

Blood transcriptomic signatures in children with tuberculosis in a treatment-shortening trial

James Seddon

`james.seddon@imperial.ac.uk`

Imperial College London <https://orcid.org/0000-0002-2296-2302>

Vanessa Mwebaza Muwanga

University of Cape Town

Claire Dunican

Imperial College London <https://orcid.org/0000-0001-5835-263X>

Simon Mendelsohn

University of Cape Town <https://orcid.org/0000-0002-4054-2766>

Ortensia Vito

Imperial College London

Stanley Mbandi

University of Cape Town

Mzwandile Erasmus

University of Cape Town

Marieke van der Zalm

Stellenbosch University

Megan Palmer

Stellenbosch University

Nicole Bilek

University of Cape Town

Anne Marie Demers

Stellenbosch University

Diana Gibb

MRC Clinical Trials Unit at University College London

Anna Turkova

University College London

Anneke Hesseling

Stellenbosch University

Myrsini Kaforou

Imperial College London <https://orcid.org/0000-0001-9878-4007>

Thomas Scriba

Article

Keywords: Mycobacterium tuberculosis, blood, transcriptomic, mRNA, biomarker, children, paediatric, TB, treatment monitoring, non-sputum

Posted Date: August 18th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10579385/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: **Yes** there is potential Competing Interest. Thomas Scriba has a patent WO2016123058A1 WIPO (PCT): Biomarkers for detection of tuberculosis risk, and a patent WO2017081618A9 WIPO (PCT): Biomarkers for prospective determination of risk for development of active tuberculosis. The other authors disclose no competing interests.

Blood transcriptomic signatures in children with tuberculosis in a treatment-shortening trial

Vanessa Mwebaza Muwanga^{1*}, Claire Dunican^{2,3*}, Simon C Mendelsohn¹, Ortensia Vito², Stanley Kimbung Mbandi¹, Mzwandile Erasmus¹, Marieke M van der Zalm⁴, Megan Palmer⁴, Nicole Bilek¹, Anne-Marie Demers^{4,5}, Diana Gibb⁶, Anna Turkova⁶, Anneke C. Hesselink⁴, Myrsini Kaforou^{2,3*}, Thomas J Scriba^{1*}, and James A Seddon^{2,3,4*}, on behalf of the SHINE trial team

*Equal contribution

Affiliations

¹South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine and Department of Pathology, University of Cape Town, Cape Town, South Africa

²Department of Infectious Disease, Imperial College London, London, United Kingdom

³Center for Paediatrics and Child Health, Imperial College London, London, UK

⁴Desmond Tutu TB Centre, Department of Paediatrics and Child Health, of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

⁵Service de Microbiologie, Centre Hospitalier Universitaire Sainte-Justine, Université de Montréal, Montréal, Canada

⁶Medical Research Council Clinical Trials Unit, University College London, London, United Kingdom

Corresponding author: James A Seddon

Email: james.seddon@imperial.ac.uk

Telephone: +44 (0)20 7589 5111

Postal address: Department of Infectious Disease, Imperial College London, South Kensington Campus, Exhibition Road, London SW7 2AZ

Key words: *Mycobacterium tuberculosis*; blood; transcriptomic; mRNA, biomarker; children; paediatric; TB; treatment monitoring; non-sputum

Word Count

Abstract: 282 words

Main manuscript: 3,872 words

1 **Abstract**

2 The SHINE trial demonstrated that 4 months of treatment was non-inferior to 6 months for children
3 with non-severe tuberculosis drug-susceptible (DS-TB) as assessed on chest radiograph. However,
4 the optimal duration likely varies between individuals. We evaluated blood transcriptomic signatures
5 as non-sputum biomarkers of treatment response and explored their potential to inform
6 individualised treatment duration in children. This sub-study included children (<16 years) with non-
7 severe TB enrolled at a trial site in Cape Town, South Africa. Participants were classified as confirmed,
8 unconfirmed, or unlikely TB by a blinded endpoint review committee. Whole blood was collected in
9 PAXgene tubes at enrolment (baseline), week 2, week 8, and at the End of Treatment (EoT; week 16
10 or 24). We measured 88 gene transcripts by microfluidic RT-qPCR, evaluated 20 published
11 transcriptomic signatures, and used a feature selection algorithm to derive a novel treatment-
12 monitoring signature which was evaluated in this cohort. We then evaluated this in an independent
13 validation cohort of Indian children. Among 198 children (median 3.4 years, range 0.4-14.8), 37
14 (18.7%) had confirmed, 129 (65.2%) unconfirmed, and 32 (16.2%) unlikely TB. Baseline signature
15 scores were the highest in children with confirmed TB and increased with radiographic severity.
16 Scores declined within 2 weeks, with minimal change thereafter. Of the literature derived signatures,
17 the Kaforou22 signature best differentiated baseline from EoT in confirmed TB (AUC 0.85, 95%CI
18 0.76-0.94) TB. We then discovered a novel 4-gene signature (*FCGR1C*, *FLVCR2*, *LTF*, and *CDKN1C*) that
19 best differentiated baseline from EoT with an AUC of 0.89 (95%CI 0.81-0.97), with an AUC of 0.81
20 (95%CI 0.64-0.98) in an independent paediatric dataset. Blood transcriptomic signatures show
21 promise as non-sputum tools for monitoring treatment response in children and may support
22 biomarker-guided individualized TB treatment.

1 **Introduction**

2 In 2024, an estimated 1.2 million children (<15 years) developed tuberculosis (TB)—11% of the
3 global burden (1). Paediatric TB has heterogeneous clinical manifestations and roughly two thirds of
4 children have non-severe disease which is (2) classified as ‘which includes peripheral lymph node TB
5 and/or paucibacillary intra-thoracic TB without airway compression/deviation, cavities, miliary
6 infiltrates, complicated pleural effusion or consolidation ≥ 1 lobe’ (3). Until recently, children were
7 treated with a standard 6-month regimen, to ensure that all children achieved relapse-free cure. It
8 has since been acknowledged that this has led to the over-treatment in a substantial proportion of
9 children (4).

10 In 2022, the results of the SHINE (Shorter treatment for minimal TB in children) trial were published.
11 SHINE was an open-label, randomised controlled trial that compared 4- versus 6-month standard TB
12 treatment in children with non-severe pulmonary TB (4). SHINE demonstrated non-inferiority of the
13 shorter regimen. These findings informed the 2022 World Health Organization (WHO) guideline,
14 which now recommends a 4-month treatment regimen for children with non-severe TB.

15 Despite this important advance in treatment stratification for childhood TB, a standardised approach
16 of 4 months for non-severe TB and 6 months for more severe disease may still provide insufficient
17 treatment for some children and/or excessive treatment for others. In adults with TB,
18 microbiological parameters during treatment, such as sputum smear or culture positivity and
19 conversion to negative, are used to assess response to treatment and identify individuals at risk of
20 poor outcomes (5). In adults, it is increasingly recognised that baseline microbiological findings and
21 microbiological changes during treatment can predict which individuals are likely to respond
22 successfully to shorter regimens (5). In contrast, children have paucibacillary TB which is easier to
23 treat given the low bacterial burden.

24 The frequent absence of microbiological confirmation in paediatric TB complicates diagnosis and
25 treatment response monitoring. Young children rarely expectorate sputum and commonly present
26 with paucibacillary disease. Microbiological confirmation in non-severe paediatric TB is low, even in
27 trial settings, at around 14% of individuals (4,6). Clinicians frequently, and appropriately, initiate
28 treatment empirically for symptomatic children with non-microbiologically-confirmed presumptive
29 TB and in the absence of sputum-based diagnostics. WHO operational handbook recommends
30 treatment response to be used as indicator for children with assumed non-severe TB but without
31 NAATs or CXR images performed at diagnosis.

1 While relapse and failure rates are low in appropriately treated children, reducing treatment
2 duration has important implications for reducing pill burden, burden on families and health services,
3 improving adherence, as well as cost-effectiveness for health programs, and minimising adverse
4 effects.

5 Host blood transcriptomic signatures reflecting inflammatory and interferon responses have
6 emerged as promising diagnostic or prognostic treatment-monitoring tools in adults with TB. These
7 RNA signatures correlate with disease burden, as measured by PET-CT FDG uptake (7,8), and track
8 response to TB treatment (7–12). To date, few paediatric studies have evaluated transcriptomic
9 biomarkers for the specific purpose of identifying children who may benefit from shorter treatment
10 durations (13–18). Blood transcriptomic signatures capable of reflecting paediatric TB disease
11 severity, and increasing confidence in TB diagnosis, could support individualised treatment and
12 potentially guide shorter regimens. Furthermore, monitoring biomarkers that signal resolution of
13 inflammation may indicate when treatment can safely be stopped.

14 Serial samples collected as part of the SHINE trial offered a unique opportunity to assess
15 transcriptomic changes over the course of therapy. We aimed to evaluate the performance of
16 published blood transcriptomic signatures for monitoring TB treatment response in children. We
17 also applied feature selection methods to identify a novel signature of TB treatment response.

18 **Methods**

19 *Study design*

20 SHINE enrolled children younger than 16 years across sites in India, Uganda, Zambia, and South
21 Africa with non-severe, symptomatic, sputum smear-negative pulmonary TB, presumed drug-
22 susceptible (4). Children from the South African site were part of this analysis given limited
23 resources. Non-severe TB was defined as peripheral lymph node TB or pulmonary TB restricted to
24 a single lobe, without cavities, miliary disease, complex pleural effusion, or airway obstruction.
25 Children were randomly assigned to a 4- or 6-month treatment regimen. All participants received
26 isoniazid, rifampicin, and pyrazinamide, with or without ethambutol, during the 8-week intensive
27 phase. The continuation phase included isoniazid and rifampicin for an additional 8 or 16 weeks,
28 depending on the treatment arm. All drugs were provided according to WHO-recommended
29 dosing. All children were enrolled with a real-time clinical diagnosis of TB and were retrospectively
30 classified as having confirmed, unconfirmed, or unlikely TB by an independent group expert review

committee, blinded to treatment arm allocation, using established consensus criteria. Criteria were guided by clinical case definitions for classification of intrathoracic TB in children, as outlined by Graham and colleagues (19). All children had a chest X-ray (CXR) at baseline and were randomized in real time based on the local clinician's assessment of TB status and disease severity. These were also retrospectively read by at least two independent child TB or child radiology experts, blinded to clinical details. Consensus for the likelihood of TB and of disease severity were generated for each CXR (4,20). Adherence was evaluated through pill counts conducted at each treatment visit, and through adherence questionnaires administered during intensive phase (first 8 weeks) and at the end of therapy (4).

For a South African subset of the study. whole blood was collected in PAXgene RNA tubes (Qiagen, Germany) and cryopreserved at recruitment, week 2, week 8, and the End of Treatment (EoT, week 16 or week 24, dependent on study arm).

Ethical considerations

The study protocol for the trial was approved by the individual Human Research Ethics Committees at each of the participating sites and regulatory authorities in all participating countries (**Supplementary Methods**) and published(20). The transcriptomic signatures sub-study protocol was reviewed and approved by the University of Cape Town Human Research Ethics Committee (522/2020).

Transcriptomic signature panel design and measurement of gene expression

Twenty whole blood transcriptomic signatures were selected for evaluation based on diagnostic performance reported in systematic reviews that conducted head-to-head comparisons of published RNA signatures (21–24). The signatures were re-parameterised to RT-qPCR and measured as previously described (**Supplementary Table 1** and **Supplementary Methods**) (25,26). Briefly, RNA was extracted from the cryopreserved PAXgene blood RNA tubes using the Maxwell HT simplyRNA Custom Kit (Promega, USA), and cDNA synthesis was performed using EpiScript reverse transcriptase (Lucigen, UK), in a semi-automated manner in 96-well plate format on the Freedom EVO 150 robotics platform (Tecan, Switzerland). Transcript expression was quantified by RT-qPCR using 96.96 microfluidic gene expression chips (Standard BioTools, USA) on the Biomark HD system (Standard BioTools, USA), which multiplexes 96 samples across 96 TaqMan primer-probe assays (**Supplementary Table 2**). Each chip included an internal positive control to assess inter-chip

1 variability. Gene expression quality control, batch correction, and signature score calculation was
2 performed using a locked-down R script as described in the **Supplementary Methods**.

3 *Statistical analyses of published signatures*

4 Statistical analyses were conducted in R Statistical Software version 4.1.3 (R Foundation for Statistical
5 Computing, Vienna, Austria). Baseline characteristics were compared using Pearson's Chi-squared
6 test for categorical variables and the Kruskal-Wallis test for continuous variables. Transcriptomic
7 signature scores were compared using the Wilcoxon Signed Rank and Mann-Whitney U tests, for
8 paired and unpaired data, respectively. Receiver operating characteristic (ROC) curve analyses were
9 performed using the pROC R package (27). Area under the curve (AUC) values were reported with
10 95% confidence intervals (CI), calculated using the DeLong method (27).

11 *Discovery of new signatures*

12 Genes with cycle threshold (Ct) values <5 or >35 in more than 50 samples were excluded. Samples
13 were also excluded if Ct values for any of the three reference genes (*TMBIM6*, *USF2*, and *ACTR3*)
14 were outside this range. For each sample, the geometric mean of the three reference genes was
15 calculated and subtracted from the Ct value of the remaining 85 genes to normalise expression
16 levels, used for downstream analysis.

17 Differential expression analysis (DEA) was performed to identify genes distinguishing baseline (n=35)
18 vs EoT (n=37) timepoints in the confirmed TB group for treatment-monitoring signature discovery.
19 DEA was conducted using the R function LimmaCtData() function from the HTqPCR R package. Age
20 (mean-centred and scaled), sex, and batch were included as covariates. Participant identity was
21 incorporated using the function dupcor() to account for repeated measurements. P-values were
22 adjusted for multiple testing using the Benjamini-Hochberg procedure. Volcano plots were
23 generated using the EnhancedVolcano R package.

24 Feature selection was conducted using an in-house Forward Selection–Partial Least Squares (FS-PLS)
25 method to derive the novel treatment-monitoring signatures (28). Differentially expressed genes
26 (DEGs) with Benjamini-Hochberg-adjusted p-values <0.05 and absolute log fold-change (logFC) >0.5
27 from each DEA were input into FS-PLS. Ct values for the selected genes were fit to a generalised
28 linear model (glm() R function), with disease group as the outcome ($y \sim \text{gene 1} + \text{gene 2} + \dots$). Gene-
29 specific coefficients (weights) were extracted and used to calculate participant-level signature scores
30 with the predict() R function (type="link"). Signature performance was evaluated using ROC curve

1 analysis (roc() R function) and AUC values were compared between treatment arms using DeLong's
2 test. To assess generalisability, we applied the model-derived gene weights to samples from children
3 with unconfirmed TB at baseline (n=125) and EoT (n=128), and calculated AUC for both diagnostic
4 discrimination against unlikely TB and treatment response.

5 *Treatment monitoring signature score*

6 For each gene in the treatment-monitoring signature, the distribution of normalised Ct values at the
7 EoT was used to calculate group-specific (confirmed, unconfirmed, and unlikely TB) standard
8 deviations. These values defined expression ranges much closer to an assumed "healthy level"
9 (relative to the level at enrolment) for each gene (± 1.5 standard deviations from the EoT mean). At
10 earlier timepoints, participants were assigned a point for each gene whose normalised Ct value fell
11 within this range. These gene-level points were summed to generate an overall monitoring score per
12 participant, ranging from 0 (no genes within range) to 4 (all genes within range). The number of
13 participants achieving each possible score at each timepoint was summarised by TB classification
14 group and visualised in heatmaps.

15 *Validation in external publicly available RNA-Seq cohort*

16 Raw fastq files from the Tornheim et al., 2020 (18) RNA-Seq dataset (SRA accession: PRJNA588242)
17 were downloaded using SRA-tools (timepoints: baseline, month 1, and month 6). Quality control was
18 performed using FastQC and summarised with MultiQC. Reads were mapped using STAR (mean
19 alignment: 84.9%, range 67.1-90.5%; 144,939,927 uniquely mapped reads per sample, range
20 94,340,397-227,572,707) and quantified using featureCounts. Genes with zero counts across all
21 samples or annotated as rRNA biotype were excluded. Remaining counts were normalised using
22 edgeR and $\log_2(+1)$ transformed prior to analysis.

23 The treatment-monitoring signature genes were extracted from the normalised gene expression
24 matrix. Gene weights were recalculated using a generalised linear model, and participant-level
25 signature scores were computed. Performance to distinguish baseline (n=16) from month 6 (n=10)
26 samples were assessed using ROC curve analysis, and AUC was calculated as previously described.

27 **Results**

28 *Participant characteristics*

Of the 1,204 participants enrolled into the SHINE trial, 315 were enrolled in South Africa. This biomarker sub-study included 198 South African children, selected based on availability of complete PAXgene RNA samples at all four time points: baseline (week 0), week 2, week 8, and EoT (week 16 for the 4-month arm, week 24 for the 6-month arm). Among these participants, 91 were randomised to the 4-month treatment arm and 107 to 6 months. Based on pre-specified case definitions adjudicated by the endpoint review committee, 37 (18.7%) of 198 participants were classified as having confirmed TB, 129 (65.2%) as unconfirmed, and 32 (16.2%) as unlikely TB (**Table 1**).

Children with confirmed TB were predominantly older than three years (78.4%), while most children with unlikely TB were younger than 3 years (62.5%). The unconfirmed group was evenly distributed across age strata. Clinical presentation also differed as children with unconfirmed (58.1%) and unlikely (81.3%) TB most commonly presented with pulmonary TB, whereas children with confirmed TB more frequently had mixed pulmonary and peripheral lymph node involvement (59.5%). CXR features varied significantly by diagnostic classification (χ^2 $p < 0.001$). Typical TB CXR features were observed in 68.6% of children with confirmed compared to 45.3% of those with unconfirmed TB. Due to the small number of children who relapsed or had treatment failure ($n=3$), signature scores were not compared by treatment outcome. Overall adherence to treatment was high, with no differences seen across TB categories.

Host blood transcriptomic signatures for diagnosis and classification of disease severity

Signature scores were lowest in the unlikely TB group, intermediate in unconfirmed TB, and highest in confirmed TB (**Figure 1**). Of the 20 preselected host blood transcriptomic signatures, 18 (90%) significantly differentiated confirmed from unlikely TB at baseline, with AUCs ranging from 0.59 (95%CI: 0.45–0.73) to 0.81 (95%CI: 0.70–0.92; **Supplementary Table 3** and **Supplementary Figure 1**). Discrimination between children with unconfirmed and unlikely TB was weaker for all signatures; Gliddon4 was the only signature with a significant difference in scores between children with unconfirmed and unlikely TB (Mann-Whitney U p -value=0.0047; AUC 0.67, 95%CI: 0.58–0.75; **Supplementary Figure 1**).

Signature scores also correlated with chest radiographic severity. Median scores for all signatures increased with more severe CXR findings (**Supplementary Figure 2**). Children with typical TB features, both severe and non-severe, had significantly higher scores than those with normal CXR. In exploratory multivariable linear regression (**Supplementary Methods**), TB classification group was

1 the only variable independently associated with signature scores (**Supplementary Tables 4-5**);
2 children with confirmed TB had higher scores than children with unconfirmed and unlikely TB.

3 *Changes in transcriptomic signature scores over time*

4 To assess changes in transcriptomic signature scores over time, longitudinal analyses were
5 performed within each TB classification group. Given the use of both 4- and 6-month treatment
6 regimens, we also examined whether treatment arm allocation influenced signature trajectories.

7 Among children with confirmed TB, 13 out of 20 evaluated signatures showed differences in end-of-
8 treatment scores between arms, but no differences were observed at earlier timepoints
9 (**Supplementary Figure 3**, only Kaforou22 signature shown). There were no differences in signature
10 scores between treatment arms at any timepoint for children with unconfirmed or unlikely TB.

11 Trajectories of the Gliddon4 and Kaforou22 signatures (**Figure 2**) exemplify the overall pattern
12 observed through treatment for the 20 signatures. Scores decreased in the first two weeks of
13 treatment among children with confirmed (Gliddon4, Wilcoxon signed-rank test, p-value=0.0013;
14 Kaforou22, p-value=0.0002), unconfirmed (Gliddon4, p-value=0.011; Kaforou22, p-value=0.0002),
15 and unlikely (Gliddon4, p-value=0.062; Kaforou22, p-value=0.013) TB. No further significant changes
16 in scores were observed beyond week 2 in any group.

17 *Transcriptomic signatures over time*

18 To assess the potential of the 20 published signatures as treatment-monitoring biomarkers, we
19 evaluated their ability to distinguish between different treatment timepoints within each TB
20 diagnostic group. Across all three groups, signature performance was best when differentiating
21 baseline from the EoT samples, compared to baseline from week 2 or week 8 samples. For confirmed
22 TB, Kaforou22 showed the strongest performance for discriminating between baseline and EoT (AUC
23 0.85, 95%CI: 0.76–0.94; **Figure 3** and **Supplementary Table 6**).

24 *Discovery and validation of new signatures*

25 Most of the 20 signatures we assessed were discovered in adult cohorts, which may be suboptimal
26 for application in children. We next aimed to identify novel signatures of treatment response and
27 diagnosis in this paediatric cohort. Following gene filtering, 771 samples and 88 genes (including the
28 three reference genes) were retained for downstream analyses. DEA between baseline (n=35) and
29 end-of-treatment (n=37) samples in the confirmed TB group then identified 59 DEGs (**Figure 4a**).

Using FS-PLS, this was reduced to a four-gene signature (*FCGR1C*, *FLVCR2*, *LTF*, and *CDKN1C*) which discriminated between baseline and EoT samples with an AUC of 0.89 (95%CI: 0.81–0.97; **Figure 4b-c**) in the confirmed TB group. Signature performance did not differ by treatment arm (DeLong’s test p-value=0.76; **Supplementary Figure 4**). For this same confirmed TB group, the AUC between baseline and the week 2 (n=37) samples was 0.77 (95%CI: 0.65–0.88). Between baseline and week 8 (n=36), the AUC was 0.83 (95%CI: 0.74–0.93). For the unconfirmed TB group, the AUC for differentiating between baseline (n=125) and EoT (n=128) was 0.73 (95%CI: 0.67–0.79).

Validation in an independent external publicly available RNA-Seq cohort of Indian children with TB (18) demonstrated similar performance to the discovery cohort, with an AUC of 0.81 (95%CI: 0.64–0.98) for discriminating between enrolment (n=16) and 6-month (EoT, n=10) samples (**Figure 4d-e**).

Expression levels of each gene changed over the course of treatment (*FCGR1C* shown as an example in **Figure 5a**, see **Supplementary Figure 5** for all genes). This is reflected in decrease in the DRS over time (**Supplementary Figure 5**). Interestingly, the one confirmed TB patient, whose treatment was extended past 16 weeks has slightly different gene expression dynamics indicating that this signature is also detecting alternative disease trajectories (arrows, **Supplementary Figure 6**). Consistent with published signatures in the previous analysis, the most dramatic change appeared between baseline and week 2. The proportion of confirmed TB participants with *FCGR1C* expression within ± 1.5 standard deviations of their end-of-treatment value increased from 37.9% at baseline to 75.9% by week 2 (**Figure 5b**).

Building on this observation, we developed a treatment-monitoring scoring system: for each of the four genes, participants received 1 point if their Ct value fell within ± 1.5 standard deviations of the end-of-treatment mean, resulting in a score from 0 (no genes within range) to 4 (all genes within range). Over half of confirmed TB participants reached a score of 4 by week 2 (**Figure 5C**, **Supplementary Figure 5**).

Discussion

We evaluated 20 published blood transcriptomic signatures as non-sputum-based biomarkers to monitor treatment response in children with non-severe TB. Across all diagnostic groups, signature scores declined rapidly within the first two weeks of treatment, with the greatest reductions observed in children with confirmed, followed by unconfirmed and unlikely TB. There was little to no significant change in scores after week 2 in the confirmed and unconfirmed groups for most

signatures, and no changes from week 2 to EoT in the unlikely group. We also identified a novel four-gene treatment-monitoring signature for paediatric TB, which we validated in an independent external paediatric TB dataset. Furthermore, we demonstrated a proof-of-concept scoring system based on this signature, which could help determine when treatment might be safely shortened.

Baseline signature scores were associated with certainty of TB diagnosis, consistent with findings of Anderson and colleagues (13), and were higher in children with features that were radiologically more severe TB on CXR. In adults, several signatures have been shown to correlate with total glycolytic activity on PET-CT among adult TB patients, a surrogate for pulmonary inflammation (7,8,29). Together, these findings support that blood transcriptomic signatures reflect TB-associated disease burden, highlighting their potential as non-sputum-based biomarkers for TB treatment monitoring.

Consistent with findings from adult TB treatment response studies (30–36), significant declines in transcriptomic signature scores were observed as early as two weeks after treatment initiation. This rapid decline likely reflects the period of most intense mycobacterial killing. Such early changes may serve as a useful surrogate marker of effective treatment response and could be leveraged in future clinical trials to evaluate treatment efficacy or support treatment-shortening strategies. We found that median baseline scores were highest in older children in the confirmed TB group, versus younger children in the unconfirmed and unlikely groups. This supports the hypothesis that more extensive, adult-like TB, which is typically more common among older children, is indicative of greater systemic inflammation. Children with unlikely TB had lowest baseline scores and showed the smallest decline during treatment, suggesting lower levels of inflammation. Some participants in this group may not have had TB at all, which could explain the limited transcriptomic response and raises the possibility of overtreatment in children with unlikely TB. In such cases, changes in transcriptomic signatures early in treatment may have value in identifying children who could discontinue therapy early.

Despite known differences in immune responses to *Mycobacterium tuberculosis* between children and adults, shared host transcriptomic pathways are evident. In a study by Tornheim et al (18), 25.9–37.5% of genes in paediatric diagnostic and treatment-response monitoring signatures overlapped with those derived in adult populations (17,29,34). In our analysis, several adult-cohort derived signatures outperformed the GjØen7 paediatric signature across diagnostic categories. Notably, the Kaforou22 signature, which showed the highest overlap with paediatric DEGs in the Tornheim study

(18), consistently differentiated children with confirmed from unlikely TB across multiple treatment timepoints in our cohort. This is most likely due to the Kaforou22 signature being made up of more transcripts and thus more likely to pick up subtle changes. While there is value in identifying paediatric-specific signatures, our findings support the potential utility of adult-derived signatures for both diagnosis and treatment monitoring in children, given the shared biological underpinnings of the host response.

As demonstrated, transcriptomic biomarkers that monitor treatment response may also help identify children who could benefit from shorter regimens, supporting efforts to personalise paediatric TB treatment duration. In our study, end-of-treatment signature scores were marginally yet significantly higher in the 4-month compared to the 6-month arm among children with confirmed TB, while no differences were observed between treatment arms in the unconfirmed and unlikely groups. These higher scores likely reflect residual pulmonary inflammation rather than persistent viable mycobacteria but may suggest that four months of treatment was insufficient for a subset of children who might have benefitted from extended treatment. Of note, very few children in the South African cohort were living with HIV. Only 3% of participants in the SHINE trial experienced unfavourable outcome after month 4 (37), limiting our ability to assess whether transcriptomic signatures can predict treatment failure or relapse. Although more children had their treatment extended in the 4-month arm, this was likely influenced by the open-label design of the SHINE trial, a limitation previously acknowledged by the investigators (37).

Strengths of our study include the comprehensive and largely unbiased evaluation of 20 candidate transcriptomic signatures, as well as the discovery of a novel paediatric-derived signature, in a well-characterised cohort of children from the only paediatric TB treatment-shortening trial completed to date. To our knowledge, this is the first study to directly compare transcriptomic signatures across confirmed, unconfirmed, and unlikely TB in children for treatment monitoring. However, several limitations should be acknowledged. First, the absence of PAXgene sample collection at the week 16 and week 24 visits for the 6- and 4-month arms, respectively, precluded direct comparison of signature scores at these timepoints between the two groups. Second, the lack of matching samples from Indian, Zambian and Ugandan SHINE sites limited assessment of geographic generalisability. Third, the absence of a healthy control group prevented comparison of end-of-treatment scores to those of children without TB. Fourth, the small number of unfavourable outcomes limited our ability to evaluate the predictive value of transcriptomic signatures for treatment failure or relapse. Finally, the re-weighting of the novel 4-gene monitoring signature for the Indian validation cohort could lead

1 to over-fitting. However, this was required due to these datasets measuring RNA across different
2 platforms, RT-qPCR and RNA-Sequencing respectively.

3 In conclusion, we present the first head-to-head comparison of multiple blood transcriptomic
4 signatures for TB treatment monitoring in children and report the discovery of a new treatment-
5 response signature. Our findings support the potential utility of transcriptomic biomarkers as non-
6 sputum tools to track treatment response in paediatric TB. Future studies of shortened or novel
7 paediatric TB regimens should incorporate transcriptomic signatures as early indicators of treatment
8 response, laying the groundwork for future biomarker-guided, individualised paediatric treatment
9 strategies and treatment-shortening trials.

10 **Author contributions**

11 JAS conceived the idea. JAS, ACH and TJS raised funds and/or provided the resources. VMM
12 developed the statistical analysis plan with input and final approval from JAS, TJS, SCM, MP and
13 MMvdZ, ACH. MP, AM, MMvdZ and ACH were responsible for all site-level activities, including
14 recruitment, clinical management, and sample and data collection. SCM, SKM, NB, KH, MMvdZ, AM,
15 ACH, DG, and AT provided technical, clinical, operational or laboratory support and project
16 management. ME and RR performed the RNA extractions and cDNA synthesis for all the samples
17 received. VMM carried out all the subsequent gene expression experiments along with the data
18 analyses. VMM and SCM verified the data. VMM and CD performed analyses and interpreted results
19 with input from SCM, MK, TJS, and JAS. VMM and CD prepared the initial drafts of the manuscript.
20 All authors have read and approved the manuscript.

21 **Declaration of interests**

22 TJS has a patent WO2016123058A1 WIPO (PCT): Biomarkers for detection of tuberculosis risk, and
23 a patent WO2017081618A9 WIPO (PCT): Biomarkers for prospective determination of risk for
24 development of active tuberculosis. The other authors disclose no competing interests.

25 **Data sharing**

26 Deidentified signature scores, PCR probe data, clinical metadata, and TB endpoint data have been
27 deposited in Zivahub, an open-access data repository hosted by the University of Cape Town's
28 institutional data repository powered by Figshare for Institutions.

29 **Acknowledgements**

1 We thank Mr Miguel Rodo and Dr Denis Awany for providing statistical support. We acknowledge
2 the substantial contributions of the SHINE study teams (lists of consortia members and their
3 affiliations appear in the supplementary information). SHINE was funded by a grant (MR/L004445/1)
4 from the U.K. Medical Research Council (MRC) and the Department for International Development
5 (DFID) Wellcome NIHR Joint Global Health Trials, by a U.K. Research and Innovation Covid-19 Grant
6 Extension Allocation Award, by a U.K. Research and Innovation MRC grant (MC_UU_00004/04), and
7 by a Clinician Scientist Fellowship (to JAS), jointly funded by the MRC and the Department for
8 International Development under an MRC–DFID Concordat agreement (MR/R007942/1). This study
9 was supported by a Clinician Scientist Fellowship (to JAS), jointly funded by the MRC and the
10 Department for International Development. The funders had no role in study design, data collection
11 and analysis, the decision to publish, or manuscript preparation. VMM is a recipient of PhD funding
12 from the University of Cape Town through the incoming international students’ bursary scheme.

13 **Funding sources**

14 MMVDZ is supported by a career development grant from the EDCTP2 program supported by the
15 European Union (TMA2019SFP-2836 TB lung-FACT2), the Fogarty International Centre of the
16 National Institutes of Health under Award Number K43TW011028. ACH is supported by a National
17 Research Foundation South Africa Sarchi Chair in Paediatric Tuberculosis.

18 **References**

- 19 1. World Health Organisation. Global Tuberculosis Report 2024 [Internet]. 1st ed. Geneva: World
20 Health Organization; 2024 [cited 2025 Jun 5]. 1 p. Available from:
21 <https://www.who.int/publications/i/item/9789240101531>
- 22 2. Marais BJ, Gie RP, Schaaf HS, Hesselning AC, Enarson DA, Beyers N. The spectrum of disease in
23 children treated for tuberculosis in a highly endemic area [Unresolved Issues]. *Int J Tuberc Lung*
24 *Dis.* 2006 Jul 1;10(7):732–8.
- 25 3. World Health Organisation. WHO Consolidated Guidelines on Tuberculosis. Module 3:
26 Diagnosis. Tests for TB Infection. 1st ed. Geneva: World Health Organization; 2022. 1 p.
- 27 4. Turkova A, Wills GH, Wobudeya E, Chabala C, Palmer M, Kinikar A, et al. Shorter Treatment for
28 Nonsevere Tuberculosis in African and Indian Children. *N Engl J Med.* 2022 Mar
29 10;386(10):911–22. doi:10.1056/NEJMoa2104535 PubMed PMID: 35263517.
- 30 5. Imperial MZ, Nahid P, Phillips PPJ, Davies GR, Fielding K, Hanna D, et al. A patient-level pooled
31 analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. *Nat*
32 *Med.* 2018 Nov;24(11):11. doi:10.1038/s41591-018-0224-2
- 33 6. Perez-Velez CM, Marais BJ. Tuberculosis in Children. *N Engl J Med.* 2012 Jul 26;367(4):348–61.
34 doi:10.1056/NEJMra1008049 PubMed PMID: 22830465.

- 1 7. Penn-Nicholson A, Mbandi SK, Thompson E, Mendelsohn SC, Suliman S, Chegou NN, et al.
2 RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response.
3 Sci Rep. 2020 May 25;10(1):1. doi:10.1038/s41598-020-65043-8
- 4 8. Thompson EG, Du Y, Malherbe ST, Shankar S, Braun J, Valvo J, et al. Host blood RNA signatures
5 predict the outcome of tuberculosis treatment. Tuberculosis. 2017 Dec 1;107:48–58.
6 doi:10.1016/j.tube.2017.08.004
- 7 9. Bayaa R, Ndiaye MDB, Chedid C, Kokhraidze E, Tukvadze N, Banu S, et al. Multi-country
8 evaluation of RISK6, a 6-gene blood transcriptomic signature, for tuberculosis diagnosis and
9 treatment monitoring. Sci Rep. 2021 Dec;11(1):13646. doi:10.1038/s41598-021-93059-1
- 10 10. Doorn CLR van, Eckold C, Ronacher K, Ruslami R, Veen S van, Lee JS, et al. Transcriptional
11 profiles predict treatment outcome in patients with tuberculosis and diabetes at diagnosis and
12 at two weeks after initiation of anti-tuberculosis treatment. eBioMedicine. 2022 Aug 1;82.
13 doi:10.1016/j.ebiom.2022.104173 PubMed PMID: 35841871.
- 14 11. Eckold C, van Doorn CLR, Ruslami R, Ronacher K, Riza AL, van Veen S, et al. Impaired resolution
15 of blood transcriptomes through tuberculosis treatment with diabetes comorbidity. Clin Transl
16 Med. 2023;13(9):e1375. doi:10.1002/ctm2.1375
- 17 12. Mendelsohn SC, Andrade BB, Araújo-Pereira M, Amorim GC, Erasmus M, Andrade AM, et al.
18 Blood transcriptomic signatures predict poor treatment outcomes in drug-susceptible
19 pulmonary TB in Brazil [Internet]. medRxiv; 2025 [cited 2025 Nov 26]. p. 2025.10.07.25337480.
20 Available from: <https://www.medrxiv.org/content/10.1101/2025.10.07.25337480v1>
21 doi:10.1101/2025.10.07.25337480
- 22 13. Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, et al. Diagnosis of
23 Childhood Tuberculosis and Host RNA Expression in Africa. New England Journal of Medicine.
24 2014. p. 1712–23.
- 25 14. Gjøen JE, Jenum S, Sivakumaran D, Mukherjee A, Macaden R, Kabra SK, et al. Novel
26 transcriptional signatures for sputum-independent diagnostics of tuberculosis in children. Sci
27 Rep. 2017 Jul 19;7(1):1. doi:10.1038/s41598-017-05057-x
- 28 15. Verhagen LM, Zomer A, Maes M, Villalba JA, del Nogal B, Eleveld M, et al. A predictive
29 signature gene set for discriminating active from latent tuberculosis in Warao Amerindian
30 children. BMC Genomics. 2013 Feb 1;14(1):74. doi:10.1186/1471-2164-14-74
- 31 16. Bobak CA, Botha M, Workman L, Hill JE, Nicol MP, Holloway JW, et al. Gene Expression in Cord
32 Blood and Tuberculosis in Early Childhood: A Nested Case-Control Study in a South African
33 Birth Cohort. Clin Infect Dis. 2023 Aug 14;77(3):438–49. doi:10.1093/cid/ciad268
- 34 17. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, et al. A blood RNA
35 signature for tuberculosis disease risk: a prospective cohort study. The Lancet. 2016 Jun
36 4;387(10035):2312–22. doi:10.1016/S0140-6736(15)01316-1
- 37 18. Tornheim JA, Madugundu AK, Paradkar M, Fukutani KF, Queiroz ATL, Gupte N, et al.
38 Transcriptomic Profiles of Confirmed Pediatric Tuberculosis Patients and Household Contacts
39 Identifies Active Tuberculosis, Infection, and Treatment Response Among Indian Children. J
40 Infect Dis. 2020 Apr 27;221(10):1647–58. doi:10.1093/infdis/jiz639

- 1 19. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi M, Detjen AK, et al. Clinical Case
2 Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update. *Clin Infect*
3 *Dis.* 2015 Oct 15;61(suppl_3):S179–87. doi:10.1093/cid/civ581
- 4 20. Chabala C, Turkova A, Thomason MJ, Wobudeya E, Hissar S, Mave V, et al. Shorter treatment
5 for minimal tuberculosis (TB) in children (SHINE): a study protocol for a randomised controlled
6 trial. *Trials.* 2018 Apr 19;19(1):237. doi:10.1186/s13063-018-2608-5 PubMed PMID: 29673395;
7 PubMed Central PMCID: PMC5909210.
- 8 21. Gupta RK, Turner CT, Venturini C, Esmail H, Rangaka MX, Copas A, et al. Concise whole blood
9 transcriptional signatures for incipient tuberculosis: a systematic review and patient-level
10 pooled meta-analysis. *Lancet Respir Med.* 2020 Apr 1;8(4):395–406. doi:10.1016/S2213-
11 2600(19)30282-6 PubMed PMID: 31958400.
- 12 22. Mulenga H, Zauchenberger CZ, Bunyasi EW, Mbandi SK, Mendelsohn SC, Kagina B, et al.
13 Performance of diagnostic and predictive host blood transcriptomic signatures for Tuberculosis
14 disease: A systematic review and meta-analysis. *PLOS ONE.* 2020 Aug 21;15(8):e0237574.
15 doi:10.1371/journal.pone.0237574
- 16 23. Turner CT, Gupta RK, Tsaliki E, Roe JK, Mondal P, Nyawo GR, et al. Blood transcriptional
17 biomarkers for active pulmonary tuberculosis in a high-burden setting: a prospective,
18 observational, diagnostic accuracy study. *Lancet Respir Med.* 2020 Apr 1;8(4):407–19.
19 doi:10.1016/S2213-2600(19)30469-2 PubMed PMID: 32178775.
- 20 24. Warsinske H, Vashisht R, Khatri P. Host-response-based gene signatures for tuberculosis
21 diagnosis: A systematic comparison of 16 signatures. *PLOS Med.* 2019 Apr 23;16(4):e1002786.
22 doi:10.1371/journal.pmed.1002786
- 23 25. Muwanga VM, Mendelsohn SC, Leukes V, Stanley K, Mbandi SK, Erasmus M, et al. Blood
24 transcriptomic signatures for symptomatic TB in an African multi-cohort study. *Eur Respir J.*
25 2024 Jan 1. doi:10.1183/13993003.00153-2024 PubMed PMID: 38964778.
- 26 26. Mendelsohn SC, Andrade BB, Mbandi SK, Andrade AMS, Muwanga VM, Figueiredo MC, et al.
27 Transcriptomic signatures of progression to TB disease among close contacts in Brazil. *J Infect*
28 *Dis.* 2024 May 6;jiae237. doi:10.1093/infdis/jiae237
- 29 27. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more
30 correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.*
31 1988 Sep;44(3):837–45. PubMed PMID: 3203132.
- 32 28. A flexible framework for minimal biomarker signature discovery from clinical omics studies
33 without library size normalisation | PLOS Digital Health [Internet]. [cited 2025 Oct 4]. Available
34 from: <https://journals.plos.org/digitalhealth/article?id=10.1371/journal.pdig.0000780>
- 35 29. Sweeney TE, Braviak L, Tato CM, Khatri P. Genome-wide expression for diagnosis of pulmonary
36 tuberculosis: a multicohort analysis. *Lancet Respir Med.* 2016 Mar;4(3):213–24.
37 doi:10.1016/S2213-2600(16)00048-5 PubMed PMID: 26907218; PubMed Central PMCID:
38 PMC4838193.

- 1 30. Bloom CI, Graham CM, Berry MPR, Wilkinson KA, Oni T, Rozakeas F, et al. Detectable Changes
2 in The Blood Transcriptome Are Present after Two Weeks of Antituberculosis Therapy. PLOS
3 ONE. 2012 Oct 2;7(10):e46191. doi:10.1371/journal.pone.0046191
- 4 31. Cliff JM, Lee JS, Constantinou N, Cho JE, Clark TG, Ronacher K, et al. Distinct Phases of Blood
5 Gene Expression Pattern Through Tuberculosis Treatment Reflect Modulation of the Humoral
6 Immune Response. J Infect Dis. 2013 Jan 1;207(1):18–29. doi:10.1093/infdis/jis499
- 7 32. Darboe F, Mbandi SK, Naidoo K, Yende-Zuma N, Lewis L, Thompson EG, et al. Detection of
8 Tuberculosis Recurrence, Diagnosis and Treatment Response by a Blood Transcriptomic Risk
9 Signature in HIV-Infected Persons on Antiretroviral Therapy. Front Microbiol. 2019;10.
10 doi:10.3389/fmicb.2019.01441
- 11 33. Heyckendorf J, Marwitz S, Reimann M, Avsar K, DiNardo AR, Günther G, et al. Prediction of
12 anti-tuberculosis treatment duration based on a 22-gene transcriptomic model. Eur Respir J.
13 2021 Sep;58(3):2003492. doi:10.1183/13993003.03492-2020
- 14 34. Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, et al. Detection of Tuberculosis
15 in HIV-Infected and -Uninfected African Adults Using Whole Blood RNA Expression Signatures:
16 A Case-Control Study. PLOS Med. 2013 Oct 22;10(10):e1001538.
17 doi:10.1371/journal.pmed.1001538
- 18 35. Penn-Nicholson A, Mbandi SK, Thompson E, Mendelsohn S, Suliman S, Chegou NN, et al. RISK6,
19 a universal 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment
20 response. medRxiv. 2019 Sep 9;19006197. doi:10.1101/19006197
- 21 36. Thompson EG, Du Y, Malherbe ST, Shankar S, Braun J, Valvo J, et al. Host blood RNA signatures
22 predict the outcome of tuberculosis treatment. Tuberculosis. 2017 Dec 1;107:48–58.
23 doi:10.1016/j.tube.2017.08.004
- 24 37. Turkova A, Wills GH, Wobudeya E, Chabala C, Palmer M, Kinikar A, et al. Shorter Treatment for
25 Nonsevere Tuberculosis in African and Indian Children. N Engl J Med. 2022 Mar
26 10;386(10):911–22. doi:10.1056/NEJMoa2104535

1 Figures

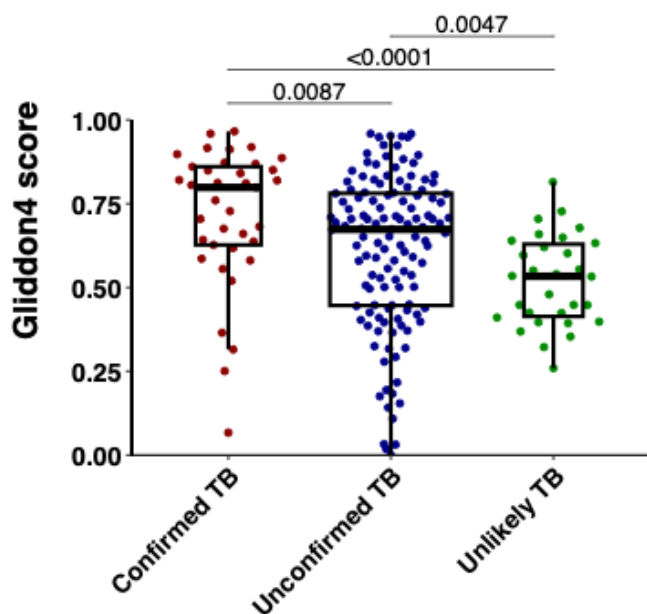
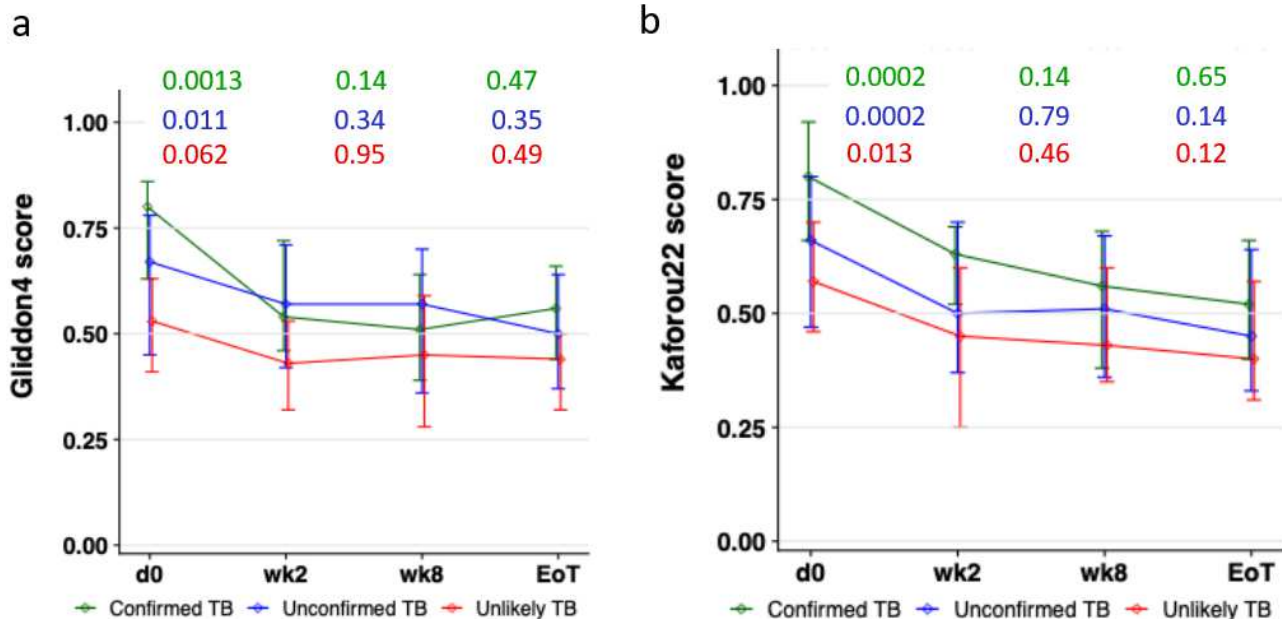


Figure 1. Signature score distributions of transcriptomic signatures at baseline in children with confirmed, unconfirmed, and unlikely TB.

Box-and-whisker plot of the signature with the highest area under the curve for distinguishing children with confirmed from those with unlikely TB at baseline (Gliddon4). The plot shows signature score distribution (each dot represents a participant) in children with confirmed (n = 37), unconfirmed (n = 128), and unlikely (n = 30) TB at baseline. P-values for comparison of median signature scores between groups were calculated using the Mann–Whitney U test. Boxes depict the IQR, the midline represents the median, and whiskers represent 1.5 × IQR. The ROC curves and summary graphs showing performance of these signatures at baseline in children with confirmed, unconfirmed, and unlikely TB are shown in **Supplementary Figure 1**.

1



2

3 **Figure 2. Longitudinal signature scores trajectories during TB treatment.**

4 Line graphs depicting median and interquartile range for (a) Gliddon4 and (b) Kaforou22 signatures
 5 for children with confirmed (green), unconfirmed (blue), or unlikely TB (red) at baseline (d0), week
 6 2 (wk2), week 8 (wk8), and end of TB treatment (EoT). P-values above the graphs, calculated using
 7 the Wilcoxon Signed Rank test, represent the comparison of paired timepoints (e.g. baseline, d0 vs
 8 week 2, wk2).

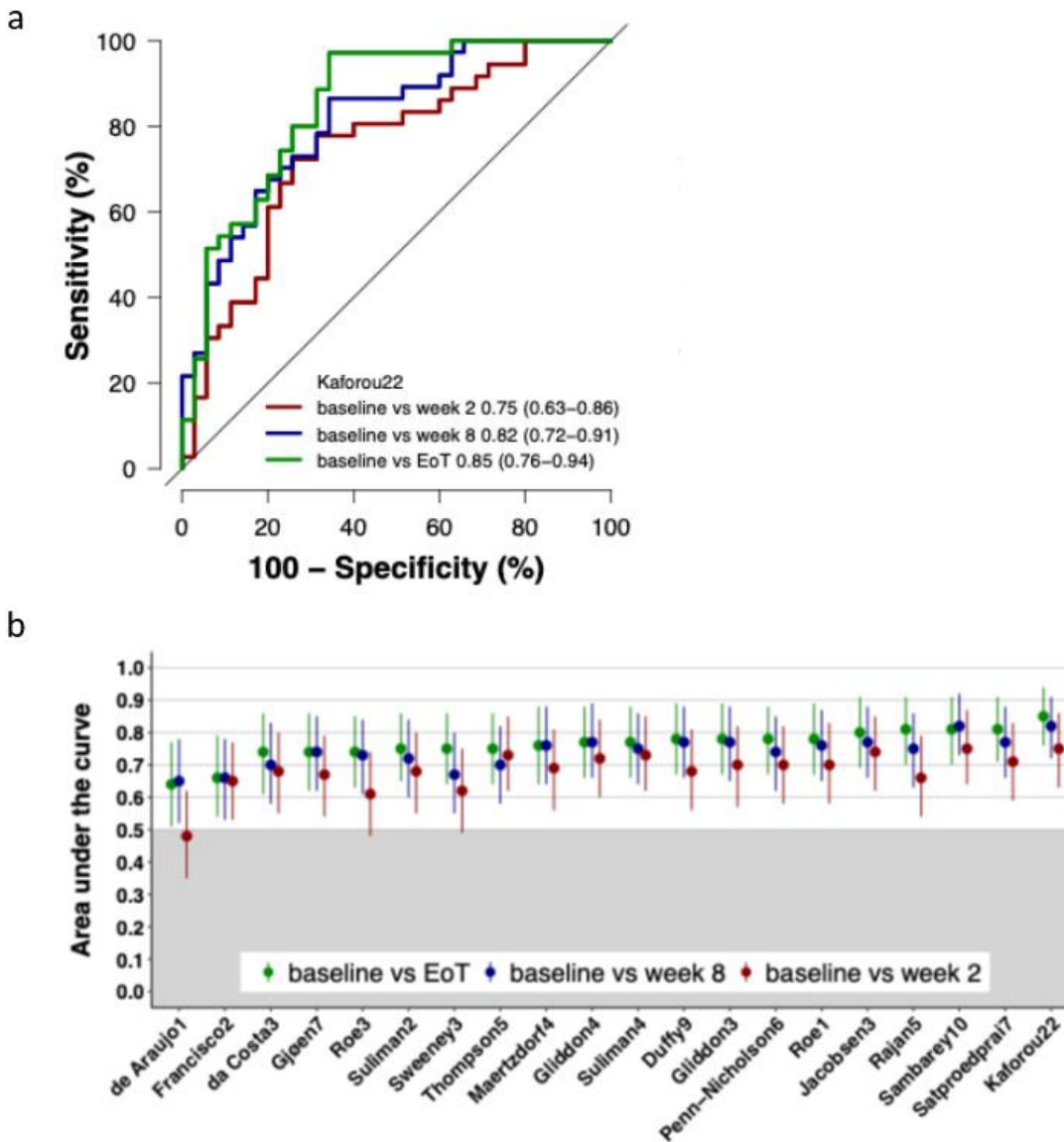


Figure 3. Performance of transcriptomic signatures as treatment monitoring biomarkers in children with confirmed TB.

a | Receiver operating characteristic (ROC) curve depicting performance (area under the curve, AUC, with 95% CI) of the Kaforou22 signature in children with confirmed TB comparing baseline signature scores across all time points during treatment. The Kaforou22 signature performed best for distinguishing children with confirmed TB at baseline from end of treatment (EoT). **b** | Summary of signature performance in children with confirmed TB comparing baseline signature scores across all time points during treatment, ranked by AUC for baseline versus EoT scores. Error bars depict 95% confidence intervals.

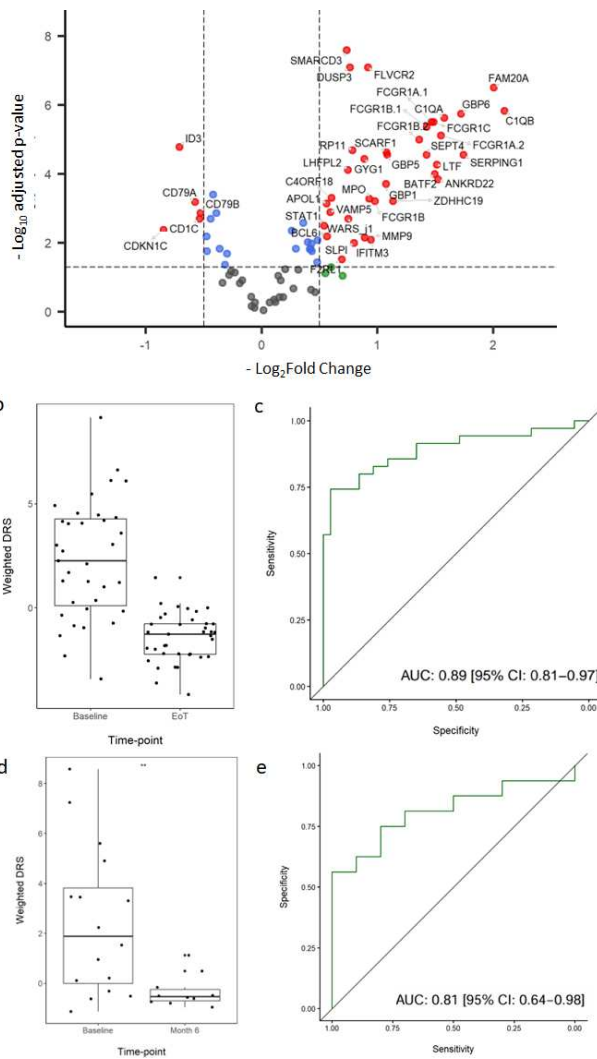


Figure 4. Discovery of a new TB monitoring score from a subset of the SHINE trial participants.

a | Volcano plot showing the genes differentially expressed between the enrolment (n=35) and end-of-treatment (n=37) samples from the confirmed TB group. Each point represents a single gene. Gray points are not statistically significant (Benjamini-Hochberg-adjusted p-value>0.05) and have small absolute log₂ fold change (FC) values (<0.5). Green points represent non-significant genes with absolute log₂ fold change (FC) values > 0.5. Blue points represent genes with significant differential (adjusted p-value<0.05) expression but small log₂ fold change (<0.5). Red points represent genes with significant differential expression and high (>0.5) absolute log₂ fold change values. **b** | Weighted Disease Risk Score (DRS) of the new 4 gene signature (*FCGR1C*, *FLVCR2*, *LTF*, and *CDKN1C*) among participants with confirmed TB at enrolment and end of treatment. **c** | Receiver operating characteristic (ROC) curve depicting performance (Area Under the Curve, AUC, with 95% CI) of the 4-gene monitoring signature score in these same participants. DRS boxplots (**d**) and ROC curves (**e**) demonstrating the performance of this 4-gene monitoring biomarker in an independent validation cohort of Indian children between enrolment (n=16) and 6-months later (EoT, n=10). **b** and (**d**) Boxes depict the IQR, the midline represents the median, and whiskers represent 1.5 × IQR.

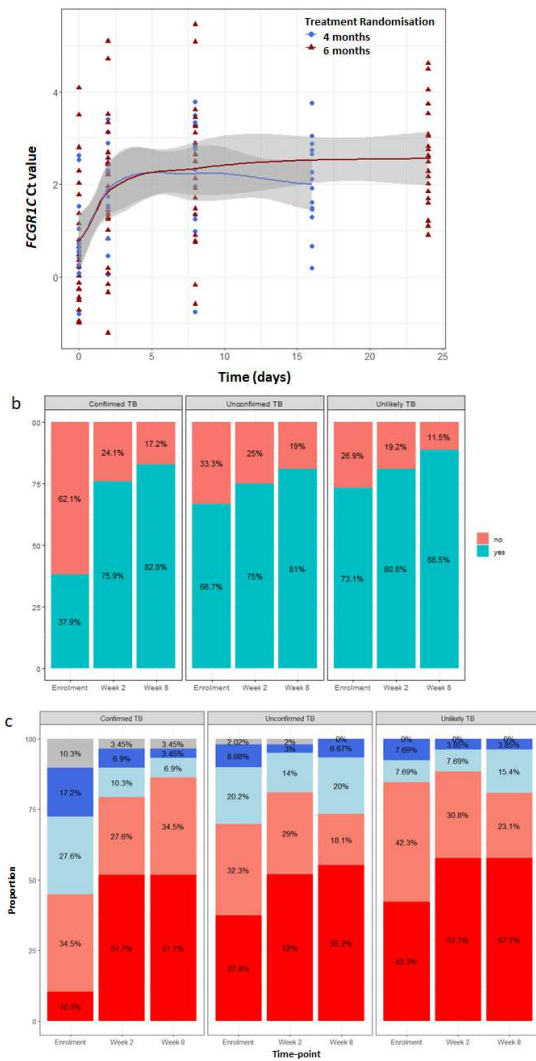


Figure 5. Development of a treatment-monitoring scoring system from the 4-gene monitoring score.

a | The change in normalised cycle threshold values of *FCGR1C* over time among participants with confirmed TB (n=37). Dots represent sampling timepoint for each participant and are coloured and shaped by treatment randomization group (4-month: blue circles, 6-month: red triangles). Lines represent Locally Estimated Scatterplot Smoothing (LOESS) regression with 95% confidence interval shading. **b** | The proportion of participants with *FCGR1C* expression within ± 1.5 standard deviations of the TB classification group (confirmed, unconfirmed, and unlikely TB) specific end-of-treatment value at each time-point. Each box shows these proportions by TB diagnostic classification. The proportion who fall within these ranges are represented by the red bar (“yes”) and those who do not by the blue bar (“no”). **c** | The treatment-monitoring scoring system developed from the 4-gene monitoring signature. For each gene, participants received 1 point if their Ct value fell within ± 1.5 standard deviations of the end-of-treatment mean, resulting in a score from 0 (no genes within range) to 4 (all genes within range). Each score value is represented by different colours, with the proportion of participants that make up these bars segregated by TB diagnostic group (confirmed, unconfirmed, and unlikely TB) at each treatment timepoint (enrolment, week 2, and week 8).

1 Tables

2 **Table 1. Participant characteristics.**

Characteristic	Confirmed TB N = 37	Unconfirmed TB N = 129	Unlikely TB N = 32	P value [†]
Treatment arm, n (%)				0.31
4-month arm	14 (37.8)	59 (45.7)	18 (56.3)	
6-month arm	23 (62.2)	70 (54.3)	14 (43.8)	
Age, years, n (%)				0.002
≥ 3 years	29 (78.4)	66 (51.2)	12 (37.5)	
< 3 years	8 (21.6)	63 (48.8)	20 (62.5)	
Female sex, n (%)	16 (43.2)	66 (51.2)	17 (53.1)	0.65
Median weight at diagnosis, kg (IQR)	18.0 (12.2–24.4)	13.0 (10.5–16.9)	12.5 (9.5–17.4)	0.002
BCG vaccination, n (%)	37 (100.0)	125 (96.9)	31 (96.9)	0.66
History of prior TB treatment, n (%)				0.28
Not previously treated for TB	34 (91.9)	123 (95.3)	32 (100.0)	
Treated for TB more than 2 years prior	3 (8.1)	6 (4.7)	0 (0.0)	
HIV positive, n (%)	1 (2.7)	2 (1.6)	0 (0.0)	0.73
Clinical presentation, n (%)				0.003
Mixed TB	22 (59.5)	54 (41.9)	6 (18.8)	
Respiratory TB	15 (40.5)	75 (58.1)	26 (81.3)	
CXR characteristics, n (%)				<0.001
Normal	4 (11.4)	35 (33.0)	19 (63.3)	
Non-typical TB	7 (20.0)	23 (21.7)	11 (36.7)	
Typical TB, non-severe	20 (57.1)	47 (44.3)	0 (0.0)	
Typical TB, severe	4 (11.4)	1 (0.9)	0 (0.0)	
Missing	2	23	2	
Treatment outcome, n (%)				0.78
Favourable	36 (97.3)	122 (94.6)	31 (96.9)	
Unfavourable	0 (0.0)	2 (1.6)	1 (3.1)	
Unassessed	1 (2.7)	5 (3.9)	0 (0.0)	

TB = tuberculosis. CXR = chest radiograph. BCG = Bacillus Calmette-Guérin. IQR = interquartile range.

† Comparisons across the three groups were performed using Pearson's Chi-squared test for categorical variables and the Kruskal-Wallis test for numeric variables.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.pdf](#)