Diagnostic Performance of Host-based Gene Expression Diagnostics in Children With Extrapulmonary Tuberculosis

A Systematic Review

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Background: Diagnosing extrapulmonary tuberculosis (EPTB) in children is challenging due to nonspecific presentations and poor diagnostic yield from conventional microbiologic tests. Host gene expression signatures offer a non-sputum-based diagnostic alternative. This systematic review evaluates their diagnostic performance in pediatric EPTB.

Methods: We systematically reviewed host-based gene expression diagnostics for pediatric EPTB. PubMed, Embase and Cochrane Library (January 1965–May 2025) were searched for studies in children (0–18 years) with EPTB. Exclusions were adult-only studies, mixed data on pulmonary TB and EPTB without disaggregation, pulmonary TB-only studies, reviews and abstracts. Two reviewers screened data, resolving disagreements by discussion.

Results: Of 830 records, 2 studies met the inclusion criteria: Pan et al. (2017) and Olbrich et al. (2024), both in low and middle-income countries, enrolling a total of 891 children under 15 years. Olbrich et al.'s 3-gene MTB-HR prototype showed 59.8% sensitivity against a strict culture-confirmed reference standard and 50.0% in isolated EPTB with a low risk of bias. Using a microbiologic, clinical and radiologic composite standard, Pan et al.'s miRNA-29a assay achieved 67.2% sensitivity, 88.5% specificity in peripheral blood mononuclear cells; 81.1% sensitivity, 90.0% specificity in cerebrospinal fluid; 84.4% sensitivity, 95.4% specificity in combined peripheral blood mononuclear cell/cerebrospinal fluid with a high risk of bias.

Conclusions: Evidence for host gene expression diagnostics in pediatric EPTB is limited by few studies, small sample sizes, bias and lack of disaggregated data, with accuracy falling short of the World Health Organization targets.

Key Words: tuberculosis, extrapulmonary TB, gene expression, signature, host-response, diagnostics, pediatrics

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The World Health Organization (WHO) estimates that 10.8 million people developed tuberculosis (TB) in 2023. Of these, approximately 1.3 million were children and young adolescents (0–14 years), accounting for 12% of all new cases worldwide. In 2023, TB caused an estimated 166,000 deaths among HIV-negative children and young adolescents and 25,000 deaths among those with HIV.[1] While pulmonary TB (PTB) is the most common presentation, extrapulmonary tuberculosis (EPTB), defined as TB infection outside the lungs, accounts for around 16% of TB cases, with a higher incidence in children.[2,3] EPTB can affect lymph nodes, the meninges, bones and spine, genitourinary tract or abdominal organs. The incidence of different forms of EPTB varies and so does the morbidity and mortality, with the mortality of pediatric tuberculosis meningitis (TBM) close to 20% and over half of survivors experience long-term neurologic consequences even with treatment.[4–6]

Timely diagnosis of pediatric EPTB remains challenging, as clinical features such as prolonged fever, failure to thrive or unexplained neurologic signs often overlap with other common pediatric illnesses, contributing to missed or delayed diagnoses.[7] The poorest outcomes are observed in children who received a delayed diagnosis.[6] Conventional microbiologic diagnostics, including microscopy, culture and nucleic acid amplification tests, often require invasive procedures in EPTB, such as lymph node biopsy or lumbar puncture, which may be technically challenging in children and they have limited sensitivity.[8,9] Although the introduction of GeneXpert MTB/RIF has improved diagnostic performance in EPTB, it varies substantially by specimen type.[10] While rapid diagnostics such as Xpert MTB/RIF or Xpert MTB/RIF Ultra are recommended by the WHO as the initial diagnostic test for all forms of TB, including EPTB, it is still not accessible where patients often first present.[11]

Host-based gene expression profiling has emerged as a promising diagnostic approach. This method measures TB-specific genes expressed signatures, potentially offering a less invasive and more sensitive test than microbiologic assays in EPTB. Host transcriptional signatures of 3 genes [guanylate-binding protein (GBP5), dual specificity phosphatase 3 (DUSP3) and krupple-like factor 2 (KLF2)] have shown encouraging accuracy for active PTB diagnosis and can discriminate against TBM from other brain infections in adults.[12,13] However, children mount distinct

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Data extraction forms and search strategies are available upon reasonable request to the corresponding author.

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immune responses and transcriptional responses to TB.[14–16] A 2018 systematic review evaluating biomarkers for pediatric PTB concluded that host-based diagnostics show promise, but their applicability to extrapulmonary forms remains underexplored.[17]

Therefore, this systematic review synthesizes the existing evidence on host gene expression signatures for diagnosing pediatric EPTB, evaluates their diagnostic performance and explores their potential to improve timely and accurate diagnosis in this vulnerable population.

METHODS

Objectives

The primary objective of this review is to evaluate the diagnostic performance of host-based gene expression diagnostics in children with EPTB, focusing on accuracy, feasibility and utility.

Inclusion and Exclusion Criteria

We included studies assessing host-derived gene expression for diagnosing EPTB in children (0–18 years) using blood or extrapulmonary clinical specimens. Eligible study designs included cross-sectional, case-control, cohort and clinical trials. We excluded adult-only studies, studies including both adults and children with non-disaggregated data, PTB-only studies, reviews, commentaries and conference abstracts.

Search Strategy

A systematic search was performed in PubMed/MEDLINE, Embase and the Cochrane Library for articles published between January 1, 1965, and May 17, 2025. The search strategy included terms related to EPTB, gene expression, diagnostics and pediatric populations. The PubMed search strategy included terms such as "extrapulmonary tuberculosis", "RNA", "gene expression", "diagnostic accuracy" and "children". The terms were adapted for each database. The full search strategy is included in Supplemental Digital Content 1, https://links.lww.com/INF/G371.

Study Selection

All identified studies were imported into Rayyan (http://rayyan.qcri.org) systematic review software (Rayyan Systems, Inc, Cambridge, MA).[18] Two reviewers (M.C.S. and M.S.) independently screened titles and abstracts, followed by full-text reviews to determine eligibility. Any disagreements were resolved through discussion or with the input of a 3rd and 4th reviewer (R.B. and J.P.S.). The selection process adhered to Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines.[19]

Data Extraction

Two reviewers (M.C.S. and M.S.) extracted data using a predesigned Excel template, capturing study design, setting, participants, EPTB type, diagnostics, reference standards, biomarkers and diagnostic accuracy metrics such as sensitivity, specificity and area under the curve (AUC). Where reported, data on implementation challenges and clinical impact were also collected. Disagreements were resolved by discussion.

Assessment of Bias/Methodologic Quality of Study

Three reviewers (M.C.S., M.S. and J.P.S.) used QUADAS-2 to assess risk of bias across patient selection, index test, reference standard and flow/timing, rating each domain as low, high or unclear.[20] Reporting quality was assessed by adhering to Standards for Reporting Diagnostic accuracy Studies guidelines.

Predictive Performance

When available, diagnostic performance metrics such as sensitivity, specificity, positive predictive value, negative predictive value and AUC were extracted and analyzed. These measures were used to assess the clinical diagnostic potential of each host gene expression signature for pediatric EPTB. The systematic review was prospectively registered with PROSPERO (CRD42024577767), and this acted as the review protocol. No amendments were made to the registered protocol.

RESULTS

A total of 830 studies were identified (PubMed, Cochrane and Embase), published between January 1965 and May 2025, shown in Figure 1 (Preferred Reporting Items for Systematic reviews and Meta-Analyses). After removing 71 duplicates, 759 titles/abstracts were screened, and 8 full-text articles were retrieved and assessed for eligibility. Two studies met the inclusion criteria and were included in the systematic review. Due to the heterogeneity, no meta-analysis was performed.

Study Characteristics

We identified 2 pediatric EPTB studies collectively enrolling 891 children under 15 years. A 2024 prospective diagnostic accuracy trial by Olbrich et al.,[21] published as part of the Rapid and Accurate Diagnosis of Pediatric Tuberculosis study group, enrolled children with presumptive TB across 5 high-burden settings (South Africa, Malawi, India, Tanzania and Mozambique). Among 639 children evaluated for primary accuracy, 202 had culture-confirmed TB and 207 were classified by clinicians as unlikely TB after negative microbiologic investigations. 71 children had severe acute malnutrition, 89 were HIV-positive and 13 had both. Extrapulmonary involvement alone was identified in 14% (59 of 418) of cases, whereas coexisting pulmonary and extrapulmonary disease was present in 18% (74 of 418).[22]

A 2017 multicenter case-control study by Pan et al.[23] enrolled 122 children diagnosed with TBM, of whom 112 were included in the analysis, compared against 130 healthy controls, from 3 hospitals in China.

Diagnostic Approaches

The 2024 Olbrich et al. study evaluated a 3-gene host-response messenger RNA (mRNA) signature looking at GBP5, DUSP3 and KLF2 via an RT-qPCR cartridge assay [cepheid mycobacterium tuberculosis host response prototype cartridge (MTB-HR)] run on capillary whole blood sampling taken from a finger (fingerstick) using the GeneXpert platform. Both approaches leverage reverse-transcription quantitative PCR but differ in their target analytes [microRNA (miRNA) vs. mRNA signatures] and specimen types [peripheral blood mononuclear cell (PBMC)/cerebrospinal fluid (CSF) vs. capillary blood] (Table 1). The selection of GBP5, DUSP3 and KLF2 in Olbrich et al.'s MTB-HR cartridge builds on multicohort evidence of their discriminatory power for active TB. The 3-gene host RNA signature (GBP5, DUSP3 and KLF2) was first proposed through a pooled transcriptomic analysis of adult PTB datasets by Sweeney et al.[12] Huynh et al. (2024) applied the 3-gene host RNA signature to whole blood samples using RNA sequencing in adults with TBM, compared with other brain infections, reporting an AUC of 0.66 and 0.74 for GBP5 alone, supporting its EPTB relevance. HIV coinfection improved diagnostic performance in TBM.[13] Pan et al. measured the expression of a single noncoding host miRNA, miR-29a, using RT-qPCR on PBMCs and CSF samples. MicroRNAs are small noncoding RNAs that regulate gene expression post-transcriptionally by promoting mRNA degradation or translational repression. Pan et al.

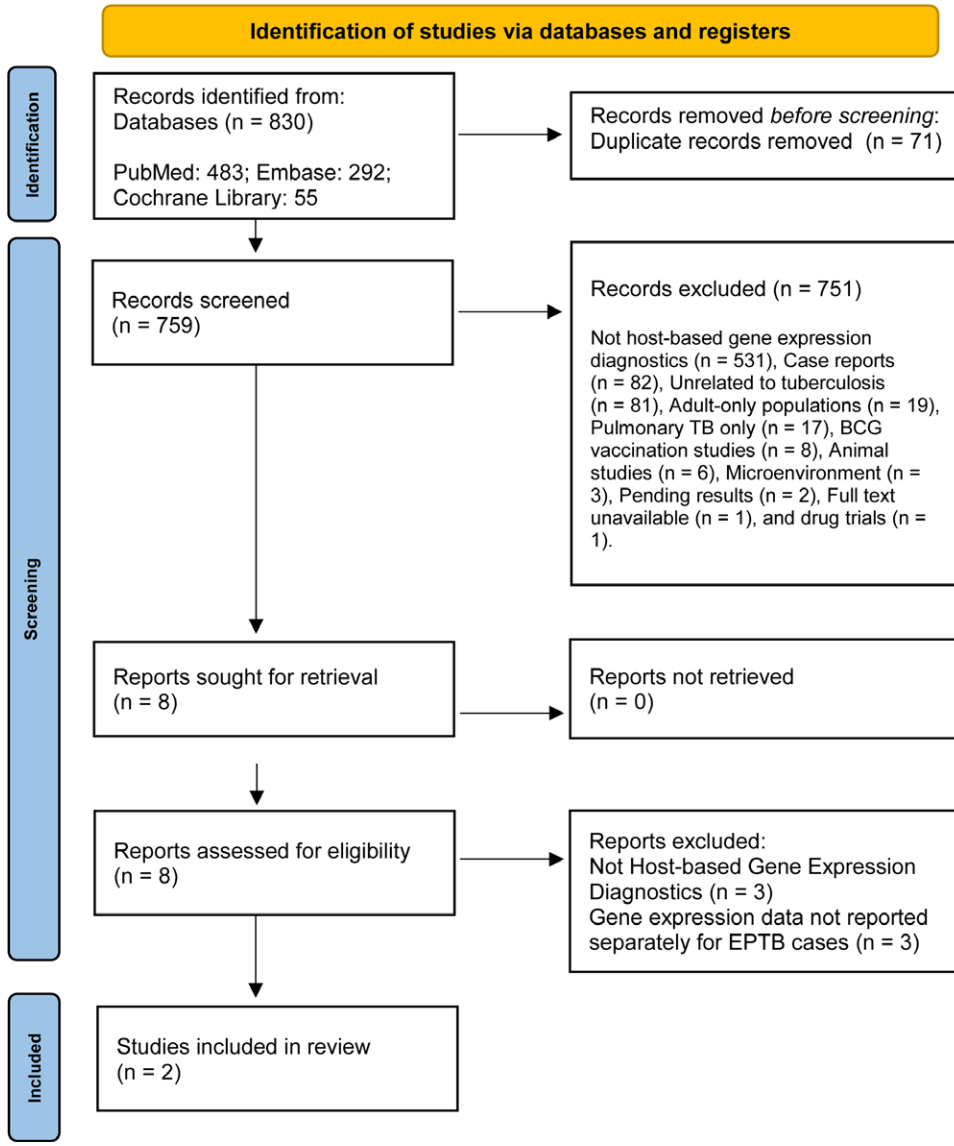

FIGURE 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses flow diagram. Adapted from Page et al.[19]

hypothesized that the single microRNA miR-29a was worth evaluating as a biomarker for TBM, due to its role in immune regulation, levels in patients with active PTB and relevance with the neurologic system.[23–26]

Signature Performance

Against their respective reference standards, both assays demonstrated higher specificity than sensitivity for pediatric EPTB. The 3-gene MTB-HR fingerstick blood cartridge by Olbrich et al. (1.5 cutoff) reached an overall sensitivity of 59.8% [95% confidence interval (CI): 50.8–68.4] and an AUC of 0.85 (95% CI: 0.80–0.89) when evaluated against a strict culture-confirmed reference standard (SRS). Sensitivity decreased with broader reference standards, with 41.6% (95% CI: 34.7–48.7) and an AUC of 0.71 (95% CI: 0.66–0.76) using the microbiologic reference standard (culture and/or Xpert Ultra), and 29.6% (95% CI: 25.4–34.2) and an AUC of 0.65 (95% CI: 0.61–0.69) using the composite clinical reference standard (CRS). Specificity was 90.3%. Diagnostic performance did not differ significantly by age, HIV status or nutritional status. Sensitivity was highest in children with combined pulmonary and extrapulmonary disease (75.0%, 95% CI: 57.8–87.9, n = 36), intermediate in isolated PTB (56.5%, 95% CI: 44·0–68·4, n=69) and lowest in pure EPTB (50.0%, 95% CI: 26.0–74.0, n = 18). Sensitivities were 70.0% (95% CI: 45.7–88.1, n = 20) for lymph node TB and 66.7% (95% CI: 34.9–90.1, n = 12) for TBM. Random-effects meta-analysis included in the original paper showed minimal heterogeneity across sites, yielding sensitivity and specificity like the fixed-effects model. The MTB-HR assay frequently returned negative results in children with very low or trace bacillary loads as determined by Xpert Ultra,[22] representing a significant limitation in real-world settings and suggesting potential value in combining MTB-HR with Xpert Ultra to improve diagnostic yield. In Pan et al., diagnostic accuracy varied by specimen type. miR-29a testing in PBMC showed 67.2% sensitivity and 88.5% specificity (AUC

TABLE 1. Characteristics of Included Studies

Study	Design	Setting	Period	Participants	Age (yr)	Sex (M/F)	Reference Standard	Cases	Comparator	Biomarker(s)	Specimen	Method
Pan et al.[23]	Case-control diagnostic accuracy study	Three hospitals, Henan Province, China	January 2012–February 2015	242(122 children with TBM; 130 healthy controls)	Median 3.5 (SD 1.5), range 1–8	TBM: 65M/57F; HC: 70M and 60F	Diagnostic reference standard based on Thwaites et al. (2002), including CSF isolation of MTB, negative bacterial/fungal cultures, clinical signs of meningitis, chest radiograph consistent with TB and CT findings.	122 children with TBM	130 healthy controls Control recruitment and CSF sampling rationale unclear; despite ethics approval.	miR-29a	PBMC and CSF	qRT-PCR
Olbrich et al.[22]	Prospective multicentre diagnostic accuracy study	Malawi (n = 50), Mozambique (n = 151), South Africa (n = 176), Tanzania (n = 178) and India (n = 84)	January 2019–June 2021	639 (202 culture-confirmed, 230 unconfirmed, 207 unlikely TB)	Median 5.4 (IQR 1.8–9.2)	333M/306F	SRS (culture, n = 127); MRS (culture/Ultra, n = 202); CRS (confirmed + unconfirmed, n = 432). Based on US NIH pediatric TB diagnostic criteria.	202 Culture-confirmed MTB. 230 Unconfirmed TB (No bacteriological confirmation, TB-compatible symptoms, negative bacteriology, clinical diagnosis and treatment response).	207 unlikely TB children (symptomatic children with negative bacteriology, clinical assessment as unlikely TB, spontaneous symptom resolution without TB treatment).	GBP5, DUSP3 and KLF2 mRNA	Fingerstick blood (100μL)	GeneXpert RT-PCR cartridge

MRS: microbiological reference standard, US NIH: United States National Institutes of Health. This table summarizes the design, setting, participant demographics, reference standards, comparator groups, biomarkers evaluated, specimen types and diagnostic methods of the included studies assessing host gene expression diagnostics for pediatric extrapulmonary tuberculosis.

TABLE 2. Diagnostic Accuracy for Each Specimen Type

Study	Biomarker	Specimen	Reference Standard	Subgroup	Comparator	Sensitivity (%)	Specificity (%)	AUC
Pan et al.[23]	miR-29a	PBMC	Thwaites composite (TBM)	–	130 healthy controls Control recruitment and CSF sampling rationale unclear.	67.20	88.50	0.852
	miR-29a	CSF	Thwaites composite (TBM)	–		81.10	90.00	0.890
	miR-29a	PBMC + CSF	Thwaites composite (TBM)	–		84.40	95.38	0.934
Olbrich et al.[22]	GBP5, DUSP3 and KLF2 mRNA	Fingerstick blood	SRS	–	207 unlikely TB children (symptomatic children with negative bacteriology, clinical assessment as unlikely TB, spontaneous symptom resolution without TB treatment)	59.80 (95% CI: 50.8–68.4)	90.30 (95% CI: 85.5–94.0)	0.850
	GBP5, DUSP3 and KLF2 mRNA	Fingerstick blood	SRS	EPTB only (n = 18)		50.00 (95% CI: 26.0–74.0)	90.30*	–
	GBP5, DUSP3 and KLF2 mRNA	Fingerstick blood	SRS	PTB and EPTB (n = 36)		75.00 (95% CI: 57.8–87.9)	90.30*	–
	GBP5, DUSP3 and KLF2 mRNA	Fingerstick blood	SRS	Lymph node TB (n = 20)		70.00 (95% CI: 45.7–88.1)	90.30*	–
	GBP5, DUSP3 and KLF2 mRNA	Fingerstick blood	SRS	TBM (n = 12)		66.70 (95% CI: 34.9–90.1)	90.30*	–

SRS: Children with culture-confirmed Mycobacterium tuberculosis, compared against children classified as having unlikely tuberculosis. MRS (Microbiological Reference Standard): Children with a positive result by culture, Xpert MTB/RIF Ultra, or both, compared against children classified as having unlikely tuberculosis. CRS: Children with either confirmed or unconfirmed tuberculosis based on clinical evaluation, compared against children classified as having unlikely tuberculosis.

*Specificity values marked with an asterisk remained constant across subgroups due to the use of a shared comparator group.

TABLE 3. Quality Assessment of Included Papers Using the QUADAS-2 Tool

Study	Patient Selection	Index Test	Reference Standard	Flow & Timing	Overall Bias
Pan et al.[23]	✗ High risk	✗ High risk	⚠ Some concerns	⚠ Some concerns	✗ High risk
Olbrich et al.[22]	✓ Low risk	✓ Low risk	✓ Low risk	⚠ Some concerns	✓ Low risk

Symbols: ✓ Low risk, ⚠ Some concerns, ✗ High risk.

Summary of risk of bias assessments for each included study using the QUADAS-2 tool across four domains (patient selection, index test, reference standard, flow and timing) and overall risk of bias.[20]

0.852), while CSF testing achieved 81.1% sensitivity and 90.0% specificity (AUC 0.890). Combining results from PBMC and CSF samples increased sensitivity to 84.4% and specificity to 95.4% (AUC 0.934) (Table 2).

Bias/Quality Assessment

We evaluated both papers using the QUADAS-2 tool (Table 3).[20] The prospective diagnostic accuracy study by Olbrich et al. carries a low risk of bias. This study prospectively enrolled consecutive children under 15 years with presumptive TB, using well-defined inclusion and exclusion criteria. The MTB-HR cartridge was performed uniformly with a prespecified threshold, and blinding was ensured. Reference standards included microbiologic (culture and/or Xpert MTB/RIF Ultra), radiologic and clinical criteria with standardized classification. Although some data were missing (due to lost samples or inconclusive results), these exclusions were transparent and explained. Overall, Olbrich et al. maintained low bias with robust methods and clear reporting, enhancing confidence in the findings.

In contrast, Pan et al. was assessed as having a high risk of bias. The case-control design compared children with TBM to healthy controls, without clarifying the source or assessment of the control group, limiting clinical relevance since elevated miRNA-29a in TBM versus healthy individuals does not demonstrate diagnostic utility. Index test bias was also high; the receiver operating characteristic curve was used to evaluate the sensitivity and specificity of miR-29a level in PBMC and CSF. It is unclear what the thresholds represent and how they were derived. Confidence intervals of results were not stated in the paper and laboratory blinding was not reported. Pan et al. used the Thwaites criteria as the reference standard, an adult scoring system based on clinical and CSF features to differentiate TBM from bacterial meningitis,[27] which may not be applicable to the pediatric population. The Marais criteria incorporate clinical, CSF, imaging and TB evidence and are applicable in pediatrics, reducing reference standard applicability concerns compared with adult-derived diagnostic scores for childhood TBM.[28,29] Pan et al. modified the published Thwaites criteria, complicating comparison with other studies and the use of healthy controls as the comparator group, making miRNA-29a's clinical diagnostic relevance uncertain.

DISCUSSION

WHO's 2024 peripheral-level target product profile (TPP) for rapid TB detection sets >98% specificity and sensitivity thresholds of ≥80% for low-complexity assays, ≥75% for near-point of-care, and ≥65% for true-point of-care formats. The TPP is not pediatric-EPTB-specific; children and EPTB fall under the optimal target population. These benchmarks apply to peripheral, non-invasive diagnostics. The TPP also requires <60-minute time-to-result, ≤3 manual steps and ≥8 tests/day throughput, with <5% invalid or indeterminate results required for reliability.[30]

Both host-RNA approaches fell short of these benchmarks. At its prespecified cutoff of 1.5, the MTB-HR fingerstick assay achieved 59.8% sensitivity, below the WHO's 75% near-POC threshold. Pan et al.'s PBMC-based miR-29a assay, aligned with the low-complexity assay category, achieved 67.2% sensitivity and 88.5% specificity, below the required WHO thresholds of ≥80% sensitivity and >98% specificity. Although CSF-based testing showed 81.1% sensitivity and 90.0% specificity, and combined PBMC/CSF testing reached 84.4% sensitivity and 95.4% specificity, CSF sampling is not TPP-compatible due to its invasive nature and unsuitability for peripheral-level implementation.

Operationally, the MTB-HR cartridge uses a simple capillary blood sample (100μL), delivers results within an hour, and requires minimal hands-on time, supporting feasibility. In contrast, miRNA testing requires central lab infrastructure and qRT-PCR platforms, making it incompatible with point of-care use. Neither assay meets WHO's triage-test profile (≥90% sensitivity and ≥70% specificity) without trade-offs. While MTB-HR's operational fit and miR-29a's combined performance are promising, neither fulfills WHO's minimum criteria for peripheral, non-sputum pediatric TB diagnostics.

The GBP5, DUSP3, KLF2 3-gene signature was first identified by Sweeney et al.[12] in predominantly adult PTB datasets. The +1.5 TB-score cutoff was adopted in Olbrich et al.'s pediatric MTB-HR study without pediatric-specific recalibration. In Olbrich et al.'s cohort, recalibration using the SRS and Youden's index demonstrated how threshold adjustments affect sensitivity-specificity the trade-offs, underscoring the need for pediatric adaptation, particularly for EPTB, where host responses and disease localization differ significantly from pulmonary forms. In Olbrich's study, MTB-HR sensitivity was 75.0% in children with both pulmonary and EPTB, dropping to 50.0% in isolated extrapulmonary disease, highlighting disease localization's measurable effect. The assay detected 59.8% of culture-confirmed EPTB cases (AUC 0.85), falling to 29.6% sensitivity (AUC 0.65) when evaluated against a broader CRS. This drop likely reflects misclassification within CRS and the inclusion of lower-burden, paucibacillary cases where immune activation may not reach detection thresholds for transcriptomic assays.

HIV and malnutrition did not significantly affect MTB-HR performance, supporting feasibility in high-burden settings. The Rapid and Accurate Diagnosis of Pediatric Tuberculosis study reported that 16% of children with suspected TB were living with HIV and 11% had severe malnutrition, reflecting substantial inclusion of these groups within the cohort who are at greater risk for severe, life-threatening manifestations, including disseminated disease and TBM.[21,22]

Combining the MTB-HR with a single Xpert Ultra test on respiratory samples detected over two-thirds of microbiologically confirmed pediatric TB cases, with minimal added yield from culture, suggesting limited value for routine culture after molecular testing. Neither MTB-HR nor miR-29a studies reported predictive values (positive predictive value/negative predictive value), essential for interpreting diagnostic performance across different epidemiologic settings.

An adult TBM study by Huynh et al. used the same 3-gene signature but, unlike the present study, employed RNA sequencing of archived blood samples and calculated the TB score as previously defined for PTB datasets. The study reported higher diagnostic performance in HIV-positive adults and observed a significant age-dependent decline in GBP5 expression, reinforcing the idea that transcriptomic biomarkers vary with age and immune status.[13]

Real-world applicability of the miR-29a assay investigated by Pan et al. is limited by several methodological concerns. The lack of differential diagnostic controls undermines the assessment of clinical specificity and raises the possibility that miR-29a may also be elevated in non-TB conditions associated with similar neurologic complications. Furthermore, miR-29a expression was significantly higher in children with severe clinical features, suggesting that its diagnostic signal may reflect acute illness rather than TB-specific immune responses. The absence of comorbidity data, including HIV status and nutritional indicators, further limits generalizability, especially in high-burden settings where such factors are prevalent.

Kathirvel et al. added further support for circulating miRNAs in pediatric PTB, achieving AUCs of 0.903 (miR-146a) to 0.978 (miR-31), while miR-29a was not discriminatory. However, they included only 5 TBM cases and did not stratify performance by EPTB form.[31] Similarly, Anderson et al. validated a 51-transcript RNA signature with excellent performance in African children (83% sensitivity and 84% specificity in the validation cohort), but again did not report outcomes separately for EPTB cases.[32] The SURE study offers a promising step to generate robust diagnostic and operational data.[33]

Limitations

This review has limitations. Critically, the number of children with different forms of EPTB for whom published diagnostic performance data are available remains very small, underlining the paucity of the current evidence base. In the included studies, there were limited children with suspected EPTB enrolled, limiting the potential to further analyze the data and come to robust conclusions on diagnostic application in this subgroup. Variability in reference standards, diagnostic thresholds and small sample sizes increases imprecision and limits generalizability. We did not search gray literature or trial registries, which may have excluded relevant unpublished data. Although our protocol planned inclusion of English, Spanish, Mandarin and Greek studies, only English-language studies met the inclusion criteria, potentially introducing language bias. Assessment of reporting bias and formal certainty assessment using GRADE were not feasible due to the limited number of included studies.

CONCLUSIONS

There remains a disconnect between the high-burden and clinical complexity of pediatric EPTB and the limited diagnostic research available. Existing host gene expression signatures, largely derived from PTB studies, may not capture the distinct immunopathology of pediatric EPTB. The included studies begin to address this gap, but highlight limitations in subgroup representation, threshold calibration and diagnostic generalizability. While the MTB-HR assay demonstrates operational advantages and miR-29a combined testing shows promising sensitivity and specificity, neither meets WHO criteria for non-sputum-based pediatric TB diagnostics. Further studies are urgently needed to prospectively enroll children with suspected EPTB, using appropriate controls, robust reference standards and report subgroup-specific performance. Only through such research can host-gene diagnostics evolve to meet WHO criteria and meaningfully improve care for children with EPTB.

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