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DIAGNOSTICS

Diagnosis of active tuberculosis by e-nose analysis of exhaled air

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SUMMARY

Tuberculosis (TB), a highly infectious airborne disease, remains a major global health problem. Many of the new diagnostic techniques are not suited for operation in the highly-endemic low-income countries. A sensitive, fast, easy-to-operate and low-cost method is urgently needed.

We performed a Proof of Principle Study (30 participants) and a Validation Study (194 participants) to estimate the diagnostic accuracy of a sophisticated electronic nose (DiagNose, C-it BV) using exhaled air to detect tuberculosis. The DiagNose uses a measurement method that enables transfer of calibration models between devices thus eliminating the most common pitfall for large scale implementation of electronic noses in general. DiagNose measurements were validated using traditional sputum smear microscopy and culture on Löwenstein-Jensen media.

We found a sensitivity of 95.9% and specificity of 98.5% for the pilot study. In the validation study we found a sensitivity of 93.5% and a specificity of 85.3% discriminating healthy controls from TB patients, and a sensitivity of 76.5% and specificity of 87.2% when identifying TB patient within the entire test-population (best-case numbers).

The portability and fast time-to-result of the DiagNose enables a proactive screening search for new TB cases in rural areas, without the need for highly-skilled operators or a hospital center infrastructure.

1. Introduction

It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*, often leading to active tuberculosis (TB).[1] In 2010 there was an estimated incident case count of 8.8 million active TB infections, resulting in 1.5 million deaths. The primary detection technique is the 125-year old Ziehl-Neelsen (ZN) staining[2] combined with microscopy. The major drawback of microscopy is that it only allows for detection of pulmonary disease cases in an advanced stage, meaning that often the disease has already been transmitted to close contacts. Chest X-ray and microbiological culture have been added to the diagnostic arsenal in developing countries while other, more advanced techniques such as nucleic acid amplification, serology, and cytokine release assays[3] are becoming available in the developed countries while research is still ongoing. In 2010 the World Health Organization (WHO) endorsed the use of the Cepheid Xpert MTB/RIF system for use in endemic areas. This polymerase chain reaction system, however, relies on a constant power supply making it not ideally suited for portable operation.

Using exhaled air as potential diagnostic indicator is an emerging activity.[4–8] In previous studies gas chromatography combined with mass spectrometry (GC/MS) was used to identify volatile biomarkers unique to *M. tuberculosis*.[9–12] From these studies it appeared that volatile biomarkers may be used to discriminate TB-patients from healthy controls. However, GC/MS is not viable as a diagnostic tool due to the complex settings of the equipment and operation skills needed and using animals[7,8] introduces a whole other set of challenges.

A viable diagnostic tool which uses volatile biomarkers to differentiate between people with and without TB should be based on an easy-to-use method. Electronic noses have already been used for different medical purposes[13–15] including the diagnostics of asthma,[4] chronic obstructive pulmonary disease,[4,5] urinary tract infection,[16] wound infection[17] and even cancer.[18,19] In the past, we used an electronic nose for the laboratory-based identification of bacterial pathogens.[20]

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In this study, we used three independently produced electronic nose units and determined the device-independent diagnostic accuracy in detecting TB. We started with a proof of principle (PoP) study to determine the possibility to discriminate between severely TB-infected and healthy people. Subsequently, we conducted a validation study (VS) to substantiate the PoP results and extend the variance between the examined groups.

2. Methods

2.1. Experimental equipment

The DiagNose (C-it BV, Zutphen, The Netherlands) is an electronic nose device incorporating 12 metal-oxide sensors, being 4 different sensor types (AS-MLC; AS-MLN; AS-MLK; AS-MLV, Applies Sensors Gmbh) in triplicate.

The Diagnose is equipped with a pump, where the inlet is controlled by a solenoid switching between 2 different inlets, to create an active airflow across the sensors. One inlet is connected to an active carbon filter to provide a baseline free from environmental influence while the second inlet is attached to the sample-bag. The DiagNose measures the air composition every 20 s using a 32-step sinusoidal modulation of the sensor surface temperature between 260 and 340 °C, thus resulting in a vector of 32 values each 20 s for each of the twelve sensors.

2.2. Study design

The studies were conducted at the Tuberculosis laboratory of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in collaboration with National TB Control Program.

The PoP study was setup as a retrospective study and conducted in October 2009; all patient samples were collected at the National Institute of Disease of Chest and Hospital (NIDCH). The Sputum Smear Microscopy (SSM) was used to select the patients meeting the TB status inclusion criteria. The PoP study was approved by the Research Review Committee (RRC) and Ethical Review Committee (ERC) of ICDDR,B under number PR-09034.

The VS was setup as a prospective study and conducted from May to October, 2010. All patients provided 3 samples at 2 consecutive days at Shyamoli Chest Disease Clinic (SCDC). The SSM result of the first sample was used to approach their neighbors to volunteer as socio-economic healthy controls. The VS study was approved by the RRC and ERC of ICCDR, B under number PR-10023. In the VS, patients with a non-TB medical condition were included.

For both studies, the breath samples were measured on-site and analyzed immediately. All sputum samples were transported to the TB laboratory at ICDDR,B where SSM and cultivation on solid Löwenstein-Jensen media were performed within 3 h. The culture results obtained were used as diagnostic reference standard since this is still the current gold standard in TB-diagnostics.

2.3. Patient characteristics and case definitions

The PoP study consisted of two groups. The first group contained 15 patients enrolled at NIDCH meeting the inclusion criteria; the second group contained 15 employees of ICDDR,B.

The inclusion criteria for the PoP study were as follows:

1. aged over 18,
2. agreement to participate through informed consent,
3. ability to produce sputum and air sample,
4. not having received anti-TB-medication for at least 7 days,
5. having two of the three provided sputum samples indicated as 3+ by SSM,
6. controls should have no health complaints.

The VS consisted of 4 groups. The first two groups consisted of patients enrolling at SCC and discriminated on basis of the TB culture results. The first group (PT, Patient confirmed TB) contained all TB positive patients while the second group (PN, Patient No TB) contained all TB culture negative patients. The third group (HC, Healthy controls) consisted of neighbors having no health complaints of the first 20 patients that were diagnosed with a TB confirmation as defined in the PoP study. These neighbors most likely have comparable socio-economic status and act as socio-economic control. The fourth group (EC, Employee control) consisted of randomly selected healthy employees of ICDDR,B.

The inclusion criteria for the VS were as described above for the PoP with the differences that patients should be aged over 15 and that the criterium number (5) mentioned above was not applicable.

2.4. Sample collection and measurement

All participants were asked to breathe through the sampling set-up that consisted of a non-rebreathing T-valve with an active-carbon filter attached to the inlet. During sampling, a nose clamp was placed on the nose of the participant to avoid entry of non-filtered air.

The first 5 min were used to flush the environmental influences from the lungs after which the sample bag was attached and filled with at least 1 L of exhaled air. After collection, the sample bag was attached to the sample inlet of the DiagNose.

Each patient visited the hospital on two consecutive days and provided three sputum samples. The first sample was taken during the first visit, the second one was collected by the patient in the early-morning at home and a third one was taken during the second visit. These three samples are needed for the routine TB diagnostics (smear microscopy and culture). During the PoP, we first needed the smear results to select eligible patients and therefore we could only obtain one breath sample per person during their second visit while during the VS the patients provided a sample on both consecutive days. The content of the sample bag was measured twice during the PoP to act as duplicate while during the VS both samples were measured independently but only once.

A single measurement took 10 min in which the first five minutes consisted of the exposure of the sensors to the breath sample, actively taken from the sample bag, and the last five minutes consisted of the recovery phase where the adsorbed chemicals are released from the system under influence of the clean reference air. Thus each single measurement comprises of the chemical adsorption and desorption dynamics at the sensor surface, caused by the sample, during these 10 min.

The DiagNose sample collection and measurements during the PoP were conducted by the authors and prior to the VS a local research associate was trained by the authors. For both studies trained staff members of ICDDR,B performed the sputum collection, sputum smear preparation, SSM, and culture according to well accepted microbiological guidelines[21] without any knowledge of the DiagNose results.

2.5. DiagNose measurements and analysis

A single measurement of the DiagNose results in 12 matrices of exposure/recovery dynamics, one for each of the sensors. These 12 sets can be regarded as a multi-way dataset in which the first dimension consists of the temperature cycle applied to the sensor, the second dimension is the time (the exposure and recovery