In Search for a Better Tuberculosis Diagnostic Test

Tuberculosis (TB) remains one of the most common deadly infectious diseases on the planet and the diagnosis, treatment and prevention of this disease present serious global public health challenges.¹

The TB treatment regimen takes too long to cure and is complicated to administer, and anti-TB drugs can be toxic.² There is no efficacious vaccine for TB.³

Rates of new infections are still rising in many endemic areas where TB co-infects those with HIV/AIDS.⁴

Treating this co-infection is further complicated by the surge of multi-drug resistant strains of *Mycobacterium tuberculosis* (*Mtb*).⁵ TB diagnosis remains antiquated and inadequate in most parts of the world.⁶,⁷

Given that one-third of the world’s population is assumed to have been exposed to *Mtb* and to have developed latent infection, diagnostic assays based on the host immune response (whether cellular or humoral) often fail to distinguish active TB from latent cases.⁸,⁹

Unfortunately, identifying individuals with active pulmonary disease (the source of disease transmission) and distinguishing them from those with latent (non-transmissible) infection is critical to provide prompt and appropriate therapy to minimize the spread of TB. Active pulmonary TB is typically diagnosed by finding *Mtb* in sputum by acid-fast smear, a labor-intensive process requiring trained personnel and with widely varied sensitivity in different settings (20-60%).
Moreover, sputum smear has limited utility in patients with paucibacillary TB (e.g. HIV co-infected patients) and those unable to produce sputum samples (e.g. children).\(^\text{10}\) In young children, testing often requires repeated gastric lavage, an invasive and unpleasant method of sample collection.\(^\text{11}\)

In more developed regions of the world, sputum smear is complemented by culture (a process that takes up to eight weeks) and, in some laboratories, by the Cepheid Xpert \textit{MTB}/RIF automated nucleic acid amplification test.\(^\text{12}\) In view of these worldwide diagnostic limitations, the World Health Organization (WHO) and several other governmental and non-governmental institutions have recently emphasized the urgent need for better TB diagnostics.\(^\text{13}\)

One study concluded that a rapid and widely available diagnostic test for TB, with $\geq 85\%$ sensitivity for smear-positive and smear-negative cases and $97\%$ specificity, could save $\sim 400,000-600,000$ lives annually.\(^\text{14}\)

To overcome these obstacles and advance the TB diagnostics field, we are working on the development of a diagnostic test based on the presence of \textit{Mtb} protein antigens in human bodily fluids.\(^\text{15-17}\) While our focus in the current work is on the detection of \textit{Mtb} antigens in urine, it is possible to interrogate other specimen types like blood, spinal fluid, pleural fluid, saliva, and feces (latter primarily for children) to allow diagnosis of individuals with pulmonary as well as a range of other clinical manifestations of active TB. Our antigen detection test will distinguish between those with active and latent disease and thus will represent an important new strategy for control of this global disease.

The test will diagnose infection with both drug-sensitive and drug-resistant strains of \textit{Mtb}. It is important to note that we expect the test will also be a powerful tool to monitor the efficacy of TB treatment, a need made much greater by the alarming increase in rates of multi-drug resistant TB (MDR-TB) and, more recently, extensively drug resistant TB (XDR-TB).\(^\text{18}\)

Currently, standard methods for monitoring TB treatment efficacy are based on culture, causing extensive diagnostic delays.

**Brief synopsis of preliminary data**

Our laboratory has used a state-of-the-art and powerful strategy to identify \textit{Mtb} proteins that were produced \textit{in vivo} in patients with active pulmonary TB and were subsequently excreted in the urine. Using a mass spectroscopy-based technique to analyze the urine of patients with active pulmonary TB (collected in Brazil, Colombia, Peru, and Zimbabwe), we identified several \textit{Mtb} proteins, of which five were initially selected as potential pathogen-derived biomarkers for assay development and clinical validation (Table 1).

| Table 1. Selected \textit{Mtb} Biomarkers Identified in the Urine of Patients with Pulmonary TB |
|---------------------------------------------------------------|
| **Putative identification**                                     | **Rv Annotation** |
| MoaA-related protein                                           | Rv1681            |
| Ornithine carbamoyltransferase                                  | Rv1656            |
| Homoserine O-acetyltransferase                                  | Rv3341            |
| Putative S-adenosyl-L-methionine-dependent methyltransferase    | Rv1729c           |
| Hypothetical protein                                           | Unassigned        |

Revista Ciências em Saúde v6, n 2, 2016
Recombinant proteins were produced, purified and used for antibody production in rabbits. Antibodies were purified and used to assemble capture ELISAs specific for each selected biomarker. A detection limit of approximately 10-100pg/ml was achieved for each recombinant biomarker.

**Key points of validation attained to date**

Following the assay’s optimization, we began the clinical validation of the capture ELISA for the Rv1681 biomarker using banked urine specimens assembled through collaborative studies sited in Texas/Mexico, Boston, and Brazil. The Rv1681 assay detected the protein in unconcentrated urine specimens from 11/25 (44%) culture-confirmed pulmonary TB patients and 1/21 (4.8%) subjects in whom TB was initially clinically suspected but then ruled out by conventional methods. Rv1681 protein was not detected in urine specimens from non-TB patients with *E. coli*-positive urine cultures, subjects with confirmed non-TB tropical diseases (schistosomiasis, Chagas’ disease, and cutaneous leishmaniasis), or healthy subjects.

**REFERENCES**

1. World Health Organization. Global Tuberculosis Report 2015. 20th ed. WHO; 2015.
2. Laurenzi M, Ginsberg A, Spigelman M. Challenges associated with current and future TB treatment. Infect Disord Drug Targets. 2007;7(2):105-19.
3. Reed SG, Campos-Neto A., Vaccines for parasitic and bacterial diseases, Curr Opin Immunol. 2003;15(4):456-60.
4. Dye C, Lönnroth K, Jaramillo E, Williams BG, Raviglione M. Trends in tuberculosis incidence and their determinants in 134 countries. Bull World Health Organ. 2009;87(9):683-91.
5. Nahid P, Kim PS, Evans CA, Alland D, Barer M, Diefenbach J, et al. Clinical research and development of tuberculosis diagnostics: moving from silos to synergy. J Infect Dis. 2012;205 Suppl 2:S159-68.
6. Perkins MD. New diagnostic tools for tuberculosis, Int J Tuberc Lung Dis. 2000 Dec;4(12 Suppl 2):S182-8.
7. Pollock NR, Campos-Neto A, Kashino S, Napolitano D, Behar SM, Shin D, et al. Discordant QuantiFERON-TB Gold test results among US healthcare workers with increased risk of latent tuberculosis infection: a problem or solution? Infect Control Hosp Epidemiol. 2008;29(9):878-86.
8. Pollock NR, Kashino SS, Napolitano DR, Sloutsky A, Joshi S, Campos-Neto A, et al. Evaluation of the effect of treatment of latent tuberculosis infection on QuantiFERON-TB gold assay results. Infect Control Hosp Epidemiol. 2009;30(4):392-5.
9. Foulds J, O’Brien R. New tools for the diagnosis of tuberculosis: the perspective of developing countries. Int J Tuberc Lung Dis. 1998;2(10):778-83.
10. Eamranond P, Jaramillo E. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. Int J Tuberc Lung Dis. 2001;5(7):594-603.
11. McNerney R, Zumla A. Impact of the Xpert MTB/RIF diagnostic test for tuberculosis in countries with a high burden of disease. Curr Opin Pulm Med. 2015;21(3):304-8.
12. Gardiner JL, Karp CL. Transformative tools for tackling tuberculosis. J Exp Med. 2015;19:212(11):1759-69.
13. Keeler E, Perkins MD, Small P, Hanson C, Reed S, Cunningham J, et al. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature. 2006;444 Suppl 1:49-57.
14. Kashino SS, Pollock N, Napolitano DR, Rodrigues V Jr, Campos-Neto A. Identification and characterization of Mycobacterium tuberculosis antigens in urine of patients with active pulmonary tuberculosis: an innovative and alternative approach of antigen discovery of useful microbial molecules. Clin Exp Immunol. 2008;153(1):56-62.
15. Napolitano DR, Pollock N, Kashino SS, Rodrigues V Jr, Campos-Neto A. Identification of Mycobacterium tuberculosis ornithine carbamoyltransferase in urine as a possible molecular marker of active pulmonary tuberculosis. Clin Vaccine Immunol. 2008;15(4):638-43.
16. Pollock NR, Macovei L, Kanunfre K, Dhiman R, Restrepo BI, Zarate I, et al. Validation of Mycobacterium tuberculosis Rv1681 protein as a diagnostic marker of active pulmonary tuberculosis. J Clin Microbiol. 2013;51(5):1367-73.
17. Matteelli A, Roggi A, Carvalho AC. Extensively drug-resistant tuberculosis: epidemiology and management. Clin Epidemiol. 2014;6:111-8.