POINT-OF-CARE BREATH TEST FOR BIOMARKERS OF ACTIVE PULMONARY TUBERCULOSIS

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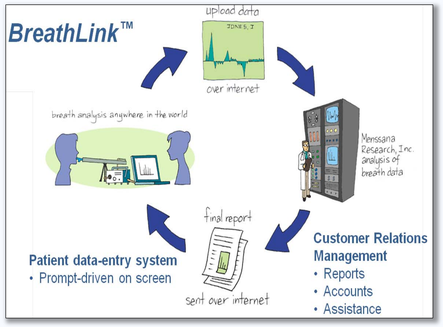
Background

Active pulmonary tuberculosis (TB) is a leading cause of death from infectious disease throughout the world. Two billion people – one third of the world's population – are infected with Mycobacterium tuberculosis, and 1.6 million died from the disease in 2005[1]. There has been little progress in detection of TB in recent decades: microscopy and culture remain the mainstay of laboratory diagnosis and there is an urgent need for new diagnostic tools, especially in high-burden countries. An ideal diagnostic test would be sensitive and specific for active pulmonary TB, as well as rapid, cost-effective, non-invasive, and suitable for use in developing countries.

A breath test could potentially detect persons with active pulmonary TB because *M. tuberculosis* manufactures volatile metabolites *in vitro*, and a number of these volatile organic compounds (VOCs) can be detected in the breath as apparent biomarkers of infection[2]. In a multicenter international study, breath VOCs identified patients with active pulmonary tuberculosis (TB) with 85% accuracy[3]. Laboratory-based GC/MS has established proof of principle of a breath tests for active pulmonary TB, but this technology is limited by high costs and slow turnaround time. A new point-of-care desktop breath testing system, BreathLink™, has dramatically reduced both the cost and the turnaround time of a breath test. We report here the preliminary findings from a multicenter international study employing BreathLink™ at sites in India, UK, and the Philippines.

The BreathLink™ system

The Breathscanner 3.2 collects and concentrates the VOCs in a sample of alveolar breath, separates them by gas chromatography (GC), and detects them with a surface acoustic wave detector (SAW). A sample of room air VOCs are analyzed in the same way, and the clinical and chromatographic data are transmitted to a central laboratory via the internet. The process is completed within 7 minutes. When algorithms are finalized, test results will be reported to the point-of-care.



Experimental design

Clinical sites

- DeLa Salle Health Sciences Institute, Cavite, Philippines
- Homerton University Hospital, London, UK
- Hinduja Hospital, Mumbai, India
- University of Santo-Tomas, Manila, Philippines

Human subjects

Disease group

Inclusion criteria

1. Subject is older than 13 years of age
2. Clinical suspicion of pulmonary TB based on:
   symptoms and signs e.g. cough, sputum production, night sweats, weight loss or hemoptysis
   OR: history of known recent exposure to infection
   OR: chest X-ray abnormalities
   OR: positive sputum smear consistent with active pulmonary TB
   OR: sputum culture results positive or pending

Exclusion Criteria

1. Subject is currently taking anti-TB therapy or has received more than 7 days of anti-TB therapy in the past six months

Control group

Inclusion criteria

1. Subject is older than 13 years of age
2. Subject is undergoing screening for pulmonary TB without clinical evidence of active TB

Exclusion criteria

1. Clinical suspicion of pulmonary TB based on:
   symptoms and signs e.g. cough, sputum production, night sweats, weight loss or hemoptysis
   OR: history of known recent exposure to infection
   OR: chest X-ray abnormalities consistent with active pulmonary TB

Analysis of data

- Subtraction chromatogram determined for each subject (breath VOCs minus room air VOCs)
- Chromatograms segmented into a series of time slices
- Time slices compared in disease group and control group
- Significant non-random times slices identified with multiple Monte Carlo simulations
- Non-random times slices combined into a predictive algorithm with weighted digital analysis (WDA)
- Outcome of predictive algorithm displayed in receiver operating characteristic (ROC) curve

Results

Human subjects

191 subjects were included in the analysis (TB/controls): Mumbai 39/16, Manila 51/38, Cavite 1/34, London 2/10, Total: 93/98.

Analysis of data

Multiple Monte Carlo simulations identified 10 significant non-random times slices that distinguished TB patients from controls. The accumulated area under curve (AUC) of the ROC curve was 0.80.

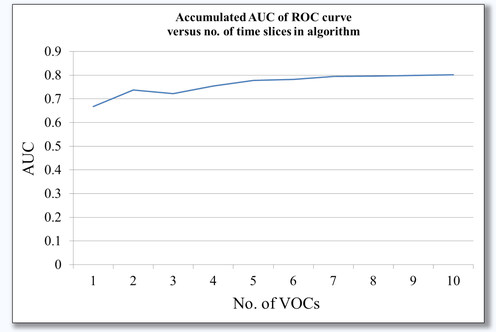


Figure 1: Accuracy of the breath test for active pulmonary TB increased with the number of VOCs identified by Monte Carlo analysis.

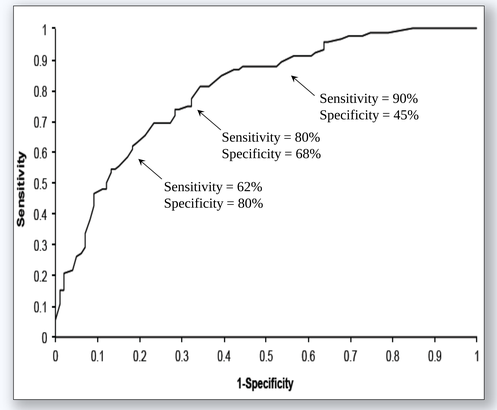


Figure 2: Receiver operating characteristic (ROC) curve of the breath test for active pulmonary TB when inclusion criteria fulfilled.

Conclusions

The BreathLink™ point-of-care breath test identified pulmonary TB with 80% accuracy. This was a preliminary analysis of data from an ongoing study, and results may improve as more subjects are entered into the analysis of data, and the subset with a positive sputum culture is analyzed separately.

References

1. Anon., World TB Day --- March 24, 2008. MMWR, 2008. 57 (11): p. 281
2. Phillips, M., et al., *Volatile biomarkers of pulmonary tuberculosis in the breath.* Tuberculosis (Edinb), 2007. **87**(1): p. 44-52.
3. Phillips, M., et al., *Breath biomarkers of active pulmonary tuberculosis.* Tuberculosis, 2010. **90**(2): p. 145-51