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Stool Xpert MTB/RIF and urine lipoarabinomannan (LAM) for diagnosing tuberculosis in hospitalized HIV-infected children

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Abstract

Background—Tuberculosis (TB) causes substantial morbidity and mortality in HIV-infected children. Sample collection and paucibacillary nature of TB in children makes diagnosis challenging. Rapid diagnostic tools using easily obtained specimens are urgently needed.

Methods—Hospitalized, HIV-infected children ≤12 years enrolled in a randomized controlled trial (NCT02063880) comparing urgent to post-stabilization ART initiation in Kenya underwent TB evaluation. At enrollment, sputum or gastric aspirates (GA) were collected for TB culture and Xpert, stool for Xpert, and urine for lateral flow lipoarabinomannan (LAM). When possible, a second sputum/GA culture was obtained. Stool Xpert and urine LAM performance were compared to reference sputum/GA culture.

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Results—Among 165 HIV-infected children, median age was 24 months (IQR 13–58), median CD4% was 14.3% (IQR 8.9–22.0%), and 114 (69.5%) had severe immunosuppression. Thirteen (7.9%) children had confirmed TB (positive culture and/or Xpert). Sputum/GA Xpert, stool Xpert, and urine LAM sensitivities were 60% (95% CI 26–88%), 63% (95% CI 25–92%), and 43% (95% CI 10–82%), respectively. Specificity was 98% (95% CI 94–100%) for sputum/GA Xpert, 99% (95% CI 95–100%) for stool Xpert, 91% (95% CI 84–95%) for urine LAM. Stool Xpert and urine LAM sensitivity increased among children with severe immunosuppression (80% [95% CI 28–100%] and 60% [95% Cl 15–95%]).

Conclusions—Stool Xpert had similar performance compared to sputum/GA Xpert to detect TB. Urine LAM had lower sensitivity and specificity, but was higher among children with severe immunosuppression. Stool Xpert and urine LAM can aid rapid detection of TB in HIV-infected children using easily accessible samples.

Keywords

tuberculosis; HIV; children; GeneXpert MTB/RIF®; stool; lipoarabinomannan; urine

Introduction

The World Health Organization (WHO) estimates there are one million new cases of tuberculosis (TB) and 136,000 TB-associated deaths in children each year.[1] TB-related morbidity and mortality among HIV co-infected children is particularly high.[2] Pediatric TB incidence and mortality are likely underestimated,[3] as evidenced by pediatric post-mortem studies.[4–7] Young children are unable to expectorate sputum, the standard specimen for diagnostic work-up, and frequently have paucibacillary disease, further delaying the gold-standard diagnostic of mycobacterial culture to provide a confirmatory result.[8,9] HIV adds complexities to TB diagnosis. HIV-infected children have a higher likelihood of co-morbidities, and both younger and HIV-infected children are more likely to develop disseminated disease, likely in part due to developmentally- or disease-related relative immunosuppresion.[10] Challenges in obtaining respiratory or gastric aspirate samples from children and rapid TB/HIV disease progression prior to obtaining culture results can lead to treatment delays and poor outcomes.[11] Thus, diagnostic tools for rapid detection of TB disease in children using less invasive specimens than sputum or gastric aspirates are urgently needed.[12]

GeneXpert MTB/RIF® (Xpert) and lateral flow urinary lipoarabinomannan (LAM) are rapid diagnostic tools that may be useful for diagnosing pediatric TB. Xpert is currently endorsed by WHO for pulmonary TB diagnosis in children and in selected non-pulmonary specimens for extra-pulmonary TB diagnosis.[13] The point-of-care urinary LAM assay is sensitive and specific in highly immunosuppressed (CD4 ≤100 cell/μl) HIV-infected adults.[14] WHO recommends urine LAM be used in HIV-infected adults with low CD4 or who are seriously ill, extending the recommendation to HIV-infected children based primarily on adult data.[15] Recent studies suggest stool Xpert may have good diagnostic performance to detect pediatric TB.[16–21] However, there is a paucity of data regarding stool Xpert and urine LAM in HIV-infected children with severe illness, a population in whom early diagnosis and treatment is lifesaving.

We prospectively assessed the performance of stool Xpert and urine LAM compared to the reference standard of culture and/or Xpert on sputum or gastric aspirates (GA), in a cohort of hospitalized HIV-infected children in Kenya.

Methods

Study setting and participants

This tuberculosis diagnostic study was nested within the Pediatric Urgent Start of HAART (PUSH) randomized controlled trial (NCT02063880) comparing urgent (<48 hours of enrollment) to post-stabilization (7 to 14 days) initiation of antiretroviral therapy (ART) in HIV-infected children hospitalized for acute illness at four hospitals in Kenya: Kenyatta National Referral Hospital and Mbagathi District Hospital in Nairobi, and Kisumu County Hospital and Jaramogi Oginga Odinga Teaching and Referral Hospital in western Kenya.[22] Children were eligible if they were ≤12 years, HIV-infected, ART-naïve (other than ART for prevention of maternal-to-child transmission), and ART-eligible per WHO guidelines.[23] Children with suspected or confirmed central nervous system (CNS) infections were excluded.

Study procedures

Clinical procedures and sample collection—At enrollment, children provided early morning sputum samples for Xpert and culture, clean-catch urine, stool, and blood samples. When sputum could not be produced, early morning GA were performed by trained medical or clinical officers. A second sputum or GA sample was obtained for culture as close to enrollment as possible. Clinical history and sociodemographic information were collected from caregivers, and anthropometric measurements (weight, height, and mid-upper arm circumference) were assessed. Chest x-rays (CXR) were read by a hospital radiologist using standardized reporting forms to identify findings suggestive of TB.[24] For tuberculin skin testing (TST), 5 units (0.1 mL) of purified protein derivative (RT23 solution; Sanofi Pasteur, Lyon, France) were injected subcutaneously and induration measured between 48 and 72 hours by a study nurse. A positive TST was defined as induration of ≥5 mm. For the parent trial, medical history and physical examination were performed at enrollment, one and two weeks post-ART initiation, then monthly for six months.

Sample Processing and Testing

Sputum/gastric aspirate Xpert and culture—Sputum and GA samples were sent to the lab within one hour of collection for Xpert testing and mycobacterial culture. For Xpert testing, sample reagent was added to sputum in a 2:1 ratio, manually agitated, and 2 mL added to the Xpert MTB/RIF cartridge and inserted into the platform. Sputum/GA for culture was decontaminated using N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH), centrifuged, suspended in phosphate buffer, and inoculated into liquid culture medium (BACTEC MGIT [mycobacteria growth indicator tube] 960 culture). Flagged bottles were removed, mixed gently, and sub-cultured to blood agar plates and Ziehl–Neelsen (ZN) smears. Positive ZN smears were sub-cultured onto Löwenstein-Jensen solid medium and evaluated for 3 additional weeks. Flagged bottles that were ZN negative but positive on brain heart infusion agar were deemed contaminated. Unflagged bottles at 60

days were considered negative. Sputum/GA culture and Xpert testing were performed at Lancet Laboratories© in Nairobi, and Kenya Medical Research Institute (KEMRI)/Center for Global Health Research (CGHR) TB laboratory in western Kenya.

Stool Xpert—Stool processing techniques were adapted from procedures developed previously.[25] Immediately after collection, stool samples were suspended in equal volume with sterile phosphate buffered saline solution (0.9%), manually homogenized, refrigerated at 2–5°C for at least 12 hours, but <48 hours, then passed through a fine filter maintaining only the liquid. Liquid and supernatant were vortexed and incubated with equal parts NaOH-NALC, centrifuged twice with phosphate buffer, pellet decanted, and additional phosphate buffer added and vortexed again to re-suspend. Sample reagent was added to the re-suspended pellet (0.7mL) in a 2:1 ratio, manually agitated and incubated, then used for Xpert testing. Stool Xpert was performed at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) laboratory in Nairobi.

Urine LAM—Fresh urine was collected using specimen bags (unless children could produce urine on demand into specimen cups) and tested within 2 hours of sample collection using Alere Determine™ TB LAM Ag Test Cards (Alere, Waltham, MA, USA) according to manufacturer recommendations.[26] A 60 μl of sample was added to the assay and results read 25–35 minutes later by a trained laboratory technician. Bar color intensity was graded using the manufacturer reference scale (grade 1 lowest intensity and grade 5 highest intensity). A bar intensity of grade ≥1 was considered positive per manufacturer recommendations at the time of the study.

Statistical Analysis

For the primary analyses, we determined diagnostic performance of stool Xpert and urine LAM to detect TB, compared to the gold standard of sputum/GA culture on two possible sputum/GA samples. For the intention-to-diagnose approach, stool Xpert and urine LAM were compared to a combined reference of sputum/GA culture or Xpert for children who had at least one reference test (culture or Xpert) performed. For the diagnosed-per-protocol approach, stool Xpert and urine LAM were again compared to the combined reference of sputum/GA culture and Xpert, but limited to the subset of children with all three reference tests (2 sputum/GA culture and 1 Xpert) per study protocol. For all scenarios, detection of MTB in at least one reference sample was considered positive. Additionally, we compared stool Xpert and urine LAM to sputum/GA Xpert. To account for possible differences in diagnostic performance in populations of children with a higher risk of disseminated TB, we stratified results by HIV immunosuppression status using WHO-defined age-specified CD4% cut-offs[27] and age less than 24 months.

Children were categorized as having confirmed, unconfirmed, or unlikely TB based on international consensus clinical case definitions for pediatric TB.[28] For the purposes of this study, response to TB treatment was defined as an increase in weight-for-age z-score (WAZ) or resolution of the symptom present at the time of TB diagnosis, at 6 months follow-up.

Participant characteristics were summarized by frequency and proportion for categorical variables, and by median and interquartile range (IQR) for continuous variables. We

estimated the performance of assays by sensitivity, specificity, negative (NPV) and positive predictive value (PPV) using 95% confidence intervals (CI) assuming a binomial distribution. Height-for-age z-scores (HAZ), weight-for-age z-scores (WAZ), and weight-for-height z-scores (WHZ) were calculated using WHO ANTHRO software.[29] Data were entered in REDCap[30] and analyzed with STATA version 12 (StataCorp, College Station, TX, USA).

Ethics Approval

Written informed consent was obtained from primary caregivers of all children. Study procedures were approved by the University of Washington Institutional Review Board, Kenyatta National Hospital/University of Nairobi Ethics and Research Committee, and the Kenya Pharmacy and Poisons Board. The study is registered at ClinicalTrials.gov (NCT02063880).

Results

Between April 2013 and May 2015, 181 children were enrolled in the clinical trial, 165 of whom had at least one sputum or GA collected for culture or Xpert (165 Xpert, 164 culture, and 146 with two consecutive culture results) (Figure 1).

Among these 165 HIV-infected ART-naïve children, median age was 24 months (IQR 13–58) and 90 (54.6%) were male (Table 1). Median CD4% was 14.3% (IQR 8.9–22.0%) and 114 (69.5%) had HIV-associated immunosuppression by age-defined CD4% or CD4 count. Nearly half (58, 46.4%) were acutely malnourished. Seven children (4.6%) had a positive TST and 20 (12.4%) reported a TB exposure in the past year. The majority had at least one sign or symptom suggestive of TB (118, 71.5%), with failure to thrive as the most common symptom (103, 64.0%). More than half had an abnormal CXR suggestive of TB (83, 57.2%), and TB treatment was initiated in 61 (37.4%) during the course of the study.

Prevalence of TB by reference tests and clinical criteria

Overall, 13 (7.9%) children had microbiologically-confirmed pulmonary TB from reference sputum/GA (6 positive by both culture and Xpert, 4 by culture alone, and 3 by Xpert alone). Seventy-two (43.6%) were considered to have unconfirmed TB, and 80 (48.5%), were considered unlikely to have TB per consensus case definitions (Table 1).[28] Figure 1 illustrates the flow of participant with at least one sputum/GA culture or Xpert including the number of children with stool Xpert and urine LAM results by the intention-to-diagnose (at least 1 reference test performed) and diagnosed-per-protocol (all 3 reference tests performed: 2 sputum/GA culture and 1 Xpert) scenarios. Compared to the gold standard of culture, sputum/GA Xpert had a sensitivity of 60% (95% CI 26–88%), specificity of 98% (95% CI 94–100), with PPV and NPV of 67% (95% CI 30–93%) and 97% (95% CI 94–99%), respectively (Table 2).

Stool Xpert performance

Among 147 children with valid stool Xpert and sputum/GA culture results, stool Xpert identified 5 of 8 children with positive sputum/GA culture (sensitivity: 63% [95% CI 25–

92%]; PPV: 71% [95% CI 29–96%], and classified 137 children as negative (specificity: 98% [95% CI 94–100%]; NPV: 98% [95% CI 94–100%]) (Table 2). Stool Xpert performance was similar when compared to a combined reference of sputum/GA culture or Xpert for children with at least one reference test result available (intention to diagnose protocol) and for those children with all three reference tests (diagnosed-per-protocol: 2 sputum/GA culture and 1 sputum/GA Xpert), as well as compared to Xpert sputum/GA alone (Supplemental Table 1). Stool Xpert sensitivity was higher among children with WHO-defined severe HIV immunosuppression compared to those without severe HIV immunosuppression (80% [95% CI 28–100%] vs. 33% [95% CI 1–91%]), but lower in children under 24 months (33% [95% CI 1–91%]) compared to older children (80% [95% CI 28–100%]) (Table 2). Specificity remained similar across these groups (Table 2).

Urine LAM performance

Among 129 children with valid urine LAM and sputum/GA culture results, LAM identified 3 of 9 children with culture-confirmed TB (sensitivity: 43% [95% CI 10–82%]; PPV: 21% [95% CI 5–51%], and classified 111 children as negative (specificity: 91% [95% CI 84–95%]; NPV: 97% [95% CI 91–99%]) (Table 2). Urine LAM performance improved when compared to a combined reference of sputum/GA culture or Xpert for children with at least one reference test result available (intention to diagnose protocol), and for those children with all three reference tests (diagnosed-per-protocol), as well as compared to Xpert sputum/GA alone, however due to small numbers formal statistical comparison was not performed (Supplemental Table 1). Urine LAM was positive in 2 (3.5%) of the 57 children with unconfirmed TB and in 7 (10.9%) of the 64 children with unlikely TB (Figure 1). The median LAM grade was 3 (range 2–5) among 5 children with confirmed TB, 1.5 (range 1–2) in 2 with unconfirmed TB, and 2 (range 1–4) in 7 children in the unlikely TB category (data not shown). Four children with confirmed TB were urine LAM negative. The performance of urine LAM improved among children with severe HIV immunosuppression (sensitivity: 71% [95% Cl 29–96%], specificity: 93% [95% Cl 85–97%]) compared to those without severe immunosuppression (sensitivity: 0% [95% Cl 0–84], specificity: 92% [95% Cl 78–98%] using the combined reference of sputum/GA aspirate culture or Xpert (Supplemental Table 1). LAM had slightly higher sensitivity (67% [95% CI 9–99%]) but lower specificity (87% [95% CI 76–95%]) in children under 24 months of age compared to older children (sensitivity: 50% [95% CI 12–88%], specificity: 97% [95% CI 90–100%]).

Figure 2 shows the overlap of urine LAM and stool Xpert positives with reference sputum/GA Xpert or culture. One child with a positive stool Xpert, and one child with a positive urine LAM did not have sputum/GA collected (Figure 2).

Discussion

In this prospective TB diagnostic study of HIV-infected hospitalized children in Kenya, stool Xpert had moderate sensitivity and high specificity for MTB detection compared to a reference of sputum/GA culture. Urine LAM had lower sensitivity and specificity in general, but improved among children with severe HIV-immunosuppression. Importantly, stool Xpert had similar performance to Xpert on sputum/GA aspirate, the assay currently recommended

for rapid detection of TB in children, with the added benefit of utilizing non-invasive sample collection.

Pediatric studies of stool Xpert from South Africa[16,17], Zimbabwe[18], Pakistan[19], Egypt[20], as well as a multi-center study[21] (Burkino Faso, Cambodia, Cameroon, and Vietnam) report sensitivities of 31–90% with specificities of 98–100%. In general, sensitivity was higher in HIV-infected compared to HIV-uninfected children,[16,18,21] and among children who were hospitalized or with more severe disease. The wide range and variability of sensitivities may be due to different populations studied (HIV-infected, HIV-uninfected, or combined), differences in sample sizes leading to variably precise estimates, as well as variability in reference standards. Sensitivity of stool Xpert was lower in studies with increased number of reference respiratory/GA samples collected, which favor the reference test when multiple reference samples are collected, and likely underestimate sensitivity of stool Xpert when only one stool sample is collected. The current WHO recommendation for Xpert on respiratory samples for pediatric pulmonary TB was based on a pooled sensitivity of 66% compared to a reference of culture on sputum/GA.[13] Our estimated sensitivity of 63–75% for stool Xpert (depending on reference used) is comparable to this and other pooled sensitivity estimates of Xpert on respiratory samples compared to culture.[31] Importantly, the performance of stool Xpert was similar to sputum/GA Xpert in our study.

Data regarding the performance of urine LAM test in children is inconsistent. In one of the first published reports pediatric urine LAM testing, Nicol et al. reported poor performance in HIV-infected and HIV-uninfected children in South Africa and recommended against its use to diagnose pulmonary TB in children.[32] Their study population may have been less severely ill, as no children recruited from clinic required hospitalization, and no children recruited from hospital died. Subsequent studies in Tanzania have demonstrated improved performance in HIV-infected children compared to HIV-uninfected children.[33] The WHO recommendation that LAM be used for the sickest adult patients, primarily HIV-infected inpatients with low CD4 counts, was extended to children based on the generalization of adult data.[15] In our study of HIV-infected ART-naive children hospitalized for acute illness, performance increased substantially among children with HIV-associated severe immunosuppression (from sensitivity 43–63% in general depending on the reference, to 60–71% when limited to those with severe immunosuppression). LAM specificity did not vary by immunosuppression status in our study (ranging from 91–93% depending on the reference, overall, and in the subset of those with severe immunosuppression) but did vary by age (ranging from 85–88% in children under 24 months and 96–97% in older children). Despite concerns raised in previous studies regarding low specificity,[32] the WHO recommendation to use LAM in this population, is based on the thought that the net benefit of using LAM in the subpopulation at high risk of mortality, outweighed the harms associated with false positive diagnosis.[15] False positive LAM results can occur from oral flora,[34,35] candida, and other non-tuberculosis mycobacteria.[34,36,37] However, an evaluation of LAM among cystic fibrosis patients with known pulmonary non-tuberculosis mycobacteria demonstrated low-cross-reactivity.[38] Our reference samples/tests used (sputum/GA Xpert and mycobacterial culture) could also have missed true cases of TB that were detected by LAM either because of the paucibacillary nature of MTB in children or

from its extra-pulmonary dissemination, which LAM may detect, contributing to spuriously low estimates of specificity.

Strengths of our study included well-characterized presentations and standardization of TB investigations in HIV-infected children. This population of HIV-infected ART-naïve children presenting to hospital with acute illness is the population most likely to benefit from early diagnosis and treatment of TB. Collecting more than one respiratory sample and testing with both Xpert and culture likely identified more children with TB. All samples were tested in-real time, as opposed to stored or frozen samples. We provided estimates of performance compared to the gold-standard of culture, and in scenarios more reflective of research settings (diagnosed-per-protocol requiring all 3 reference samples). We also summarized performance compared to Xpert on sputum/GA and among children with at least 1 reference samples (intention-to diagnose) which is likely more reflective of clinical settings, improving the generalizability of our findings.

Children with suspected or confirmed CNS infections were excluded, based on primarily adult data that early antiretroviral therapy in the context of CNS co-infection (tuberculosis or cryptococcus) resulted in poor outcomes including increase risk of mortality and immune reconstitution inflammatory syndrome (IRIS).[39,40] Our study population was relatively young, with median age of 24 months. Younger children, especially those with HIV, may be more likely to have disseminated disease. Exclusion criteria and the younger age of participants may limit the generalizability of our study findings. Since we used respiratory aspirates as our gold standard, there may have been issues with ascertainment bias, with under-detection of TB in cases of disseminated disease.

Although stool Xpert and urine LAM reduce sample collection issues, further improvements are needed regarding standardization for stool processing. Processing steps prior to running the Xpert test, including allowing the stool and MTB bacilli to homogenize into saline and passing through a filter, make the testing procedures slightly more logistically challenging than processing sputum samples. If stool becomes a recommended sample type for Xpert, further studies could be done to identify optimal processing of stool samples prior to testing. We did not conduct reference culture testing of stool and urine samples, nor additional non-pulmonary samples (including blood), which could have provided important insight into whether stool Xpert and urinary LAM detect extra-pulmonary or disseminated TB. Previous pediatric urine LAM studies have evaluated the performance of the LAM ELISA test, which requires batch testing and significantly more sample processing than the point-of-care Determine LAM strip used in this study, though may be more sensitive. We did not evaluate the performance of LAM ELISA, and may have missed children who would have been identified by this test. LAM test results were read by a single lab technician. We were unable to obtain all samples from all participants making comparisons between tests difficult. Samples were missed if a child was too sick or died before a sample could be collected, and this group of children was potentially at highest risk of TB.

Children are less likely to benefit from Xpert scale-up in high-burden settings due to sample collection challenges. The current WHO-recommended samples for Xpert include sputum which cannot be feasibly collected in young children, gastric aspirates which require fasting

and often hospitalization, and induced sputum which can result in adverse events and potential transmission risk to other patients and health care workers. From surveys of 651 pediatric HIV care sites in sub-Saharan Africa, <10% had capacity to perform induced sputum or gastric aspirates.[41] Continuing developments of rapid TB diagnostics including Xpert Ultra, with increased sensitivity over Xpert and potentially superior to culture, will aid in improved detection of MTB in frequently paucibacillary populations, including children and HIV-infected individuals. However, improvements in detection limits of these diagnostics will not overcome the challenges of pediatric respiratory sample collection. This highlights the need for continued investigation of alternative sample types that can be easily collected for TB detection in children. Although low sensitivity of urine LAM assays compromises its utility as a replacement for mycobacterial culture or Xpert on respiratory samples, the ease of the urine dipstick underscores its potential utility as either an add-on test in severely ill HIV-infected children, or in settings where Xpert and/or culture are not available.[42,43] This study adds to the growing body of literature supporting the use of stool Xpert in children, and provides data specifically regarding hospitalized HIV-infected ART-naïve children, a particularly high-risk population in whom clinical manifestations of TB are protean and complex, and in which rapid and accurate diagnosis can be life-saving.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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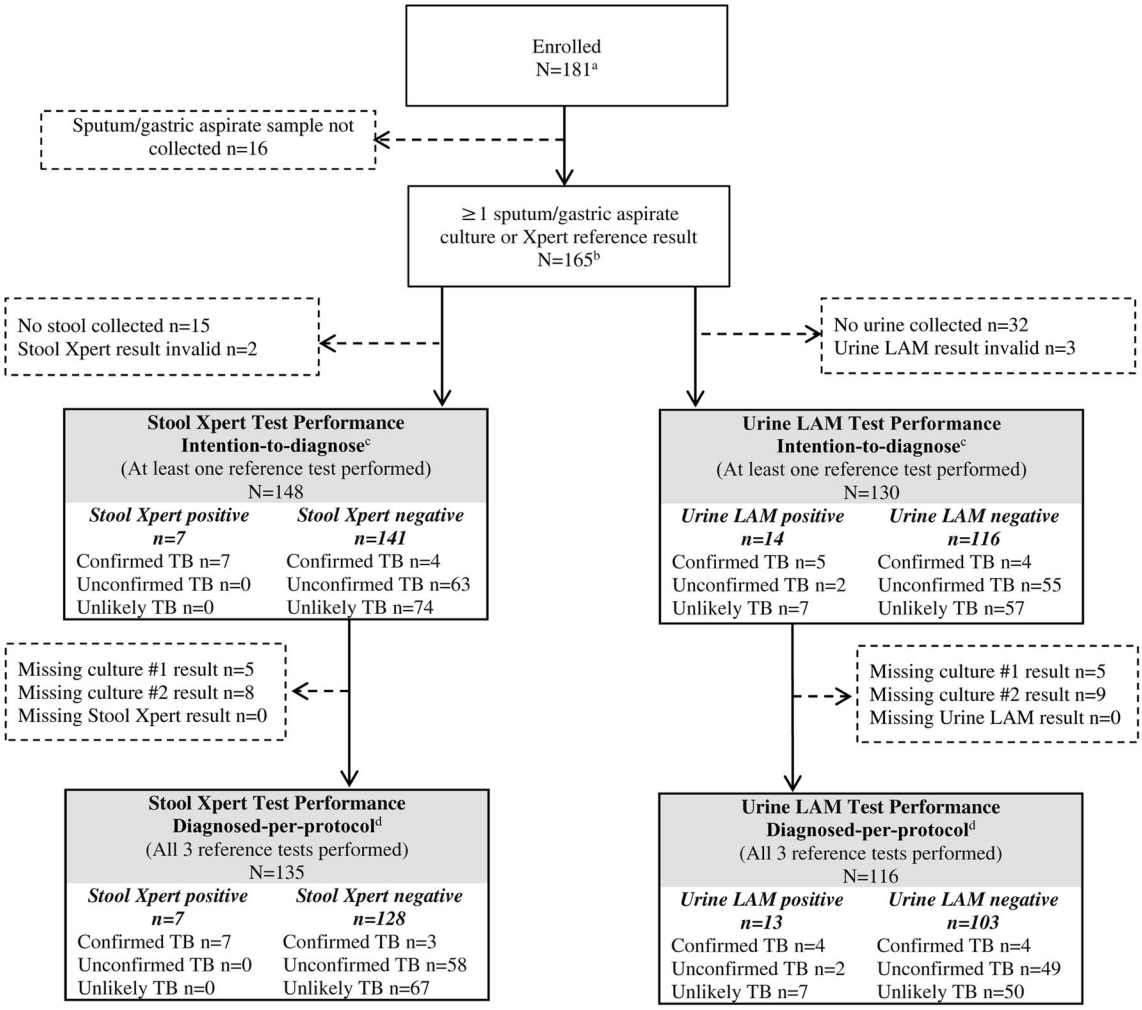

Figure 1.

Participant flow of children in TB diagnostic sub-study evaluating performance of stool Xpert and urine LAM compared to culture and Xpert on induced sputum or gastric aspirate among hospitalized newly diagnosed HIV-infected children.

[a]Parent trial randomized 183 participants, 2 of which were later excluded (1 due to TB meningitis, 1 due to false positive HIV-test) and therefore not included in the enrolled population of the sub-study.

[b]Of 165 children with at least 1 sputum/gastric aspirate culture, 165 had Xpert and 164 had at least one culture (146 of whom had results from two consecutive cultures). Children were intended to have both stool Xpert and urine LAM performed, however 148 had a valid stool Xpert result, 130 had a valid urine LAM result, and 116 had both.

[c]Intention-to-diagnose: At least one reference test performed (culture or Xpert) on sputum or gastric aspirate

[d]Diagnosed-per-protocol: All three reference tests (2 consecutive cultures and 1 Xpert) performed on sputum or gastric aspirate

Confirmed, Unconfirmed, and Unlikely TB per international consensus clinical case definitions for pediatric TB (Graham et al. 2015)

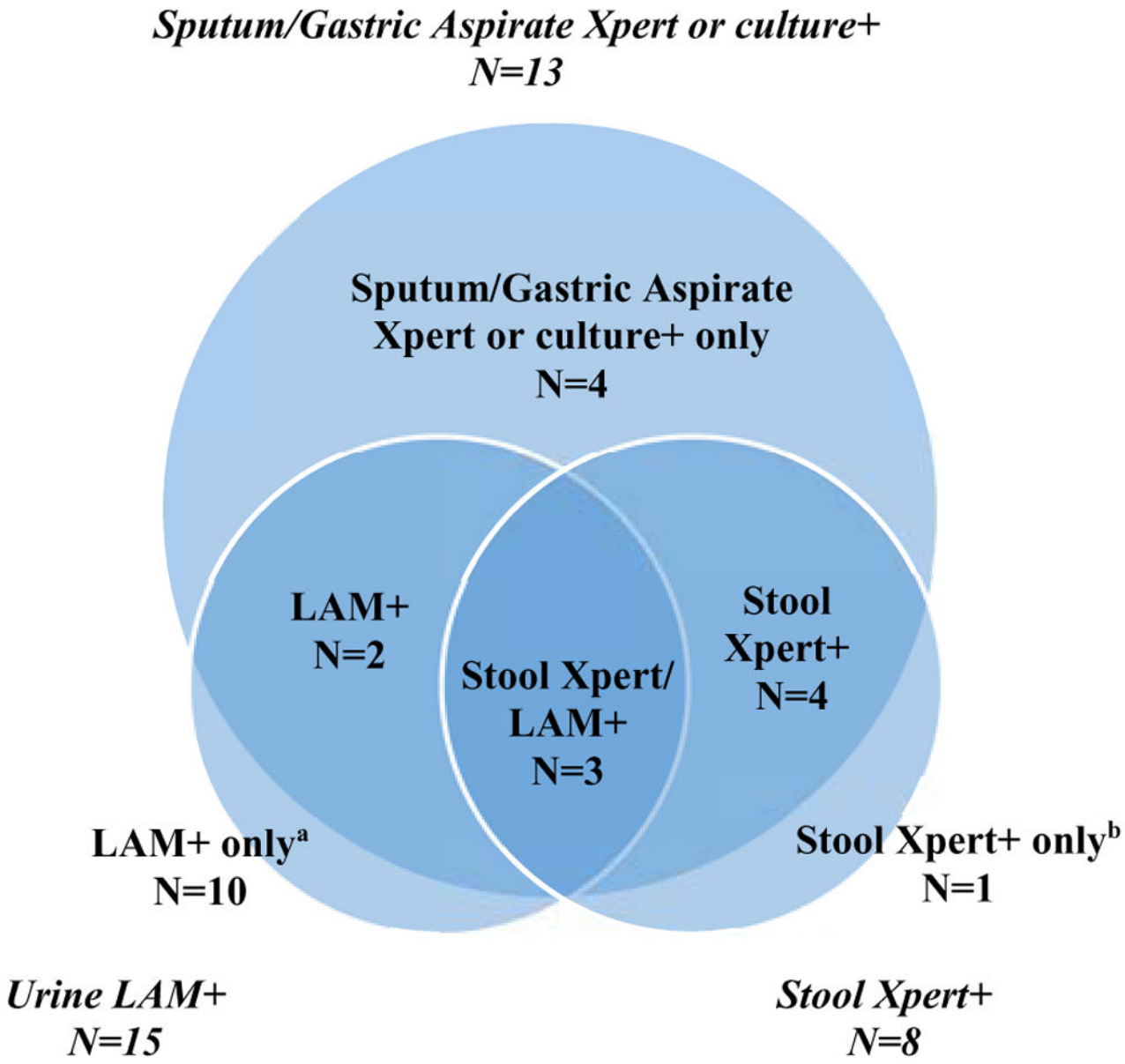

Figure 2.

Overlap of urine LAM and stool Xpert with reference sputum/gastric aspirate Xpert and/or culture.

[a]Includes nine children with sputum/gastric aspirate Xpert or culture results negative (2 with Unconfirmed TB and 7 with Unlikely TB) and one child who did not have sputum/gastric aspirate sample collected who was urine LAM positive.

[b]Includes one child who did not have sputum/gastric aspirate sample collected who was stool Xpert positive.

Table 1

Characteristics of hospitalized newly diagnosed HIV-infected children analyzed in the TB diagnostic sub-study (N=165)

Characteristic	n (%)[i] Median (IQR)
Sociodemographic	
Median age (months)	24(13–58)
<24	84(50.9%)
24–59	40(24.2%)
60+	41(24.9%)
Male	90(54.6%)
Household crowding[ii]	126(76.4%)
Clinical Presentation	
CD4 cell count (cells/μl)	699(288–1227)
CD4%	14.3(8.9–22.0)
HIV-associated immunosuppression[iii]	114(69.5%)
Wasted[iv] (WHZ<-2 or MUAC<12.5)	58(46.4%)
Underweight (WAZ<-2)	102(63.4%)
Any sign/symptom suggestive of TB	118(71.5%)
Persistent cough (≥14 days)	25(15.2%)
Persistent fever (≥7 days)	34(20.6%)
Failure to thrive[vi]	103(64.0%)
Persistent lethargy (≥7 days)	31(18.8%)
Evidence of BCG immunization[vii]	163(98.8%)
TST positive[vii] (n=151)	7(4.6%)
TB exposure in last year (n=162)	20(12.4%)
CXR suggestive of TB[viiii] (n=145)	83(57.2%)
TB treatment initiated (n=163)	61(37.4%)
TB Clinical Case Classification[ixi]	
Confirmed TB[xi]	13(7.9%)
Unconfirmed TB	72(43.6%)
Unlikely TB	80(48.5%)

[i] Calculated based on those with complete data

[ii] 2 people per room living in house

[iii] Defined in terms of World Health Organization age-specified CD4% cut-offs (<12 months: <25%, 12–35 months: <20%, >36 months: <15%) or, in absence of CD4 % data, in terms of CD4 count (age <12 months: <1500 cells/mm^3, 12–35 months: <750 cells/mm^3, >36 months <350 cells/mm^3)

[iv] Among children 5 years and under

[vi] Wasted (WHZ<-2 or MUAC <12.5) or underweight WHZ<-2 at enrollment (growth trajectories unavailable before enrollment)

[vii] Per immunization record

[viii] Induration >5 mm

viiii: CXR read by using standardized reporting forms to identify those suggestive of TB (Graham et. al 2015)

[ixi] Based on international consensus clinical case definitions for pediatric TB (Graham et. al 2015)

[xi] Thirteen (7.9%) children had microbiologically-confirmed pulmonary TB from reference sputum or gastric aspirates (6 positive by culture and Xpert, 4 by culture alone, and 3 by Xpert alone).

Table 2

Diagnostic accuracy of stool Xpert, urine lipoarabinomannan (LAM), and sputum/gastric aspirate Xpert for microbiologically-confirmed pulmonary tuberculosis in hospitalized HIV-infected Kenyan children

		Sputum/Gastric Aspirate Culture 1st or 2nd sample N=164[a]		
		n/total	%	95%CI
Sputum/Gastric Aspirate Xpert[b] N=164	Sensitivity	6/10	60	(26–88)
	Specificity	151/154	98	(94–100)
	PPV	6/9	67	(30–93)
	NPV	151/155	97	(94–99)
	ROC area	-	79	(63–95)
Stool Xpert N=147	Sensitivity	5/8	63	(25–92)
	Specificity	137/139	99	(95–100)
	PPV	5/7	71	(29–96)
	NPV	137/140	98	(94–100)
	ROC area	-	81	(63–99)
Urine LAM N=129	Sensitivity	3/7	43	(10–82)
	Specificity	111/122	91	(84–95)
	PPV	3/14	21	(5–51)
	NPV	111/115	97	(91–99)
	ROC area	-	67	(47–87)
HIV Immunosuppression Status[c]				
Stool Xpert Severe N=100	Sensitivity	4/5	80	(28–100)
	Specificity	93/95	98	(93–100)
	PPV	4/6	67	(22–96)
	NPV	93/94	99	(94–100)
	ROC area	-	89	(69–100)
Stool Xpert Not Severe N=46	Sensitivity	1/3	33	(1–91)
	Specificity	43/43	100	(92–100)
	PPV	1/1	100	(3–100)
	NPV	43/45	96	(85–100)
	ROC area	-	67	
Urine LAM Severe N=90	Sensitivity	3/5	60	(15–95)
	Specificity	77/85	91	(82–96)
	PPV	3/11	27	(12–74)
	NPV	77/79	98	(6–61)
	ROC area	-	75	(51–100)
Urine LAM Not Severe N=39	Sensitivity	0/2	0	(0–84)
	Specificity	34/37	92	(78–98)
	PPV	0/3	0	(0–71)
	NPV	34/36	94	(81–99)

			Sputum/Gastric Aspirate Culture 1st or 2nd sample N=164[a]		
			n/total	%	95%CI
		ROC area	-	46	(42–50)
Age					
	Stool Xpert <24 months N=75	**Sensitivity**	1/3	33	(1–91)
		Specificity	71/72	99	(93–100)
		PPV	1/2	50	(1–99)
		NPV	71/73	97	(91–100)
		ROC area	-	66	(33–99)
	Stool Xpert ≥24 months N=72	**Sensitivity**	4/5	80	(28–100)
		Specificity	66/67	99	(92–100)
		PPV	4/5	80	(28–100)
		NPV	66/67	99	(92–100)
		ROC area	-	89	(70–100)
	Urine LAM <24 months N=57	**Sensitivity**	1/2	50	(1–99)
		Specificity	47/55	86	(73–94)
		PPV	1/9	11	(0–48)
		NPV	47/48	98	(89–100)
		ROC area	-	68	(19–100)
	Urine LAM ≥24 months N=72	**Sensitivity**	2/5	40	(5–85)
		Specificity	64/67	96	(88–99)
		PPV	2/5	40	(5–85)
		NPV	64/67	96	(88–99)
		ROC area	-	68	(44–92)

[a] Among those with at least one culture result

[b] Only first sputum/gastric aspirate sample tested by Xpert

[c] Defined in terms of World Health Organization age-specified CD4% cut-offs (<12 months: <25%, 12–35 months: <20%, >36 months: <15%) or, in absence of CD4 % data, CD4 count (<12 months: <1500 cells/mm^3, 12–35 months: <750 cells/mm^3, >36 months <350 cells/mm^3)