New technologies for diagnosing active TB: the VANTDET diagnostic accuracy study

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Declared competing interests of authors: Alice Halliday has a patent pending entitled 'A cellular immune signature for risk stratification of latent tuberculosis infection'. Robert Parker reports having the following patents pending: 1719853.2 and 2017904359. Lachlan Coin reports having the following patents pending: WO2014067943A1 and US20150284780A1. In addition, Lachlan Coin has one patent issued: EP2914740B1. Jon Deeks reports grants from National Institute for Health Research (NIHR) during the conduct of the study and receipt of a NIHR Senior Investigator Emeritus award and that he is supported by the NIHR Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and University of Birmingham. Peter White reports grants from the Medical Research Council and the NIHR during the conduct of the study, and grants from Otsuka Pharmaceutical (Tokyo, Japan) outside the submitted work. Onn Min Kon is chairperson of the UK Joint Tuberculosis Committee. Ajit Lalvani reports issued patents underpinning interferon gamma release assays (IGRAs) and next-generation IGRAs, some of which were assigned by the University of Oxford to Oxford Immunotec Global plc (Abingdon, UK), resulting in royalty entitlements for the University of Oxford and Ajit Lalvani. Ajit Lalvani is also inventor of issued and pending unlicensed patents underpinning flow-cytometric diagnosis of tuberculosis.

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Scientific summary

The VANDTET diagnostic accuracy study

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Scientific summary

Background

Tuberculosis (TB) is an important global disease, with millions of cases reported, including 1.3 million deaths, annually. In order to reduce the burden of TB disease, new tools are needed, including improved diagnostic tests. The currently available toolkit lacks a test that can detect all cases of TB with high accuracy and speed. The gold-standard tests for active TB are those that detect the causative bacteria, *Mycobacterium tuberculosis* (*Mtb*), but these have limitations. The smear test is quick but lacks sensitivity for all TB cases and does not achieve 100% specificity; *Mtb* culture is more sensitive than the smear test and has greater specificity, but lacks speed. The new nucleic acid amplification tests are an improvement on current tests; they have a greater sensitivity than smear, are rapid and provide information about drug sensitivity. However, these tests are unable to detect culture-negative TB cases. There are two major clinical unmet needs for active TB diagnostics: a triage rule-out test for TB and a rule-in test for hard-to-diagnosis TB cases (including culture-negative TB).

There has recently been an explosion in the discovery of biomarkers for TB based on measurements of a wide range of host responses using genome-wide gene expression microarrays, proteomic techniques and characterisation of functional T-cell subsets by flow cytometry. The resulting biomarker and cellular immunological signatures have high diagnostic sensitivity and specificity for active TB and distinguish active from latent infection. The feasibility of these novel approaches for diagnosis of TB has now been established and the apparent accuracy of the signatures has the potential to revolutionise TB diagnosis. In addition to the above, we have recently developed a highly sensitive whole-blood molecular test that can reliably rule out the diagnosis of TB based on measurement of interferon gamma (IFNγ)-dependent chemokine release in response to *Mtb*-specific antigens.

Thus, there are a number of new, promising biomarkers that now need urgent prospective and rigorous validation in routine clinical practice.

Aim

To validate promising new technologies [namely whole-blood transcriptomics, proteomics, flow cytometry and quantitative reverse transcription-polymerase chain reaction (qRT-PCR)] and existing signatures for detection of active TB in samples obtained from individuals suspected of active TB.

Objectives

Primary objectives

- To define the role of previously identified whole-blood gene expression signatures in the diagnostic evaluation of active TB using host transcriptomic microarray.
- To define the role of previously identified serum proteomic signatures in the diagnostic evaluation of active TB using liquid chromatography–mass spectrometry (LC–MS) and expanded to surface-enhanced laser desorption ionisation time-of-flight mass spectrometry (SELDI-TOF).
- To define the role of previously established cellular immunological signatures in the diagnostic evaluation of active TB using flow cytometry.
- To validate our candidate-stimulated whole-blood chemokine-based quantitative reverse transcription-polymerase chain reaction assay for rapid patient triage by excluding the diagnosis of active TB.

Secondary objectives

- To evaluate the use of the previously identified tests in the diagnosis of key subpopulations of active TB, namely human immunodeficiency virus positive/human immunodeficiency virus negative (HIV–), culture-confirmed/highly probable TB and pulmonary tuberculosis (PTB)/extrapulmonary tuberculosis (EPTB).
- To define optimal diagnostic algorithms that delineate the role of these new tests in the routine diagnostic assessment of patients with suspected active TB in the NHS.
- To assess the cost-effectiveness of the best-performing novel tests validated in this study for the diagnostic work-up of TB patients in routine NHS practice.
- To identify novel signatures of active TB (using the same technologies described in the primary objectives), selected to provide optimal diagnostic performance (i.e. beyond those previously proposed).

Methods

This project used the biobank of samples from the interferon gamma release assay (IGRA) in the Diagnostic Evaluation of Active TB (IDEA) study, in which individuals with suspected TB were recruited as part of routine clinical practice in 12 hospital sites in England (2011–14). The Validation of New Technologies for the Diagnostic Evaluation of active Tuberculosis (VANTDET) study comprised four laboratory subprojects, each evaluating proposed tests using the following new technologies/approaches: transcriptomics, proteomics, flow cytometry and candidate rule-out tests. For each of the subprojects, the reference standard used was the final diagnosis as allocated in the IDEA study, in which clinicians used the Dosanjh classification to stratify patients based on the diagnostic and clinical data available (excluding the IGRA results) into the following groups: culture-confirmed TB, highly probable TB, clinically indeterminate TB and active TB excluded. Signatures were first assessed according to their ability to detect all active TB cases (i.e. culture-confirmed TB and highly probable TB combined), from the TB excluded group [other diseases (ODs)]. Subanalyses assessing the performance of tests/signatures for specific types of TB (i.e. culture-confirmed TB, highly probable TB, PTB and EPTB) were also performed.

For the validation of transcriptomic signatures from whole-blood samples, a total of 628 individuals were selected who reflected the full IDEA study cohort and were profiled using genome-wide gene expression microarray using the Illumina platform. For validation of proteomic signatures, we were restricted to a total of 90 individuals for SELDI-TOF analysis owing to the lack of consumable supply; meanwhile, 166 individuals infected with HIV, from the IDEA study cohort were profiled using LC–MS. For both of these 'omic' approaches, signatures were evaluated for their discriminatory ability by calculating the score as previously described, using raw expression/abundance data, or using linear discriminant analysis (LDA).

To validate previously reported cellular immune signatures for discrimination of active TB from individuals with latent tuberculosis infection (LTBI) and ODs, we took a nested case–control approach to select the cohort. Peripheral blood mononuclear cells (PBMCs) from 184 individuals were stimulated with *Mtb* antigens overnight, stained with antibodies for functional and phenotypic markers, and enumerated using flow cytometry.

For validation of candidate rule-out tests, we took a GO/NO-GO approach to select the cohort. The GO/NO-GO cohort of 100 patients was selected to artificially inflate the proportion of individuals with TB who are negative with standard IGRA tests. If high test accuracy was achieved using this cohort, we planned to carry out the tests on remaining samples. We developed protocols to evaluate the performance of two candidate immune-based rule-out tests: either using qRT-PCR to detect IFNγ-dependent target genes in *Mtb*-stimulated PBMCs or using Meso Scale Discovery to detect chemokines in QuantiFERON® GOLD In-Tube supernatants (QIAGEN, Hilden, Germany).

For the health economic analysis, the best-performing tests were evaluated. Costs were estimated based on similar assays currently available in NHS practice. Decision tree models were developed to calculate the incremental costs and incremental health utilities [quality-adjusted life-years (QALYs)] of changing from current practice to using the candidate test as either rule-in or initial rule-out of active TB. The models were parameterised using the IDEA study clinical patient records and relevant current literature.

Results

The project and four substudies assessed each of the new technologies and evaluated the performance of previously published signatures. In addition, best-performing tests were evaluated for cost-effectiveness in a health economic analysis.

Principal findings in the transcriptomic substudy

We assessed six previously reported gene expression signatures, using LDA. Three of these signatures could also be valuated using the score methods as previously described. For all TB, the optimal diagnostic accuracy for all signatures ranged from an area under the curve (AUC) = 0.81–0.84; inclusion of HIV-infected individuals in the validation cohort reduced diagnostic accuracy. The diagnostic accuracy for detecting culture-confirmed TB or PTB was better than for highly probable TB or EPTB.

Principal findings in the proteomics substudy

We assessed the performance of one four-protein signature using SELDI-TOF and LC–MS, and three additional previously reported proteomic signatures using LC–MS. The optimal performance for the four-protein signature was found using SELDI-TOF, for which the AUC for detecting culture-confirmed TB was 0.74. Using LDA, the performance of all signatures for detecting all TB cases using LC–MS ranged from AUC 0.62 to 0.68.

Principal findings in the cellular immune signatures substudy

Four of six previously described cellular immune signatures provided a reasonable level of diagnostic accuracy (AUC = 0.78–0.92) for discriminating all TB from those with ODs/LTBI in HIV– TB suspects. Two of these assays may be useful in the IGRA-positive population and can provide high positive predictive value (PPV).

Principal findings in the candidate rule-out test substudy

Improved diagnostic accuracy for all TB over IGRA was achieved using the qRT-PCR detection of chemokine (C-X-C motif) ligand 9 (*CXCL9*), chemokine (C-X-C motif) ligand 10 (*CXCL10*) or basic leucine zipper atf-like transcription factor 2. None of the tests was able to provide > 95% sensitivity and > 65% specificity for active TB and, therefore, would not be suitable for a rule-out test. The assay also resulted in a high failure rate and is complex. *CXCL9* may provide good discriminatory ability for TB and LTBI in the T-SPOT.*TB* (Oxford Immunotec Global plc, Abingdon, UK) test-positive population.

None of the evaluated new tests for TB can be considered cost-effective for use in detecting all TB patients with their current cost and diagnostic performance.

Conclusions

None of the previously reported signatures using new technologies was able to provide diagnostic accuracy at a level that could be considered clinically useful for either all TB cases or for culture-positive TB. However, in specific patient groups, there may be a use for the best-performing new tests. Specifically, although the diagnostic accuracy for detection of highly probable TB was suboptimal (AUC = 0.8), the Sweeney *et al.* four-gene transcriptomic signature may be useful in this microbiologically unconfirmed population (Sweeney TE, Braviak L, Tato CM, Khatri P. Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis. *Lancet Respir Med* 2016;**4**:213–24), as it could provide a

high negative predictive value (0.95) and a result quickly. We identified two cellular-immune signatures that could provide a high PPV (0.88–0.95) for all TB, and for culture-confirmed TB, which may be useful in the IGRA-positive population of TB suspects.

In general, the performance of all signatures we evaluated was suboptimal compared with previous publications, probably as a result of the heterogeneous cohort of TB and OD patients included in this study, which reflects the full complexity of TB and its differential diagnoses presenting in real-life clinical settings. The secondary objective of defining novel signatures for active TB using the data sets generated is outstanding, but will build the bases for future studies. We recommend that future studies investigating blood-based tests for TB using these technologies focus on specific subgroups of TB patients and the OD they are clinically confused with. In particular, new tests for culture-negative TB and EPTB are needed most.

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