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

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Word count: 2,670

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 1990–2020. 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 extra-pulmonary 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.

PROSPERO registration: CRD42021224155

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 (PRISMA-P) 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 (QUADAS-2) assessment tool and Grading of Recommendations Assessment, Development and Evaluation (GRADE) 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.

INTRODUCTION

In 2019 44% of the estimated 815,000 global incident tuberculosis cases amongst people living with HIV (PLHIV) went unreported or undiagnosed, with an estimated case fatality rate of 26% amongst 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 sub-clinical 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 amongst PLHIV than amongst 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 predicting[8] progression from asymptomatic quiescent or incipient infection to active disease. A recent systematic review[9] 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 the World Health Organization (WHO) Target Product Profile (TPP) minimum performance criterion for a tuberculosis triage test (sensitivity 90%; specificity 70%)[10] and one signature[11] 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 to 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 review and Meta-Analysis Protocols (PRISMA-P)[14,15] guidelines (Supplementary File). The systematic review will adhere to the Preferred Reporting Items for Systematic reviews and Meta-Analysis of Diagnostic Test Accuracy Studies (PRISMA-DTA)[16] recommendations. Significant amendments made to the protocol will be documented and published alongside the results of the systematic review. This systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 02 January 2021 with registration number CRD42021224155.

Definitions and study eligibility criteria

Study design

Study eligibility criteria are summarised in **Table 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.

Index test

We define diagnostic blood transcriptomic signatures of tuberculosis as host whole-blood or peripheral blood mononuclear cell (PBMC) 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 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 for acid-fast bacilli (auramine and Ziehl-Neelsen stains). Microbiologically-confirmed extra-pulmonary 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 (e.g. lymph node and cerebrospinal fluid aspirates) chemistry, symptomatology, and composite non-microbiological endpoints. Latent tuberculosis infection is defined by a positive tuberculin skin test (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 in separate figures 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 one month of conducting the index test is presumed to be prevalent disease (diagnostic studies); incident tuberculosis is defined as tuberculosis disease diagnosed more than one 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 four weeks to respond to email requests.

Table 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 in test and/or validation cohort. Studies including human participants of all ages, geographic locations, and settings will be considered.
4. Index test: Study evaluates whole-blood or peripheral blood mononuclear cell (PBMC) diagnostic transcriptomic signatures of tuberculosis consisting of one or more host transcripts
5. Control group: Includes healthy individuals, individuals with *Mtb* 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 *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, or do not stratify results by control group definition
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

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 2**. 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 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.

Table 2: 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	

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 a reviewer for adjudication. Study selection will be summarised in a PRISMA flow diagram.

Data extraction

Data elements (**Table 3**) 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.

Table 3: 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 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, 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 >5mm, TST >10mm, 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 geographic 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% confidence intervals for all estimates

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

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 (e.g. Author2019a) and signatures by first author and number of transcripts (e.g. Author11).

Quality appraisal

The methodological quality of included studies will be assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-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 with 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" (GRADE) approach[20] 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 is 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 sub-clinical 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 focussed 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 pre-specifying 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 sub-group 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 under-estimation 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-word health-care 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.

AUTHORS' CONTRIBUTIONS

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 TJS. SCM, HM, SKM, FD, MS, TJS, 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 STATEMENT

TJS is a co-inventor of two patents of host-blood transcriptomic signatures of tuberculosis risk.

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

Supplementary File: PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al**:** Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Location
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	Line 2, Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☐	N/A
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	Line 57, Page 2; lines 135-136 page 5
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	Page 1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	Lines 327-331, Page 12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☒	☐	Lines 133-134, Page 5
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	Lines 333-341, Page 12
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☐	N/A
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☐	N/A
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	Lines 96-111, Page 4
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	Lines 120-129, Page 5

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

Supplementary File: PRISMA-P 2015 Checklist

Section/topic	#	Checklist item	Information reported		Location
			Yes	No	
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	Table 1; Lines 140-209, Pages 5-8
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	Lines 192-209, Page 7-8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	Table 2, Page 8
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	Lines 213-221, Pages 8-9
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	Lines 213-221, Pages 8-9
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	Lines 223-236, Page 9
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	Table 3, Page 9
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	Lines 186-194, Pages 6-7; Table 3, Page 9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	Lines 238-248, Page 10

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

Supplementary File: PRISMA-P 2015 Checklist

Section/topic	#	Checklist item	Information reported		Location
			Yes	No	
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	☐	Lines 255-266, Page 10
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☐	☐	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☒	☐	Lines 264-266, Page 10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☒	☐	Lines 255-266, Page 10
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☒	☐	Lines 306-319, Pages 11-12
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	Lines 250-253, Page 10

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-048623.R1
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Date Submitted by the Author:	14-Jun-2021
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	HIV/AIDS, Immunology (including allergy), Diagnostics, Global health, Respiratory medicine
Keywords:	Tuberculosis < INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES, Molecular diagnostics < INFECTIOUS DISEASES

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

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Keywords: Human, host, blood, diagnostic, prognostic, predictive, transcriptomic, mRNA, gene expression, biomarker, signature, tuberculosis, *Mycobacterium*, TB, HIV

Word count: 3,174

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 1990–2020. 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 extra-pulmonary 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.

PROSPERO registration: CRD42021224155

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 (PRISMA-P) 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 (QUADAS-2) assessment tool and Grading of Recommendations Assessment, Development and Evaluation (GRADE) 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.

INTRODUCTION

In 2019 44% of the estimated 815,000 global incident tuberculosis cases amongst people living with HIV (PLHIV) went unreported or undiagnosed, with an estimated case fatality rate of 26% amongst 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 sub-clinical 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 amongst PLHIV than amongst 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 predicting[8] progression from asymptomatic quiescent or incipient infection to active disease. A recent systematic review[9] 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 the World Health Organization (WHO) Target Product Profile (TPP) minimum performance criterion for a tuberculosis triage test (sensitivity 90%; specificity 70%)[10] and one signature[11] 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 to 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 review and Meta-Analysis Protocols (PRISMA-P)[14,15] guidelines (Supplementary File). The systematic review will adhere to the Preferred Reporting Items for Systematic reviews and Meta-Analysis of Diagnostic Test Accuracy Studies (PRISMA-DTA)[16] recommendations. Significant amendments made to the protocol will be documented and published alongside the results of the systematic review. This systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 02 January 2021 with registration number CRD42021224155.

Definitions and study eligibility criteria

Study design

Study eligibility criteria are summarised in **Table 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 (PBMC) 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 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 extra-pulmonary 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 (e.g. lymph node and cerebrospinal fluid aspirates) chemistry, symptomatology, and composite non-microbiological endpoints. Latent tuberculosis infection is defined by a positive tuberculin skin test (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 one month of conducting the index test is presumed to be prevalent disease (diagnostic studies); incident tuberculosis is defined as tuberculosis disease diagnosed more than one 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 four weeks to respond to email requests.

Table 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 in test and/or validation cohort. Studies including human participants of all ages, geographic locations, and settings will be considered.
4. Index test: Study evaluates whole-blood or peripheral blood mononuclear cell (PBMC) diagnostic transcriptomic signatures of tuberculosis consisting of one or more host transcripts
5. Control group: Includes healthy individuals, individuals with *Mtb* 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 *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

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 2**. 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 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.

Table 2: 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	

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 a reviewer for adjudication. Study selection will be summarised in a PRISMA flow diagram.

Data extraction

Data elements (**Table 3**) 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.

Table 3: 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 >5mm, TST >10mm, 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 geographic 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% confidence intervals for all estimates

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

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 (e.g. Author2019a) and signatures by first author and number of transcripts (e.g. Author11).

Quality appraisal

The methodological quality of included studies will be assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-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 with 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" (GRADE) approach[20] 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 is 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 sub-clinical 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 focussed 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 pre-specifying 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 sub-group 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 under-estimation 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-word health-care 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.

AUTHORS' CONTRIBUTIONS

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 TJS. SCM, HM, SKM, FD, MS, TJS, 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 STATEMENT

TJS is a co-inventor of two patents of host-blood transcriptomic signatures of tuberculosis risk.

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Supplementary File: PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al**:** Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Location
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	Line 2, Page 1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☐	N/A
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	Line 57, Page 2; lines 135-136 page 5
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	Page 1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	Lines 329-333, Page 12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☒	☐	Lines 133-134, Page 5
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	Lines 335-343, Page 12
Sponsor	5b	Provide name for the review funder and/or sponsor	☐	☐	N/A
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	☐	N/A
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	Lines 94-109, Page 4
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	Lines 118-127, Page 5

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

Supplementary File: PRISMA-P 2015 Checklist

Section/topic	#	Checklist item	Information reported		Location
			Yes	No	
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	Table 1; Lines 138-208, Pages 5-7
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	Lines 197-207, Page 7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	Table 2, Page 8
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	Lines 211-219, Pages 8
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	Lines 213-219, Pages 8
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	Lines 221-234, Page 9
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	Table 3, Page 9
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	Lines 184-193, Pages 6-7; Table 3, Page 9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	Lines 238-248, Page 10
DATA					

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

Supplementary File: PRISMA-P 2015 Checklist

Section/topic	#	Checklist item	Information reported		Location
			Yes	No	
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	N/A: See lines 261-264, Page 10
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☒	☐	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☒	☐	Lines 263-266, Page 10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☒	☐	Lines 255-266, Page 10
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☒	☐	Lines 306-319, Pages 11-12
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	Lines 250-253, Page 10