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Performance of host blood transcriptomic signatures for diagnosing and predicting progression to tuberculosis disease in HIV-negative adults and adolescents: a systematic review protocol

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ABSTRACT

Introduction One-quarter of the global population, including the majority of adults in tuberculosis (TB) endemic countries, are estimated to be *Mycobacterium tuberculosis* (MTB) infected. An estimated 10 million new TB cases occurred in 2017. One of the biggest challenges confronting TB control is the lack of accurate diagnosis and prediction of prevalent and incident TB disease, respectively. Several host blood transcriptomic messenger RNA (mRNA) signatures that reflect the host immune response following infection with MTB and progression to TB disease in different study populations have recently been published, but these TB biomarkers have not been systematically described. We will conduct a systematic review of the performance of host blood transcriptional signatures for TB diagnosis and prediction of progression to TB disease.

Methods and analysis This systematic review will involve conducting a comprehensive literature search of cohort, case–control, cross-sectional and randomised-controlled studies of the performance of host blood transcriptomic signatures for TB diagnosis and prediction of progression to TB disease. We will search Medline via PubMed, Scopus, Web of Science and EBSCO libraries, complemented by a search of bibliographies of selected articles for other relevant articles. The literature search will be restricted to studies published in English from 2005 to 2018 and conducted in HIV-uninfected adults and adolescents (≥12 years old). Forest plots and a narrative synthesis of the findings will be provided. The primary outcomes will be sensitivity, specificity, as well as true/false positives and true/false negatives. Heterogeneity resulting from differences in the design, composition and structure of individual signatures will preclude meta-analysis and pooling of results.

Ethics and dissemination Ethics approval is not required for this systematic review protocol. The results of this review will be disseminated through a peer-reviewed journal as well as conference presentations.

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Strengths and limitations of this study

- This will be the first systematic review of the performance of host blood transcriptomic signatures for the diagnosis of prevalent tuberculosis (TB) and prediction of incident TB disease in adults and adolescents.
- Data reporting will adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for reviews and protocols.
- Included studies will be restricted to those published in English, which may introduce publication and language bias.
- The design/composition/structure of individual signatures are expected to be significantly heterogeneous, precluding meta-analysis and pooling of results.

INTRODUCTION

Tuberculosis (TB) is the most common cause of infectious disease mortality worldwide, yet TB control remains a major public health challenge, because it is difficult to predict and prevent, diagnose and treat. TB disease is caused by the bacillus *Mycobacterium tuberculosis* (MTB) and is transmitted by inhaled droplet spread from individuals with active disease. Healthy individuals who are exposed to aerosolised MTB bacilli may develop infection, which may be cleared, contained as latent MTB infection, or, if containment is unsuccessful, progress to active TB disease known as primary TB. Latent MTB infection may also progress to active TB disease at a later stage known as postprimary TB.[1] The ultimate result of exposure to MTB bacilli is

determined by a range of environmental, sociological, mycobacterial and host immune factors.[2]

An estimated 1.7 billion individuals or 23% of the world population, including the majority of adults in TB-endemic countries, are MTB infected.[3] There were 10 million new cases of TB disease in 2017, of which 90% occurred in adults.[4] One of the 2030 Sustainable Development Goals adopted by the United Nations in 2015 is to end the global TB epidemic. The End TB Strategy demands that new cases of TB should be reduced by 80% from 2015 levels by the year 2030, and deaths occurring due to TB should be reduced by 90% for the same period.[5] In order to reduce new TB cases and deaths to meet the set targets, major advances in TB drugs, vaccines and diagnostics are critical.

Currently available TB diagnostic tests have important drawbacks especially if applied as a screening test, thus making TB diagnosis difficult.[6–9] Sputum smear microscopy, still used in many high burden TB countries, has low sensitivity[9] ranging from 32% to 89%[10] resulting in a considerable number of active pulmonary TB (PTB) patients being missed.[8] Xpert MTB/RIF has considerably better diagnostic performance with sensitivity of 77% and specificity of 99%.[11] However, Xpert MTB/RIF is relatively unaffordable in resource-limited settings and has technical limitations such as the need for special equipment as well as a reliable power supply, thereby impeding routine screening in TB-endemic resource-limited settings.[12] MTB culture, the gold standard, delays TB diagnosis as it usually takes more than 2 weeks (up to 42 days) to get a confirmatory result, and this is not ideal for rapid patient management.[1 13] Furthermore, MTB culture requires a reference laboratory and is relatively costly. A chest radiograph (CXR) is inconclusive for PTB diagnosis as it may yield false-negative results particularly when the disease is in its initial phases.[14] It may also yield false positives in individuals with lung damage from prior TB disease or other lung diseases. The inability of CXR to accurately differentiate between the many abnormalities consistent with TB from those of other lung pathologies restricts its specificity, which ranges between 46% and 89%.[14–16] Furthermore, readout of a CXR is highly dependent on a skilled interpretation and a level of subjectivity, which is problematic for low resource settings. Symptom screening alone has a low specificity in diagnosis of PTB, especially in HIV-infected individuals. In HIV-uninfected individuals and individuals of unknown HIV status, symptom screening has a sensitivity and specificity of about 77% and 68%, respectively.[16]

Latently MTB infected individuals, identified by a positive tuberculin skin test (TST) or interferon-gamma release assay (IGRA), have a higher risk of developing TB disease than uninfected people.[7 17] However, IGRA and TST have poor specificity for incident TB disease (49.3% and 45%, respectively) and hence predicting incident TB disease remains difficult.[17] This problem is further compounded by the fact that, in TB-endemic populations, up to 90% of people who test IGRA or TST positive will not go on to develop active TB disease.[6 18] A systematic review showed that the positive predictive values (PPVs) for these current predictive tools are too low to have clinical utility in directing use of preventive therapy[19] for high TB burden settings. The PPVs for progression from latent MTB infection to TB disease in all settings were 2.7% and 1.5% for IGRA and TST, respectively. In high risk groups, the PPVs increased marginally to 6.8% for IGRA, and 2.4% for TST.[19] Although prevention of TB disease arising from latent MTB infection is key to achieving WHO elimination targets,[20] mass preventive therapy based on IGRA or TST screening in TB-endemic countries would need to treat 50% to 80% of the population, most of them unnecessarily. Many incident TB cases would also be missed due to poor sensitivity (IGRA=75% and TST=77%).[17] Mass preventive therapy for all MTB infected people using current tools would not be feasible, affordable or effective, because reinfection would likely occur before programmatic coverage was complete. More specific predictive tools are needed to identify those individuals who would most benefit from preventive therapy. Given the inadequacies of current diagnostic tools, more sensitive, highly specific, quicker and much more affordable tests that differentiate active TB from healthy individuals, latent MTB infection and other diseases, as well as predict progression from latent MTB infection to active disease, are needed. Advances in TB prevention, prediction, diagnosis and treatment are impeded by the fact that the immunological basis for progression from MTB infection to disease is poorly understood.[2 21]

In recent years, host blood transcriptomic (mRNA) signatures have provided a promising alternative for both TB disease diagnosis and prediction of progression to TB disease. Transcriptional signatures of TB have also provided better understanding of the TB-specific immune mechanisms[22] in individuals with MTB infection[23] and those with active TB disease.[24] Several studies of host blood transcriptomic signatures have shown that individuals with prevalent TB disease can be discriminated from those who are uninfected, latently MTB infected, or suffering from another disease.[25] Diagnostic sensitivity has ranged between 61% and 100% while specificity has ranged between 75% and 97% for active TB versus latent MTB infection, or active TB versus other diseases.[12 26] Recent work has also shown that transcriptomic signatures can predict the development of TB disease in individuals with MTB infection. A 16-gene signature of risk predicted progression from latent MTB infection to TB disease with a sensitivity of 66.1% and a specificity of 80.6% in the 12 months prior to TB diagnosis.[27] Validation of this signature in an independent cohort of household contacts of active TB patients, predicted progression to TB disease with a sensitivity of 53.7% and a specificity of 82.8% in the 12 months preceding TB diagnosis.[27] Recently, Suliman *et al* reported that a four-gene signature of risk predicted progression to TB disease in household contacts of active TB disease with an area under the curve (AUC) of 0.66 in the 12 months prior to TB diagnosis.[28] However, the

performance of host blood transcriptomic signatures for diagnosis of prevalent and prediction of incident TB disease has not been synthesised and examined systematically. Consequently, we will conduct a systematic review aiming to describe and summarise the performance of the currently available host blood transcriptomic signatures for diagnosing and predicting TB disease.

RESEARCH QUESTION AND AIMS

Research question

What are the performance characteristics of host blood transcriptomic signatures for diagnosing prevalent TB disease and predicting incident TB disease in HIV-negative adolescents and adults?

Objectives

1. To describe the performance of host blood transcriptomic signatures for diagnosis of TB disease in HIV-negative adolescents and adults.
2. To describe the performance of host blood transcriptomic signatures for predicting progression to TB disease in HIV-negative adolescents and adults.

METHODS AND ANALYSIS

This protocol conforms to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P)[29] (online supplementary file 1) and the Cochrane Collaboration's diagnostic test accuracy methods for evidence searching and synthesis.[30 31] Our review methodology will include a thorough literature search, examination of studies identified and selection of studies using predefined criteria. We will then extract data from the included studies, evaluate methodological quality, summarise it and rate the quality of evidence from our systematic review. The statistical analysis, evidence synthesis and reporting of findings will be performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[32] The study will be conducted and completed between February 2019 and June 2019.

Definitions and study inclusion criteria

(i) Definitions

- Predictive studies: prospective studies of progression to TB disease in which biomarker status is assigned at enrolment and TB case/control status is assigned at a later time point, after at least 6 months of follow-up.
- Diagnostic studies: studies in which both biomarker status and TB case/control status are assigned at the same time point.
- Latent MTB infection is defined as a positive TST ≥5 mm; or positive IGRA conforming to the manufacturer's instructions and cut-off point.
- TB disease is defined as either or both pulmonary TB (PTB) and extrapulmonary TB (EPTB) diagnosed with microbiological confirmation by MTB culture or Xpert MTB/RIF or smear microscopy.
- A TB contact is defined as a person in close contact with, or living in the same household as, an individual diagnosed with active TB disease within the past 6 months.

We will select studies meeting all the following criteria:

(ii) General inclusion criteria

- Cohort, case–control, cross-sectional and randomised control studies conducted in HIV-uninfected humans.
- Studies using host blood transcriptomic (mRNA) signatures for diagnosis or prediction of TB disease with a microbiological reference standard of either MTB culture or Xpert MTB/RIF or smear microscopy.
- Studies using either TST or IGRA for the diagnosis of latent MTB infection.
- Studies comparing TB disease cases versus controls with or without other diseases, and with or without latent MTB infection. Both PTB and EPTB cases will be included.
- Studies reporting either discovery or validation of a host blood transcriptomic (mRNA) signature.
- Studies conducted in adults or adolescents (≥12 years old).
- Studies published both as abstracts and full articles after 2005–2018.
- Studies published in English regardless of location or country of origin.
- Studies reporting sensitivity and specificity; or reporting results enabling the recreation of a 2×2 table for test performance calculation, or studies where we receive a response on test performance data within 4 weeks of inquiry.

(iii) Additional inclusion criteria for TB predictive studies

- Studies with a follow-up period of at least 6 months from enrolment.
- Studies enrolling TB contacts, latently MTB infected individuals or healthy individuals.
- Randomised controlled trials or prospective cohort studies.

Literature search

The primary electronic searches will be conducted in Medline via PubMed, Scopus, Web of Science and EBSCO databases. The search strategy will employ a combination of database specific Medical Subject Heading terms and other key words that include but not limited to TB, Tuberculosis, *Mycobacterium tuberculosis*, *M. tuberculosis*, MTB, diagnosis, diagnostic, detect, prognosis, prognostic, predict, blood, host, human, biomarker, signature, bio-signature, transcriptome, transcriptomic, RNA, sensitivity and specificity, accuracy, diagnostic accuracy, performance, area under the curve, AUC, receiver operating characteristic and ROC. The initial PubMed search strategy is availed as online supplementary file 2. The finalised PubMed search strategy will be adapted to other databases and will be published in the systematic review. Furthermore, bibliographies of included papers will be scrutinised for potential papers to include in the review

that would otherwise have been missed by the search term. Unpublished reports and conference proceedings/papers will not be included due to absence of peer review and difficulties in obtaining data. We recognise that this shortcoming may result in publication bias.

Data management

The first author (HM) will conduct the data management activities. A Google drive account will be created and maintained for the systematic review. All documents relating to the conduct of this review, such as a record of the search strategy and identified articles, protocol, individual study quality assessment records and other supplementary material will be uploaded to this Google drive folder. Additionally, a database will be developed using Microsoft SqlServer 2012 as the back-end and forms in Microsoft Access 2010 as the user interface, to manage individual data metrics extracted from the articles. This will enable electronic and quick comparison of the extracted data as well as inclusion/exclusion decisions between HM and EWB. EndNote referencing software will be used to manage the titles of identified articles and references during study selection and write up. A backup of all the records will also be kept on South African Tuberculosis Vaccine Initiative (SATVI's) server as well as the laptop from which this work will be carried out.

Study selection

Two reviewers, HM and EWB will independently screen the search outputs for potentially qualifying studies. The selection process will initially involve importing all articles returned by the search strategy into EndNote software using distinct groups (folders) for each literature source. Once all articles have been imported into their respective groups, another group will be created which will contain all articles from these subgroups, including duplicates. Duplicates will then be removed by creating the final group into which distinct titles will be stored. HM will then import all distinct studies into the Microsoft SqlServer database and assign a unique study identification number. Only the article title, first-author name and publication year will be imported into the database, while the rest of the information will be captured as the studies are screened. HM and EWB will separately screen titles and abstracts first, and thereafter, read the full text of all potentially qualifying studies to assess eligibility. Only studies meeting all the inclusion criteria will be included in the systematic review. HM and EWB will independently categorise articles into one of the three groups; (1) selected, (2) not selected and (3) pending. Thereafter, the two reviewers, HM and EWB, will compare their results and resolve any disagreements by discussion. Articles categorised as 'selected' and 'not selected' by both reviewers will be included and excluded in the review, respectively, while articles categorised as pending will be discussed by both reviewers in order to reach consensus. If consensus cannot be reached, discrepancies will be discussed with a third reviewer (BK). The search process and selection of studies will be summarised and presented as a flow chart in conformance with PRISMA guidelines for reviews.

Data extraction

Data from selected studies will be recorded into an electronic data extraction form (online supplementary file 3) developed using Microsoft Access-2010 forms, in order to enable assessment of study quality and evidence synthesis. Because the reviewers will independently extract the data, this form will be piloted on a sample of at least five randomly selected studies to assess the concordance level between the two data extractors. HM and EWB will then compare the results of the extracted data and resolve any differences by discussion, with arbitration from (BK) for any unresolved differences. We will request missing data from study authors through email and exclude studies where the author does not respond to two email requests over a period of 4 weeks. Data elements to be extracted will include, but are not limited to the following:

- *Study characteristics*: first author, title, publication year, sample type, country, design, type (diagnostic vs predictive) case definition and specimen used for reference standard tests.
- *Population characteristics*: age category, number of study participants, cohort type (test vs validation) proportion of adolescents, gender composition and number by disease status (TB disease, latent MTB infection, healthy control or other disease).
- *Transcriptomic signature characteristics:* signature name and number of genes, sample type, signature discovery method (microarray, RNA sequencing and or PCR), model (random forest, pairwise, support-vector machine (SVM) and so on) and threshold score.
- Gold standard: the reference standard tests used to diagnose TB disease or latent MTB infection will include MTB culture, smear microscopy or Xpert MTB/RIF for the diagnosis of TB; and TST or IGRA for the diagnosis of latent MTB infection.
- Outcomes: primary outcome measures will include sensitivity and specificity, true/false positives and true/false negatives. Secondary outcome measures will include positive likelihood ratio (LR+) and negative likelihood ratio (LR−) and area under the receiver operating characteristic curve (AUC).

Quality appraisal

The quality of the studies included in the systematic review will be evaluated using a customised form (online supplementary file 4) based on the Quality Assessment of Diagnostic Accuracy Studies[33] assessment tool as well as the 'Standards for the Reporting of Diagnostic Accuracy Studies'[34] guidelines. HM and EWB will independently assess the risk of bias and compare their evaluations. If the two reviewers cannot reach consensus, a third reviewer (BK) will adjudicate. The reviewers will not be blinded to the journal titles, study authors or institutions.

Data analysis and synthesis

We will provide a narrative synthesis of the findings from the included studies, focused on the performance of the transcriptomic signatures for diagnosis of prevalent TB disease (diagnostic performance) or prediction of incident TB disease (predictive performance), and the target population characteristics. Data on diagnostic performance will be analysed and presented separately from that of predictive performance. The individual index tests (transcriptomic signatures) will be compared against a reference test (MTB culture or Xpert MTB/RIF or smear microscopy). For each test, the reported true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) will be retrieved. If these values are not reported, but data are available within the paper or can be obtained from the authors, we will calculate these values based on the reported sensitivity and specificity. Similarly, sensitivity and specificity will be computed where it is not reported but data allowing the calculation of such performance values is available. Separate evidence summary tables and forest plots for diagnostic and predictive studies will be reported for each signature. Signatures used for both diagnosis and prediction of TB disease will be presented in both sets of analysis. The forest plots will be produced using Review Manager (RevMan),[35] the Cochrane Collaboration's software for preparation and maintenance of reviews. The forest plot will show the data for the sensitivity and specificity of the index test and the corresponding 95% CI. Data from the test and validation sets, including for previously published signatures being evaluated in a different cohort, will be analysed and compared separately for each signature and cohort.

Each study will likely have used a different transcriptomic signature incorporating a number of different genes for the diagnosis or prediction of TB disease and consequently, we anticipate considerable clinical and methodological heterogeneity. We therefore anticipate that meta-analysis of synthesised data would not usually be appropriate.[36] However, if the same signature is evaluated in more than one study, then meta-analysis might be possible. For such analysis, a bivariate random effects model will be used to calculate pooled sensitivity and specificity with the corresponding 95% CI.[37] The Higgins (I^2 test, which quantifies the degree of inconsistency in the results of studies, will be used to assess statistical heterogeneity.[38] This test explains the percentage of total variation across studies that is attributable to heterogeneity rather than chance. I^2 values less than 25% and those between 26% and 50% will be considered as low and moderate, respectively, while those between 51% and 75% and above 75% will be considered high and very high, respectively. Very high inconsistency will preclude meta-analysis. For prospective studies of predictive performance of mRNA transcriptomic signatures, we will categorise performance over prospective time points reported in the studies. Rate ratios with corresponding 95% CI will also be shown for the predictive studies. If there is sufficient data available, we will conduct subgroup analyses by age category, signature discovery method, signature model, infection/exposure category (household contacts or latent MTB infection) and for predictive studies only, time to TB disease diagnosis.

Ethics and dissemination

Given that this is a systematic review that will use peer-reviewed, published and publicly available anonymised data, ethical approval of this protocol is not required. This review will be reported as much as possible in conformance with the PRISMA statement. The findings from this study will be published in a peer-reviewed journal and presented at conferences. This review will also form part of a doctoral thesis at the University of Cape Town.

Assessing cumulative evidence

Assessing the quality of the body of evidence is recommended by PRISMA-P. We will attempt to rate the quality of our review evidence as either high, moderate, low or very low, by applying 'The Grading of Recommendations Assessment, Development and Evaluation' (GRADE) methodology.[39] Using the GRADE development software, GRADEpro, we will create a summary table of the evidence. HM and EWB will independently evaluate the body of evidence for each gradable outcome with regard to study design, risk of bias, directness and precision,[40 41] and then compare the results afterwards so as to arrive at a grading decision. The quality of the evidence will be applied to test performance estimates of TP, TN, FP and FN using a previously published GRADE guideline.[41]

Patient and public involvement

Patients and or public were not involved in this systematic review protocol of published peer-reviewed articles.

DISCUSSION

Considering the massive global burden of TB disease, low-cost, rapid and easy-to-use tests that will accurately diagnose TB disease are urgently needed to ensure early diagnosis and treatment of patients, improve treatment outcomes and interrupt transmission. Similarly, tests that will accurately predict which individuals with latent MTB infection will develop active TB disease are also urgently needed to ensure that treatment is targeted to those people at increased risk of incident TB disease, thereby avoiding the huge expense and unfeasibility of treating a quarter of the world's population as well as the unnecessary treatment and adverse events in people that will otherwise remain healthy. Host blood transcriptomic signatures are a candidate for such rapid biomarker-based non-sputum-based tests.

This systematic review will generate up-to-date information on the performance levels of present host blood transcriptomic signatures for diagnosis of prevalent TB and prediction of incident TB and help us compare the performance characteristics of the individual signatures to the target product profiles (TPPs) for new rapid

non-sputum TB diagnostic and predictive tests proposed by WHO, Foundation for Innovative New Diagnostics and the New Diagnostics Work Group.[42 43] Comparing the performance levels of these host blood transcriptomic signatures for diagnosis of prevalent TB and prediction of incident TB to the TPP is important because it will allow scientists to focus their research and development efforts on the signatures that are closest to meeting the TPP and may translate into practice and have impact on the epidemic. Based on the evidence from this systematic review, we will discuss the differences and similarities of the signatures, as well as knowledge gaps identified.

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Contributors MH and TS conceived the study. HM wrote the protocol under supervision from MH and TS. HM, EWB, SKM, SCM, BK, AP-N, TS and MH, reviewed, revised and approved the final version of protocol and will be involved in analysis and interpretation of the results.

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Competing interests TS and AP-N are inventors of blood transcriptomic signatures of risk of TB.

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