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Rapid and Accurate Diagnosis of Pediatric Tuberculosis Disease: A Diagnostic Accuracy Study for Pediatric Tuberculosis

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Introduction: An estimated 1.2 million children develop tuberculosis (TB) every year with 240,000 dying because of missed diagnosis. Existing tools suffer from lack of accuracy and are often unavailable. Here, we describe the scientific and clinical methodology applied in RaPaed-TB, a diagnostic accuracy study.

Methods: This prospective diagnostic accuracy study evaluating several candidate tests for TB was set out to recruit 1000 children <15 years with presumptive TB in 5 countries (Malawi, Mozambique, South Africa, Tanzania, India). Assessments at baseline included documentation of TB signs and symptoms, TB history, radiography, tuberculin skin test, HIV testing and spirometry. Respiratory samples for reference standard testing (culture, Xpert Ultra) included sputum (induced/spontaneous) or gastric aspirate, and nasopharyngeal aspirate (if <5 years). For novel tests, blood, urine and stool were collected. All participants were followed up at months 1 and 3, and month 6 if on TB treatment or unwell. The primary endpoint followed NIH-consensus statements on categorization of TB disease status for each participant. The study was approved by the sponsor's and all relevant local ethics committees.

Discussion: As a diagnostic accuracy study for a disease with an imperfect reference standard, Rapid and Accurate Diagnosis of Pediatric Tuberculosis Disease (RaPaed-TB) was designed following a rigorous and complex methodology. This allows for the determination of diagnostic accuracy of novel assays and combination of testing strategies for optimal care for children, including high-risk groups (ie, very young, malnourished, children living with HIV). Being one of the largest of its kind, RaPaed-TB will inform the development of improved diagnostic approaches to increase case detection in pediatric TB.

Key Words: tuberculosis, children, diagnosis, diagnostic accuracy study

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L.O and M.N shared first authorship to this article.

The project received ethical approval from the Ethics commission of the Ludwig Maximilian University of Munich (Projekt Nr: 18-205) and ethics committees of participating sites as follows: The College of Medicine and Research Ethics Committee (COMREC) (REF: P.10/18/2495) in Malawi; Comité Nacional de Bioética para a Saúde (CNBS) (REF- 559/CNBS/18) in Mozambique; The Human and Research Ethics Committee, Faculty of Health Sciences, University of Cape Town (REF: 429/2018) in South Africa; The Ethics Committee of the National Institute of Medical Research in Tanzania (REF: NIMR/HQ/R.8a/Vol IX/2855); and the Institutional Review Board of Christian Medical College, Vellore, India (REF: 11,638). Parents or legal guardians of participating children provided signed written informed consent for having children participate in the study.

Data generated from this project will be made available on request by contacting Dr Heinrich Norbert through e-mail: Norbert.Heinrich@med.uni-muenchen.de.

The study and its protocol was designed by N.H., L.O., M.H., H.J.Z., M.N., N.E.N., I.S., C.K., J.S.M., V.P.V., S.M.G., and E.L.C., while the clinical and laboratory procedures were designed and implemented by C.G., H.J.Z., T.M., P.N., A.T., D.B., and R.S. Additional methodological contribution was given by S.M.G., R.S., U.E., S.H.S., and J.M. L.O., M.N., N.H., and I.S. drafted the article. All authors contributed to revisions of the manuscript, all authors reviewed and agreed with the final version.

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Tuberculosis (TB) in children remains a significant cause of morbidity and mortality worldwide. In 2020, of the estimated 1.3 million TB deaths, 208,000 (16%) occurred in children below 15 years of age and over 80% of these deaths were in young children (<5 years).[1,2] Once diagnosed, TB treatment outcomes are excellent with a mortality of <1%; hence, undiagnosed TB cases account the vast amount of pediatric TB deaths.[2] The 2018 United Nations General Assembly High Level Meeting on the Fight Against TB committed to diagnosing and treating 3.5 million children with TB by 2022[3]; however, over half of the incident TB cases in children are still not diagnosed nor reported annually.[1]

Addressing the diagnostic challenge that pediatric TB poses is central and critical to progress in achieving targets for prevention, detection and treatment.[3] Children often present with nonspecific clinical and radiological findings.[4,5] Obtaining respiratory samples for microbiological confirmation is difficult in children, and the paucibacillary nature of pediatric TB results in cases being missed.[1] Thus, sample collection is often not attempted and most children are initiated on TB treatment based on clinical grounds.[6,7] In clinical studies, microbiological confirmation of TB is established in only 2–50% of evaluated children, highlighting the need for more sensitive and accurate diagnostics, limiting misdiagnosis and enabling rapid initiation of appropriate therapy.[8–11]

Recently, there has been increased attention towards evaluating novel diagnostics for pediatric TB. In 2014, the World Health Organization (WHO) target product profiles (TPPs) were developed to define and align priority needs of the end users with the optimal performance characteristics of new tests that should be met by developers.[12] The development of TPPs coupled with increased advocacy has led to several new tests being developed with potential to become game changers for pediatric TB diagnostics.[13]

In this article, we describe the methodology of RaPaed-TB, a multicountry consortium set up to evaluate novel TB diagnostic tests and sample collection strategies in children investigated for TB. In the absence of a reliable reference standard for pediatric TB, a rigorous methodological approach was chosen, and a highly standardized diagnostic workup was implemented across sites. The primary objective was to evaluate diagnostic accuracy of novel candidate tests and accessible sample types for diagnosing TB in children <15 years as well as key subgroups of children in India and 4 African countries. The study also aimed to assess accuracy and efficiency of combining various novel tests and sample types in algorithms composed of screening (ie, rule-out) tests together with confirmatory tests. The goal was to identify candidate tests that meet the TPPs for impact on TB diagnosis in children globally.

MATERIALS AND METHODS

Study Partners and Setting

The RaPaed-TB consortium included several academic and research institutions, stakeholders and industry partners. The consortium was coordinated by researchers from the Division of Infectious Diseases and Tropical Medicine at the University of Munich (LMU), Germany. The recruiting sites were hospitals or tertiary pediatric care centers in 5 high-TB burden countries, namely India, Malawi, Mozambique, South Africa and Tanzania. In India, the study center was located at the Christian Medical College, Vellore (Tamil Nadu, India), and recruitment was based at the Pediatric Infectious Diseases Department, including inpatients and outpatients. In Malawi, the Kamuzu University of Health Sciences and The Malawi-Liverpool-Wellcome Trust clinical research program oversaw the participant recruitment in the Queen Elizabeth Central hospital, the only major public health facility for Blantyre city and the teaching hospital for the University of Health Sciences. In Mozambique, the Instituto Nacional de Saúde (INS) recruited at 2 research units, in Mavalane Health Centre (City of Maputo) and in Machava General Hospital (Province of Maputo), primarily recruiting outpatients. In South Africa, recruitment was conducted at Red Cross War Memorial Children's Hospital, a major pediatric tertiary hospital and referral center for the Western Cape. In Tanzania, the study was led by the National Institute of Medical Research, Mbeya Centre, and participant recruitment was conducted at the outpatient and inpatient department of the Mbeya Regional Referral hospital.

The consortium also included the Foundation for Innovative New Diagnostics (FIND), a not-for-profit organization based in Geneva, Switzerland, which facilitates the development of novel diagnostics for diseases of poverty, and brings together country partners, academia, industry partners and public funders, and ensures access to promising novel diagnostic tests. Representatives of the coordinating institute (LMU), principal investigators of participating sites, partners and invited scientists formed the steering committee of the consortium.

Study Design

RaPaed-TB was a prospective single-gate diagnostic accuracy study evaluating several novel index tests and diagnostic approaches in children <15 years with presumptive TB. The aim was to recruit 1000 children investigated for TB disease and follow them up during TB treatment or, if not diagnosed with TB, until symptom resolution (Fig. 1). Participant recruitment was conducted between January 21, 2019, and July 1, 2021, analysis of laboratory test results is ongoing.

Participant Eligibility

Eligibility was assessed and recorded following the criteria listed in Table 1. Before any study-specific procedure, signed written consent by the parent or legal guardian and assent by the child was obtained. The threshold for child assent was guided by local institutional review boards. In case of illiteracy, witnessed oral consent/assent was obtained.

Recruitment

Recruitment of children was conducted in participating health facilities, with collaboration from the National TB control Programs (NTPs). In communities, recruitment was enhanced by individual and community awareness through advertisement, posters, radio announcements, community sensitization and so on depending on local legal requirements and approval by ethics committees.

Study Procedures

The study consisted of a baseline visit and follow-up visits at month 1 and month 3, if children were started on TB treatment or clinically unwell, an additional visit at month 6 was performed. The sites in Mozambique enrolled participants in a spirometry substudy with additional visits at months 9 and 12. Study procedures for each study visit were conducted according to the schedule of events (Table 2). While all children were followed for the time periods as outlined above, treatment (both preventive as well as for TB disease) was provided by the local NTPs.

Study Procedures at Baseline

After informed consent, study-specific diagnostic procedures were performed and documented. Data from standard diagnostic procedures were recorded in electronic Case Report Forms (eCRFs). The baseline visits included collection of demographic information,

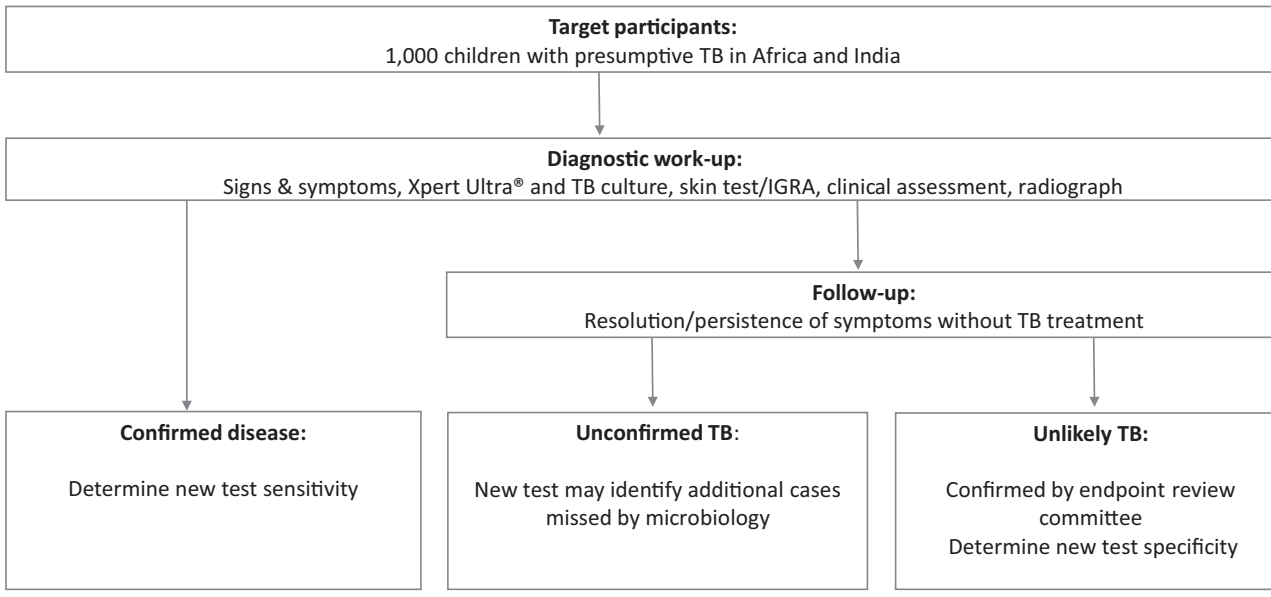

FIGURE 1. Diagnostic workup and clinical case definitions in RaPaed-TB.

TABLE 1. Inclusion and Exclusion Criteria for RaPaed-TB Study

Inclusion Criteria	Exclusion Criteria
1) **Consent and Assent (if applicable):** signed written consent/assent, or witnessed oral consent/assent in the case of illiteracy, before undertaking any study-specific activity. **Of the following, either criterion 2), OR criterion 3), or both, has to be met:** 2) **Confirmation of TB disease:** microbiological confirmation of TB disease by positive smear AND/OR culture AND/OR PCR (e.g., Xpert MTB/RIF®); e.g., in a nonstudy health facility **AND/OR** 3) **Signs and Symptoms:** suspicion of TB disease (one or more criteria): a. Chest radiograph suggestive of TB: cavity AND/OR hilar/mediastinal lymph node enlarged AND/OR miliary pattern b. Weight loss** or failure to thrive within the previous 3 months that, in the investigator's opinion, is not solely because of inadequate feeding; or to another non-TB cause. c. Any cough combined with: • Loss of weight**; • Evidence of *Mycobacterium tuberculosis* infection: TST AND/OR IGRA positive d. Cough alone: persistent unremitting cough duration of ≥ 14 days e. Repeated episodes of fever within 14 days not responding to course of antibiotics AND positive TST or IGRA, (for malaria endemic areas: AND after malaria has been excluded by at least a negative rapid test) f. Signs & symptoms of extrapulmonary TB: • Unilateral nonpainful lymph node(s) visibly enlarged ≥ 1 month; • Gibbus (especially of recent onset) • Nonpainful enlarged joint • Pleural effusion • Pericardial effusion g. CSF examination findings in line with TB meningitis with at least elevated protein and low glucose (in relation to serum glucose); OR signs and symptoms in line with TB meningitis/CNS TB if lumbar puncture is contraindicated, in the view of the investigator at least one of the following 2: • palsy of oculomotoric nerves of recent onset • focal neurological symptoms indicating elevated intracranial pressure OR CNS lesions, of recent onset AND/OR at least two of the following less-specific signs of TB meningitis/CNS TB (for malaria endemic areas: AND a negative malaria rapid diagnostic test*): • Lethargy • Convulsion • Meningism (neck stiffness) • Headache	1) Critical condition (if study procedures seem like an undue risk to the participant's life), such as hypovolemic shock or clinically relevant anemia (tachypnoea, tachycardia) 2) Body weight less than 2 kg 3) Children of 15 years of age or more 4) Are currently receiving anti-TB drug(s): ideally, eligible participants should not have received any anti-TB treatment. 5) In exceptions, up to three daily doses given since treatment start before first study blood draw are acceptable for study inclusion

CNS indicates central nervous system; CSF, cerebrospinal fluid; IGRA, interferon-gamma release assay; TST, tuberculin skin test.

TABLE 2. Schedule of Visits and Assessments

Schedule of Events	Visit 1: Day 1	Visit 1: Days 2 and/or 3	Visit 2: Week 4/ Day 28± 4 days	Visit 3: Week 12/ Day 84 ± 7 days	Visit 4: Week 26/Day 162 ± 14 days (if on TB treatment or unwell at Month 3)	Visit 5: End of Treatment (if EOT is later than Week 26 + 14 days)	Visit 6[A]: Week 40/ Day 280 ± 14 days (Follow-up spirometry)	Visit 7[A]: Week 52/ Day 365 ± 14 days (Follow-up spirometry)
Clinical Assessment								
Informed consent	X							
Questionnaire		X						
Physical examination & Symptoms		X	X	X	X	X	$_{x}$A	$_{x}$A
Chest radiograph (other radiology if indicated)		X						
Treatment compliance			X	X	X	X		
Spirometry[A]		$_{x}$A		$_{x}$A	$_{x}$A	$_{x}$A	$_{x}$A	$_{x}$A
TB Reference Standard Microbiology								
Sputum/sample for microbiology[B]	X Culture (LJ[B] + MGIT) Pellet: GeneXpert Ultra®	X Culture (LJ[B] + MGIT) Pellet: cryostorage	1 X[C] *(if sputum produced spontaneously, and initial bacteriology positive)*	1 X[C]	1 X[C] *(if sputum produced spontaneously, and initial bacteriology positive)*	1 X[C] *(if sputum produced spontaneously, and initial bacteriology positive)*		
Separate Sputum for storage	X *(if possible during scheduled visits)*							
Children ≤ 5yr: nasopharyngeal aspirate	X (Xpert MTB/RIF Ultra®)							
Laboratory (blood)								
Routine diagnostics								
Incl. hematology/storage/ EDTA and biochemistry, if applicable		X	$_{x}$D	$_{x}$D	$_{x}$D	$_{x}$D		
Malaria testing								
HIV serology[F]	X/ Cd4 if HIV positive							
Tuberculin Skin Test[H]		X						
Experimental tests								
Serum for storage		X	X	$_{x}$D	$_{x}$D	$_{x}$D		
PaxGene® RNA tube		X	X	$_{x}$D	$_{x}$D	$_{x}$D		
TAM-TB[H]		X	X	$_{x}$D	$_{x}$D	$_{x}$D		
IGRA[H]		$_{x}$A	$_{x}$D	$_{x}$D	$_{x}$D	$_{x}$D		
Total maximum volume (blood)					*According to body weight* [I]			
Laboratory (urine)								
Urine analysis by dipstick		X	$_{x}$D	$_{x}$D	$_{x}$D	$_{x}$D		
FujiFilm® (LAM)		1 ml	1 ml[D]	1 ml[D]	1 ml[D]	1 ml[D]		
Uri-TB direct (LAM)		20 ml	20 ml[D]	20 ml[D]	20 ml[D]	20 ml[D]		
Total maximum volume (urine)[J]		**21 ml**	**21 ml[D]**	**21 ml[D]**	**21 ml[D]**	**21 ml[D]**		
Laboratory (stool)								
Stool (Xpert MTB/RIF Ultra®)		X						
Stool (storage)		X						

Study Schedule of Events

Please note: sample volumes between individual tests may vary, however maximum volumes given will not be exceeded. The standard diagnostic schedule is a nonbinding recommendation and based on current practice in study sites.

[A].Optional visits/procedures for substudies: in a subset of children and not in all sites.

[B].Sputum or induced sputum according to patient age and center preference. Other diagnostic samples; e.g., bronchial secretion, or fine needle aspirate biopsy according to decision of attending clinician and best medical practice. Standard microbiology assessments include culture in MGIT and LJ media, smear, PCR (GeneXpert Ultra®). LJ culture may be omitted in centers with a low contamination rate if agreed with the sponsor.

Cultures positive for AFBs should be analyzed by HAIN LPA for species, and molecular drug resistance testing. Isolates are to be cryopreserved in glycerol.

[C].Sputum culture after visit 1 only in patients on TB treatment who were sputum positive in any microbiological test initially, and who are able to produce sputum spontaneously.

[D].Samples for assessing treatment response – only to be taken in children who are started on anti-TB treatment; and not at all sites.

[E].Only in malaria endemic settings

[F].To be omitted if HIV status was ever documented positive, or documented negative less than 3 months ago.

[G].Before day 8, only if HIV positive: Cd4 count, HIV viral load.

[H].IGRA: Interferon-gamma release assay. TST: tuberculin skin test.

TST is to be applied **after** blood for TAM-TB is taken, to avoid interaction between TST antigen and TAM-TB.

[I].Maximum blood volumes

[J].Minimum total urine volume should be not less than 10 ml, otherwise repeated collection is advised

IGRA indicates Interferon-gamma release assay; LAM , Lipoarabinomannan assay; LJ, Lowenstein Jensen medium; MGIT, Mycobacterium growth indicator tube; TAM-TB, T-cell activation marker assay.

medical history and physical examination. Clinical investigation included HIV testing, tuberculin skin tests (TST) and chest radiograph or other imaging, if clinically indicated. If not performed before enrollment as part of the screening procedures, TST testing was conducted after blood collection for novel tests following established guidelines.[1] In short, 0.1 mL (2T.E. = 0.04 µg Tuberculin PPD RT23) was applied intradermally and the widest lateral induration was marked with a pen and read within 48–72 hours by 2 independent readers.

Baseline procedures occurred on 2–3 days according to the preference of the center. Participants' demographic, clinical and laboratory data were recorded on paper worksheets and concurrently entered onto eCRFs using OpenClinica software (OpenClinica Version: 3.12; OpenClinica LLC, Waltham, MA, United States). For confidentiality, participant ID numbers were used (ie, pseudonymization) throughout the study.

Study Procedures at Follow-up Visits

At each follow-up visit, the following procedures were performed: TB symptoms assessment, physical examination, radiological assessment and treatment documentation. Collection of respiratory samples and other samples for TB microbiology were performed if clinically indicated. For children on TB treatment, index tests were conducted to evaluate their use for monitoring treatment (Table 2).

Sample Collection

Sample collection was standardized across study sites and included blood, urine, respiratory specimens (sputum/nasopharyngeal aspirate) or gastric aspirate, stool and, if applicable and feasible, extrapulmonary specimens.

For each child, 2 respiratory samples were to be obtained as reference sample. All staff was trained in respiratory sample collection using well-established procedures.[16] Consecutive sputum samples were collected in sterilized, well-lockable labeled sputum container. If possible, spontaneous (ie, voluntarily expectorated) sputum samples were collected; in younger children, sputum induction (ie, at the African sites) or gastric aspirate (ie, at the Indian site) were performed as previously described.[16,17] For sputum induction, children inhaled hypertonic saline (up to 6%) until they started coughing productively. Nasopharyngeal suctioning using a sterile catheter with an appropriate diameter was performed to collect the sample with a mucus trap. In children under 5 years of age, a nasopharyngeal aspirate was collected in addition.

Other specimens for TB investigations, such as pleural fluid, ascites or cerebrospinal fluid (CSF) and fine needle aspiration biopsies or tissue from biopsies, were collected when clinically indicated following local standard operating procedures (SOPs).

Blood samples were collected for evaluation of new tests, such as T-cell activation marker assay (TAM-TB), RNA transcriptomics, and serum for analysis of potential host-biomarkers. Other blood tests for routine hematology and clinical chemistry were performed as clinically indicated to exclude TB or diagnosis of coinfections. Maximum volumes of blood collected depended on the participant's weight in accordance with the WHO recommendations for clinical studies in children.[18] Urine and stool samples were collected into designated containers. Children, guardians, and study staff were instructed to ensure a clean collection of urine samples. To reduce contamination, the study staff was instructed to clean the genitals with water before applying adhesive collection bags in younger and noncompliant children. As comparator, Alere Determine TB-LAM Ag (AlereLAM; Abbott, Palatine, IL, United States) was performed.

TB-specific Laboratory Procedures

All laboratory procedures (apart from specific assays planned in the substudies) were performed at each study site. Each procedure was outlined in the detailed RaPaed-TB laboratory manual and the respective staff were trained thoroughly. At the study laboratory, the standard TB diagnostic tests included Xpert MTB/RIF Ultra assay (Cepheid Inc., United States), liquid (BACTEC Mycobacterial Growth Indicator Tube 960 automated system, Becton Dickinson (BD) Microbiology Systems, Sparks, MD, United States) and solid culture (Löwenstein–Jensen). In case growth was detected, all culture samples underwent further testing, including Ziehl-Neelsen smear to identify acid-fast bacilli. If positive, further speciation was performed using the MPT64 Ag test, an immunochromatographic detection of the mycobacterial protein MPT64.[19] In all patients with positive cultures, at least 1 GenoType MTBDRplus V2 line-probe assay (Hain Lifescience, Germany) was done.

For characterization of MTB strains, sputum samples were stored for mycobacterial DNA extraction and further analysis will be performed centrally using classical molecular MTB typing

TABLE 3. New Assays Being Investigated in RaPaed-TB Study

Name of Assay and Developer	Assay Characteristics	Sample Types	Relevant References
Stool processing kit coupled with Xpert MTB/RIF Ultra	Novel method to process stool (≥0.2 g–1.2 g) specimens for direct detection of MTB using Xpert MTB/RIF Ultra	Stool	Banada, 2016[40] Walters, 2018[41]
Xpert MTB Host Response [MTB-HR] prototype	Analyses messenger RNA (mRNA) expression levels of 3 genes (GBP5, DUSP3, and KLF2) to determine a score for discrimination between active TB and other diseases	Fingerstick blood	Sweeney, 2016[22] Sutherland, 2021[23]
T-cell activation marker assay	Immunodiagnostic test using phenotypic characterization of MTB-specific T-cells. Uses flow cytometry technique	Blood	Portevin, 2014[21] Ahmed, 2018[20]
Uri-TB direct, Karolinska Institutet, Sweden	Detection of lipoarabinomannan, a cell wall constituent of MTB using a lateral flow assay	Urine	Hamasur, 2015[24]
SILVAMP TB-LAM FujiLAM, FujiFilm and FIND	Detection of lipoarabinomannan, a cell wall constituent of MTB using a lateral flow assay	Urine	Broger, 2019[25] Nicol, 2021[26]
Host biomarker signature (University of Cape Town and FIND)	Proteomic signatures of TB disease risk	Blood/plasma	Penn-Nicholson[42]
Host biomarker combination (University of Stellenbosch)	Seven-marker host serum protein signature	Serum/fingerstick blood	Chegou, 2016[43]
Host RNA biomarker (LMU)	16-gene signature to predict TB progression	Blood	Zak, 2016[44]

FIND indicates Foundation for Innovative New Diagnostics; LAM, lipoarabinomannan; LMU, University of Munich.

TABLE 4. Case Definitions by Which the Reviewers' Classifications Are Assessed

Diagnostic Classification	Description of Definition
Confirmed tuberculosis	Bacteriologic confirmation obtained *Requires Mycobacterium tuberculosis to be confirmed (culture or Xpert® MTB/RIF (Ultra®) assay) from at least 1 specimen*
Unconfirmed tuberculosis	Bacteriologic confirmation NOT obtained AND at least 2 of the following: •Symptoms suggestive of TB •CXR consistent with TB •Recent exposure or immunologic evidence of MTB infection (TST and/or IGRA positive) •Positive response to TB treatment *Requires documented positive clinical response on tuberculosis treatment - no time duration specified* oWith *M. tuberculosis* infection *Immunologic evidence of M. tuberculosis infection (TST and/or IGRA positive)* oWithout *M. tuberculosis* infection *No immunologic evidence of M. tuberculosis infection*
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND criteria "unconfirmed TB" not met oWith *M. tuberculosis* infection *Immunologic evidence of M. tuberculosis infection (TST and/or IGRA positive)* Without *M. tuberculosis* infection *No immunologic evidence of M. tuberculosis infection*

methods (genotyping) as well as next generation sequencing. This will allow for a detailed classification of circulating MTB lineages in the pediatric study population and the alignment or comparison of sequences with results of rapid molecular tests.

Laboratory Procedures of Novel Tests

The performance of several new tests and possible biomarkers for TB was assessed in different compartments of the body (sputum, blood, urine). Staff conducting the new tests were blinded to the diagnostic classification of the children. An overview of new tests assessed in the RaPaed-TB study is given in Table 3.

Sample Storage

Several samples were stored, including urine, sputum, mycobacterial isolates from positive liquid or solid culture, sputum pellet and blood (PAXgene, EDTA-whole blood, serum; see Table, Supplemental Digital Content 1, http://links.lww.com/INF/E932). Stored specimens will be used for the evaluation of future emerging TB diagnostics, genetic markers and biomarkers, or new diagnostics for other infectious diseases. Consent for storage of samples was obtained at recruitment.

Data Management

Data entry was monitored and reviewed regularly during the course of the study, including comparing source data with entries in OpenClinica. OpenClinica is a web-based Electronic Data Capture software and was used in this study as Clinical Data Management System (CDMS). The subject data was entered by study site personnel directly into electronic Case Report Forms.

Primary Outcome and Clinical Case Definitions

Disease classification followed the clinical case definitions of intrathoracic TB in children as outlined in an National Institutes for Health (NIH) consensus statement.[27] These were adapted to allow for the inclusion of extrapulmonary TB. The primary endpoint of the study was the confirmation/rule-out of TB disease, with the degrees of certainty "confirmed TB," "unconfirmed TB" or "unlikely TB" (Table 4). Children were classified using information on microbiological testing, clinical signs and symptoms suggestive of TB, radiological findings, TB contact history, determination of MTB infection, and treatment response/clinical trajectory. The latter included attendance of visits, treatment compliance, resolution of symptoms, and anthropometrics over time. Allocation into respective diagnostic categories followed the criteria as outlined in Table 4, all datapoints were recorded separately to facilitate in-depth analysis.

External radiologic experts reviewed the baseline chest radiographies of all participants (see Supplemental Digital Content 2, http://links.lww.com/INF/E933, for the datapoints collected). These experts were blinded to the participants' clinical information to prevent bias. For overall diagnostic classification, an independent endpoint review committee (ERC) was set in place to review all children recruited in RaPaed-TB (Fig. 2). A Microsoft Access ERC review tool was used to standardize classification approach between reviewers. Information provided included demographic information, clinical history (ie, TB history, exposure and medication history, baseline symptoms follow-up, physical examination and results of routine TB and non-TB investigations), and the results from the external radiological review, as well as the images themselves. Members of the ERC were chosen based on their clinical experience. Two experts reviewed clinical data from all children and assigned diagnostic categories (confirmed, unconfirmed, or unlikely TB) based on their clinical judgement. In case of disagreement, a third reviewer was consulted, and rule of majority applied.

Sample Size

The study aimed to recruit 1000 participants across 5 sites (200 each) over a 2-year period. The aim was to achieve 25% microbiological confirmation; this approach would allow to enroll at least 250 with confirmed TB across all sites. The number of 250 participants with confirmed TB would allow for detection of

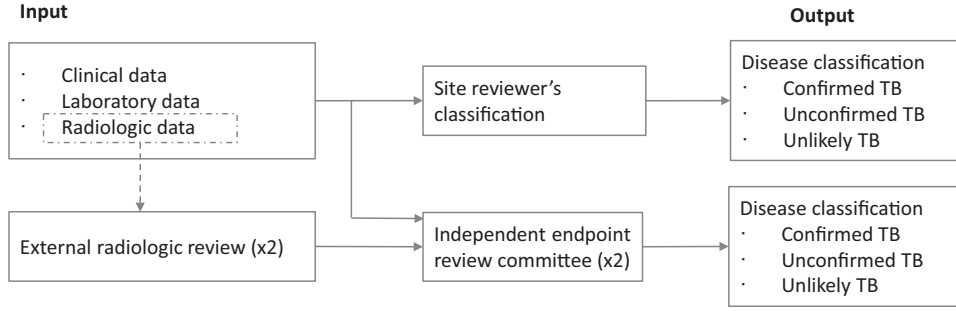

FIGURE 2. Demonstration of the review process among the site investigators and the endpoint review committee.

a sensitivity increase from 62% (Xpert MTB/RIF) to 82%, with more than 90% power at the 95% confidence level. Importantly, this sample of 250 participants with confirmed TB disease would allow for meaningful subgroup analyses within and between age groups, HIV, and nutritional status. For example, assuming HIV coinfection in approximately 40% of the children, and new test sensitivity of 82% in HIV-negative children, the minimum detectable difference to a lower sensitivity in HIV-positive children is 17.7% (absolute sensitivity 64.8%), with a power of 80% at the 95% confidence level. Comparing age groups, at assumed sensitivity of 82% in the age group of 9–14 years (30% of children); the minimum detectable difference in sensitivity to the age group of 0–4 (40% of children) years is –22% (ie, 60% sensitivity in this group).

Ethical Consideration

This study was performed in accordance with the study protocol, the declaration of Helsinki,[28] as well as any other applicable national and other regulatory guidelines. The protocol and the informed consent document used in this study were submitted to the coordinators and all local institutional review board (see Table, Supplemental Digital Content 3, http://links.lww.com/INF/E934).

DISCUSSION

The RaPaed-TB study was 1 of the largest TB diagnostic studies in children, evaluating multiple sampling strategies and novel tests in children investigated for TB with innovative mechanisms of action. In this article, we provide an in-depth description of the study procedures implemented and share the tools, definitions, and approaches chosen to facilitate standardization of methods for diagnostic accuracy studies in children.

In the past, many diagnostic studies for pediatric TB were characterized by heterogeneous methodologies and in the absence of a reliable reference standard, studies were mostly reported using varying case definitions, impeding cross-comparison and pooling of data.[29] The RaPaed-TB study used multiple sites, with standardization of clinical and laboratory procedures, intensive investigations for confirmation of TB disease, and thorough participant follow-up, all ensuring better diagnostic case classification in line with published consensus statements.[27] In addition, standard clinical case definitions were used and only slightly adjusted to include children with extrapulmonary TB. In addition, an independent endpoint review committee provided an extra layer of confidence regarding the diagnostic classification of participants. In this article, we present a through description of the study activities implemented to ensure transparency and standardization of future studies.

The design of the RaPaed-TB study, including screening, sampling, and clinical approaches, was aimed to achieve a high number of microbiologically confirmed children to allow for the generation of reliable and solid diagnostic test accuracy estimates in this well-characterized cohort. Conducting diagnostic studies with an imperfect reference standard results in several challenges. Strict criteria were applied to classify children following published clinical case definitions, which subsequently informed reference standards for diagnostic test accuracy estimates.[27,29,30] Previous projects evaluating novel diagnostics in children often suffered from a relatively low number of microbiologically confirmed children, ranging from 5.2% to 35.6%.[31–35] In RaPaed-TB, thorough sampling of reference samples was implemented with at least 2 respiratory specimens collected, including induced sputum or gastric aspirate. Most of the RaPaed-TB study sites were affiliated with tertiary care centers, which often provide care to children with advanced disease, and with expected high rate of microbiological confirmation for TB. As a result, data generated here are not necessarily representative of those populations undergoing testing in primary health care facilities, where most children investigated for TB present first. However, this approach is needed to generate solid data accuracy estimates on novel tests at this stage of evaluation, which will need to be evaluated in less stringently recruited cohorts in the future.[29]

The WHO declared a high-priority need for new TB diagnostics, particularly for children and extrapulmonary disease.[12] These were outlined in the TPPs for new TB diagnostics. More importantly, a new test should at least be as specific as Xpert MTB/RIF (98%) and at least ≥66% sensitive. In children, diagnostic yield represents both the diagnostic accuracy of a test and the feasibility of obtaining a specimen and is improved by the availability of a specimen such as blood, urine or stool, compared with sputum alone.[36] As diagnosis of pediatric TB in health programs is limited, in part because available sampling and testing strategies are delivered mostly in tertiary-level facilities, the WHO highlighted the requirement for rapid biomarker-based nonsputum tests for TB suitable for children[14] but also for a community-based point of care triage or referral test.[15] Thus, the RaPaed-TB study evaluated primarily noninvasive sampling approaches such as urine, stool and nasopharyngeal aspirate, which would allow TB diagnostic services to be decentralized to lower levels of the health system. Additionally, it would enable reaching more children, similar to other projects focusing on the aspect of expanding access to diagnosis using these easy-to-collect samples in secondary and primary care facilities in low-resource settings.[37–39]

The determination of accuracy of each individual assay in diagnostic studies is only the first step in ensuring that novel assays have an actual impact on patient care. As part of future analysis of the RaPaed-TB study, we will assess whether individual tests being investigated can be combined in algorithms for maximal impact on correct diagnosis of pediatric TB. Furthermore, the RaPaed-TB study served as a platform for substudies, which included an evaluation on lung outcome applying spirometry and extended evaluation of the role of coinfections such as with cytomegalovirus. Finally, within RaPaed-TB, a large repository of samples was created to facilitate future test evaluation within this well-characterized cohort.

As one of the largest diagnostic cohorts in low- and middle-income countries, the RaPaed-TB study will generate important evidence on diagnostic accuracy of promising novel tests for pediatric TB, contribute to new test development using its established biorepository, and contribute to a harmonization of research methodologies.

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