A Novel Blood-Based Assay for Treatment Monitoring of Tuberculosis

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Research note

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Abstract

Objectives

A novel 3-gene host transcriptional signature (*GBP5*, *DUSP3* and *KLF2*) that has been validated for TB diagnosis. The signature was recently translated by Cepheid into a prototype cartridge-based test that can be run on the GeneXpert instruments. In this study, we prospectively evaluated the change in the expression of the cartridge-based 3-gene signature with time following treatment initiation among pulmonary TB patients who were microbiologically cured at the end of treatment.

Results

Our results show that the 3-gene signature expression level (TB score) changes significantly over time with respect to baseline levels. The greatest increase in TB score occurred within the first month of treatment (median fold-increase in TB score: 1.08 [IQR 0.54-1.52]) and plateaued after 4 months of treatment (median TB score: 1.97 [IQR: 1.03-2.33]). The rapid and substantial increase of the TB score in the first month of treatment holds promise for the early identification of patients that respond to TB treatment. The plateau in TB score at 4 months may indicate early clearance of disease and could direct treatment to be shortened. These hypotheses need to be further explored with large prospective treatment monitoring and test of cure studies.

Introduction

Closing the diagnostic gap is a priority in the response to TB. In 2019, approximately one third of the estimated 10 million new tuberculosis (TB) cases were undiagnosed.(1) The underdiagnosis of patients presenting at health facilities must be addressed through the scale up more accurate, non-sputum-based biomarkers and diagnostics. Treatment monitoring tools are particularly limited and smear and culture-based methods remain the mainstay. And no Test of Cure is available.

Genetic host transcriptional signatures are promising biomarkers for active disease diagnosis, predicting progress to active TB disease, and treatment monitoring.(2) The most widely validated signature is a parsimonious combinatorial 3-gene score (TB score) validated by Sweeney et al. (*GBP5*, *DUSP3* and *KLF2*), which was found to be associated with disease severity and normalized after treatment initiation.(3) A prospective study by Warsinske et al. validated the 3-gene signature as a diagnostic and treatment monitoring tool while Francisco et al. demonstrated that two of the three genes (*GBP5* and *KLF2*) could be useful for monitoring treatment response in whole blood. (4)(5)

In order to move this 3-gene signature to scale, it must be integrated into a testing platform. Cepheid (Sunnyvale, CA, USA) has recently developed an early-prototype cartridge-assay ("Xpert MTB Host Response" or Xpert-MTB-HR-Prototype) to detect the signature expression levels in whole blood using GeneXpert.

The first study to assess the prototype by Södersten et al. evaluated the diagnostic accuracy of the Xpert-MTB-HR-Prototype as a triage test for TB among people living with HIV. The area under the curve (AUC) for the Xpert-MTB-HR-Prototype was 0.89 (95% CI 0.83, 0.94) against a comprehensive microbiological reference standard.(6)

In this exploratory study, we assessed whether the Xpert-MTB-HR-Prototype can detect changes in TB score after the onset of treatment among microbiologically cured patients. Our findings could inform future studies to investigate the potential application of the Xpert-MTB-HR-Prototype as a treatment monitoring tool for the early identification of patients that respond to TB treatment and potentially also for treatment shortening.

Methods

We obtained a total of 185 PAXgene blood samples from 31 patients all ≥ 18 years with presumptive pulmonary TB symptoms and a positive smear and/or Xpert result and no history of anti-TB therapy in the 60 days prior. Patients were enrolled at the Phthisiopneumology Institute in Moldova (n=16), Universidad Peruana Cayetano in Peru (n=10) and the University of Cape Town in South Africa (n=5) between November 2015 and April 2017.

No resistance was identified and participants were started on first-line TB treatment after enrollment with 5 (16.1%) completing treatment by 6 months and the remaining 26 (83.9%) by 12 months. Patients were considered cured if treatment was completed by 6 months or 12 months, microbiological testing was negative at 6 or latest at 12 months, and symptoms were improved or resolved.

Patients were assessed at initiation of treatment and followed-up at 1, 2, 4, 6, and 12 months. All sputum samples were tested on MGIT liquid culture (Becton Dickinson, Franklin Lakes, USA), solid culture on Löwenstein-Jensen (LJ) medium, and smear microscopy. Blood samples were collected at every follow up in PAXgene tubes and stored at -70°C at the Foundation for Innovative New Diagnostics (FIND) specimen bank (Zepmetrics, USA). Testing using the Xpert-MTB-HR-Prototype (Cepheid Sunnyvale) was performed from biobanked samples at Cepheid (Solna, Sweden) in 2019 as described previously.(6) One cured patient was missing a 3-gene TB score value at the 12-month follow-up.

The 3-gene TB score was calculated using the algorithm established by Sweeney et al. for reverse transcription-polymerase chain reaction (RT-qPCR), defined as (3):

$$\text{3-gene TB score} = ((\text{GBP5} + \text{DUSP3}) / 2) - \text{KLF2}$$

Results

The median age across the 31 participants was 41 years (IQR: 23-57), 19 (61.3%) were male, 27 (87.1%) had a history of BCG vaccination, and 5 (16.1%) were HIV-positive. The median TB scores increased over the follow-up period from 0.13 (IQR: -0.69 - 0.59) prior to treatment initiation to 2.03 (IQR: 1.58-2.41) by 12

months (Figure 1A-B). The greatest increase occurred within the first month of treatment initiation with a median increase in TB score of 1.08 (IQR: 0.54-1.52). The median TB score plateaued after 4 months of treatment at 1.97 (IQR: 1.03-2.33). These findings are in line with published findings on the 3-gene host signature when performed using laboratory-based RNA sequencing platforms.(3)(4)

Discussion

In this study, we demonstrate that the Xpert-MTB-HR-Prototype assay detects changes in the 3-gene signature over time with respect to treatment initiation. These findings are in line with published findings on the host signature when performed in laboratory-based RNA sequencing platforms.(3)(4) The rapid and substantial increase of the TB score in the first month of treatment holds promise for the early identification of patients that respond to TB treatment. The plateau in TB score at 4 months may indicate early clearance of disease and could direct treatment to be shortened. These hypotheses need to be further explored.

The current 6-month regimen for drug susceptible TB has several challenges including adverse effects and poor adherence. Shorter regimens are needed to overcome these limitations, and the WHO has put forth a Target Regimen Profile that seeks regimens that are at most 4 months in length. (7) A major roadblock in drug development trials is the dependence of long follow up periods for recurrent TB to evaluate treatment effect. A more dynamic tool, such as the Xpert-MTB-HR-Prototype has potential for early up-selection of more promising regimens in shorter and safer trials.

The diagnostic landscape lacks tools that accurately predict TB treatment outcomes within the early stages of treatment initiation. Such tools would allow patients to receive personalized therapies that ensure relapse-free cure in a timely manner. We demonstrate that the 3-gene TB score detected with the Xpert-MTB-HR-Prototype correlates well with treatment response in drug susceptible TB patients, however further studies are needed to assess its ability to monitor treatment effects and predict treatment outcomes.

Limitations

There were several limitations to our study. First, the small sample size may have affected the reliability of our findings. Second, our sample did not include patients with treatment failure, preventing us from comparing the TB score trajectories between success and failure cases. Third, we were not able to perform diagnostic accuracy analyses since reference standard testing (i.e., culture) were not available at follow-up times other than at enrollment, month six, and month 12.

Abbreviations

AUC Area under the curve

Ct Cycle threshold

HIV Human immunodeficiency virus

LJ Löwenstein-Jensen

MGIT Mycobacterium Growth Indicator Tube

TB Tuberculosis

WHO World Health Organization

Declarations

Ethics approval and consent to participate

Ethical approvals for the study were obtained respectively from the Phthisiopneumology Institute Chiril Draganiuc (PPI-NRL, Moldova), the Faculty of Health Sciences UCT Human Research Ethics Committee (UCT, South Africa), and the Ethics Committee of the Universidad Peruana Cayetano Heredia (UPCH, Peru). The study was undertaken in accordance with the principles of the Helsinki Declaration. Informed written consent was obtained from patients who agreed to participate.

Consent for publication

Not applicable.

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

P.K. is a co-inventor on a 3-gene TB score pending patent owned by Stanford University, which has been licensed for commercialization. P.K. is a consultant with Cepheid. E.S., S.B., L.S.M., J.P., D.G., and D.H.P. are employees of Cepheid.

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Author's contributions

A.J.Z. wrote the first manuscript draft. A.J.Z. and P.K. did the statistical analyses. All authors contributed to interpretation of data and editing of the article and approved the final version of the manuscript.

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Figures

Figure 1

(A) TB score at each time point from treatment initiation (month 0) over a 12-month follow-up period among microbiologically cured patients (n=31) at each time point. Cycle threshold (Ct) values were used which already exist on the log2-scale, thus the TB score trends appear inverted compared to the data presented by Sweeney et al.(3) (B) TB score trajectories for individual patients over a 12-month follow-up period among microbiologically cured patients (n = 31). Grey shading represents the 95% confidence interval.