

BMJ Open Host blood transcriptomic biomarkers of tuberculosis disease in people living with HIV: a systematic review protocol

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ABSTRACT

Introduction Current tuberculosis triage and predictive tools offer poor accuracy and are ineffective for detecting asymptomatic disease in people living with HIV (PLHIV). Host tuberculosis transcriptomic biomarkers hold promise for diagnosing prevalent and predicting progression to incident tuberculosis and guiding further investigation, preventive therapy and follow-up. We aim to conduct a systematic review of performance of transcriptomic signatures of tuberculosis in PLHIV.

Methods and analysis We will search MEDLINE (PubMed), WOS Core Collection, Biological Abstracts, and SciELO Citation Index (Web of Science), Africa-Wide Information and General Science Abstracts (EBSCOhost), Scopus, and Cochrane Central Register of Controlled Trials databases for articles published in English between 1990 and 2020. Case-control, cross-sectional, cohort and randomised controlled studies evaluating performance of diagnostic and prognostic host-response transcriptomic signatures in PLHIV of all ages and settings will be included. Eligible studies will include PLHIV in signature test or validation cohorts, and use microbiological, clinical, or composite reference standards for pulmonary or extrapulmonary tuberculosis diagnosis. Study quality will be evaluated using the 'Quality Assessment of Diagnostic Accuracy Studies-2' tool and cumulative review evidence assessed using the 'Grading of Recommendations Assessment, Development and Evaluation' approach. Study selection, quality appraisal and data extraction will be performed independently by two reviewers. Study, cohort and signature characteristics of included studies will be tabulated, and a narrative synthesis of findings presented. Primary outcomes of interest, biomarker sensitivity and specificity with estimate precision, will be summarised in forest plots. Expected heterogeneity in signature characteristics, study settings, and study designs precludes meta-analysis and pooling of results. Review reporting will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies guidelines.

Ethics and dissemination Formal ethics approval is not required as primary human participant data will not be collected. Results will be disseminated through peer-reviewed publication and conference presentation.

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INTRODUCTION

In 2019, 44% of the estimated 815 000 global incident tuberculosis cases among people

Strengths and limitations of this study

- This systematic review will be the first to synthesise the published literature on host-response blood transcriptomic biomarkers for diagnosing prevalent and predicting progression to incident tuberculosis disease in people living with HIV.
- Data reporting will adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols guidelines.
- Strengths of this protocol include a clear research question with explicit and reproducible methodology, comprehensive eligibility criteria with a stringent microbiological reference standard as well as clinical and composite reference standards for tuberculosis disease, inclusion of participants of all ages and recruitment settings, a rigorous and inclusive search strategy of multiple databases, and structured evaluation of study bias and evidence quality using the Quality Assessment of Diagnostic Accuracy Studies-2 assessment tool and Grading of Recommendations Assessment, Development and Evaluation approach.
- Inclusion will be restricted to published studies in English, which may introduce publication and language bias.
- Anticipated limitations of this review include heterogenous signature, study, and cohort designs, precluding meta-analysis.

living with HIV (PLHIV) went unreported or undiagnosed, with an estimated case fatality rate of 26% among all PLHIV.¹ We currently rely on symptom screening, which performs poorly as a triage test in PLHIV, to find these missing cases.² A test which could detect *Mycobacterium tuberculosis* (Mtb) infected individuals at highest risk of progression to disease, so-called incipient tuberculosis, or asymptomatic, minimal, or subclinical tuberculosis disease prior to symptom onset, facilitating earlier treatment and Mtb clearance, may reduce morbidity and mortality in PLHIV, and help to interrupt transmission. Tuberculin skin testing (TST) and the interferon gamma release assay (IGRA), which reflect a

memory T-cell response following Mtb sensitisation, are unable to distinguish current versus cleared Mtb infection and are thus not sufficiently specific for predicting progression to tuberculosis disease.^{3 4} In tuberculosis-endemic settings, very high rates of Mtb exposure and consequent TST or IGRA positivity limit the utility of these tests to guide administration of tuberculosis preventive therapy (TPT). IGRA also has lower sensitivity and produces more indeterminate results among PLHIV than among those without HIV.⁵ There is, therefore, a need for more specific, rapid, non-sputum tuberculosis triage and prognostic tools to direct further diagnostic testing and TPT in PLHIV.

Host-response blood transcriptomic biomarkers show potential for diagnosing^{6 7} prevalent tuberculosis and predicting⁸ progression from asymptomatic quiescent or incipient infection to active disease. A recent systematic review⁹ found 20 studies evaluating 25 predominantly interferon-stimulated gene (ISG) transcriptomic signatures of tuberculosis in adults without HIV; 17 signatures met at least one of WHO Target Product Profile (TPP) minimum performance criterion for a tuberculosis triage test (sensitivity 90%; specificity 70%)¹⁰ and one signature¹¹ predicted progression to tuberculosis disease through 6 months with performance meeting the minimum WHO TPP criteria for a test predicting progression to active disease (sensitivity and specificity 75%).¹² Although these results bode well for translation to a point-of-care transcriptomic triage test for people without HIV, there is evidence that HIV infection may affect signature score through induction of ISGs.¹³ An unsuppressed HIV viral load may thus erode diagnostic accuracy of ISG-dominant transcriptomic biomarkers. There are currently no systematic reviews evaluating diagnostic and prognostic performance of host-response blood transcriptomic tuberculosis biomarkers in PLHIV. Biomarkers selected for further development as point-of-care tests and field implementation studies in high-tuberculosis-risk groups should ideally perform well in people without HIV and in PLHIV, before and during antiretroviral therapy (ART).

We aim to systematically review the published literature on host-response blood transcriptomic biomarkers for diagnosing prevalent and predicting progression to incident tuberculosis disease in PLHIV. Our objectives are to provide an evidence synthesis of existing transcriptomic host-response biomarkers of tuberculosis disease evaluated in PLHIV; to appraise the quality of evidence, describe study design and biomarker characteristics, and compare the diagnostic and prognostic performance of the biomarkers with the WHO TPP criteria.

Research question

How do host blood transcriptomic signatures of tuberculosis perform in diagnosing prevalent and predicting progression to incident tuberculosis disease in PLHIV compared with the WHO TPP criteria?

Population

PLHIV of all ages and from all settings.

Index test

Blood transcriptomic biomarkers.

Reference standard

Microbiologically confirmed tuberculosis (primary endpoint) or non-microbiologically confirmed, presumptive clinical tuberculosis (secondary endpoint).

Comparator

WHO TPP criteria.

Outcome

Diagnosis of prevalent and prediction of progression to incident tuberculosis disease.

METHODS AND ANALYSIS

This protocol was developed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA)^{14 15} guidelines (online supplemental file 1). The systematic review will adhere to the PRISMA of Diagnostic Test Accuracy Studies¹⁶ recommendations. Significant amendments made to the protocol will be documented and published alongside the results of the systematic review.

Definitions and study eligibility criteria

Study design

Study eligibility criteria are summarised in [box 1](#). We will consider cross-sectional and case-control studies, prospective and retrospective cohort studies, and randomised control trials of human host diagnostic or prognostic transcriptomic signatures of tuberculosis that report test or validation cohort performance data. Studies that only report signature discovery cohort performance, or treatment response and failure monitoring cohorts, will not be considered.

Study participants and setting

We will consider study participants living with HIV of all ages, ethnicities, and settings, and include ART-naïve and ART-experienced individuals. Eligible studies must include participants living with HIV in either the signature test or validation cohorts. If the study encompasses both PLHIV and HIV-uninfected individuals, the study will only be included if the data are stratified by HIV subgroups.

Index test

We define diagnostic blood transcriptomic signatures of tuberculosis as host whole-blood or peripheral blood mononuclear cell biomarkers consisting of one or more host transcripts which are able to diagnose or predict progression to tuberculosis disease and have been validated in external cohorts. Studies which only evaluate

Box 1 Study eligibility criteria

Study inclusion criteria

1. Study design: Cross-sectional, case-control, prospective/retrospective cohort, or randomised control.
2. Study reports test and/or validation cohort diagnostic or prognostic performance data.
3. Study participants include people living with HIV (PLHIV) in test and/or validation cohort. Studies including human participants of all ages, geographical locations, and settings will be considered.
4. Index test: Study evaluates whole-blood or peripheral blood mononuclear cell diagnostic transcriptomic signatures of tuberculosis consisting of one or more host transcripts.
5. Control group: Includes healthy individuals, individuals with *Mycobacterium tuberculosis* infection and/or individuals with other diseases.
6. Tuberculosis endpoint: Studies will provide clearly defined microbiological tuberculosis reference standard or presumptive clinical diagnosis definitions (see the section Tuberculosis endpoints).
7. Outcome measures: Host tuberculosis transcriptomic signature sensitivity and specificity in test or validation cohorts, or reported data which enable the reconstruction of a two-by-two table for test accuracy calculation.

Study exclusion criteria

1. Study design: Statistical or mathematical modelling articles, cost-effectiveness studies, opinion pieces, narrative reviews, case studies, case series, and letters to editors which do not include original data will not be considered.
2. Study only reports signature discovery cohort performance, or treatment response, or failure monitoring cohorts.
3. Study participants do not include PLHIV, or it is not possible to stratify results by HIV status.
4. Index test: Study evaluates non-host (mycobacterial) transcriptional profiles only.
5. Control group: Studies which do not report a definition of the control group.
6. Tuberculosis endpoint: Studies which do not clearly state the case definition of tuberculosis disease, or do not separate clinically from microbiologically diagnosed cases.
7. Outcome measures: Studies which do not report any measures of signature performance, or do not separately report signature performance in PLHIV.
8. Article not available in English.
9. Full-text article not available.
10. Study published before 1 January 1990 or after 31 December 2020.
11. Studies conducted in animals.

non-host (mycobacterial) transcriptional profiles as diagnostic biomarkers will be excluded.

Tuberculosis endpoints

The primary tuberculosis disease endpoint is defined by a positive microbiological test from sputum or other bodily fluids, such as solid and liquid mycobacterial culture, Xpert MTB/RIF assay, or smear microscopy for acid-fast bacilli (auramine and Ziehl-Neelsen stains). Microbiologically confirmed extrapulmonary tuberculosis disease (such as disseminated tuberculosis and tuberculosis meningitis) will also be included. The secondary tuberculosis disease

endpoint is defined by non-microbiologically confirmed, presumptive clinical tuberculosis diagnoses through techniques such as chest radiography, ultrasonography, fluid aspirate (eg, lymph node and cerebrospinal fluid aspirates) chemistry, symptomatology, and composite non-microbiological endpoints. Latent tuberculosis infection is defined by a positive TST or interferon-gamma release assay (IGRA).

Eligible studies will use the primary microbiological tuberculosis reference standard endpoint or secondary presumptive clinical diagnosis endpoint for tuberculosis disease cases. Studies which do not separate clinically from microbiologically diagnosed cases will be excluded. Studies which use smear microscopy as a reference standard will be reported separately due to reduced diagnostic certainty. Eligible studies must include healthy individuals, individuals with latent Mtb infection, or individuals with other diseases as a control group. Tuberculosis disease diagnosed within 1 month of conducting the index test is presumed to be prevalent disease (diagnostic studies); incident tuberculosis is defined as tuberculosis disease diagnosed more than 1 month following study enrolment or measurement of index test. Prognostic studies are defined as prospective studies in which participants are followed up for progression to incident tuberculosis disease with prospective or retrospective measurement of a transcriptomic biomarker from blood RNA samples collected at enrolment.

Outcome measures

Outcome measures of interest will include reported host tuberculosis transcriptomic signature sensitivity and specificity in test or validation cohorts, or reported data which enable the reconstruction of a two-by-two table for test accuracy calculation for PLHIV. Studies which do not report any measures of signature performance, do not clearly state the case definition of tuberculosis disease, do not report primary data, lack explicit description of methodology, or do not separately report signature performance in PLHIV, will be excluded. If data supplied in the papers are not sufficient to reconstruct two-by-two tables, we will contact the corresponding authors to request additional data. Corresponding authors will be given up to 4 weeks to respond to email requests.

Search strategy

We will systematically search for published full-text articles using Medical Subject Headings (MeSH) and keyword search terms as outlined for our PubMed (MEDLINE) search in [table 1](#). Our systematic literature search will be adapted to WOS Core Collection, Biological Abstracts, and SciELO Citation Index (via Web of Science), Africa-Wide Information and General Science Abstracts (via EBSCOhost), Scopus and Cochrane Central Register of Controlled Trials databases. We will review reference lists of eligible articles and perform forward citation tracking using a citation index (such as Scopus or Science Citation Index via Web of Science) to identify further articles and

Table 1 PubMed Search strategy, modified as needed for other electronic databases

Diagnostic search terms:		
#1	MeSH terms:	Diagnosis [MeSH] Diagnosis [subheading]
#2	Text word:	diagnose OR diagnostic OR diagnosis OR detect OR detection OR predict OR prediction OR predictive OR prognose OR prognostic OR prognosis OR receiver operating characteristic OR receiver operator characteristic OR ROC OR risk OR screening OR sensitivity OR specificity OR area under the curve OR AUC OR accuracy
#3		#1 OR #2
Transcriptomic:		
#4	MeSH terms:	RNA, Messenger [MeSH]
#5	Text word:	gene OR genes OR mRNA OR messenger ribonucleic acid OR messenger RNA OR transcription OR transcriptome OR transcriptional OR transcriptomic
#6		#4 OR #5
Biomarker:		
#7	MeSH terms:	Biomarkers/blood [MeSH]
#8	Text word:	assay OR assays OR biomarker OR biomarkers OR bio-signature OR bio-signatures OR expression OR marker OR markers OR profile OR profiling OR profiles OR signature OR signatures OR surrogate endpoint OR test OR tests OR tool OR tools
#9		#7 OR #8
Tuberculosis:		
#10	MeSH terms:	Tuberculosis [MeSH] Mycobacterium, Tuberculosis [MeSH]
#11	Text word:	tuberculosis OR TB OR MTB
#12		#10 OR #11
HIV:		
#13	MeSH terms:	HIV(MeSH) Acquired Immunodeficiency Syndrome [MeSH]
#14	Text word:	HIV OR Human Immunodeficiency Virus OR AIDS virus OR Acquired Immune Deficiency Syndrome Virus
#15		#13 OR #14
#16		#3 AND #6 AND #9 AND #12 AND #15
#17		Filter 1990–2020
#18		Filter to English only

reports missed by the electronic database search.¹⁷ Only full-text articles will be considered. Statistical or mathematical modelling articles, cost-effectiveness studies, opinion pieces, narrative reviews, case studies, case series, and letters to editors which do not include original data will not be considered. We will consider articles published in English between 1 January 1990 and 31 December 2020.

Data management

EndNote bibliographic software will be used to manage, and screen references and full-text articles as previously described.¹⁸ Two reviewers will independently conduct the literature search and screen the search outputs for potential inclusion. After removal of duplicates, the selection process will include an initial screening of article titles and abstracts (include, exclude, or unsure), followed by full-text review for eligibility. Only studies meeting the eligibility criteria will be included in the systematic review. The two reviewers will compare their

results and resolve any disagreements or uncertainties by discussion. If consensus cannot be reached, the discrepancies will be referred to a third reviewer for adjudication. Study selection will be summarised in a PRISMA flow diagram.

Data extraction

Data elements (table 2) of included studies will be independently extracted and coded by the two reviewers using an electronic data collection form and results will be collated. The data extraction form will be piloted on the first five studies selected for inclusion to assess agreement between the two reviewers and need for amendments to the data collection form.

A study may evaluate multiple signatures using several validation cohorts. Studies and cohorts will be designated by the first author name and year of publication (eg, Author 2019a) and signatures by first author and number of transcripts (eg, Author 11).

Table 2 Summary of data extraction

Study identification	Study first author; article title; journal title; publication year; study type (discovery and/or validation; diagnostic and/or prognostic);
Cohort identification and methodology	Cohort first author; journal title; publication year; GEO and/or ArrayExpress database; country or geographic region of the study; cohort type (discovery, test or validation); study design (cross-sectional, case-control, prospective cohort, randomised control trial or other); study setting; age groups of participants (child, adolescent, adult or mixed); sample size; sampling method and participant selection (consecutive, convenience, random, other); sample representative of target population (were participants with suspected but unconfirmed tuberculosis excluded introducing spectrum bias); control group definition (LTBI, healthy control and/or other disease); microbiological reference standard(s) used to diagnose tuberculosis disease; clinical and/or composite non-microbiological methods of tuberculosis diagnosis; method of LTBI diagnosis (TST >5 mm, TST >10 mm, IGRA: T-Spot.TB or QuantiFERON); duration of follow-up for prediction of progression to incident tuberculosis; signature measurement method (RNA sequencing, microarray, PCR or other) and sample type (whole blood or PBMC); flow and timing of index and reference test measurement; study blinding
Signature characteristics	Signature discovery author; publication year; country or geographical region of discovery cohort; study design; signature discovery method (RNA sequencing, microarray, PCR or other) and sample type (whole blood or PBMC); transcripts included in the signature; signature model; intended use of signature.
Outcome data	True and false positives; true and false negatives; sensitivity; specificity; area under the curve; signature positivity rate (prevalence) in study population; signature cut-off/threshold applied (if reported); 95% CI for all estimates.

GEO, gene expression omnibus; IGRA, interferon-gamma release assay; LTBI, latent tuberculosis infection; PBMC, peripheral blood mononuclear cells; ; RNA, ribonucleic acid; TST, tuberculin skin test.

Quality appraisal

The methodological quality of included studies will be assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 assessment tool,¹⁹ a widely used tool for classification of the quality of the evidence from diagnostic accuracy studies. Risk of bias and applicability concerns for individual study patient selection, index test, reference standard, and study flow and timing will be reported as low risk, high risk, or unclear risk.

Two independent reviewers will assess the methodological quality of eligible trials and score the selected studies. Disagreements will be resolved through discussion and/or a third reviewer. The risk of bias for each outcome across individual studies will be summarised in a risk of bias table. A review-level narrative summary of the risk of bias will also be provided.

We will assess the cumulative quality of evidence synthesised by the systematic review using the ‘Grading of Recommendations Assessment, Development and Evaluation’ approach²⁰ with classification based on study design and limitations, indirectness, inconsistency, imprecision, and publication bias.²¹

Data analysis and reporting

Narrative synthesis of the findings from the eligible studies, including study design and signature characteristics, discovery and validation population characteristics, and performance of each signature, stratified by diagnostic (prevalent tuberculosis) and prognostic (incident tuberculosis) tests, study design, site of disease (pulmonary or extra-pulmonary), microbiological or composite clinical reference standards, and control group (healthy, latent-Mtb infected or other disease) will be provided. We anticipate considerable clinical and methodological heterogeneity between studies, with each study evaluating different transcriptomic signatures for the diagnosis of tuberculosis disease. In addition, signature score cut-off values will not be standardised for calculating signature sensitivity and specificity. As such, we do not plan to

perform a meta-analysis. If sufficient data are available, subgroup analysis by CD4 cell count, HIV plasma viral load, TPT and ART status may be undertaken. Signature sensitivity and specificity will be summarised using forest plots.

Patient and public involvement

As this research will be based on previously published data, there will be no patient and public involvement in the design, interpretation, or dissemination of the findings.

Ethics and dissemination

This systematic review protocol does not require formal ethics approval as primary human participant data will not be collected. The results will be disseminated through a peer-reviewed publication and conference presentation.

DISCUSSION

Transcriptomic biomarkers hold promise as markers of incipient, asymptomatic, minimal, or subclinical tuberculosis for targeted screening of high-risk populations, guiding targeted TPT and intensified follow-up.²² There is also need for non-sputum-based triage tests for detection of sub-clinical and clinical tuberculosis, to trigger further intensified investigation and therapeutic intervention.²³

While several studies have recently systematically evaluated transcriptomic biomarker performance for incipient and prevalent tuberculosis,^{7–9 24 25} none have specifically focused on PLHIV. As highlighted in the introduction, PLHIV are over-represented in global tuberculosis incidence and have a particularly high case-fatality rate. PLHIV are also less likely to expectorate sputum while paucibacillary tuberculosis is more common, factors that make diagnosis even more challenging in PLHIV.²⁶ As such, it is important that non-sputum tuberculosis biomarkers selected for further development and commercialisation are efficacious in this high-risk population. This



systematic review will be the first to provide synthesis of transcriptomic signature performance in diagnosing prevalent and predicting progression to incident tuberculosis in PLHIV.

A rigorous protocol acts as a roadmap to the reviewers; by prespecifying and registering a detailed systematic review protocol, we aim to reduce bias in selection of studies and reporting of results, reducing arbitrary decision-making in data extraction, quality assessment and analysis. This protocol will allow journal editors, peer reviewers, and readers to critically gauge the review completeness and transparency, identify deviations from planned methods, and identify biased interpretation of review results and conclusions, holding accountability to the reviewers.¹⁴ Specific strengths of this systematic review protocol include a clear research question, explicit and reproducible methodology, comprehensive eligibility criteria with a stringent microbiological reference standard, as well as clinical and composite endpoints for tuberculosis disease, inclusion of participants of all ages and recruitment settings, a rigorous and inclusive search strategy of multiple databases, and structured evaluation of study bias and evidence quality.

Potential limitations of this study include the heterogeneity of measures and outcomes reported by biomarker discovery and validation studies, with few studies applying a priori biomarker thresholds across cohorts or one that is relevant to the WHO TPP criteria. We anticipate scant reporting of signature performance stratified by ART and TPT status, CD4 cell count, and HIV viral load, limiting subgroup analysis. We are also aware that much of the tuberculosis biomarker literature in PLHIV emanates from sub-Saharan Africa, potentially limiting generalisability of findings. We expect significant heterogeneity in signature, study and cohort designs, precluding meta-analysis. Inclusion of studies published in English only may introduce publication bias. Diagnosing tuberculosis in PLHIV can be particularly challenging due to more common paucibacillary disease and difficulties in expectorating sputum in advanced HIV; we, thus, chose to include clinical and composite diagnostic endpoints which are still used in many settings to presumptively initiate tuberculosis treatment. However, this may lead to overdiagnosis of tuberculosis and underestimation of transcriptomic biomarker performance. Clinically diagnosed symptomatic disease without microbiological confirmation remains an enigma which merits further attention beyond the scope of this review.

This review will inform further optimisation and development of transcriptomic signatures as they progress through the clinical implementation pipeline. Transcriptomic signatures discovered and validated in high quality studies with well-designed cohorts and meeting or approaching the WHO TPP criteria may be considered for advancement for further prospective validation in real-world healthcare settings and development as point-of-care tests for PLHIV who are at elevated risk of tuberculosis and its sequelae. The review may also inform

whether current WHO TPP benchmarks can realistically be attained in PLHIV, and whether they need to be revisited.

Contributors SCM and MH conceived the idea and planned the study protocol. SCM, MS and MH undertook a scoping search and designed the search strategy. SCM wrote the protocol under supervision from MH and TS. SCM, HM, SKM, FD, MS, TS, and MH have contributed to, reviewed, and approved the final protocol, and will participate in the interpretation of the results.

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Competing interests TS is a coinventor of two patents of host-blood transcriptomic signatures of tuberculosis risk.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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