A prospective evaluation of a three-gene host response signature to classify tuberculosis severity in children

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Abbreviations:

AFB – acid fast bacilli

AUC – area under the curve

CI – confidence interval

COMBO – Childhood 'Omics' and Mycobacterium tuberculosis-derived BiOsignatures

Ct – cycle threshold

CXR – chest X-ray

DUSP3 – dual specificity phosphatase 3

GBP5 – guanylate binding protein 5

HR – host response

IQR – interquartile range

MTB – mycobacterium tuberculosis

NPV – negative predictive value

PCR – polymerase chain reaction

PPV – positive predictive value

PTB – pulmonary tuberculosis

ROC – receiver operating curve

RIF – rifampicin

SAM – severe acute malnutrition

STARD – Standards for Reporting Diagnostic Accuracy Studies

TB – tuberculosis

TBP – TATA-binding protein

TPP – target product profile

WHO – World Health Organization

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Conflicts of Interest:

The authors declare no conflicts of interest.

Summary:

We assessed the performance of a three-gene signature to stratify TB severity in children. We found that accuracy was highest among older children and those with Confirmed TB but had lower sensitivity in young children and with culture-negative disease.

ABSTRACT

Background: Children with non-severe TB may benefit from short-course treatment, but point-of-care tools are needed to stratify disease severity. We prospectively evaluated the Cepheid Xpert MTB-Host Response (HR) prototype cartridge for distinguishing TB severity in children with pulmonary TB (PTB) in The Gambia and Uganda.

Methods: We included children <15 with microbiologically confirmed or clinically diagnosed unconfirmed PTB. Severity was defined using the World Health Organization (WHO) guidelines for a four-month, drug-susceptible regimen. Capillary or venous blood was tested with the HR cartridge for PCR-based detection of three mRNA genes and calculation of a TB score from cycle thresholds. We generated receiver operating characteristic curves with the TB score to classify severe TB and assessed if Xpert-HR could achieve the WHO target accuracy for treatment optimization (≥90% sensitivity, ≥70% specificity).

Results: Among 106 children, the median age was 4 years (IQR 1-7), 56.6% were female, and 13.2% were living with HIV. In all children with PTB, Xpert-HR achieved an AUC of 0.67 (95% CI 0.55-0.78), with 89.3% sensitivity (95% CI 71.8-97.7) and 29.5% specificity (95% CI 19.7-40.9, cut-off≤-0.60). By confirmation status, Xpert-HR approached the target accuracy in children with Confirmed TB, with 62.5% specificity (95% CI 24.5-91.5) at 91.7% sensitivity (95% CI 61.5-99.8, cut-off≤-1.349). Among children with Unconfirmed TB, specificity was lower (24.3% 95% CI 14.8-36.0) at 93.8% sensitivity (95% CI 69.8-99.8, cut-off≤-0.450). Target accuracy was almost achieved in children 5-9 regardless of confirmation status (100% sensitivity [95% CI 71.5-100], 66.7% specificity [95% CI 43.0-85.4], cut-off≤-1.35), but specificity (28.2%, 95% CI 18.6-39.5) was lower for children <5 (92.9% sensitivity [95% CI 76.5-99.1], cut-off≤-0.550).

Conclusions: Xpert-HR approached the target accuracy to stratify PTB severity in older children and those with Confirmed TB but had lower specificity in children with Unconfirmed TB. Child-specific signatures may be needed to improve performance in younger children with paucibacillary disease.

Keywords:

tuberculosis; child; severity; gene signature; host-response

INTRODUCTION

Tuberculosis (TB) is a curable infectious disease afflicting 1.3 million children globally each year [1]. Although children frequently present with less severe and paucibacillary TB disease [2], available anti-TB drug regimens were developed to treat more severe disease and adults, subjecting many children to a longer treatment. These regimens impose a significant strain on children and their families, demanding the daily administration of multiple medications and frequent visits to health care centers. Some children also experience adverse drug reactions, such as hepatoxicity, further compromising their prognosis [3, 4].

The SHINE trial found that four-month treatment was non-inferior to six-months for children with non-severe TB and has been endorsed by the World Health Organization (WHO) [5-7]. However, implementation is challenging due to the reliance on radiographic imaging to identify non-severe TB. Chest x-ray (CXR) is frequently unavailable in primary care settings and can be challenging to interpret given heterogenous features in children. Acid-fast bacilli (AFB) smear microscopy and Xpert MTB/RIF semiquantitative results may be used but are frequently falsely negative in children who have paucibacillary disease. Without a feasible approach, health workers may resort to unvalidated algorithms or opt against abbreviated regimens altogether. The WHO has highlighted a need for tests to identify children who require less versus more intensive treatment regimens, and indicated the minimum accuracy needed (≥90% sensitivity and ≥70% specificity) [8].

RNA expression signatures for TB screening have been associated with disease severity [9, 10]. The Cepheid® Xpert® MTB-Host Response (HR) prototype cartridge is an automated polymerase chain reaction (PCR) assay (Sunnyvale, CA, USA) that detects the three-gene signature (*GBP5, DUSP3,* and *TBP*) associated with TB disease [11]. It requires a small amount of blood through finger-prick or venous collection and is compatible with 10-color GeneXpert platforms that can also run Xpert MTB/RIF Ultra cartridges for

sputum testing. Preliminary studies have shown an association with disease severity among adults [9, 10], but its applicability in pediatric populations remains underexplored.

We therefore evaluated the role of the Xpert-HR cartridge in detecting severe disease in children under the ages of 15 years diagnosed with pulmonary TB (PTB) from Uganda and The Gambia.

METHODS

Study design

Children less than 15 years old were consecutively enrolled as part of the Childhood 'Omics' and Mycobacterium tuberculosis-derived BiOsignatures (COMBO) study in Uganda and the Gambia [12]. Children were recruited from Mulago National Referral Hospital and surrounding community clinics and hospitals in Kampala, Uganda, and from the Medical Research Council (MRC) Unit at the London School of Hygiene and Tropical Medicine in Fajara, The Gambia, from November 4, 2021 to December 7, 2022. All caregivers completed written informed consent and children completed assent when applicable. Ethical approval was obtained from the Mulago Hospital Research and Ethics Committee, the London School of Hygiene and Tropical Medicine Observational and Interventions Research Ethics Committee, the Gambia Government/MRC Joint Ethics Committee, and the University of California San Francisco Institutional Review Board.

Participants

Eligible children had microbiologically confirmed PTB or signs and symptoms suggestive of TB, namely unexplained cough for any duration and one or more of the following: 1) Weight loss or poor weight gain/failure to thrive, 2) Unexplained fever >1 week; 3) Unexplained lethargy or reduced playfulness >1

week; 4) Abnormal chest X-ray (CXR); 5) Positive tuberculin skin test (TST); or 6) Known TB contact. Children who had received anti-TB treatment for more than three days, completed treatment in the past year, or were unable to provide specimens for culture testing were excluded. As the focus of this analysis was on detection of TB severity, we included children with microbiologically Confirmed or clinically diagnosed Unconfirmed TB, and excluded those with Unlikely TB, as defined by the NIH consensus definitions below [13], and in alignment with the SHINE trial methods [7].

Procedures

Routine care procedures involved a clinical evaluation, two-view CXR, HIV testing, and collection of two respiratory specimens (expectorated or induced sputum, gastric aspirate, or nasopharyngeal aspirate) for mycobacterial smear microscopy and culture and Xpert MTB/RIF or Ultra testing. Weight and height were obtained, and underweight was defined as a weight-for-age z-score < -2 if the child was under 5 years, or a body mass index (BMI) < 18.5 for children 5 years and older. Severe acute malnutrition (SAM) in children under 5 years was defined as a weight-for-height or -length z-score < -3 or a mid-upper arm circumference < 115 mm. CXRs were performed and evaluated for the presence of abnormalities using a standardized study form by local expert clinicians and radiologists, who were blinded to participant details and TB status. Personnel completing sample collection and testing were also blinded to participants' TB status. Initiation of TB treatment was at the discretion of the health care providers according to national guidelines.

Index tests

Prior to diagnosis and initiation of treatment, 100 microliters of blood were collected by finger-prick or venipuncture, loaded into the Xpert-HR cartridge, and tested within 24 hours on the GeneXpert® platform using the manufacturer's instructions. The Xpert-HR output provides cycle threshold (Ct) values for the

messenger RNA expression of three genes: guanylate binding protein 5 (*GBP5*), dual specificity phosphatase 3 (*DUSP3*), and TATA-binding protein (*TBP*). These Ct values are used to calculate an HR TB score with the equation of (*GBP5* + *DUSP3*)/2 – *TBP* [11]. As *GBP5* and *DUSP3* are upregulated (lower Ct value) and *TBP* is downregulated (higher Ct value) in TB disease, the HR TB score can be negative.

Definitions

Classification of Confirmed or Unconfirmed TB was assigned according to the NIH consensus definitions [13]. Confirmed TB was defined as positive molecular or culture testing for *M. tuberculosis* from at least one respiratory sample. Those with Unconfirmed TB did not have microbiological confirmation, but had at least two signs and symptoms of TB, and all were started on TB treatment.

TB disease severity was based on WHO guidelines for the determination of children eligible for a four-month, drug-susceptible regimen for PTB [5, 7]. Non-severe disease was defined as: (1) CXR findings of abnormalities confined to one lobe, with no cavities and no signs of miliary TB; and (2) negative, trace, low, or very low on Xpert MTB/RIF or Ultra; and (3) AFB smear negative; and (4) not hospitalized. CXRs were read using the zone approach, and two or more zones were used to define involvement of more than one lobe [14]. Severe TB was defined as not meeting the criteria of non-severe TB. Children younger than 6 months or with SAM are automatically ineligible for the four-month regimen per WHO guidance; for this analysis, they were included in the overall assessment and then excluded in a secondary analysis.

Statistical analysis

We used summary statistics to describe the cohort included in the analysis. We determined and compared the median HR TB scores for severe versus non-severe TB groups using the Mann-Whitney U test, overall and stratified by TB confirmation status (Confirmed or Unconfirmed TB), with significance defined as p-

value < 0.05. We generated a receiver operating characteristic (ROC) curve and calculated the area under the curve (AUC) with 95% confidence intervals (CIs) overall and by TB confirmation status. We then assessed if there was a TB score threshold that could achieve the WHO target product profile (TPP) accuracy for optimizing TB treatment, based on prediction of poor outcomes for high-risk individuals treated with less intense regimens (minimum ≥90% sensitivity and ≥70% specificity) [8]. We also conducted these analyses (i.e., ROC generation with determination of optimal threshold) by age group, gender, HIV status, and nutritional status to determine whether using subgroup-specific TB score thresholds improved accuracy compared to applying a single overall cut-off to all subgroups. The results are presented according to the Standards for Reporting Diagnostic Accuracy Studies (STARD) [15]. All analyses were performed using STATA version 18 (Statacorp, College Station, TX, USA).

RESULTS

Participant characteristics

A total of 106 children were included in the analysis (Figure 1), and key characteristics are described in Table 1. The median age was 4 years (IQR 1-7) and over half (60, 59.6%) were female. TB was microbiologically confirmed in 20 children (18.9%, 5 from the Gambia and 15 from Uganda), and all had drug-susceptible TB. Children with Confirmed TB were older than those with Unconfirmed TB (median 8.5 years versus 3 years). The proportion of children with Confirmed TB was 9.4% in the < 5 years age group, 21.9% in the 5-to-9-year age group, and 70.0% in the 10-to-14-year age group. Of those with a known HIV status (n = 91), 12 (13.2%) were positive and the median CD4 count was 402 cells/mm^3 (IQR 63-675). One child had SAM, though 57.3% (51/89) were underweight.

Twenty-eight children (26.4%) were classified as having severe TB compared to 78 (73.6%) with non-severe TB. The proportion of severe TB was higher among those with Confirmed TB (12/20, 60.0%) than

Unconfirmed TB (16/86, 18.6%). Ten children were unclassifiable for disease severity due to missing CXR information (n = 5), Xpert semiquantitative level (n = 4), or both (n = 1). Within the severe TB group (n = 28), 24 (85.7%) had CXR findings consistent with severe TB and 4 (14.3%) had both CXR findings consistent with severe TB and an Xpert semiquantitative level of medium or high or smear positive result. Three children (10.7%) met the criteria for severe TB based on inpatient status. One child met all criteria for severe TB.

Comparison of HR TB score by TB severity

The HR TB score ranged from -3.95 to 1.55 overall, with non-severe ranging from -3.90 to 1.55 and severe TB -3.95 to 0.10 (Figure 2). Overall, the median HR TB score was significantly lower in severe TB (median -1.5, IQR -1.0 to -2.30) compared to non-severe TB (median -1.0, IQR -0.50 to -1.85, $p < 0.01$). The median score was also lower in severe versus non-severe TB when stratified by Confirmed or Unconfirmed TB, but it was not significantly different (Confirmed TB: severe median -2.15, IQR -1.48 to -3.38; non-severe median -0.83, IQR -0.63 to -3.20; $p = 0.12$, Unconfirmed TB: severe median -1.33, IQR -0.72 to -1.65; non-severe median -1.0, IQR -0.45 to -1.80; $p = 0.36$).

Xpert-HR accuracy to classify severe TB for all children with PTB

We first assessed the accuracy of Xpert-HR independent of TB confirmation status (Confirmed or Unconfirmed TB). At a cut-off value of -0.60, Xpert-HR achieved an AUC of 0.67 (95% CI 0.55-0.78) at a sensitivity of 89.3% (95% CI 71.8-97.7) and specificity of 29.5% (95% CI 19.7-40.9, Table 2). The positive predictive value (PPV) was 31.2% (95% CI 21.3-42.6) and the negative predictive value (NPV) was 88.5% (95% CI 69.8-97.6). When we excluded children under 6 months or with SAM, specificity increased to 43.1% (95% CI 31.4-55.3) at 88.5% sensitivity (95% CI 69.8-97.6) at a cut-off value of -0.849.

Xpert-HR accuracy to classify severe TB, by TB confirmation status

When stratified by TB confirmation status (Figure 3), accuracy improved among children with Confirmed TB, and Xpert-HR could achieve an AUC of 0.71 (cut-off value: -1.35, 95% CI 0.43-0.99) with a sensitivity of 91.7% (95% CI 61.5-99.8) and specificity of 62.5% (95% CI 24.5-91.5, Table 2). However, accuracy in children with Unconfirmed TB was lower, with an AUC of 0.57 (cut-off value: -0.45, 95% CI 0.42-0.72), sensitivity of 93.8% (95% CI 69.8-99.8) and specificity of 24.3% (95% CI 14.8-36.0).

Subgroup analysis of Xpert-HR to classify severe TB

Xpert-HR prediction of disease severity in key subgroups was evaluated using the overall cut-off value of -0.60 (Table 3). Compared to the overall accuracy, sensitivity was lower in children under 5 years and males, although the 95% CIs overlapped with the overall estimate.

When group-specific cut-off values were applied (independent of TB confirmation status), Xpert-HR showed increased performance in children aged 5 to 9 years, achieving a sensitivity of 100% (95% CI 71.5-100) and specificity of 66.7% (95% CI 43.0-85.4). Young adolescents aged 10 to 14 years did not show greater specificity at a cut-off closest to 90% sensitivity, but the sample size was small (n = 10) with wide CIs. Stratifying by HIV status improved the specificity to detect severe TB among children with (60.0%, 95% CI 14.7-94.7) and without (45.2%, 95% CI 32.5-58.3) HIV. Subgroup performance did not significantly differ from overall performance across gender and nutritional status using overall and group-specific cut-off values.

DISCUSSION

Simple and objective tools are needed to stratify disease severity for childhood TB and support implementation of shorter treatment regimens. We performed the first known assessment of the Xpert-HR three-gene signature for classifying severe TB in children. Overall, we found that Xpert-HR had high sensitivity but low specificity to detect TB severity in children with Confirmed and Unconfirmed TB. However, it approached the target accuracy for TB treatment optimization among children with Confirmed TB and children aged 5 to 9 years. However, the low specificity in young children < 5 and those with Unconfirmed TB could lead to overtreatment in these groups.

Similar to previous studies in adults [9, 10], we found significant differences in HR TB score by severity status. Despite differences in median score, we found low specificity at 90% sensitivity when applied to all children independent of TB confirmation status. However, when stratified by TB confirmation status, the accuracy to classify severe TB disease was higher in children with Confirmed than Unconfirmed TB, and the Confirmed TB group achieved 92% sensitivity and 63% specificity. In children with Confirmed TB, the HR TB score was lower in those with severe TB compared to those with non-severe TB, although this difference was not statistically significant given the limited sample size. Although the sample size was small, this is consistent with Olbrich et al., who found that the accuracy of Xpert-HR to diagnose TB was higher in children with both culture-positive TB and severe disease per WHO criteria [16]. This also may be expected as the data used to identify the three-gene signature did not include individuals with clinically diagnosed TB [11]. Moreover, children classified as Unconfirmed TB are heterogenous, and given the lack of microbiological confirmation may have included children without true TB disease. From an implementation perspective, as the GeneXpert platform accommodates both Xpert MTB/RIF Ultra and Xpert-HR cartridges, there may be value in using Xpert-HR if a child is Xpert Ultra positive to guide treatment duration. While the Xpert Ultra semi-quantitative level could be used, we found that it was only

medium or high in 44% of children who were Xpert positive and had severe TB. For children who are Xpert Ultra negative or it is not feasible to collect a respiratory or stool sample for *Mtb* testing, they may need to rely on CXR or other risk factors including inpatient or nutritional status. As most children diagnosed with TB have Unconfirmed disease, identifying novel biomarkers for this group is critical.

We found heterogeneity in performance across key groups, suggesting that sub-group-specific thresholds were needed. Notably, in the 5-to-9-year age group, Xpert-HR achieved a sensitivity of 100% and specificity of 66.7%. While the higher accuracy compared to younger children may be in part due to a higher proportion with Confirmed TB, the majority of children (78%) had Unconfirmed TB. The three-gene signature was predominantly derived from adult data [11], and older children have more developed immune systems than younger children and a host response more similar to adults. Gene signatures derived from pediatric data are likely needed to improve accuracy in younger children [17]. While performance was lower in adolescents aged 10 to 14 years, this is difficult to interpret due to the limited sample size (n = 10). In addition, we found that stratifying by HIV status improved specificity for both children with and without HIV. HIV co-infection alters the immune response to TB [18], and supports that different thresholds are needed for each group. Xpert-HR was 100% sensitive and 60% specific to classify TB severity among children with HIV, but further validation is needed given the small sample size (n = 12).

To our knowledge, this is the first published study exploring the ability of Xpert-HR to predict disease severity in children. We prospectively enrolled children from primary and referral health centers in high-TB-burden settings with standardized approaches to TB classification and CXR reading. However, this study has some limitations. The sample size is relatively small and draws from two clinical settings in sub-Saharan Africa, which may restrict the applicability of our findings to similar populations. Our study population also comprised a small number of children with microbiologically confirmed TB, HIV co-

infection, and receiving inpatient care. Only one child had SAM, so we were unable to perform this subgroup analysis. We were also unable to compare Xpert-HR to CXR for disease severity stratification, as most children were classified as severe based on radiographic findings and only four children with severe TB had a positive Xpert Ultra result with a medium or high semiquantitative grade. We defined >1 lobe based on multi-zone involvement, which could have underestimated the proportion with severe disease. Further research is needed to determine if serial Xpert-HR testing could be beneficial.

CONCLUSIONS

Objective tools at or near the point-of-care are critically needed to rapidly assess severity in children with TB and guide treatment. Among older children aged 5 to 9 years and those with microbiological confirmation of TB, Xpert-HR may have value and has the advantage of using the same platform used for molecular testing. However, the low overall specificity suggests that a large proportion would be overtreated if Xpert-HR was performed in all children diagnosed with TB. Future studies in large, diverse cohorts are needed to validate these findings, with further work needed to identify severity biosignatures for younger children and those with paucibacillary disease.

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DATA AVAILABILITY

The data for this research are available upon reasonable request.

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TABLES

Table 1. Demographic and Clinical Summary of Cohort

	Overall (n = 106)	**Severe TB (n = 28)**	**Non-Severe TB (n = 78)**
Country, n (%)			
Uganda	92 (86.8)	22 (78.6)	70 (89.7)
The Gambia	14 (13.2)	6 (21.4)	8 (10.3)
Female, n (%)	60 (56.6)	17 (60.7)	43 (55.1)
Age category in years, n (%)			
<5	64 (60.9)	13 (46.4)	51 (65.4)
5-9	32 (30.2)	11 (39.3)	21 (26.9)
10-14	10 (9.4)	4 (14.3)	6 (7.7)
HIV positive, n (%)	12/91 (13.2)	7/23 (30.4)	5/68 (7.4)
Underweight	51/89 (57.3)	18/25 (72.0)	33/64 (51.6)
Confirmed TB, n (%)	20 (18.9)	12 (42.9)	8 (10.3)
Xpert MTB/RIF or Ultra positive	13/19 (68.4)	9/12 (75.0)	4/7 (57.1)
Xpert Semiquantitative Level – Medium/High	4/13 (30.8)	4/9 (44.4)	-
Characteristics of Severe TB			
Radiographic features, n (%)	-	24 (85.7)	-
Involvement of >1 lobe[1]	-	23/24 (95.8)	-
Cavitation	-	4/24 (16.7)	-
Miliary TB[2]	-	2/18 (11.1)	-
Inpatient, n (%)	-	8 (28.6)	-
AFB Smear Positive, n (%)	-	2/28 (7.1)	-

[1] Includes presence of any consolidation and/or infiltrates.

[2] Data only available from Uganda cohort.

Abbreviations: MTB = mycobacterium tuberculosis; RIF = rifampicin; TB = tuberculosis; AFB = acid fast bacilli.

Table 2. Accuracy of Xpert-HR for Classifying TB Severity, Overall and by TB Confirmation Status

	AUC (95% CI)	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**Cut-off Value**
Overall	0.67 (0.55, 0.78)	89.3 (71.8, 97.7)	29.5 (19.7, 40.9)	-0.60
By TB Confirmation Status				
Confirmed	0.71 (0.43, 0.99)	91.7 (61.5, 99.8)	62.5 (24.5, 91.5)	-1.349
Unconfirmed	0.57 (0.42, 0.72)	93.8 (69.8, 99.8)	24.3 (14.8, 36.0)	-0.450

Abbreviations: TB = tuberculosis; CI = confidence interval.

Table 3. Subgroup Accuracy of Xpert-HR for Classifying TB Severity

	Overall Cut-Off[1]		Group-Specific Cut-Off[2]		
	Sensitivity (95% CI)	**Specificity** (95% CI)	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**Cut-off Value**
Overall	89.3 (71.8, 97.7)	29.5 (19.7, 40.9)	-	-	-0.60
Age, years					
< 5	84.6 (54.6, 98.1)	31.4 (19.1, 45.9)	92.9 (76.5, 99.1)	28.2 (18.6, 39.5)	-0.550
5-9	100 (71.5, 100)	33.3 (14.6, 57)	100 (71.5, 100)	66.7 (43.0, 85.4)	-1.35
10-14	75 (19.4, 99.4)	0 (0, 45.9)	75.0 (19.4, 99.4)	33.3 (4.30, 77.7)	-1.449
Gender					
Female	94.1 (71.3, 99.9)	34.9 (21.0, 50.9)	88.2 (63.6, 98.5)	41.9 (27.0, 57.9)	-0.850
Male	81.8 (48.2, 97.7)	22.9 (10.4, 40.1)	90.9 (58.7, 99.8)	22.9 (10.4, 40.1)	-0.550
HIV					
HIV Positive[3]	-	-	100 (59.0, 100)	60 (14.7, 94.7)	-1.449
HIV Negative	93.8 (69.8, 99.8)	33.9 (22.3, 47)	87.5 (61.7, 98.4)	45.2 (32.5, 58.3)	-0.849
Underweight	88.9 (65.3, 98.6)	27.3 (13.3, 45.5)	88.9 (65.3, 98.6)	45.5 (28.1, 63.6)	-0.850

[1] Cut-off value of ≤ -0.60.

[2] Cut-off values that optimized sensitivity were determined for each subgroup.

[3] Analysis could not be performed due to insufficient variability, as all cases were classified as severe TB.

Abbreviations: TB = tuberculosis; CI = confidence interval.

FIGURE LEGENDS

Figure 1. Participant Flowchart.

Figure 2. Box-and-whisker plots of Xpert-HR TB scores by TB Severity in children. The box represents the interquartile range and the horizontal line within the box marks the median. Circles represent outliers. ** $p < 0.01$.

Figure 3. Receiver operating characteristic curve for the Xpert-HR TB score on TB Severity for children (a) Overall, (b) with Confirmed TB, and (c) with Unconfirmed TB. The red dot on the curve represents the cut-off value closest to 90% sensitivity. The dashed lines denote the sensitivity and specificity at this cut-off.

Figure 1

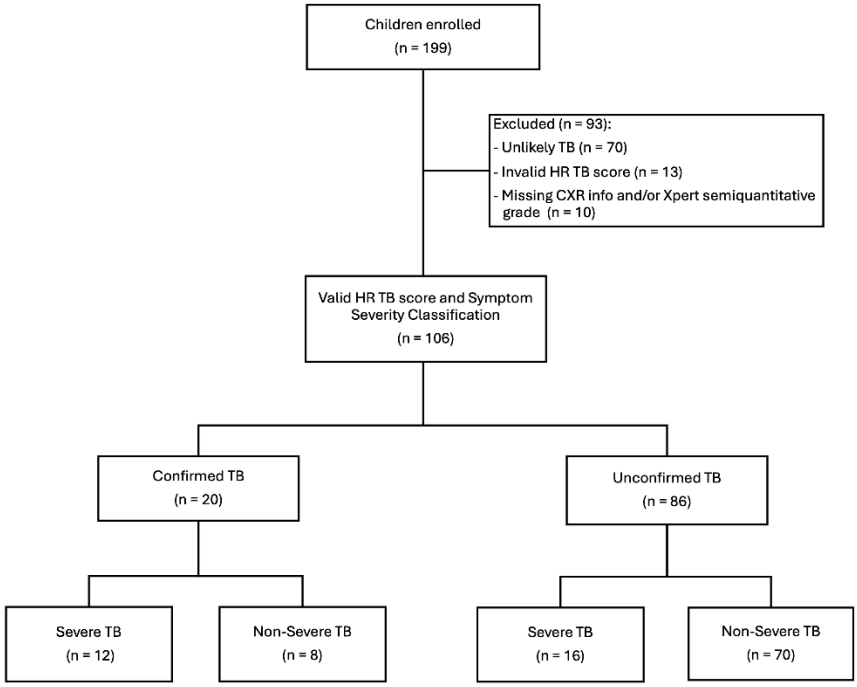

Figure 2

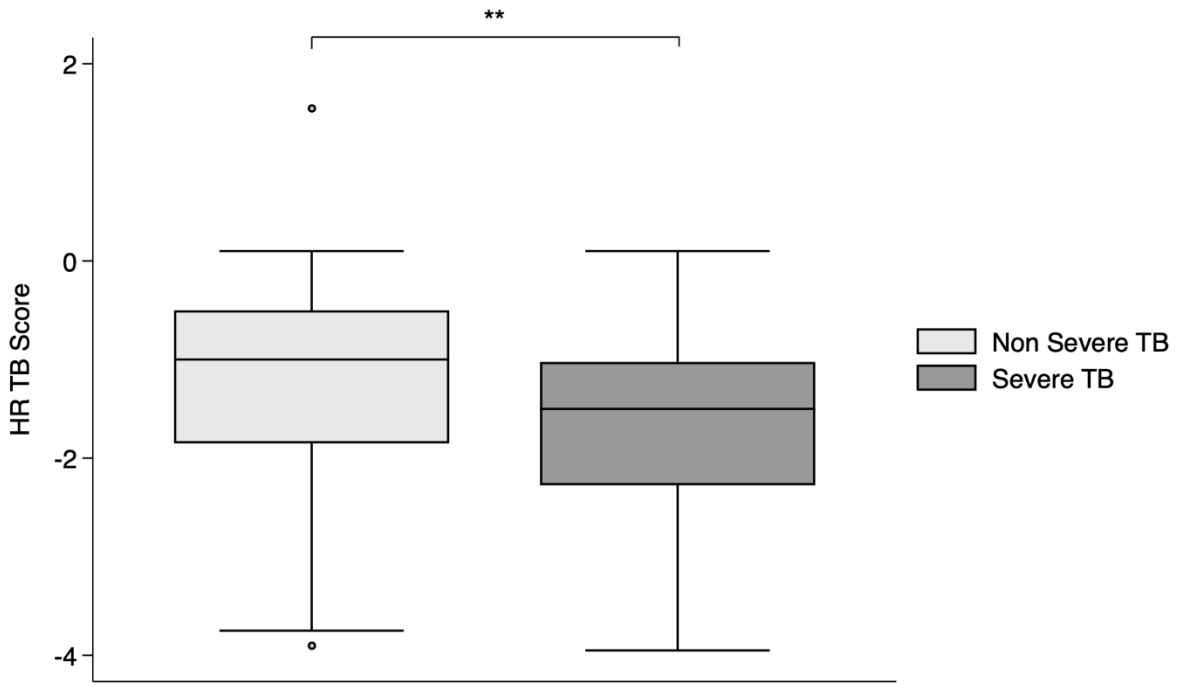

Figure 3