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Host biomarkers detected in saliva show promise as markers for the diagnosis of pulmonary tuberculosis disease and monitoring of the response to tuberculosis treatment

Ruschca Jacobs, Enock Tshehla, Stephanus Malherbe, Magdalena Kriel, Andre G. Loxton, Kim Stanley, Gian van der Spuy, Gerhard Walzl, Novel N. Chegou *

DST/NRF Centre of Excellence for Biomedical Tuberculosis Research and SAMRC Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa

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ABSTRACT

Background: There is an urgent need for new tools for the rapid diagnosis of tuberculosis (TB) disease in resource-constrained settings. Tests based on host immunological biomarkers maybe useful, especially if based on easily available samples. We investigated host biomarkers detected in saliva samples from individuals with suspected pulmonary TB disease, as tools for the diagnosis of TB disease and monitoring of the response to treatment.

Methods: We collected saliva samples from 104 individuals that presented with symptoms requiring investigation for TB disease at a primary health care clinic in the outskirts of Cape Town, South Africa, prior to assessment for TB disease. We evaluated the concentrations of 33 host markers in stored saliva samples using a multiplex cytokine platform. Using a combination of clinical, radiological and laboratory results and a pre-established diagnostic algorithm, participants were later classified as having TB disease or other respiratory diseases (ORD). The diagnostic potentials of individual analytes were analysed by the receiver operator characteristics curve approach while the predictive abilities of combinations of analytes for TB disease were analysed by general discriminant analysis, with leave-one-out cross validation.

Results: Of the 104 individuals enrolled, 32 were pulmonary TB cases. There were significant differences in the levels of 10 of the markers investigated between the patients with TB disease and those with ORDs. However, the optimal diagnostic biosignature was a seven-marker combination of salivary CRP, ferritin, serum amyloid P, MCP-1, alpha-2-macroglobulin, fibrinogen and tissue plasminogen activator. This biosignature diagnosed TB disease with a sensitivity of 78.1% (95% CI, 59.6–90.1%) and specificity of 83.3% (95% CI, 72.3–90.7%) after leave-one-out cross validation. When compared to baseline levels, the concentrations of 9 markers including granzyme A, MCP-1, IL-1β, IL-9, IL-10, IL-15, MIP-1β, ferritin and serum amyloid A changed significantly by months 2 or 6 after initiation of TB treatment, thereby indicating that they might be useful in monitoring the response to TB treatment.

Conclusion: We have identified candidate biomarkers in saliva, which may be useful in the diagnosis of TB disease and monitoring of the response to TB treatment. These results require further validation in larger studies.

1. Introduction

Tuberculosis (TB) remains a global health problem and accounts for the deaths of nearly 1.5 million people annually [1]. One of the major challenges in the control of TB is the lack of globally effective tools for early diagnosis of the disease, and for monitoring of the response to treatment. Delays in the diagnosis of the disease result in delays in treatment initiation with consequently increased chances of transmission [2]. The most widely available test for both the diagnosis of TB disease and monitoring of the response to treatment (smear microscopy), has poor sensitivity, whereas the gold standard test (culture) is not widely available especially in resource-limited settings. Furthermore, culture may take up to 42 days to yield results [3]. The GeneXpert test (Cepheid Inc., Sunnyvale, USA) delivers results within 2 h, but is not widely available in resource-constrained settings and where available, for example

* Corresponding author.
E-mail addresses: rjacobs@sun.ac.za (R. Jacobs), enockrooi@gmail.com (E. Tshehla), malherbe@sun.ac.za (S. Malherbe), daleen.kriel@gmail.com (M. Kriel), gl2@sun.ac.za (A.G. Loxton), Kstanley@sun.ac.za (K. Stanley), gvds@sun.ac.za (G. van der Spuy), gwalzl@sun.ac.za (G. Walzl), novel@sun.ac.za (N.N. Chegou).

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in South Africa, is generally offered in centralized facilities [4]. Molecular tests such as the geneXpert are not useful for monitoring of the response to TB treatment since they cannot discriminate between DNA from dead and live bacteria [5]. Furthermore, sputum-based tests are not suitable in individuals with difficulty in providing good quality sputum samples such as children and those with extrapulmonary TB [6,7]. New tests are urgently needed that could enable the rapid diagnosis of TB disease, and monitoring of the response to treatment. Immunodiagnostic approaches might be beneficial, especially if based on the detection of host biomarkers in easily available sample types such as saliva [4,8], as they may be easily adaptable to point-of-care tests.

Saliva has not been widely investigated for biomarker discovery purposes in the TB field, but has attracted interest in other fields, with saliva-based diagnostic tests currently existing for HIV and oral diseases [9]. Saliva is highly abundant in both adults and children [10], and an average adult reportedly always has about 1 ml of saliva in the buccal cavity. Furthermore, collection of saliva is not as invasive and has less risks to the patient in comparison to other sample types such as blood. In a previous study investigating the possibility of detecting different host biomarkers in saliva, up to 6-fold higher expression of some markers was observed in saliva samples from TB patients in comparison to the levels detected in serum. Furthermore, the combination host markers detected in saliva with markers detected in serum including IL-5, IL-6, IL-15, CRP and TNF-α, showed potential in the diagnosis of TB disease [11].

In an attempt to identify saliva-based host markers which might be useful in the diagnosis of TB disease and also in monitoring of the response to TB treatment, we investigated the concentrations of 33 host markers in saliva samples from individuals undergoing anti-TB treatment in addition to samples from individuals without active TB disease. We hereby show that in addition to having potential in the diagnosis of TB disease, salivary host markers may also contribute to monitoring of the response to TB treatment.

2. Methods

2.1. Study participants

We prospectively recruited individuals suspected of having pulmonary TB disease, from the Fisantekraal Community Clinic, in the outskirts of Cape Town, South Africa, as part of a larger study; the African European Tuberculosis Consortium (www.ae-tbc.eu). Participants were recruited prior to clinical or laboratory assessment for pulmonary TB disease, between November 2010 and November 2012. All study participants presented with persistent cough lasting $\geqslant$2 weeks and at least one of either fever, malaise, recent weight loss, night sweats, knowledge of close contact with a TB patient, haemoptysis, chest pain or loss of appetite. Participants were eligible for the study if they were 18 years or older and willing to give written informed consent for participation in the study, including consent for HIV testing. Patients were excluded if they were pregnant, had not been residing in the study community for more than 3 months, were severely anaemic (haemoglobin <10 g/l), were on anti-TB treatment, had received anti-TB treatment in the previous 90 days or if they were on quinolone or aminoglycoside antibiotics during the past 60 days. The study was approved by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences of the University of Stellenbosch.

2.2. Sample collection and diagnostic tests

Saliva samples were collected at first contact with the patient, into salivette tubes (Sarstedt, Numbrecht, Germany), and transported at 4–8 °C to the laboratory for further processing. After the diagnostic work-up and confirmation of TB disease, sample collection was repeated at month 2 and month 6 after the initiation of TB treatment, in confirmed TB cases. Saliva specimens were centrifuged at 1000*g* for 2 min and the supernatants harvested and stored at −80 °C until use. Sputum samples were collected from all study participants and cultured using the MGIT method (BD Biosciences). Specimens demonstrating growth of microorganisms were examined for acid-fast bacilli using the Ziehl–Neelsen method followed by Capilia TB testing (TAUNS, Numazu, Japan), to confirm the isolation of organisms of the *M.tb* complex, before being designated as positive cultures.

2.3. Classification of study participants and reference standard

Using a combination of clinical, radiological, and laboratory findings, participants were classified as definite TB cases, probable TB cases, participants with other respiratory diseases (ORD) or questionable disease status as described in Table 1. Briefly, individuals with ORD had a range of other diagnoses, including upper and lower respiratory tract infections (viral and bacterial infections, although attempts to identify organisms by bacterial or viral cultures were not made), and acute exacerbations of chronic obstructive pulmonary disease or asthma. Because there were proportionally more individuals with ORD in comparison with the TB cases, we included all TB cases recruited at the study site during the study period and randomly selected the individuals with ORD.

2.4. Luminex multiplex immunoassay

The concentrations of 33 host markers including interferon (IFN)-γ, CXCL1(GRO), interleukin (IL)-1α, IL-1β, IL-2, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-15, IL-17A, macrophage derived chemokine (MDC), tumour necrosis factor (TNF)-α, IFN-γ inducible protein (IP)-10, vascular endothelial growth factor (VEGF), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1β, fractalkine, granzyme A, soluble Fas (sFas), soluble Fas Ligand (sFasL), solubleCD-137 (sCD137) (Merck Millipore, Billerica, MA, USA), and alpha-2-macroglobulin (A2M), haptoglobin, c-reactive protein (CRP), serum amyloid P (SAP), procalcitonin (PCT), ferritin, tissue plasminogen activator (TPA), fibrinogen and

Table 1
Case definitions used in classifying study participants as definite, probable, questionable TB cases or other respiratory diseases.

Classification	Definition
Definite TB	Sputum culture positive for MTB OR 2 positive smears and symptoms responding to TB treatment OR 1 positive smear plus CXR suggestive of PTB
Probable TB	1 positive smear and symptoms responding to TB treatment OR CXR evidence and symptoms responding to TB treatment
Questionable	Positive smear(s), but no other supporting evidence OR CXR suggestive of PTB, but no other supporting evidence OR Treatment initiated by healthcare providers on clinical suspicion only. No other supporting evidence
ORD	Negative cultures, negative smears, negative CXR and treatment never initiated by healthcare providers

Abbreviations: CXR, chest X ray; MTB, *Mycobacterium tuberculosis*; TB, pulmonary tuberculosis, ORD, individuals investigated for TB disease but in whom TB was ruled out, suggesting other respiratory conditions.