
- For aerosol-generating procedures (NPA and GA collection, samples processing at the laboratory): isolation gowns, long gloves, goggles and high filtration masks

Safety boxes will be provided to dispose contaminated sharps and waste appropriately.

Triple packaging boxes will be provided to ensure biosafety during sample transportation.

12. STATISTICAL DATA ANALYSIS

12.1. Statistical analysis manager

The statistical analysis manager will be the study statistician, based at UBx.

The statistical analysis plan will be written by the study statistician and validated by the coordinating investigators.

12.2. Description of the statistical analysis plan

12.2.1. Analysis of the primary endpoint

We will use logistic regression to develop diagnostic prediction models for TB. We will handle missing data using complete-case analysis, restricting analysis to those children with data available for candidate predictors, and will consider multiple imputation. We will include as candidate predictors characteristics used in previous childhood TB scoring systems and algorithms, characteristics previously described as associated with TB in children with SAM, as well as QFT and abdominal ultrasonography results. We will include as predictors CXR features as assessed by the local reader using the simplified TB-Speed CXR reading tool. We will test various duration of symptoms (>2, >3, >4 weeks) in the models and select the one with the best Akaike Information Criterion (AIC). Alternatively automated LASSO method will be used to select predictors among all study data available for the model.

We will develop different models integrating all predictors, and excluding one at a time tests that may not be available in all resource limited settings such as QFT and abdominal ultrasonography, after discussion with the expert committee. We will obtain final models by stepwise backward selection, using a threshold of 0.135 when incorporating variables with two degree of freedom (QFT: negative, positive, and indeterminate) to maximize the AIC, or 0.157 when all variables had one degree of freedom. We will perform internal validation using bootstrap resampling to quantify model optimism, i.e. the apparent performance of the model minus its true performance, which enables to adjust the model area under the receiver-operating-characteristic curve (AUROC) to its real performance.

We will compare AUROCs of models obtained and select the models with the best discriminative ability and parsimony. We will identify the predicted probability cut-off reaching a sensitivity ≥80%. Using this cut-off, we will develop associated diagnostic scores by assigning each variable in the models to a predictive score equal to the beta coefficient.

As sensitivity analysis, we will assess diagnostic performance of the score obtained in the different countries, in children with or without HIV-infection to identify potential heterogeneity in score performances between these groups and countries.

Finally we will propose to use the score obtained in a stepwise diagnostic algorithm, including the possible first-step screening prediction score identifying children with presumptive TB, and the diagnostic prediction score for the final TB diagnosis. The stepwise algorithm will be discussed with local clinicians involved in the study to better adapt it for future use in their routine practice.

Results will be reported according to the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) guidelines for clinical prediction models [83].

The modelling strategy will be definitely elaborated according to prevalence and diagnostic accuracy of the different signs/symptoms collected and tests results. It will be detailed in a statistical analysis plan validated by the SAB.

12.2.2. Analysis of secondary endpoints

For the analysis of the secondary endpoint related to the screening prediction score to identify children with presumptive TB who would benefit from further diagnostic testing, the analysis and modelling strategy will be similar to that of the primary endpoint, using logistic regression. The predicted variable will be determined using individual predicted probability of TB using the diagnosis score dichotomized to maximise sensitivity. We will exclude the following tests from model development, considered as invasive and costly: Ultra results on microbiological samples, abdominal ultrasound, as well as CXR if possible. This choice will be discussed with the expert committee prior to analysis. Among patients classified as presumptive TB, performance of the original diagnostic score will be re-estimated or a new diagnostic score will be developed.

Analysis of other secondary endpoints will be further detailed in the statistical analysis plan.

12.2.3. Cost-effectiveness analyses

A mathematical model will be informed by the data collected during the trial and costs from a specific survey to project mortality and costs for the following strategies: 1) the standard of care with current TB diagnostics practice in children with SAM; 2) the TB-Speed approach using a new diagnostic prediction score with improved and active TB diagnostics. The projected mortality will be used to estimate Disability Adjusted Life Years saved (DALYs) for each strategy. ICERs will compare the differences in DALYs and costs between the 2 strategies. ICERs will be estimated for each country separately and will be compared to previously published estimated cost-effectiveness thresholds for each country.

The cost-effectiveness analysis will be from the health payer perspective and only direct health care costs will be included. A budget impact analysis will be conducted to evaluate the actual impact of implementing the new TB-Speed approach on health care budgets at 2- and 5-year horizons in the countries participating in the project.

Sensitivity analyses will be conducted to assess uncertainties around the estimates and the robustness of our findings. Variation of parameters such as TB prevalence, TB incidence and costs will help to simulate different scenarios of implementing the TB-Speed approach, adapted to the countries' specific contexts. The latter will be important for the generalization of the results and to inform more general TB guidelines.

13. COMMUNICATION AND PUBLICATION POLICY

13.1. Findings publication procedure

All data collected during this research are the property of the study sponsor and cannot be communicated, under any circumstances, to a third party without the written consent of the sponsor.

The results will be published after final analysis in the form of scientific articles in peer-reviewed journals, or presented at national and international conferences. To ensure respect of international standards for authorship, all publications must follow the rules contained in the publication charter defined by the TB-Speed project as part of the project communication plan. Any publication or communication (oral or written) is decided by mutual agreement between the coordinating investigators, the SAB and the sponsor, and will respect the international guidelines: "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals" (http://www.icmje.org/recommendations).

The mention of the origin of the funding, the authorizations of the competent authorities, and the consent of the participants must appear in the acknowledgments according to the model suggested below:

" / Ethics statement / * / This study is part of **** CXX-XX ** sponsored by Inserm. It was granted approval by local Ethics Committee or "Committee for the Protection of Persons" on ---- **** DATE ** ---, authorized by the French authorities (**** ANSM ** **** NB * *), and registered in a public trials registry (**** CT XXXX **)./ Funded by Unitaid and the ANRS. All study participants gave their informed written consent to participation, in line with ethical guidelines.*

13.2. Procedure for writing up the final report

The international CTU will establish the final report of the study as well as summary report within a year after the end date of the study, i.e. the last visit of the last patient. The report and its summary are established according to ICH recommendations (International Conference for Harmonisation – ICH Topic E3 – Structure and Content of Clinical Study Reports CPMP/ICH/137/95. Accessible at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E3/E3_Guideline.pdf). The report will be approved by the SAB of the TB-Speed project.

Within one year after the end of the study, the sponsor or its representative will release to the ethical and regulatory authorities of each country involved the final study report and/or summary including the results of the study and the scientific publications or communications related to these results.

13.3. Procedure for informing the study participants of the overall research findings

The final study results will be presented to the investigators and the national authorities of each participating country. A series of documents (written detailed report, and short summary) will be released to help investigators, national authorities and participants to understand the results of the study. Should the country investigator, national authorities, and patients' representatives consider it desirable, participants may be invited to attend a meeting during which the results will be presented and explained orally.

13.4. Procedure for informing the participants of their health data during and after the research

Parent(s)/guardian(s) are informed of their right during or after the research, to be given information concerning their child's health held by the investigator or, where appropriate, the qualified person who represents it.

Any clinically significant abnormality detected in the examination or test results will be communicated the parent(s)/guardian(s) and the physician selected by them unless they have objected.

13.5. Press communication procedure

A press release in collaboration with the funders and the sponsor will be developed by the TB-Speed communication group to inform the press about the study results.

14. STUDY OVERSIGHT

14.1. Output Steering Committee

The study (Output) Steering Committee (OSC) is the operational team that will undertake the day-to-day decisions related to study implementation in each country, based on the model applied in all clinical trials currently managed by the IDLIC team at UBx.

The OSC will consist of the coordinating investigators, country PIs and co-PIs, country project managers, the international trial manager, the laboratory coordinator, the international CRA and data manager, and any relevant participants invited to discuss specific issues.

The OSC will be in charge of the reporting and formulation of proposals for the Executive Committee regarding work-plan and budget reallocation and execution of the decisions taken by the Project Coordination Committee.

Members of OSC will interact once a month. Every 6 months, the OSC meeting will be opened to Protocol Writing Committee external members for a review of study progresses and results.

14.2. Scientific Advisory Board

The TB-Speed Scientific Advisory Board (SAB) is an expert consultative committee providing scientific advice to the project management teams. It gives input on the relevance and scientific validity of the project design and implementation, monitors progress and ensure scientific and ethical integrity of the project.

- **Role**

The SAB members will bring their individual expertise to review and advice on the following:

- The relevance of the project objectives within the context of the paediatric TB research landscape;
- The appropriateness of designs and methods of the proposed studies (outputs) to the research questions;
- The scientific strength, safety and feasibility to meet the stated objectives of the project;
- The complementarity of the project with other ongoing or planned external trials/studies;
- The continued relevance of the project in light of new scientific and/or clinical developments;
- The final Research Protocols, including informed consent forms, prior to their submission to relevant ethics committees;
- The project progress upon receiving of progress reports, including interim and final statistical analyses;
- Any important scientific decisions or changes made during the course of the project (e.g., major protocol amendment);
- Any publication ahead of submission to international peer-reviewed scientific journals;
- Confidential scientific reports transferred to WHO for consideration and inclusion of outcomes into development and update of WHO normative guidance.

- **Composition and appointment**

SAB members are initially appointed on an invitation basis from a list of nominees developed by the TB Speed Executive Committee.

The SAB is led by a Chairperson who is independent from the project consortium and includes independent external experts, as well as members of the protocol development teams. The committee will consist of at least 10 but no more than 12 members bringing their individual knowledge, experience and expertise. The experts will include at least two paediatric TB experts, one expert in operational research on TB diagnostic, one mycobacteriologist, one paediatric HIV expert, one paediatric pulmonologist, one health economist, one representative of NTP from a high TB burden country, and one representative from the community.

Members of the SAB will be required to meet at least once a year. Additional bi-annual meetings may be considered as needed for the project.

14.3. Independent Data Monitoring Committee

The IDMC is a consultative board for the SAB and the sponsor. It monitors the main safety and efficacy outcome measures and the overall conduct of the study, with the aim of protecting the safety and the interests of the trial participants. Its members will provide general advice on the progress of the trial, including the rate of inclusions, quality of follow-up, overall rate of AEs, changes in biological markers, overall incidence of primary outcomes, and the number of subjects needed.

It will be responsible for examining data with respect to clinical outcomes and treatment failures and for informing the SAB about any decisions it needs to take to pursue or discontinue the trial, such as:

- Premature discontinuation (because the rate of AEs is high, the trial is no longer feasible, or the available data are sufficient);
- Substantial changes to the protocol that becomes necessary during the inclusion or follow-up phases, or to account for new scientific information.

The IDMC will have access to overall safety and efficacy data, as well as to any information justifying any change affecting the course of the trial. It may request an intermediate statistical analysis.

During the trial, the IDMC may be asked to deliberate on questions relative to the scientific and ethical integrity of the trial, at the request of the SAB, the coordinating investigators, the international coordinating CTU or other participants in the trial. The IDMC will provide a formal written opinion report to the SAB and the sponsor after each IDMC meeting.

IDMC members will be selected in collaboration between the coordinating investigators and the sponsor before the beginning of the trial. All IDMC members must be free from any direct involvement with the trial. Any competing interests, both real and potential, must be declared.

The IDMC will meet soon after the beginning of the inclusion phase, and every 4 to 6 months until the end of the study. The sponsor, the SAB or the IDMC may request to increase the frequency of these meetings

14.1. Expert Committee

In each country, an expert review committee will consist of: (i) the country PI; (ii) one or several other adequately trained physicians, selected by the country PI; (iii) a representative of the country CTU.

The expert review committee will make a criterion-related validation of the TB reference diagnosis and assess causes of death in children who may die after inclusion in the study. The Coordinating Investigators and members of the international CTU will review all event validation forms and uploaded documents, as well as any relevant information in the database, in order to verify that the criterion-related validation is applied homogeneously across participating countries, and ask the country PIs for additional information whenever needed.

Safety reports will be available for periodic review by the SAB and the IDMC.

14.1. Country Project Committee

At country level, the TB-Speed Country Project Committee (CPC), without any steering role, associates all major TB and child health stakeholders in the country (e.g. implementers, political supports, local NGOs). Under the supervision of the Country PI, the CPC will act as a facilitator for national operations as well as dissemination and communication activities.

14.2. Coordination

The international coordinating CTU, in charge of overall study coordination, data monitoring and management will be the IDLIC/Mereva team at the Inserm U1219 Bordeaux Population Health, located at University of Bordeaux (France). Study implementation, monitoring and data management activities will be coordinated by an international CRA.

The study will be conducted and monitored according to a set of SOPs. Monitoring will be implemented according to the monitoring plan which is written by the international CTU and validated with Inserm, the study sponsor. Writing of the SOPs is coordinated by the International CTU.

In each country where the study will be conducted, the CTU will be based at the level of the TB-Speed Consortium partner, i.e.MU-JHU in Uganda, and University of Zambia in Zambia.

The country CTU will be in charge of study coordination, monitoring and data management in the country. Study activities will be coordinated by a country trial manager, who will work in close collaboration with the international trial (Output) manager, and monitored by a country CRA who will work in close collaboration with the international CRA.

A consortium agreement, established between UBx and TB-Speed Consortium members, defines task distribution and responsibilities of the different centres during the project.

15. CONFIDENTIALITY

15.1. Procedure for respecting the confidentiality of participants

Each study subject will be assigned a unique identification code. Every effort will be made to have this code as the only patient identifier on any document, record, report or laboratory specimen related to the trial. This will be the only identifier in the electronic trial database, including gxx and dicom files.

The study ID assignment log (only in paper form) will be kept shut-away on site under the responsibility of the investigator. Direct personal identifiers (including names, dates, demographic and contact information) will only be made available to those whose job within the operational activities of the study makes having such information absolutely essential, subject to signature of a confidentiality agreement. This includes routine hospital staff involved in the child clinical management, as well as research study staff when they may be visiting the study site for monitoring, coordination, or event validation purposes. They will not have access to direct personal identifiers outside of the trial site premises.

All documents (such as the signed consent forms) containing patients' names will be kept in a locked cabinet under the responsibility of the site investigator.

15.2. Procedure for keeping the necessary study data confidential

Individual medical information obtained as result of this study will be confidential. Study team members are subject to the obligation of professional secrecy. Individual patient data will be made available upon request to the study investigators, physicians in charge of patients' care, representatives of the sponsor, and representatives of the ethical and regulatory health authorities in case of external audit or inspection. Disclosure to other third parties is strictly prohibited. Parent(s)/guardian(s)'s consent for this is obtained as part of the consent process.

The data recorded during this study will be the subject of computer processing on behalf of the sponsor. The sponsor will declare the database to the French CNIL, in compliance with the provisions of the French Law No. 78-17, dated January 6, 1978, and amended by Law No. 2004-80, dated August 8, 2004.

16. PROTECTING RESEARCH PARTICIPANTS

16.1. Ethical justification of the protocol

16.1.1. Risks

Young children with SAM have a high risk of death, independent to the study intervention, especially if they have comorbidities like TB or HIV infection.

Risks due to para-clinical investigations are well known and will be explained to the participants. The potential risks of NPA and blood draw will be limited by insuring they are performed by trained nurses with appropriate supplies and standardized procedures detailed in the study clinical SOPs.

- NPA usually causes a reactive cough; epistaxis and discomfort can also occur;

- The NPA does not increase the risk of bronchospasm and should not increase risk of hypoxemia. However sample collection will always be done with access to salbutamol and oxygen;
- Blood draw from a vein may induce discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection, and, uncommonly, faintness from the procedure.

No new drugs will be tested during this study but parent(s)/guardian(s) will nevertheless be informed of possible TB drugs and antiretroviral side effects and other possible concomitant AEs (paradoxical reactions and IRIS).

Risks specific to study participation include the potential for breach of confidentiality. To minimize this risk, as well as stigma and emotional risks associated with TB and HIV diagnosis, testing will be confidential and performed with pre- and post-test counselling. HIV-infected children identified through the study will be referred to antiretroviral treatment programs.

16.1.2. Benefits

This study is providing the following opportunities for eligible children:

- an improved management of SAM
- an improved and earlier diagnosis of TB, especially by optimizing bacteriological specimen collection and processing;
- an enhanced prognosis thanks to timely TB treatment initiation as recommended by national health authorities;
- in case of Rifampicin resistance detected by Ultra, an access to drug susceptibility testing for *Mycobacterium tuberculosis* and the opportunity to receive an adapted treatment according to these results, including access to MDR-TB treatment;
- investigation fees per protocol paid by the study (clinical examinations, laboratory, radiology, hospitalization and transportation fees).

Together these factors will positively impact local NTPs by improving case detection rates as well as TB outcomes. It will also positively impact Nutrition Programmes and nutritional rehabilitation centres by raising awareness on TB in children with SAM and linking with NTPs. It is hoped that lessons learned from this study will help to improve management of children with SAM in high TB burden countries.

16.2. Regulatory provisions

The investigators undertake to conduct the research in compliance with the protocol and in accordance with:

- the French regulations in force, including provisions relating to research involving the human person provided for in Articles L 1121-1 *et seq.* of the Public Health Code, the Bioethics Laws, the Data Protection Act;
- Participating countries' laws and regulations relating to biomedical research on human participants;
- The Declaration of Helsinki (approved by the World Health Association on June 1964, lastly amended at the 64th WMA General Assembly, Fortaleza, October 2013);
- The Good Clinical Practice (ICH Harmonized Tripartite Guidelines for Good Clinical Practice E6 step 4 - 1996) and Good Clinical Laboratory Practice (GCLP. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases, 2009);
- The 2017 revision of the ANRS Ethics Charter for research in Developing Countries.

This study will be registered at the ClinicalTrials.gov registry and The Pan African Clinical Trials Registry (PACTR).

16.3. Ethical approvals

Before carrying out the research, the protocol, the information sheet, the consent form and any other relevant documents will be submitted to the approbation of each implementing country's National Ethics Committee, to relevant Institutional Review Boards, to the WHO Ethical Review Board, and to the Inserm Ethics Evaluation Committee.

The study will be implemented in each country only once the ethical clearance document of the Ministry of Health or relevant Health Authority is received. The research can only start when Inserm has been informed of the favourable opinion delivered by the different ERCs concerning the submitted protocol. This notice will include the title and protocol number assigned by the proponent, the documents reviewed, as well as the date of review and the list of ERC members who participated.

Once approved and authorized, the final version of the protocol will be signed by the coordinating investigators and the sponsor. All PIs will sign the protocol as a commitment to conduct the study according to the protocol, the declaration of Helsinki, the Good Clinical Practice and adhere to the procedures described in the SOPs.

The proponent will inform the different ERCs of any subsequent amendments and any serious or unexpected AEs and developments that occur during the course of the research that would likely affect the safety of those who are suitable for the research.

16.4. Additional approvals

The study will be approved by health authorities of the implementing countries, namely the National Tuberculosis Programs as implementing partners, and if relevant the Ministry of Health at a broader level.

In Zambia, approval to conduct health research projects requires to obtain an administrative clearance issued by the Ministry of Public Health.

16.5. Data protection

The data recorded during this trial will be subject to computer processing on behalf of the Sponsor. The protocol will be submitted for approval to the French data protection authority (CNIL). It will also be conducted in accordance with the African Union Convention on Cyber Security and Personal Data Protection adopted on 27 June 2014.

16.6. Insurance

Inserm, which is sponsoring this study, accepts the legal responsibility in the name of the investigator for any direct or indirect harm caused to patients by the methods used in this research.

Inserm has taken out a civil liability insurance for the entire duration of the study, in accordance with the French legal provisions and regulations on research.

The certificate of insurance relating to this Protocol constitutes Appendix 7.

16.7. Participants amenities

Study investigators will ensure that each subject receives the following benefits throughout the study: reimbursement of transportation fees to the hospital, compensation for time spent during follow-up study visits in agreement with local regulations, medical exams, tests, and medications related to the study.

If not covered by the national health system, hospital stays will be free of charge, covered by the TB-Speed project, whenever prescribed or approved by the country trial investigators, within the limits of the available budget.

17. QUALITY ASSURANCE AND MONITORING

17.1. Description of the quality assurance system

The role of quality assurance is to ensure the safety of individuals who are amenable to research involving the human person and to ensure the credibility of data derived from such research and their recognition by the medical and scientific community.

Research monitoring will be conducted according to the Good Clinical Practices (ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996) to guarantee the quality of the research and safeguard the health and the rights of the patient. The investigator is above all the guarantor of the quality of the study progress.

The monitoring plan is established by the international coordinating CTU with the sponsor and the coordinating investigators before the start of the study. Key data subject to source data verification are identified in the monitoring plan. Procedures for monitoring will be detailed in specific SOPs developed by the Mereva team at the international CTU.

17.2. Monitoring (quality control of the study)

17.2.1. General organization

Country CTUs are in charge of the monitoring of data collected in the country. The international coordinating CTU, based at the UBx IDLIC/Mereva team, coordinates and supervises monitoring performed by country CTUs and performs targeted monitoring.

17.2.2. Monitoring by the country CTU

A CRA will regularly visit each implementing site during the all study period, including at set-up, implementation, and at the end of the study. During these visits, the country CTU will be in charge of the following, according to the monitoring plan:

- Check adherence to the protocol, SOPs and Good Clinical Practice, including eligibility criteria, informed consent, patient schedule;
- establish and maintain the investigator's TMF up-to-date;
- check the completeness and the accuracy of patient key data on the CRF (source data verification);
- verify that confidentiality of data is fully respected;
- verify SAEs reporting, documentation and follow-up, and send the forms to the sponsor's pharmacovigilance and to the international coordinating CTU;
- evaluate the progress of patient enrolment;
- check the quality management of samples and biobank;
- ensure that quality controls and quality management for laboratory assessments are implemented;
- monitor CXR interpretation performance;
- follow-up with investigator sites centralized correction requests sent by the international coordinating CTU.

After each visit a report will be written by the country CRA.

Furthermore, the country CRA will also hold regular meetings with the study staff at each site to discuss any patient file international and country CTUs deem problematic, as well as practical and logistic issues in study implementation and patient or sample management.

17.2.3. Monitoring by the international coordinating CTU

During the study set-up process, an opening visit will be performed for each site by the international CRA and the project co-investigator on behalf of the sponsor. Only upon completion of equipment, training, ethical and regulatory approvals (including civil liability insurance) will a site be authorized to start enrolling patients.

A member of the international coordinating CTU will visit each study site at least once a year. The purpose of these visits will be to review with the country CTU advances and issues with the local monitoring and data management process, as well as perform a targeted/random monitoring of a limited number of files.

The following aspects will be reviewed according to the monitoring plan:

- Informed consent
- Compliance with the study protocol, SOPs and Good Clinical Practices, including eligibility criteria and reporting of SAEs
- Consistency with the source documents for key data
- Management of samples and biobanking
- Laboratory quality controls

Each visit will be recorded in a written monitoring report, sent to the co-investigators, the clinical and country project managers, the country PIs and the sponsor.

The country CTU will also be monitored on specific aspects such as the availability and maintenance of an updated TMF.

In the context of the Covid pandemic, due to travel restrictions, remote site monitoring visits might be conducted by the international CTU. Where needed, pseudonymized source documents will be forwarded by the country CRA through a ftps for monitoring purpose only; source documents will be destroyed afterwards.

A closing visit will be carried out at the end of the study by the international CRA.

17.2.4. Direct access to source data

Participating investigators should agree to allow study-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data/documents as required. Patients' agreement for this is obtained as part of the informed consent process.

17.3. Audits/inspections

All documents and data relating to the research should be made available at any time to the sponsor as well as ethical and regulatory health authorities in case of external audit or inspection. Those should be carried out in the respect of the professional secrecy and without being able to be opposed the medical confidentiality. Disclosure to other third parties is strictly prohibited.

18. ACCESS TO DATA AND FROZEN SAMPLES

18.1. Data

All data collected in relation to the study will be under the responsibility of the international coordinating CTU.

Data will be utilized according to this protocol. After expertise and opinion by the SAB, any utilization for analyses not listed in the protocol should be approved by the trial coordinating investigators and the sponsor.

Data will be held in a centralized database held at UBx. However, each of the implementing countries will have access to their own data. A Data Sharing Agreement will be signed between UBx and each consortium partner.

18.2. Frozen samples

18.2.1. Biobank governance

Samples for which parent(s)/guardian(s) have consented for storage will be under the country CTU's responsibility, during and after the end of the study. The consortium agreement defines the responsibilities of country CTUs, including management of the biobank in their own facilities, or contracting with an external laboratory with adequate biobanking capacity. Specific SOPs will describe methods and procedures for the collection of biological samples, as well as the Laboratory Quality Assurance system put in place during the TB-Speed Pneumonia study. In addition, each country CTU will be provided with a deep freezer to ensure enough space and good condition for the storage of study samples.

Biological samples will be retained for 10 years after study completion, unless objection expressed by parent(s)/guardian(s). Destruction of leftovers or unused samples will be undertaken by country central laboratories in accordance with local regulations relating to the disposal of biological specimens. In the event of samples shipped to external laboratories, those will be disposed of in accordance with applicable regulations in both recipient and supplier countries.

Any utilization for tests not listed in the protocol should be approved by the study co-investigators and the sponsor, after expertise and opinion by the SAB. Each ancillary study will seek ethics approval at national and international level.

Subject to approval by relevant Ethics Committees at the national and international level, frozen samples may be sent to external laboratories (inside or outside the country) for additional analyses performed as part of ancillary studies. A Material Transfer Agreement will be submitted to appropriate ethics committees and regulatory authorities of both recipient and supplier countries.

18.2.1. Patients' rights

In line with the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Health-related Research Involving Humans (Guideline 11), authorization from the donor (i.e. parents/guardians of the participating child) for future use of stored biological samples will be sought during the informed consent process. The Information Notice will include: the name and city of the country central laboratory; the purpose of the biobank; the foreseeable use of the samples (extending to a number of yet undefined research studies including genetic analyses); the conditions and duration of storage; the rules of access to the biobank and the protection of data confidentiality. No further consent will be sought from parents/guardians in case of post-trial studies.

The donor can retract his authorisation for sample storage at any time. In such case, biological material will be destroyed.

Biobank samples will be collected from children benefitting from a 6-month cohort follow-up. Any undiagnosed TB at baseline will most likely be detected by the end of follow-up. We therefore do not expect unsolicited findings, and will not report any results from biobank samples to the patient.

However, as mentioned in the Information Notice, knowledge generated by the research will be shared with participants if they are willing to. Site investigators will be responsible for informing study participants by using the most appropriate mean that research results are available and can be communicated to them if they wish so.

19. SUBSTANTIAL AMENDEMENTS TO THE PROTOCOL

Any change or addition to this protocol requires a written protocol amendment to be approved by each country's National Ethics Committee, the WHO Ethical Review Board, and signed by the coordinating investigators, the PIs and the Inserm before implementation.

These requirements for approval should in no way prevent any immediate action from being taken by the investigators or by the sponsor in the interests of preserving the safety of all study participants. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented by him/her for safety reasons, the Inserm should be notified and each country's National Ethics Committee should be informed within 10 working days.

20. BIBLIOGRAPHY/REFERENCES

[1] R. E. Black *et al.*, “Maternal and child undernutrition and overweight in low-income and middle-income countries,” *The Lancet*, vol. 382, no. 9890, pp. 427–451, Aug. 2013, doi: 10.1016/S0140-6736(13)60937-X.

[2] World Health Organization and Unicef, “WHO child growth standards and the identification of severe acute malnutrition in infants and children: joint statement by the World Health Organization and the United Nations Children’s Fund,” 2009.

[3] UNICEF / WHO / World Bank Group, “Joint Child Malnutrition Estimates,” 2017.

[4] I. Olofin *et al.*, “Associations of Suboptimal Growth with All-Cause and Cause-Specific Mortality in Children under Five Years: A Pooled Analysis of Ten Prospective Studies,” *PLoS ONE*, vol. 8, no. 5, p. e64636, May 2013, doi: 10.1371/journal.pone.0064636.

[5] World Health Organization, “Global Tuberculosis Report 2017,” Geneva, 2017.

[6] K. Lönnroth *et al.*, “Tuberculosis control and elimination 2010–50: cure, care, and social development,” *The Lancet*, vol. 375, no. 9728, pp. 1814–1829, 2010.

[7] D. Jaganath and E. Mupere, “Childhood Tuberculosis and Malnutrition,” *J. Infect. Dis.*, vol. 206, no. 12, pp. 1809–1815, Dec. 2012, doi: 10.1093/infdis/jis608.

[8] M. Singh, M. L. Mynak, L. Kumar, J. L. Mathew, and S. K. Jindal, “Prevalence and risk factors for transmission of infection among children in household contact with adults having pulmonary tuberculosis,” *Arch. Dis. Child.*, vol. 90, no. 6, pp. 624–628, Jun. 2005, doi: 10.1136/adc.2003.044255.

[9] R. K. Chandra, “Nutrition and the immune system: an introduction,” *Am. J. Clin. Nutr.*, vol. 66, no. 2, pp. 460S-463S, Aug. 1997, doi: 10.1093/ajcn/66.2.460S.

[10] J. K. Frediani *et al.*, “Macronutrient intake and body composition changes during anti-tuberculosis therapy in adults,” *Clin. Nutr.*, vol. 35, no. 1, pp. 205–212, Feb. 2016, doi: 10.1016/j.clnu.2015.02.007.

[11] World Health Organization, “Guidance for national tuberculosis programmes on the management of tuberculosis in children - 2nd ed.,” Geneva, 2014.

[12] R. Zachariah, M. P. Spielmann, A. D. Harries, and F. M. L. Salaniponi, “Moderate to severe malnutrition in patients with tuberculosis is a risk factor associated with early death,” *Trans. R. Soc. Trop. Med. Hyg.*, vol. 96, no. 3, pp. 291–294, May 2002, doi: 10.1016/S0035-9203(02)90103-3.

[13] R. M. Hicks *et al.*, “Malnutrition associated with unfavorable outcome and death among South African MDR-TB and HIV co-infected children,” *Int. J. Tuberc. Lung Dis.*, vol. 18, no. 9, pp. 1074–1083, Sep. 2014, doi: 10.5588/ijtld.14.0231.

[14] M. Bonnet *et al.*, “Outcome of Children With Presumptive Tuberculosis in Mbarara, Rural Uganda,” *Pediatr. Infect. Dis. J.*, vol. 37, no. 2, p. 147, Feb. 2018, doi: 10.1097/INF.0000000000001727.

[15] H. Huerga and F. Tapié de Céleyran, “Systematic tuberculosis treatment versus current TB diagnosis and treatment practices in children with severe acute malnutrition and poor response to 2 weeks of standard nutritional and medical therapy - First phase of the research study: Literature review and Program data analysis,” MSF-Epicentre, Jun. 2015.

[16] P. G. Bhat *et al.*, “Intensified Tuberculosis Case Finding among Malnourished Children in Nutritional Rehabilitation Centres of Karnataka, India: Missed Opportunities,” *PLoS ONE*, vol. 8, no. 12, p. e84255, Dec. 2013, doi: 10.1371/journal.pone.0084255.

[17] V. B. Sathenahalli, N. Minarey, V. Gornale, R. Kumar, K. Joshi, and S. H P, “ASSOCIATION OF TUBERCULOSIS WITH SEVERE ACUTE MALNUTRITION,” *J. Evol. Med. Dent. Sci.*, vol. 4, no. 68, pp. 11865–11870, Aug. 2015, doi: 10.14260/jemds/2015/1710.

[18] A. K. Arya, P. Lal, and P. Kumar, “Co-morbidities in Children with Severe Acute Malnutrition - A Tertiary Care Centre Experience,” vol. 4, no. 5, p. 3, 2017.

[19] S. M. LaCourse *et al.*, “Use Of Xpert For The Diagnosis Of Pulmonary Tuberculosis In Severely Malnourished Hospitalized Malawian Children.,” *Pediatr. Infect. Dis. J.*, vol. 33, no. 11, pp. 1200–1202, Nov. 2014, doi: 10.1097/INF.0000000000000384.

[20] T. Munthali *et al.*, “Tuberculosis caseload in children with severe acute malnutrition related with high hospital based mortality in Lusaka, Zambia,” *BMC Res. Notes*, vol. 10, no. 1, Dec. 2017, doi: 10.1186/s13104-017-2529-5.

[21] E. Kemigisha and V. Katawera, “Prevalence and clinical predictors of tuberculosis in severely malnourished Ugandan children,” *Ann. Glob. Health*, vol. 81, no. 1, p. 7, 2015.

[22] M. J. Chisti *et al.*, “A Prospective Study of the Prevalence of Tuberculosis and Bacteraemia in Bangladeshi Children with Severe Malnutrition and Pneumonia Including an Evaluation of Xpert MTB/RIF Assay,” *PLoS ONE*, vol. 9, no. 4, p. e93776, Apr. 2014, doi: 10.1371/journal.pone.0093776.

[23] P. J. Dodd, C. M. Yuen, C. Sismanidis, J. A. Seddon, and H. E. Jenkins, “The global burden of tuberculosis mortality in children: a mathematical modelling study,” *Lancet Glob. Health*, vol. 5, no. 9, pp. e898–e906, 2017.
[24] World Health Organization, “Roadmap for childhood tuberculosis: towards zero deaths,” Geneva, 2013.
[25] E. Kemigisha and V. Katawera, “Prevalence and clinical predictors of tuberculosis in severely malnourished Ugandan children,” *Ann. Glob. Health*, vol. 81, no. 1, p. 7, 2015.
[26] L. N. Patel and A. K. Detjen, “Integration of childhood TB into guidelines for the management of acute malnutrition in high burden countries,” *Public Health Action*, vol. 7, no. 2, pp. 110–115, Jun. 2017, doi: 10.5588/pha.17.0018.
[27] M. J. Chisti *et al.*, “Diagnosis of Tuberculosis Following World Health Organization–Recommended Criteria in Severely Malnourished Children Presenting With Pneumonia,” *Glob. Pediatr. Health*, vol. 4, p. 2333794X16686871, 2017.
[28] S. M. LaCourse *et al.*, “Stool Xpert MTB/RIF and urine lipoarabinomannan for the diagnosis of tuberculosis in hospitalized HIV-infected children.,” *AIDS*, vol. 32, no. 1, pp. 69–78, Jan. 2018, doi: 10.1097/QAD.0000000000001662.
[29] O. Marcy *et al.*, “Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children,” *Clin. Infect. Dis.*, vol. 62, no. 9, pp. 1161–1168, May 2016, doi: 10.1093/cid/ciw036.
[30] World Health Organization, “High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Available at: http://apps.who.int/iris/bitstream/10665/135617/1/WHO_HTM_T- B_2014.18_eng.pdf,” Geneva, 2014.
[31] A.-L. Page *et al.*, “Diagnostic and Prognostic Value of Procalcitonin and C-Reactive Protein in Malnourished Children,” *PEDIATRICS*, vol. 133, no. 2, pp. e363–e370, Feb. 2014, doi: 10.1542/peds.2013-2112.
[32] A. E. M. Kheir and B. G. Gebreel, “C-reactive protein as a marker of infection in children with severe acute malnutrition in Khartoum state, Sudan,” *Healthc. Low-Resour. Settings*, vol. 5, no. 1, Aug. 2017, Accessed: May 02, 2018. [Online]. Available: http://www.pagepressjournals.org/index.php/hls/article/view/6401.
[33] I. Latorre *et al.*, “IP-10 is an accurate biomarker for the diagnosis of tuberculosis in children,” *J. Infect.*, vol. 69, no. 6, pp. 590–599, Dec. 2014, doi: 10.1016/j.jinf.2014.06.013.
[34] R. Choudhary, “Monocyte-to-lymphocyte ratio is associated with confirmed TB disease and declines with anti-tuberculosis treatment in HIV-infected children,” presented at the 48th Union World Conference on Lung Health, Guadalajara, Mexico, Oct. 2017.
[35] G. Guadagnino, N. Serra, C. Colomba, A. Giammanco, D. Mililli, and F. Scarlata, “Monocyte to lymphocyte blood ratio in tuberculosis and HIV patients: comparative analysis, preliminary data,” *Pharmacol. Online*, vol. 1, pp. 22–33, 2017.
[36] World Health Organization, “The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV. Policy guidance.,” Geneva, 2015.
[37] T. Broger *et al.*, “Novel High Sensitivity Tuberculosis Point-of-Care Test for People Living with HIV,” Social Science Research Network, Rochester, NY, SSRN Scholarly Paper ID 3254479, Sep. 2018. Accessed: Dec. 17, 2018. [Online]. Available: https://papers.ssrn.com/abstract=3254479.
[38] S. T. Anderson *et al.*, “Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa,” *N. Engl. J. Med.*, vol. 370, no. 18, pp. 1712–1723, May 2014, doi: 10.1056/NEJMoa1303657.
[39] G. Walzl *et al.*, “Tuberculosis: advances and challenges in development of new diagnostics and biomarkers,” *Lancet Infect. Dis.*, Mar. 2018, doi: 10.1016/S1473-3099(18)30111-7.
[40] M. Zhou, G. Yu, X. Yang, C. Zhu, Z. Zhang, and X. Zhan, “Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection,” *Mol. Med. Rep.*, vol. 13, no. 6, pp. 4620–4626, Jun. 2016, doi: 10.3892/mmr.2016.5097.
[41] J. E. Gjøen *et al.*, “Novel transcriptional signatures for sputum-independent diagnostics of tuberculosis in children,” *Sci. Rep.*, vol. 7, no. 1, Dec. 2017, doi: 10.1038/s41598-017-05057-x.
[42] L. M. Verhagen *et al.*, “A predictive signature gene set for discriminating active from latent tuberculosis in Warao Amerindian children,” *BMC Genomics*, vol. 14, no. 1, p. 74, 2013, doi: 10.1186/1471-2164-14-74.
[43] E. C. Pearce, J. F. Woodward, W. M. Nyandiko, R. C. Vreeman, and S. O. Ayaya, “A Systematic Review of Clinical Diagnostic Systems Used in the Diagnosis of Tuberculosis in Children,” *AIDS Res. Treat.*, vol. 2012, pp. 1–11, 2012, doi: 10.1155/2012/401896.
[44] A. C. Hesseling, H. S. Schaaf, R. P. Gie, J. R. Starke, and N. Beyers, “A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis,” *Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis.*, vol. 6, no. 12, pp. 1038–1045, Dec. 2002.

[45] S. M. Graham *et al.*, “Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update,” *Clin. Infect. Dis.*, vol. 61, no. suppl 3, pp. S179–S187, Oct. 2015, doi: 10.1093/cid/civ581.
[46] N. Dendukuri *et al.*, “Concerns about composite reference standards in diagnostic research,” *BMJ*, p. j5779, Jan. 2018, doi: 10.1136/bmj.j5779.
[47] O. Marcy, L. Borand, and V. Ung, “A diagnostic prediction score and algorithm for anti-tuberculosis treatment decision in HIV-infected children with suspected tuberculosis.,” *submitted*, 2018.
[48] S. Bélard *et al.*, “Sonographic Findings of Abdominal Tuberculosis in Children With Pulmonary Tuberculosis,” *Pediatr. Infect. Dis. J.*, vol. 36, no. 12, pp. 1224–1226, Dec. 2017, doi: 10.1097/INF.0000000000001590.
[49] S. Bélard *et al.*, “Utility of Point-of-Care Ultrasound in Children with Pulmonary Tuberculosis,” *Pediatr. Infect. Dis. J.*, Dec. 2017, doi: 10.1097/INF.0000000000001872.
[50] D. Sculier *et al.*, “Performance of Abdominal Ultrasound for Diagnosis of Tuberculosis in HIV-Infected Persons Living in Cambodia,” *J. Acquir. Immune Defic. Syndr. 1999*, Jun. 2010, doi: 10.1097/QAI.0b013e3181e6a703.
[51] World Health Organization, *Definitions and reporting framework for tuberculosis – 2013 revision*. Geneva: World Health Organization, 2013.
[52] S. M. Graham *et al.*, “Evaluation of Tuberculosis Diagnostics in Children: 1. Proposed Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease. Consensus From an Expert Panel,” *J. Infect. Dis.*, vol. 205, no. suppl_2, pp. S199–S208, May 2012, doi: 10.1093/infdis/jis008.
[53] World Health Organization, *Guideline: updates on the management of severe acute malnutrition in infants and children.* Geneva, 2013.
[54] B. Dim, T. Tran, and L. Borand, “Feasibility, safety and tolerability of nasopharyngeal aspirates and string tests for diagnosis of TB with Xpert® MTB/RIF in HIV-infected children: ANRS 12229 PAANTHER 01 study,” presented at the 48th Union World Conference on Lung Health, Guadalajara, Mexico, Oct. 2017.
[55] S. Bharadwaj *et al.*, “Malnutrition: laboratory markers vs nutritional assessment,” *Gastroenterol. Rep.*, p. gow013, May 2016, doi: 10.1093/gastro/gow013.
[56] U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS., “Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1.,” 2017.
[57] A. R. Frydenberg and S. M. Graham, “Toxicity of first-line drugs for treatment of tuberculosis in children: review,” *Trop. Med. Int. Health TM IH*, vol. 14, no. 11, pp. 1329–1337, Nov. 2009, doi: 10.1111/j.1365-3156.2009.02375.x.
[58] Q. Li *et al.*, “Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia,” *N. Engl. J. Med.*, Jan. 2020, doi: 10.1056/nejmoa2001316.
[59] C. Huang *et al.*, “Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China,” *The Lancet*, vol. 395, no. 10223, pp. 497–506, Feb. 2020, doi: 10.1016/S0140-6736(20)30183-5.
[60] N. S. Zhong *et al.*, “Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People’s Republic of China, in February, 2003.,” *Lancet Lond. Engl.*, vol. 362, no. 9393, pp. 1353–8, Oct. 2003, doi: 10.1016/s0140-6736(03)14630-2.
[61] A. M. Zaki, S. Van Boheemen, T. M. Bestebroer, A. D. M. E. Osterhaus, and R. A. M. Fouchier, “Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia,” *N. Engl. J. Med.*, vol. 367, no. 19, pp. 1814–1820, Nov. 2012, doi: 10.1056/NEJMoa1211721.
[62] Z. Wu and J. M. McGoogan, “Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention,” *JAMA*, Feb. 2020, doi: 10.1001/jama.2020.2648.
[63] J. F.-W. Chan *et al.*, “A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster,” *Lancet Lond. Engl.*, vol. 395, no. 10223, pp. 514–523, 15 2020, doi: 10.1016/S0140-6736(20)30154-9.
[64] X. Lu *et al.*, “SARS-CoV-2 Infection in Children,” *N. Engl. J. Med.*, Mar. 2020, doi: 10.1056/NEJMc2005073.
[65] CDC MMWR, “Coronavirus Disease 2019 in Children — United States, February 12–April 2, 2020,” *MMWR Morb. Mortal. Wkly. Rep.*, vol. 69, 2020, doi: 10.15585/mmwr.mm6914e4.
[66] E. Livingston and K. Bucher, “Coronavirus Disease 2019 (COVID-19) in Italy,” *JAMA*, Mar. 2020, doi: 10.1001/jama.2020.4344.
[67] WHO Africa, “Situation reports on COVID-19 outbreak, WHO AFRO region,” *WHO | Regional Office for Africa*. https://www.afro.who.int/health-topics/coronavirus-covid-19 (accessed May 17, 2020).

[68] S. Garg, “Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 — COVID-NET, 14 States, March 1–30, 2020,” *MMWR Morb. Mortal. Wkly. Rep.*, vol. 69, 2020, doi: 10.15585/mmwr.mm6915e3.
[69] Y. Dong *et al.*, “Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China,” *Pediatrics*, Mar. 2020, doi: 10.1542/peds.2020-0702.
[70] FDA, “Coronavirus (COVID-19) Update: FDA Issues Emergency Use Authorization for Potential COVID-19 Treatment,” *FDA*, Apr. 05, 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment (accessed May 17, 2020).
[71] H. Chen *et al.*, “Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records,” *Lancet Lond. Engl.*, vol. 395, no. 10226, pp. 809–815, 07 2020, doi: 10.1016/S0140-6736(20)30360-3.
[72] J. Cai *et al.*, “A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features,” *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, Feb. 2020, doi: 10.1093/cid/ciaa198.
[73] W. Wang *et al.*, “Detection of SARS-CoV-2 in Different Types of Clinical Specimens,” *JAMA - Journal of the American Medical Association*. American Medical Association, 2020, doi: 10.1001/jama.2020.3786.
[74] Z. M. Chen *et al.*, “Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus,” *World Journal of Pediatrics*. Institute of Pediatrics of Zhejiang University, 2020, doi: 10.1007/s12519-020-00345-5.
[75] A. Tang *et al.*, “Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China,” *Emerg. Infect. Dis.*, vol. 26, no. 6, Jun. 2020, doi: 10.3201/eid2606.200301.
[76] COLSON Philippe *et al.*, “Children account for a small proportion of diagnoses of SARS-CoV-2 infection and do not exhibit greater viral loads than adults.,” *2020*. https://www.mediterranee-infection.com/wp-content/uploads/2020/03/Ms_Raoult_SARS-CoV-2_Age_Mar2020_vL.pdf (accessed Apr. 09, 2020).
[77] K. Danis *et al.*, “Cluster of coronavirus disease 2019 (Covid-19) in the French Alps, 2020,” *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, Apr. 2020, doi: 10.1093/cid/ciaa424.
[78] Y. Dong *et al.*, “Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China.,” *Pediatrics*, Mar. 2020, doi: 10.1542/peds.2020-0702.
[79] E. Livingston and K. Bucher, “Coronavirus Disease 2019 (COVID-19) in Italy.,” *JAMA*, Mar. 2020, doi: 10.1001/jama.2020.4344.
[80] A. Ayouba *et al.*, “Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human Coronaviruses SARS-CoV and MERS-CoV,” *J. Clin. Virol.*, p. 104521, Jun. 2020, doi: 10.1016/j.jcv.2020.104521.
[81] F. B. Piel, M. H. Steinberg, and D. C. Rees, “Sickle cell disease,” *New England Journal of Medicine*, vol. 376, no. 16. Massachussetts Medical Society, pp. 1561–1573, Apr. 20, 2017, doi: 10.1056/NEJMra1510865.
[82] WHO, “Unity Studies: Early Investigation Protocols,” *2020*. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/early-investigations (accessed Apr. 09, 2020).
[83] G. S. Collins, J. B. Reitsma, D. G. Altman, and K. G. M. Moons, “Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD): The TRIPOD Statement,” *Ann. Intern. Med.*, vol. 162, no. 1, p. 55, Jan. 2015, doi: 10.7326/M14-0697.

21. APPENDICES

21.1. APPENDIX 1: Protocol summary

Clinical trial ID number:

Title: DEVELOPMENT OF A DIAGNOSTIC PREDICTION SCORE FOR TUBERCULOSIS IN HOSPITALIZED CHILDREN WITH SEVERE ACUTE MALNUTRITION

Short title: TB-Speed SAM

Coordinating investigators: Dr Olivier Marcy, Dr Maryline Bonnet, Dr Eric Wobudeya

Participating countries: Uganda, Zambia

Primary Objective:

- To develop a diagnostic prediction score for TB in hospitalized children with SAM

Secondary Objectives:

1. To assess the prevalence of TB among hospitalized children with SAM
2. To describe the symptoms and clinical characteristics of TB disease in hospitalized children with SAM
3. To develop a first-step screening prediction score to identify children with presumptive TB among hospitalized children with SAM
4. To propose a stepwise diagnostic algorithm based on the score(s) developed
5. To assess the diagnostic performance and the added value in a diagnostic prediction score for TB in hospitalized children with SAM of the following tests:
    - Ultra performed on one NPA and one stool sample
    - Chest radiography features as assessed by the simplified TB-Speed CXR reading tool
    - Abdominal ultrasound
    - QuantiFERON®-TB Gold In-Tube (QFT) IGRA
    - Monocyte-to-lymphocyte ratio (MLR)
    - CRP
6. To assess the feasibility of collecting NPA and stool in children with SAM
7. To assess the safety and tolerability of NPA collection in children with SAM
8. To assess mortality and weight gain at 6 months in children with SAM, with or without anti-TB treatment
9. To assess the effect of bacteriological features (Xpert and/or culture-confirmed TB) and other key patient characteristics (age, HIV status, initial severity markers, percentage weight gain, CXR features) on TB treatment outcome
10. To evaluate the cost effectiveness of implementing the new diagnostic prediction score for TB treatment compared to the estimated effect of the standard of care in children with SAM.

Primary endpoint:

- i) Sensitivity of the score obtained using predicted probability cut-off with the prediction model for the diagnosis of TB, defined as either confirmed or unconfirmed using the updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis (see section 3.3)
  ii) Specificity of the score obtained using predicted probability cut-off with the prediction model for the diagnosis of TB

Secondary endpoints:

1. Proportion of confirmed and unconfirmed TB in the study population
2. Clinical (symptoms, anthropometric measures, physical signs), laboratory (bacteriological, haematological, immunological signs) and radiological features (chest X-ray and abdominal US) of children with tuberculosis (confirmed and unconfirmed)
3. Sensitivity and specificity of the score obtained using predicted probability cut-off with the screening prediction model for the identification of children with presumptive TB requiring further diagnostic evaluation
4. Estimated time to TB treatment decision in hospitalized children with SAM, with and without presumptive TB based on the first-step screening prediction score
5. Diagnostic accuracy measures (Sensitivity, specificity, negative and positive predictive value) of the different tests evaluated for the diagnosis of TB (Ultra performed on NPA and stools, CXR, abdominal ultrasound, QFT, MLR, CRP) and AUROC of diagnostic prediction models with and without the different tests results
   Diagnostic accuracy of Ultra performed on NPA and stool samples will also be estimated against a specific microbiological reference standard including Ultra and mycobacterial culture from gastric aspirates (GAs)
6. Feasibility of NPA and stool specimen collection: proportion of children with samples collected as per study protocol
7. i) Safety of NPA collection procedure: adverse events (AEs) collected by study nurses (vomiting, nose bleeding, low oxygen saturation, respiratory distress)

ii) Tolerability of NPA collection procedure: discomfort/pain/distress assessed from the child (Wong-Baker face scale), by the parents (visual analog scale), by the nurses (FLACC behavioural scale) (quantitative assessment) measured in a subset of children.

8. i) Mortality at 6 months, with or without TB treatment
   ii) Percentage weight gain, WHZ at 6 months
9. TB treatment outcomes as defined per WHO recommendations
10. Incremental cost-effectiveness ratio (ICER)

Study design:

- Multicentric, prospective diagnostic cohort study aiming to assess several diagnostic tests that could result in the development of a score and algorithm for TB treatment decision in hospitalised children with SAM
- **Implementing sites**: 3 tertiary healthcare hospitals in 2 countries with high and very high TB incidence: Uganda and Zambia
- **Methodology:**
- The diagnostic strategy will include an initial clinical, radiographic and bacteriological evaluation. For the purpose of the study, additional diagnostic methods will be evaluated including abdominal ultrasonography, QFT IGRA, MLR, CRP as well as alternative sample collection methods (NPA, stool samples). MLR, CRP, QFT, and abdominal ultrasound findings will be made available to the clinicians.
- TB diagnosis will be made according to national TB guidelines. Attending clinicians, i.e. not members of the study, will make the final decision to initiate TB treatment according to current National Guidelines.
- At the end of the study, children will be retrospectively classified as confirmed, unconfirmed, or unlikely TB, using the updated version of the Clinical Case Definition for Classification of Intrathoracic Tuberculosis.
- Using logistic regression, we will develop a score for TB diagnosis in hospitalized children with SAM and, if possible, an initial symptom-based screening step to identify children with presumptive TB.
- Both scores will be internally validated using resampling and will be incorporated in a stepwise algorithm to guide practical implementation of the screening and diagnosis process.
- **Follow-up**: children will be followed up for 6 months upon enrolment, regardless of their TB diagnosis with protocol visits at day 0 (TB diagnosis visit), day 15, month 1, 2, 3, and 6.

Sample size: 720 children <5 years old with WHO-defined severe acute malnutrition

Inclusion criteria:

- Children aged < 5 years
- Severe acute malnutrition defined as weight-for-height Z score (WHZ) < -3 standard deviation (SD) or mid-upper arm circumference (MUAC) < 115 mm or clinical signs of bilateral pitting oedema in children aged <5 years
- Hospitalized per hospital clinician's decision
- Parent/guardian informed consent

Non-inclusion criteria: Ongoing TB treatment or history of intake of anti-TB drugs in the last 3 months.

Diagnostic strategy:

- The diagnostic strategy will include:
  - Assessment of household TB contact history
  - Medical history and duration of symptoms in the previous 4 weeks
  - Physical examination including vital signs
  - Clinical, anthropometric and biochemical assessment of malnutrition
  - Clinical assessment for other non-dietary causes of malnutrition, including comorbidities like HIV
  - A digitalized CXR (standard anteroposterior and lateral view)
  - Ultra performed on NPA and stool samples, as well as on the first of the two GAs collected
  - Mycobacterial culture performed on two GAs
  - Abdominal ultrasonography to assess the presence of intra-abdominal lymphadenopathies or ascites
  - MLR
  - QFT
  - CRP
- TB diagnosis will be made according to national TB guidelines.

Trial agenda:

- First inclusion: 31/12/2021
- Inclusion period: 26 months
- Duration of follow-up for each participant once enrolled: 6 months
- Last visit of the last patient: 30/06/2022
- Overall duration of the study (from the first inclusion to the last visit): 32 months

Statistical analysis (primary endpoint):.

We will use logistic regression to develop diagnostic prediction models for TB. We will handle missing data using complete-case analysis and will consider multiple imputation. We will include as candidate predictors

characteristics used in previous childhood TB scoring systems and algorithms, characteristics previously described as associated with TB in children with SAM, as well as QFT and abdominal ultrasonography results. We will test various duration of symptoms (>2, >3, >4 weeks) in the models and select the one with the best Akaike Information Criterion (AIC). Alternatively automated LASSO method will be used to select predictors among all study data available for the model.
We will develop different models integrating all predictors, and excluding one at a time tests that may not be available in all resource limited settings such as QFT and abdominal ultrasonography. Final models will be obtained by stepwise backward selection. We will perform internal validation using bootstrap resampling. AUROCs will be compared to select the models with the best discriminative ability and parsimony. We will identify the predicted probability cut-off reaching a sensitivity ≥80%.
As sensitivity analysis, the diagnostic performance of the score obtained will be assessed in the different countries, in children with or without HIV-infection to identify potential heterogeneity in score performances.
Finally we will propose to use the score obtained in a stepwise diagnostic algorithm, including the possible first-step screening prediction score identifying children with presumptive TB.

Expected results:
Development of a treatment-decision algorithm for the diagnosis of TB in hospitalized children with SAM, based on easily collected clinical features, chest X-Ray, Xpert MTB/RIF, and abdominal ultrasonography. Such a prediction score improving TB diagnosis and shortening time to treatment initiation would be a key benefit in children with SAM.

21.2. APPENDIX 2: Description of study sites

UGANDA

- **Mulago National Referral Hospital** is the main National Regional Hospital for the entire country and a teaching hospital. The Mulago Paediatrics Unit has a bed capacity of 250 with a dedicated pneumonia ward of 40 beds. The unit admits between 150-200 children with severe pneumonia every month. The Nutrition Ward admits children with severe acute malnutrition exclusively, with an average of 100 children per month. Paediatric HIV services are offered by the Baylor College Center of Excellence Uganda. The center has up to 5,000 children under active care.

ZAMBIA

- **The University Teaching Hospital** is the national referral hospital in Zambia, serving a population of 2 million in the capital city of Lusaka. It offers specialized health care, research, and medical training through the University Of Zambia School Of Medicine. The Department of Paediatrics and Child Health is a 352-bed hospital attending to 35,000 children attends annually, and housing the Children with HIV in Africa, Pharmacokinetics and Acceptability/adherence of Simple antiretroviral regimen (CHAPAS) project site established in 2001. The hospital has a dedicated HIV treatment and care center with a cohort of 2,413 patients. It also holds a Nutritional Rehabilitation Unit for children with severe acute malnutrition requiring hospitalization. The Research Clinic is now a well-established research site, which has participated in a number of multicenter clinical trials. The study site is currently led by a Principal and co-Investigator and has a staff of 15 including doctors, research nurses, data and other support staff all of whom have undergone GCP training.
- **Arthur Davison Children Hospital** is a public tertiary care hospital and is the only standalone paediatric hospital in the country. It is situated in Ndola, the provincial capital of the Copperbelt Province and serves as a referral hospital for the Northern part of Zambia. The Copperbelt province is the second most populated region after Lusaka and has the highest prevalence of TB in the country. The hospital has a 250-bed capacity and attends to about 19,000 children per year.

21.3. APPENDIX 3: Summary procedure for nasopharyngeal aspirates collection

NPA consist in the collection of 2-5 ml of throat contents through a catheter tube connected to a mucus aspirator. The procedure is performed in a child in supine position on his/her back or side, or sitting on his family member/guardian's lap. In order to avoid child injury due to movement, young children are wrapped in a piece of cloth, and the child's head id hold throughout procedure.

After connecting a mucus extractor to the suction pump and catheter, the suction pressure is adjusted. Recommended pressure and catheter size are based on the child's age as follows: in children aged < 1 year, 8 CH catheter with 80-100 mmHg (0.10 bar) suction pressure; in children aged 1 to 10 years, 8 CH catheter with 100-120 mmHg (0.15 bar) suction pressure; and in children aged >10 years, 10 CH catheter with 120-150 mmHg (0.20 bar) suction pressure. After measuring the length of tube necessary to reach the posterior pharynx, equal to the distance between the tip of the nose and the external opening of the ear, the catheter is inserted into the child's nose, without applying suction, along the nostril floor to the posterior pharyngeal wall.

This usually induces cough and sputum expectoration that can be aspirated by applying suction and slowly withdrawing catheter, using a rotating movement, without pushing the catheter forward while aspirating to reduce the risk of local trauma. The catheter should remain in nasopharynx for a minimal period of time, not to exceed 10 seconds.

This procedure should aspirate 2 to 5 ml of secretions. If the volume is not reached by the first aspiration, the procedure is repeated in the other nostril. This procedure is not repeated more

than twice. After recapping and cleaning of the specimen container with alcohol/chlorhexidine to prevent cross-infection, and appropriate labeling, the specimens should be transported to the laboratory within 4 hours.

The entire procedure is performed under peripheral oxygen saturation monitoring with an oximeter.

21.4. APPENDIX 4: Summary procedure for the preparation of stool samples for Xpert MTB/RIF Ultra

(Subject to further modifications)

Stool samples are prepared for Ultra testing by emulsification of 0.5 g of material in Sheather's solution, filtering through funnel gauze and centrifugation.

Sheather's solution is prepared by dissolving 454 g of sucrose in 355 mL of distilled water over low-heat on a stove. After autoclaving for 15 min at 110°C, 10 mL aliquots of this solution are prepared in sterile 15 ml Falcon tube and kept at 4° C to prevent mold contamination.

Stool samples are processed by adding 10 mL of the 50% Sheather's solution to 0.5 g of fresh stool specimen or frozen stool specimen thawed at room temperature into a 15 ml Falcon tube, emulsifying stool manually with two wooden sticks, and vortexing for 30 seconds. The emulsion obtained is poured through funnel-gauze into a new 15 mL Falcon tube, and centrifuged at 100g for 1 minute (no brake). After careful removal of the tube from the centrifuge to avoid disturbing the suspension, 0.5 mL of suspension is retrieved from the top of the specimen and added to 1.8 mL of Xpert MTB/RIF Ultra Sample Reagent, shaken vigorously 10 - 20 times, and incubated for 15 minutes at room temperature. After 5 to 10 minutes of incubation, the specimen is shaken again vigorously 10 to 20 times. The specimen obtained is then directly tested with Ultra.

21.1. APPENDIX 5: Covid-19 suspect case definition, testing indications and management of Covid-19 in children per country

Country	Covid-19 suspect case definition	Testing indication for children with severe pneumonia	National recommendations for sample collection	Treatment guidelines (including paediatric if any) (subject to modifications based on the latest national guidelines)	Management/ isolation of paediatric cases
Uganda	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Respiratory distress management, fluid management, prevention of complications, ATB. Antiviral treatment if pneumonia: CQ + azm + high-dose vitamin C No specific paediatric guidelines	Centralised. Covid units at Mulago Hospital, Kampala, and Mbarara Regional Hospital
Zambia	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Respiratory distress management, fluid management, prevention of complications, ATB. Antiviral treatment: HCQ + azm, AND LPV/r for severe cases Children: idem	Centralised. Lusaka UTH is a transfer centre only.

ATB: antibiotics; azm: azithromycin; CQ: Chloroquine; HCQ: Hydroxychloroquine; LPV/r: lopinavir/ritonavir; INF: interferon

** WHO case definition (subject to modifications based on the latest published definitions)*

***Suspect case**:*

- A patient with acute respiratory illness, AND a history of travel to or residence in a location reporting community transmission of Covid-19 disease during the 14 days prior to symptom onset;
OR
- A patient with any acute respiratory illness AND having been in contact with a confirmed or probable Covid-19 case in the last 14 days prior to symptom onset;
OR
- A patient with severe acute respiratory illness requiring hospitalization AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

***Contact case:** A person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:*

- Face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 minutes;
- Direct physical contact with a probable or confirmed case;
- Direct care for a patient with probable or confirmed Covid-19 disease without using proper personal protective equipment; OR
- Other situations as indicated by local risk assessments.

21.2. APPENDIX 6: Information sheet and informed consent form

21.3. APPENDIX 7: Copy of the insurance policy

21.4. APPENDIX 8: Copy of the Inserm CEEI and WHO ERC approvals

21.5. APPENDIX 9: Copy of the Competent Authority's authorization

21.6. APPENDIX 10: List of required administrative and/or ethical clearance per country

(Subject to further completion)

COUNTRY	Ethical clearance	Administrative research clearance	Institutional Review Board
UGANDA	- Uganda National Council of Science and Technology (UNCST) - Joint Clinical Research Centre Research & Ethics Committee (JCRC REC/IRB)		
ZAMBIA	National Research Ethics Authority (NREA)	Administrative Research Authorization from the Ministry of Public Health	University of Zambia Biomedical Research Ethics Committee