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Blood-based diagnosis of pediatric tuberculosis: A prospective cohort study in South Africa and Dominican Republic

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SUMMARY

Objectives: Pediatric tuberculosis (TB) diagnosis is complicated by challenges in obtaining invasive respiratory specimens that frequently contain few *Mycobacterium tuberculosis* (*Mtb*) bacilli. We report the diagnostic performance of an *Mtb* antigen-derived peptide (MAP-TB) assay and its ability to monitor TB treatment response.
Methods: Study cohorts enrolled children who presented with presumptive TB at two hospitals in South Africa from 2012 to 2017 (157 children aged < 13 years) and at community-based clinics in the Dominican Republic from 2019 to 2023 (101 children aged < 18 years). Children were evaluated for TB at enrollment and six months post-enrollment and assigned confirmed, unconfirmed, or unlikely TB diagnoses using the 2015 NIH diagnostic criteria for pediatric TB. MAP-TB assay performance was evaluated using serum collected at baseline and at regular intervals post-enrollment following STARD guidelines.
Results: MAP-TB sensitivity for confirmed and unconfirmed TB was comparable to culture and Xpert sensitivity for confirmed TB, but MAP-TB specificity revealed age-dependence, decreasing from 98·1% to 78·4%, when including children aged < 1 year. MAP-TB values decreased by six months post-treatment initiation in children with symptom improvement.
Conclusions: Serum MAP-TB results can effectively diagnose pediatric TB, including unconfirmed and extrapulmonary TB missed by current methods, and correspond to effective treatment.

Introduction

The number of children < 15 years of age estimated to develop TB annually increased from 1 to 1·3 million between 2019 and 2022.[1] Most of these children, especially those < 5 years of age, are never diagnosed with TB and are thus excluded from global TB control efforts.[2] Challenges in diagnosing childhood TB can delay its treatment, resulting in increased morbidity and mortality.[3–5] Current TB diagnostics primarily utilize sputum, which is difficult to obtain from children, particularly those < 5 years of age, often leading to collection of more invasive gastric aspirates, induced sputum, or nasopharyngeal aspirate specimens.[6] Pediatric samples frequently have low bacillary loads given the paucibacillary nature of TB in children; thus, culture (gold standard) and nucleic acid amplification

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tests (NAATs) display low diagnostic sensitivity,[7,8] leading the World Health Organization (WHO) to prioritize the development of rapid, non-sputum-based assays for pediatric TB diagnosis.[1,9]

Multiple approaches have been developed to improve the diagnosis of TB in children by analyzing stool, urine, or blood specimens, but all have reduced sensitivity compared to a sputum-based reference standard, limiting their use to children with more severe disease and hence higher bacillary loads.[10–13] Xpert MTB/RIF NAAT results generated with an optimized stool specimen analysis protocol have 32–47% diagnostic sensitivity for pediatric TB,[12,13] Xpert Ultra analysis of adult blood samples exhibits only 37% sensitivity for HIV-associated severe TB, and Xpert does not detect TB when applied to analyze pediatric urine specimens.[10,14] A urine lipoarabinomannan assay has slightly higher sensitivity (48·3–64·9%) in specific pediatric populations, but poor specificity (60·8–83·8%).[15,16] None of these approaches meet the optimal performance requirement of the 2014 WHO target product profile for non-sputum pediatric TB diagnostic assays (≥66% sensitivity and ≥98% specificity).[17] Here, we therefore investigated the diagnostic performance of an ultrasensitive blood-based *Mtb* antigen-derived peptide test (MAP-TB),[18] and its ability to monitor treatment response, in independent prospective pediatric TB diagnostic cohorts enrolled in South Africa and the Dominican Republic, respectively.

Methods

Study design and oversight

This study evaluated the diagnostic performance of an *Mtb* antigen-derived peptide (MAP-TB, based on immuno-affinity liquid chromatography-tandem mass spectrometry) assay (appendix pp 3–4) that detects an *Mtb*-specific CFP10 peptide (CFP10pep) using trypsin-digested serum from children with presumptive TB enrolled in South Africa and the Dominican Republic, in adherence to Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines.[19] Study participants were enrolled under protocols approved by the Human Research Ethics Committee at Stellenbosch University (Stellenbosch, South Africa) or the Institutional Review Board of the University of Miami and the O&M Medical School (Santo Domingo, Dominican Republic). Written informed consent was obtained from participants' parents/legal guardians and assent was obtained from study participants > 7 years of age.

Samples were de-identified, archived and stored at −80 °C, then shipped to the Tulane University School of Medicine on dry ice for MAP-TB testing. All samples were analyzed by personnel blinded to study and clinical information. All authors had access to and participated in data interpretation and approved the final manuscript for submission. The authors vouch for the completeness and accuracy of the data and fidelity to the study protocol.

Study population

The SA cohort serum samples and patient data were obtained from symptomatic children with clinically presumed intrathoracic TB enrolled at Tygerberg Hospital and Karl Bremer Hospital (Cape Town, South Africa) between May 2013 and August 2017. These sites recruited children aged < 13 years with presumptive pulmonary TB based on any compatible symptom (e.g., cough, unexplained fever, poor growth or weight loss).[20] The DR cohort serum samples were prospectively collected from children aged < 18 years with presumptive TB enrolled at the Robert Reid Cabral Children's Hospital (Santo Domingo, Dominican Republic) between July 2019 and May 2023. All children were eligible if they had complete demographic information, results from all clinical testing conducted as per protocol, and at least one analyzable serum sample after excluding those with hemolysis, hyperlipidemia, or low volume (< 100 μL).

Clinical procedures

All eligible participants were screened for TB risk factors based on established local and international practice, including HIV status (HIV-positive or HIV-negative by ELISA or qPCR results), clinical features, TB exposure history over the previous 12 months, and tuberculin skin test (TST), chest X-ray (CXR), spinal CT (for the diagnosis of Pott's disease), microbiologic assay (≥ 2 *Mtb* culture, smear and/or Xpert MTB/RIF results on respiratory specimens) or histology (lymphoid, pleural and lung biopsy) results. TB treatment decisions were made by attending clinicians based on clinical/epidemiologic assessment and results of all other available investigations. All children were followed for six months and completed follow-up with routine clinical services to assess TB treatment outcomes. Blood samples from children who received antituberculosis treatment were collected at baseline (all), two weeks (DR cohort only), and two and six months after anti-TB treatment initiation (all). All other children who were not started on TB treatment and considered symptomatic controls had blood samples collected at study enrollment (SA and DR cohort) and at two weeks, two and six months follow-ups (DR cohort only).

TB definitions

Final TB disease classifications were retrospectively determined after follow-up using the 2015 NIH classification criteria.[21] Children were categorized as having confirmed TB (*Mtb* culture-positive or Xpert-positive from at least one respiratory specimen or tissue biopsy), unconfirmed TB (≥2 non-microbiological diagnostic features of TB without microbiological confirmation), or unlikely TB (symptomatic by 2015 NIH criteria irrespective of duration, with insufficient evidence of TB and improvement of symptoms without treatment for TB) (appendix p 12). EPTB was identified using previously reported criteria.[22] Effective response to treatment was defined as symptom improvement or resolution without the development of new symptoms suggestive of TB.

Primary and secondary outcomes

The two primary outcomes were MAP-TB assay superiority to the 2014 WHO target product profile for non-sputum pediatric TB diagnostic assays (≥66% sensitivity and ≥98% specificity)[17] and culture and Xpert MTB/RIF diagnostic performance, defining sensitivity as the percentage of confirmed and unconfirmed TB with positive assay results, and specificity as the percentage of unlikely TB with negative assay results (appendix p 12).

Secondary outcomes included MAP-TB sensitivity estimates based on HIV status; *Mtb* infection site (pulmonary or extrapulmonary); participant age (< 1, 1–2, 2–5, > 5 years); serial testing (baseline ± follow-up results); and combined assay results (MAP-TB, Xpert MTB/RIF, CXR and TST).

Exploratory outcomes included the evaluation of MAP-TB assay performance to monitor treatment response in children with confirmed and unconfirmed TB.

Clinical and MAP-TB evaluations

Respiratory specimen culture and Xpert MTB/RIF, CXR and TST analyses were conducted according to national guidelines as per standard of care. MAP-TB assays were performed at Tulane University's School of Medicine (appendix pp 3–4). MAP-TB assay peaks met the criteria for true CFP10pep signal if they coeluted with the internal standard peptide and had a signal-to-noise ratio ≥2·2 (to filter out electronic noise), had dot product and relative dot product similarity scores ≥0·71 and ≥0·79 (indicative of substantial target and fragment ion vector similarity to the spectral library and internal

standard peptide values of this target, respectively), exhibited negatively correlated ion pair percentages < 22% (to reject peptides with misalignments between target and internal standard ion fragments), and had significantly correlated ion pair coefficient sum values and ≥1·58 (to reject peptides without significant peak alignment).[18] CFP10pep intensity values, calculated as the peak area of a CFP10pep peak divided by the peak area of the sequence matched heavy-isotope-labeled internal standard peptide, were employed to standardize CFP10pep signal, but were not used as a threshold for CFP10pep-positive signal. Specimens that did not meet the five mass spectrometry criteria for CFP10pep signal were classified as CFP10pep-negative and by definition had CFP10pep intensity values of zero. Technicians were unaware of clinical findings until after MAP-TB results were locked and released for analysis.

Statistical analysis

This study was designed to have ≥90% power to assess all primary and secondary analyses using a paired design. We calculated that ≥66 children with TB would provide ≥90% power to evaluate MAP-TB sensitivity for pediatric TB diagnosis. Primary and secondary analyses employed all available data without imputation for missing data.

Superiority to the 2014 WHO optimum requirements for non-sputum pediatric TB diagnostic assays (≥66% sensitivity and ≥98% specificity)[17] were the coprimary outcome measures for this study. The primary hypotheses tested MAP-TB sensitivity for pediatric TB against a 66% null hypothesis, and specificity for unlikely TB against a 98% null hypothesis. Comparisons of sensitivity for confirmed and unconfirmed TB between serum-based MAP-TB assay and sputum-based tests were evaluated using exact McNemar tests in SAS software, v9·4 (SAS Institute). Potential CFP10pep signal differences between groups were analyzed in GraphPad Prism v10·2·2 or SPSS v27·0 using two-sided parametric (Brown-Forsythe and Welch) or non-parametric (Kruskal-Wallis) one-way analysis of variance (ANOVA) tests adjusted for multiple comparisons, or Student's t-tests or Mann-Whitney U tests, as appropriate. Categorical variables were analyzed by chi-square test or Fisher's Exact test. All tests were two sided with an α of 0·05.

Results

Study participants

The SA cohort enrolled and classified 157 children with valid culture, Xpert MTB/RIF, and MAP-TB results (Fig. 1A): 41 children with confirmed TB (16 with both EPTB and PTB, 25 with PTB only) (appendix p 13); 41 with unconfirmed TB, and 75 with unlikely TB (appendix p 13). Most children (84·7%) were < 5 years old at enrollment (median 20 months (interquartile range "IQR", 10–46)), although children with confirmed TB (median 39 months (IQR 17–83)) were older than those with unconfirmed TB (median 21 months (IQR 12–30)) or unlikely TB (median 15 months (IQR 10–30)), and had lower bacille Calmette-Guérin (BCG) vaccination frequency (87·8% versus 100% and 98·7%, respectively) (Table 1).

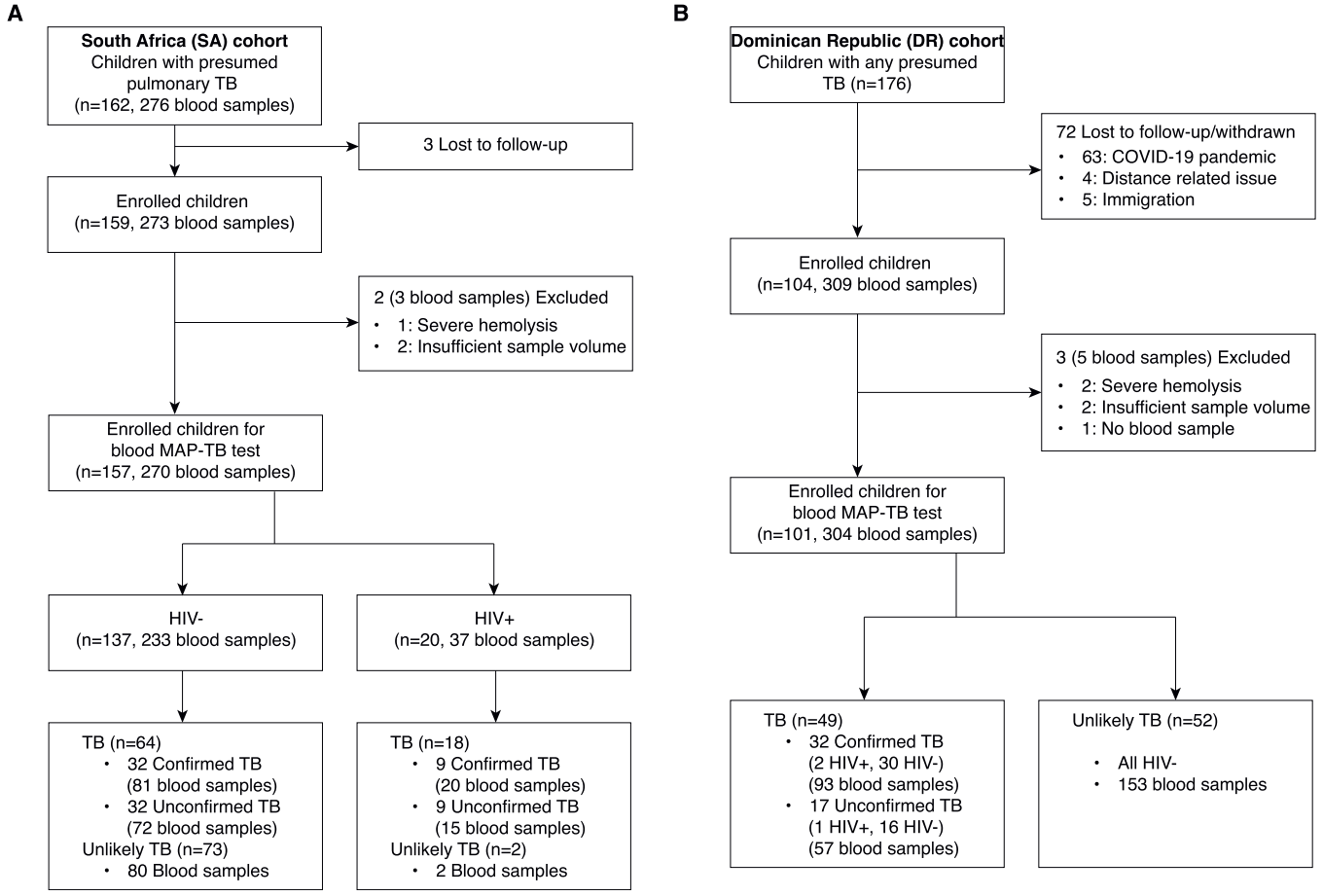

Fig. 1. Enrollment and outcomes. (**A**) A total of 157 children with presumed pulmonary TB (with 270 blood samples) who met all inclusion and exclusion criteria and had complete and valid respiratory specimen culture and Xpert MTB/RIF results were enrolled in the SA cohort, including 41 with microbiologically confirmed TB, 41 with unconfirmed TB and 75 with unlikely TB; (**B**) A total of 49 children with presumed TB (with 150 blood samples) who met all inclusion and exclusion criteria were enrolled in the DR cohort, including 32 with microbiologically confirmed TB, 17 with unconfirmed TB, as well as 52 children with unlikely TB who lacked a history of TB exposure. HIV+: HIV-positive; HIV-: HIV-negative.

Table 1
Baseline demographic and clinical characteristics of the SA and DR cohort of children with presumptive tuberculosis.

SA cohort	Total (n=157)[a]	Confirmed TB (n=41)[b]	Unconfirmed TB (n=41)[c]	Unlikely TB (n=75)[d]	*p*-value (Confirmed TB vs. Unlikely TB)	*p*-value (Unconfirmed TB vs. Unlikely TB)
Male sex — no. (%)	84 (52·5)	16 (37·2)	26 (61·9)	42 (56·0)	0·12	0·55
Age distribution — no. (%)						
< 1 yr	47 (29·9)	10 (24·4)	10 (24·4)	27 (36·0)	0·22	0·22
1–2 yr	45 (28·7)	7 (17·1)	15 (36·6)	23 (30·7)	0·13	0·54
2–5 yr	41 (26·1)	10 (24·4)	13 (31·7)	18 (24·0)	1·00	0·39
5–13 yr	24 (15·3)	14 (34·1)	3 (7·3)	7 (9·3)	0·002	1·00
Median age (IQR), mo	20 (10–46)	39 (17–83)	21 (12–30)	15 (10–30)	0·001	0·38
Median weight-for-age z score (IQR)[e]	−1·68 (−2·54 to −0·85)	−1·47 (−2·35 to −0·87)	−2·09 (−3·16 to −0·99)	−1·51 (−2·45 to −0·75)	0·90	0·25
Median height-for-age z score (IQR)	−2·01 (−2·92 to −0·83)	−1·77 (−2·76 to 0·88)	−2·31 (−3·12 to −1·29)	−1·79 (−2·81 to −0·62)	0·87	0·17
HIV Status — no.(%)						
HIV-positive	20 (12·5)	9 (22·0)	9 (22·0)	2 (2·7)	0·001	0·001
HIV-negative	137 (87·5)	32 (78·0)	32 (78·0)	73 (97·3)	0·001	0·001
Median CD4 count (IQR) — cells/μL[f]	1011 (733–1198)	1176 (1038–1240)	569 (364–860)	1269 (1116–1422)	–	–
Median CD4% (IQR) — %[f]	21(13–29)	27 (20–29)	12 (11–19)	33 (29–38)	–	–
Median plasma HIV−1 RNA (IQR) — copies/mL	81,235 (24,917–658,362)	48,827 (14,929–74,117)	658,363 (108,864–1,640,083)	N/A	–	–
Antiretroviral treatment before study entry — no. (%)[f]	10 (50·0)	7 (77·8)	1 (11·1)	2 (100)	–	–
Comorbidities — no.(%)	30 (19·1)	8 (19·5)	8 (19·5)	14 (18·7)	1·00	1·00
Neurological	9 (5·7)	3 (7·3)	2 (4·9)	4 (5·3)	0·70	1·00
Respiratory	5 (3·2)	2 (4·9)	0 (0·0)	3 (4·0)	1·00	0·55
Gastrointestinal	2 (1·3)	0 (0·0)	1 (2·4)	1 (1·3)	1·00	1·00
Other	14 (8·9)	3 (7·3)	5 (12·2)	6 (8·0)	1·00	0·52
BCG vaccination — no.(%)	151 (96·2)	36 (87·8)	41 (100)	74 (98·7)	0·02	1·00
Symptoms/Signs consistent with TB — no.(%)	122 (77·7)	34 (82·9)	37 (90·2)	51 (68·0)	0·12	0·007
Cough for ≥ 2 wk	66 (42·0)	21 (51·2)	16 (39·0)	29 (38·7)	0·24	1·00
Fever for ≥ 1 wk	20 (12·7)	8 (19·5)	7 (17·1)	5 (6·7)	0·06	0·11
Poor appetite or lethargy	104 (66·2)	27 (65·9)	30 (73·2)	47 (62·7)	0·84	0·31
Failure to thrive	68 (43·3)	17 (41·5)	23 (56·1)	28 (37·3)	0·69	0·08
Tuberculin skin test — +/total (%)	35/109 (32·1)	20/27 (74·1)	10/28 (35·7)	5/54 (9·3)	< 0·001	0·006
TB exposure — no.(%)	55 (35·0)	23 (56·1)	18 (43·9)	14 (18·7)	< 0·001	0·005
History of anti-TB treatment — no.(%)	13 (8·3)	4 (9·8)	4 (9·8)	5 (6·7)	0·72	0·72
DR cohort	**Total (n=101)**	**Confirmed TB (n=32)**	**Unconfirmed TB (n=17)**	**Unlikely TB (n=52)**	***p*-value (Confirmed TB vs. Unlikely TB)**	***p*-value (Unconfirmed TB vs. Unlikely TB)**
Male sex — no.(%)	49 (48·5)	14 (43·8)	8 (47·1)	27 (51·9)	0·51	0·79
Age distribution — no.(%)						
< 1 yr	4 (4·0)	4 (12·5)	0 (0·0)	0 (0·0)	0·02	–
1–2 yr	16 (15·8)	3 (9·4)	4 (23·5)	9 (17·3)	0·36	0·76
3–5 yr	10 (9·9)	1 (3·1)	1 (5·9)	7 (13·5)	0·15	0·67
5–17 yr	71 (70·3)	23 (71·9)	12 (70·6)	36 (69·2)	1·00	1·00
Median age (IQR), yr	10·0 (4·0–14·0)	11·5 (3·8–15·0)	10·0 (3·0–14·0)	9·0 (4·0–13·0)	0·40	0·87
Median body-mass index (IQR), kg/m^2	16·5 (14·9–19·7)	16·6 (15·3–18·2)	16·4 (14·4–21·3)	16·7 (14·6–19·9)	0·71	0·78
HIV Status — no.(%)						
HIV+	3 (3·0)	2 (6·3)	1 (5·9)	0 (0·0)	0·14	0·25
HIV-	98 (97·0)	30 (93·7)	16 (94·1)	52 (100)	0·14	0·25
Symptoms/Signs consistent with TB — no.(%)						
Cough for ≥ 2 wk	28 (27·7)	20 (62·5)	6 (35·3)	2 (3·8)	< 0·001	0·002
Fever for ≥ 1 wk	30 (29·7)	19 (59·4)	9 (52·9)	2 (3·8)	< 0·001	< 0·001
TB exposure — no.(%)	12 (11·9)	7 (21·9)	5 (29·4)	0 (0·0)	< 0·001	< 0·001
Tuberculin skin test — +/total (%)	13/79 (16·5)	7/14 (50·0)	6/14 (42·9)	0/51 (0·0)	< 0·001	< 0·001

[a] TB status was retrospectively determined using the 2015 NIH criteria.
[b] Confirmed TB: at least one positive *Mtb* culture or Xpert MTB/RIF result.
[c] Unconfirmed TB: ≥2 different categories of non-microbiological evidence of TB.
[d] Unlikely TB: insufficient evidence of TB.
[e] United Kingdom Standards were applied.[28]
[f] Values calculated for HIV-positive children only.

Median weight-for-age and height-for-age z-scores did not differ between groups (*p*=0·17–0·90, by Mann-Whitney U test). More children with confirmed and unconfirmed TB were HIV-positive (22·0% for both) than those with unlikely TB (2·7%) (*p*=0·001, by Fisher's Exact test). Anti-retroviral treatment (ART) coverage at enrolment was higher in the confirmed and unlikely TB groups (77·8% and 100%) than the unconfirmed TB group (11·1%), with corresponding effects on their viral loads and CD4 cell measures.

Table 2
Diagnostic performances of culture, Xpert MTB/RIF and MAP-TB for pediatric TB using SA and DR cohorts baseline samples.

Assay	All TB[a]	Confirmed TB	Unconfirmed TB	All unlikely TB	Unlikely TB, no NIH criteria[b]	Unlikely TB, with NIH criteria[c]
	Sensitivity, % (95% CI)			Specificity, % (95% CI)		
SA cohort						
Culture, +/total	36/82	36/41	0/41	0/75	0/24	0/51
	43·9 (33·0–55·3)	87·8 (73·8–95·9)	0·0 (0·0–8·6)	100 (95·2–100)	100 (85·8–100)	100 (93·0–100)
Xpert, +/total	18/82	18/41	0/41	0/75	0/24	0/51
	22·0 (13·6–32·5)	43·9 (28·5–60·3)	0·0 (0·0–8·6)	100 (95·2–100)	100 (85·8–100)	100 (93·0–100)
MAP-TB, +/total	67/82	34/41	33/41	12/75	1/24	11/51
	81·7 (71·6–89·4)	82·9 (67·9–92·9)	80·5 (65·1–91·2)	84·0 (73·7–91·5)	95·8 (78·9–99·9)	78·4 (64·7–88·7)
DR cohort						
Culture, +/total	7/11	7/9	0/2	0/1	–	–
	63·6 (30·8–89·1)	77·8 (40·0–97·2)	0·0 (0·0–84·2)	100 (2·5–100)	–	–
Xpert, +/total	29/42	29/30	0/12	0/0	–	–
	69·1 (52·9–82·4)	96·7 (82·8–99·9)	0·0 (0·0–26·5)	–	–	–
MAP-TB, +/total	40/49	27/32	13/17	1/52	–	–
	81·6 (68·0–91·2)	84·4 (67·2–94·7)	76·5 (50·1–93·2)	98·1 (94·3–100)	–	–

[a] Includes both confirmed and unconfirmed TB.
[b] Symptomatic but do not meet any NIH TB criteria.
[c] Symptomatic with ≥1 NIH criteria, but improved without anti-TB treatment.

Comorbidity was similar for all groups. The proportion of children with "typical TB symptoms" was higher in the confirmed and unconfirmed TB groups (82·9% and 90·2%) than the unlikely TB group (68·0%) (p = 0·12, 0·007, by Fisher's Exact test), as was the frequency of TB exposure (56·1% and 43·9% versus 18·7%) (p < 0·001 and p=0·005, by Fisher's Exact test), while positive tuberculin skin test (TST) results were more frequent in the confirmed (74·1%) vs. unconfirmed (35·7%) and unlikely TB (9·3%) groups (p < 0·001 and p=0·006, by Fisher's Exact test).

The DR cohort included 176 children with presumptive TB; 63 were lost to follow-up due to COVID-19 pandemic related disruptions; 9 were withdrawn due to inability to attend follow-up; and 3 were excluded for missing or poor quality blood samples. DR analyses thus employed data from 101 children ≤18 years-old (median 10 years (IQR 4·0–14·0)) who met study criteria: 32 with confirmed TB, 17 with unconfirmed TB, and 52 with unlikely TB (Fig. 1B). These children had low median body-mass index (BMI) scores (16·5 (IQR 14·9–19·7) kg/m^2) that did not vary by group (p=0·71, 0·78, by Fisher's Exact test), and HIV infection rates were low in all groups (0·0–6·3%) (Table 1). Cough and fever were common in the confirmed TB (62·5% and 59·4%) and unconfirmed TB (35·3% and 52·9%) groups, but not the unlikely TB group (3·8% for both). Reported TB exposure was lower in the DR than the SA cohort (11·9% vs. 35·0%) (p < 0·001, by Fisher's Exact test), as was the proportion of children with TST positivity (16·5% vs. 32·1%) (p=0·02, by Fisher's Exact test).

Primary and secondary study results

Most SA children with confirmed TB had positive MAP-TB results (82·9% sensitivity, 95% confidence interval [95%CI]: 67·9–92·9%) (Table 2, Fig. 2A), exceeding the WHO-proposed optimum sensitivity (66%) for new non-sputum-based pediatric TB diagnostics.[17] Sensitivity for unconfirmed TB was 80·5% (95% CI 65·1–91·2%), yielding 81·7% (95% CI 71·6–89·4%) overall sensitivity. Sensitivity was not affected by TB disease site (pulmonary or extrapulmonary, appendix p 14), but differed by age (< 1 year: 57·1%, 1–2 years: 81·0%, 2–5 years: 91·3%, > 5 years: 100%, and < 5 years: 76·9%) (appendix pp 15–16). MAP-TB produced false-negative results for seven children with confirmed TB, but four of five of these children with follow-up samples had at least one MAP-TB-positive sample (appendix p 5). MAP-TB also identified three children with EPTB and 33 (80·5%) with unconfirmed TB (Fig. 2B), including nine children living with HIV.

Mean MAP-TB signal was greater in the confirmed and unconfirmed TB groups than unlikely TB group (p < 0·001) but did not differ between the confirmed and unconfirmed TB (p=0·54), PTB and EPTB (p=0·93), or severe and non-severe TB (p=0·85) groups (Fig. 2C; appendix p 6). Substantial signal overlap was observed among these groups, and high values in the SA unlikely TB group were comparable to those of the SA confirmed and unconfirmed TB groups. The reason(s) for overlap are unclear, but these false-positives could represent subclinical or missed TB cases, although none of these children had TB diagnoses within six-months of follow-up, and subsequent samples and clinical results were not available for MAP-TB analysis and TB evaluation.

Baseline MAP-TB signal and diagnostic sensitivity did not differ in the HIV-positive confirmed and unconfirmed TB groups (88·9% (95% CI 51·8–99·7%) for both) despite different ART initiation rates pre-enrollment (7/9 vs. 1/9) (Fig. 2D, appendix p 17). Nor did sensitivity estimates differ for the HIV-negative confirmed and unconfirmed TB groups (81·3% (95% CI 63·6–92·8%) vs. 78·1% (95% CI 60·0–90·7%)) (appendix p 18). Diagnostic sensitivity estimates and area under the receiver operating characteristic (ROC) curve (AUC) values were higher for HIV-positive versus HIV-negative children (0·94 vs. 0·82) (Fig. 2E), but sensitivity estimates for these groups did not differ (88·9% (95% CI 65·3–98·6%) vs. 79·7% (95% CI 67·8–88·7%) (p=0.50, by Fisher's Exact Test) (appendix pp 17,18).

MAP-TB sensitivity estimates were similar in the DR (81·6% (95% CI 68·0–91·2%)) and SA cohorts (81·7% (95% CI 71·6–89·4%) despite the DR cohort's greater median age (10·0 (4·0–14·0) vs. 1·7 (0·8–3·8) years) (Table 2). MAP-TB sensitivity was comparable in the DR confirmed and unconfirmed TB groups (84·4% (95% CI 67·2–94·7%) vs. 76·5% (95% CI 50·1–93·2%)) (Fig. 2F, Table 2), but higher for the EPTB (100% (95% CI 69·2–100%)) and PTB + EPTB (100% (95% CI 39·8–100%)) than PTB groups (74·3% (95% CI 56·7–87·5%)) (p=0.045, by Fisher's Exact Test) (appendix p 19). However, MAP-TB signal intensity did not differ among these subgroups (Fig. 2G, H). For children with both results, MAP-TB detected ten children with unconfirmed TB missed by Xpert, but missed five children with confirmed TB (Fig. 2I). ROC AUC values were higher in the DR vs. SA cohort (0·91 vs. 0·83) (Fig. 2J, E), where sensitivity did not differ with age (appendix p 20–21). Three SA cohort and one DR cohort children were diagnosed with confirmed TB based on follow-up culture/Xpert results but had MAP-TB-positive baseline serum specimens.

Including positive MAP-TB results from one or both follow-up samples increased the SA sensitivity estimates at baseline from 81·7% (95% CI 71·6–89·4%) to 90·2% (95% CI 81·7–95·7%) and 92·7% (95% CI 84·8–97·3%), and similar effects were observed in the DR cohort. No comparable effects were detected when baseline culture and Xpert results were considered with their follow-up results in either the SA or DR cohort (appendix pp 7, 22–23). Combining SA

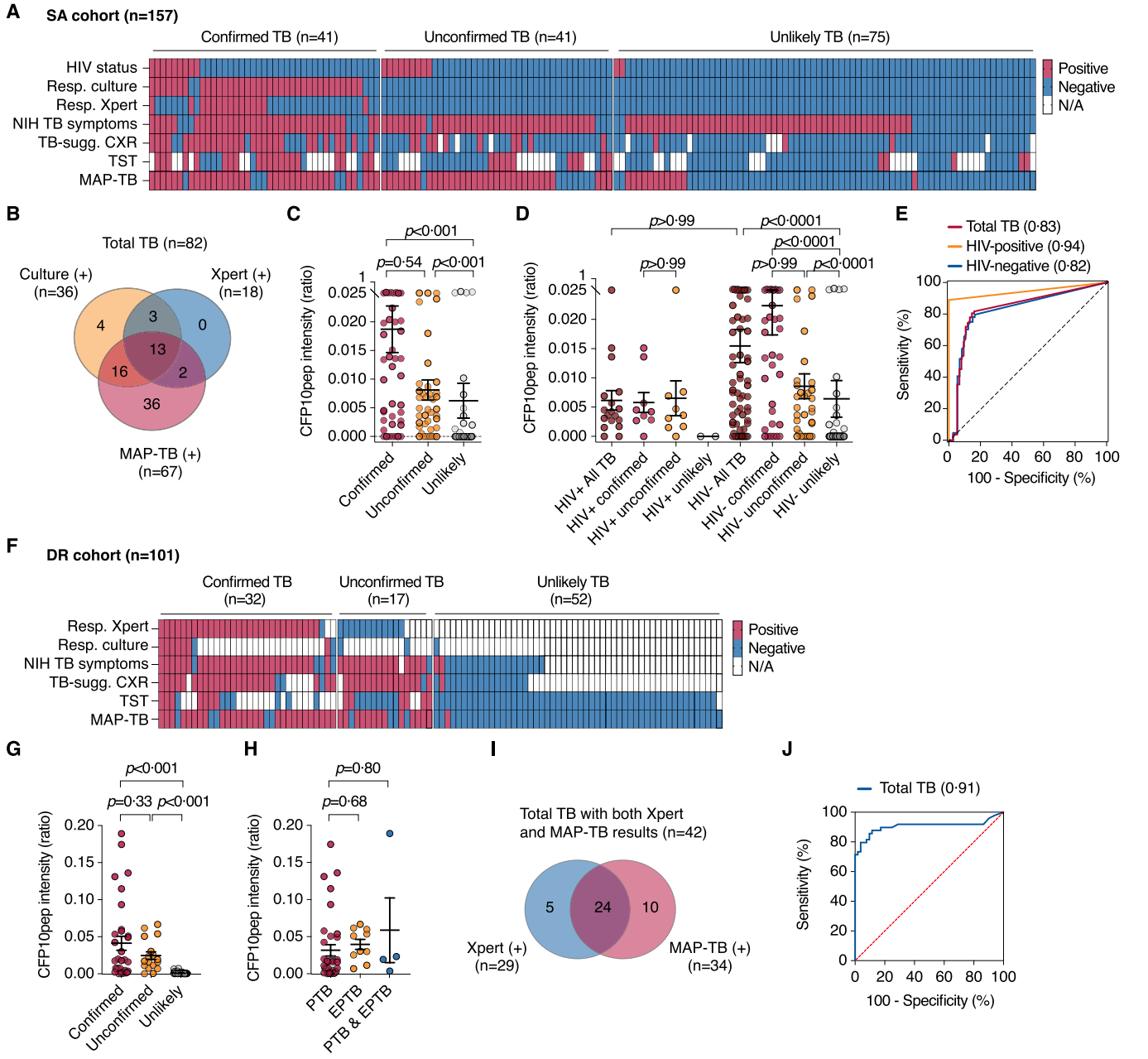

Fig. 2. Diagnostic sensitivity and specificity of the MAP-TB blood-based assay. (**A, F**) Baseline respiratory culture, Xpert MTB/RIF, NIH TB symptoms (persistent cough > 14 d, fever > 7 d, failure to thrive, or lethargy > 7 d), CXR, TST and MAP-TB results of the (**A**) SA cohort and (**F**) DR cohort; (**B, I**) Venn diagram of the number of confirmed and unconfirmed TB identified by culture, Xpert and/or MAP-TB results of the (**B**) SA cohort and (**I**) DR cohort; (**C, G, H**) CFP10pep signal intensities detected in confirmed, unconfirmed and unlikely TB of the (**C**) SA cohort and (**G**) DR cohort and (**H**) PTB, EPTB and PTB plus EPTB of the DR cohort; (**D**) CFP10pep signal intensities detected in SA cohort HIV-positive and HIV-negative confirmed, unconfirmed and unlikely TB; (**E, J**) ROC values for MAP-TB diagnosis of HIV-positive, HIV-negative, and all pediatric TB in the SA cohort (**E**) and all pediatric TB in the DR cohort (**J**). Children with negative respiratory culture and Xpert results in the SA and DR cohort confirmed TB groups were diagnosed with EPTB using results from tissue biopsy specimens. Specimens were determined to be CFP10pep-positive if their MAP-TB signal met five previously reported criteria, as described in Methods. CFP10pep signal intensity ratios were calculated as the ratio of the peak areas of the CFP10pep and its sequence-matched stable-isotope-labeled internal standard peptide, so that all CFP10pep-negative samples had zero values. Error bars indicate Mean ± SEM.

MAP-TB results with positive results from Xpert increased the TB identification rate from 81·7% to 85·4% without reducing specificity, but including results from other tests increased the TB detection rate while decreasing specificity (appendix pp 8, 24–25). Missing data prevented replication of this analysis with DR cohort results.

Most SA children with unlikely TB were MAP-TB-negative (84·0% (95% CI 73·7–91·5%) specificity), but 68% (51 of 75) had TB-consistent symptoms and could represent incipient, subclinical, or undiagnosed TB. Excluding these symptomatic children yielded 95·8% (95% CI 78·9–99·9%) specificity, short of the WHO target product profile's 98% optimal specificity target, unlike the DR cohort's MAP-TB specificity estimate (98·1% (95% CI 94·3–100%)) (Table 2). The WHO target product profile did not specify age ranges, however, and our results suggest that MAP-TB has reduced specificity in very young children with developing immune systems as 10 of the 12 SA children with false-positive results were < 5 years old (median 1·99 years (IQR: 1·41–3·94 years) (appendix pp 15–16).

Exploratory analyses

Most SA cohort children who received antituberculosis treatment had valid MAP-TB results (65 of 82; 79·3%) at both follow-up visits,

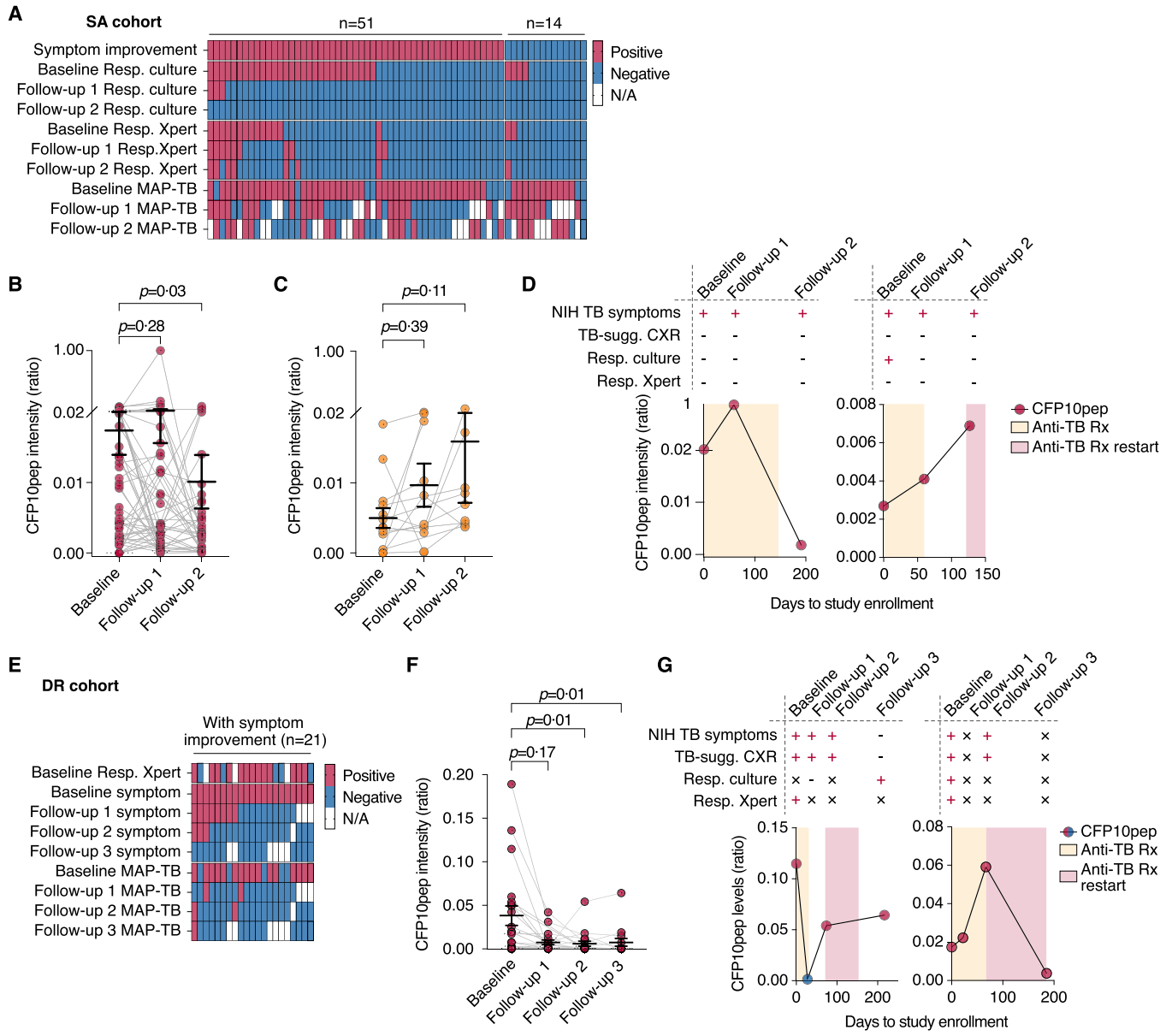

Fig. 3. Serum MAP-TB signal responses decrease during effective TB treatment. (**A, E**) Follow-up respiratory culture, Xpert MTB/RIF and serum MAP-TB results of (**A**) SA cohort and (**E**) DR cohort; (**B, C, F**) CFP10pep signal intensity in baseline and follow-up serum of SA cohort TB (**B**) with and (**C**) without symptom improvement, and (**F**) DR cohort TB with symptom improvement; (**D, G**) CFP10pep signal intensity changes during anti-TB treatment for children in (**D**) the SA cohort who had TB lymphadenitis (left) or HIV/TB coinfection and premature discontinuation of their anti-TB treatment (right) and (**G**) the DR cohort who had premature discontinuation of their anti-TB treatment (left), or rifampin-resistant TB (right).

improved symptoms (51 of 65; 78·5%), and valid MAP-TB, Xpert, and culture results at their first and second follow-up visits (80% and 72%) (Fig. 3A, appendix p 9). MAP-TB signal did not differ between enrollment and the first follow-up visit for children with and without symptom improvement (p=0·28 and 0·39), and decreased at the second follow-up visit only for children with symptom improvement (Fig. 3B, C). Most children with poor treatment responses showed CFP10pep signal increases at follow-up (85·7%, 12 out of 14) (appendix p 10, Fig. 3D, Right), suggesting MAP-TB signal may distinguish successful and unsuccessful treatment responses.

Individual analyses also suggest that MAP-TB results could guide treatment monitoring. CFP10pep signal increased and markedly decreased at the first and second follow-up visits of one child with EPTB (TB lymphadenitis) (Fig. 3D, Left) consistent with symptom improvement. Conversely, a child living with HIV whose anti-TB treatment was stopped after only 63 days revealed a continuous CFP10pep signal increase consistent with no symptom improvement (Fig. 3D, Right).

Fourteen DR cohort children with confirmed TB and seven with unconfirmed TB had MAP-TB results and clinical information available at two- and six-months follow-up. Most lacked follow-up culture and/or Xpert results, but all had symptom improvement consistent with a positive treatment response by their final follow-up visit (Fig. 3E). Seventeen children had positive CFP10pep signals (81·0%), and all but one child revealed rapid CFP10pep decreases that yielded positive-to-negative conversions after treatment initiation (Fig. 3F) and tended to inversely relate to BMI increases (appendix p 11). One child with confirmed TB who stopped treatment at discharge 25 days post-hospitalization was culture-negative and MAP-TB-negative at discharge but relapsed one month later, and was MAP-TB positive at the start and end of a second three-month treatment regimen that was again stopped for unknown reasons

(Fig. 3G, Left). Notably, MAP-TB signal increased in one child with rifampin-resistant TB until they were switched to alternate treatment at their second follow-up visit (Fig. 3G, Right).

Discussion

MAP-TB overall diagnostic sensitivity (confirmed and unconfirmed TB) in the SA and DR cohorts (81·7% and 81·6%), exceeded the sensitivity of Xpert (22·0% and 69·1%) and culture (43·9% and 63·6%) reference tests employing respiratory specimens for TB diagnosis,[21] and the optimum sensitivity threshold proposed by the WHO target product profile (≥66%). MAP-TB sensitivity for SA and DR children with confirmed TB (82·9% and 84·4%) was comparable to culture and Xpert, and to MAP-TB sensitivity for SA and DR children with unconfirmed TB (80·5% and 76·5%). MAP-TB also met the WHO-proposed optimum specificity threshold in the DR cohort but not in the younger SA cohort. No children with false-positive MAP-TB results developed TB within the six-month follow-up period but subsequent samples and clinical results were not available for MAP-TB analysis and TB evaluation. CFP10pep-positive signal can precede TB diagnosis in at-risk infants,[23] and future studies should thus evaluate whether it can predict TB development in young children.

MAP-TB sensitivity did not markedly vary between confirmed vs. unconfirmed TB, PTB vs. EPTB, and HIV-positive vs. HIV-negative TB subgroups. Culture and Xpert do not detect unconfirmed TB, require invasive samples to diagnose EPTB, and have reduced sensitivity in HIV-positive children, who are more prone to develop EPTB.[24] MAP-TB had comparable sensitivity in SA cohort children aged 1–2 and 2–5 years (81·0% and 91·3%) but reduced sensitivity for children aged < 1 year (57·1%), while culture and Xpert had variable sensitivity when stratified by age. However, statistical power limits these analyses. Xpert reportedly has 48·6% (32·5–65·0%) sensitivity versus culture when analyzing induced sputum from children aged 0–4 years, while Xpert Ultra exhibits 69·8% (54·6 to 81·6%) sensitivity, but only 20·7% (15·7 to 26·6%) sensitivity versus a composite standard that includes confirmed and unconfirmed TB.[25,26]

Young children are more likely to present with disseminated TB or EPTB, which can rapidly progress in the absence of appropriate treatment, likely due to a failure to contain *Mtb* within stable granulomas.[27] This could explain reduced respiratory Xpert sensitivity in this group, but not reduced serum MAP-TB sensitivity. MAP-TB sensitivity for SA and DR cohort PTB+EPTB (87·5% and 100%) and EPTB participants (100%) enrolled only in the DR cohort exceeded sensitivity thresholds proposed for adult EPTB diagnosis from lymph node aspirates or tissue biopsies (≥85%) or cerebrospinal fluid (≥80%).[17]

New approaches are needed to evaluate TB treatment response in children to inform care and assess novel treatment shortening regimens that are particularly relevant to children with paucibacillary disease. Most children with pulmonary TB (≥70%) are culture-negative and Xpert-negative at diagnosis,[7] radiographic findings have low specificity and may not normalize until well after treatment completion, and signal from specific immunologic tests does not decrease within a useful timeframe. MAP-TB signal, however, rapidly decreased in children with confirmed or unconfirmed TB who had good clinical responses after appropriate treatment initiation, indicating its potential to monitor treatment response.

More data is required to confirm both primary and exploratory results, and more children with unlikely TB are needed to accurately evaluate MAP-TB specificity against the WHO target product profile's optimal specificity threshold. Future studies should also evaluate the feasibility of translating MAP-TB for central laboratory testing of samples shipped from resource limited areas, and its utility in diagnostic algorithms. Finally, this study did not employ Xpert Ultra, which might have altered the diagnosis of some children, but would have likely not have affected the composite reference category.

Despite these limitations, we believe our findings indicate MAP-TB has potential utility to improve pediatric TB diagnosis and treatment monitoring since it detects confirmed and unconfirmed TB, PTB and EPTB, and HIV-positive vs. HIV-negative TB with similar sensitivity and can distinguish children who are and are not responding to treatment.

Author contributions

LL and CJL analyzed and interpreted data and drafted the manuscript. LM, SL, and QS performed the MAP-TB experiments. MP, PG, HSS, SN, AH, MMVDZ and EG were involved in data curation and project administration of the SA cohort. JO, CV, AMD, and EPT were involved in data curation and project administration of the DR cohort. CDM, EG, MMVDZ, and TYH conceived, designed, and acquired funding for the conduct of the study. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data availability

Data supporting the major results in this study are available within this article and its appendix. Raw datasets generated and analyzed during the current study are available from the corresponding author on reasonable request following publication.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: TYH Patent Number PCT US20/37785; founder of NanoPin Technologies; owns stock in NanoPin Technologies. CJL, officer at NanoPin Technologies; inventor on NanoPin Patent Application Number: 63/006.822; owns stock in NanoPin Technologies.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106404.

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