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The electronic nose as a rule-out test for tuberculosis in an indigenous population

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Abstract. Coronel Teixeira R, IJdema D, Gómez C, Arce D, Roman M, Quintana Y, González F, Jiménez de Romero N, Pérez Bejarano D, Aguirre S, Magis-Escurra C (National Institute of Respiratory Diseases and the Environment (INERAM), Asunción, Paraguay; Radboud University Medical Centre - TB Expert Centre Dekkerswald, Nijmegen - Groesbeek, The Netherlands; Medical Health Center, Puerto Casado, Chaco; Central Public Health Laboratory (LCSP), Asunción; and National Tuberculosis Control Program (PCNT), Asunción, Paraguay). The electronic nose as a rule-out test for tuberculosis in an indigenous population. *J Intern Med* 2021; **290**: 386–391. https://doi.org/10.1111/joim.13281

Introduction. To end the tuberculosis (TB) epidemic, efficient diagnostic tools are needed. In a previous calibration study, a portable 'point of care' electronic nose device (Aeonose^{TM}) proved to be a promising tool in a hospital setting. We evaluated this technology to detect TB in an indigenous population in Paraguay.

Methods. A total of 131 participants were enrolled. eNose results were compared with anamnesis, physical examinations, chest radiography and mycobacterial cultures in individuals with signs and symptoms compatible with TB. The eNose analysis was performed in two stages: first, the training with a combination of a previous study population plus 47 participants from the new cohort (total n = 153), and second, the 'blind prediction' of 84 participants.

Results. 21% of all participants (n = 131) showed symptoms and/or chest radiography abnormalities suspicious of TB. No sputum samples resulted culture positive for *Mycobacterium tuberculosis* complex. Only one patient had a positive smell print analysis. In the training model, the specificity was 92% (95% confidence interval (CI): 85%-96%) and the negative predictive value (NPV) was 95%. In the blind prediction model, the specificity and the NPV were 99% (95% CI: 93%-99%) and 100%, respectively. Although the sensitivity and positive predictive value of the eNose could not be assessed in this cohort due to the small sample size, no active TB cases were found during a one year of follow-up period.

Conclusion. The eNose showed promising specificity and negative predictive value and might therefore be developed as a rule-out test for TB in vulnerable populations.

Keywords: breath analysis, electronic nose, tuberculosis, volatile organic compounds, vulnerable groups.

Introduction

To curb the tuberculosis (TB) epidemic, the World Health Organization (WHO) promotes early detection and development of new techniques to improve TB diagnosis, especially in vulnerable populations, including indigenous people [1]. The Maká indigenous community in Paraguay had a TB incidence 66 times higher than the general population [2]. Due to cultural barriers, remoteness and poor healthcare policies, TB case finding and treatment are extremely difficult in indigenous populations [3]. Current diagnostic methods for TB, as smear microscopy and the complementary test; chest radiography (CXR), are not widely available due to lack of resources and/or skilled personnel [4]. To improve the detection of TB cases, especially in hard-to-reach communities, it is imperative to have

a point-of-care test (POC) that is non-invasive, cost-efficient, non-sputum-based and without requirement of highly trained personnel or constant electrical power supply. The portable electronic nose (Aeonose^{TM}, *eNose Company*, Zutphen, the Netherlands) may meet these requirements. It detects 'Volatile Organic Compounds' (VOCs) in breath samples and has demonstrated a sensitivity and specificity to detect TB of 91% (95% CI: 70%-98%) and 93% (95% CI: 81%-98%) in a hospital setting in Paraguay [5]. Until now, the device was never tested in remote areas in which conditions concerning extreme temperatures, dust and potential reluctancy of its future users were interesting new issues to evaluate. The objective of this current pilot study was to determine its utility and accuracy as a screening tool to detect TB in an indigenous population in a remote area.

Methods

From November 2015 to February 2016, we performed a cross-sectional study to identify TB in an isolated indigenous population (Maskoy ethnic group, consisting of a total of 160 adults) in Livio Fariña, Department of Alto Paraguay, 650 km from Asunción (Fig. 1). Ethical approval was obtained from the Ethics Committee (CEI) of the Central Public Health Laboratory (LCSP) (International Certification No. FWA00020088), with code CEI-LCSP No. 66/40915. All adult community members (>15 years) were approached by their leader after we informed her first about the aim and objectives of the study. Subsequently, all adults received oral information from their leader about the study because widespread of illiteracy within the community. After signing informed consent, participants were included. Respiratory failure was an exclusion criterion. Clinical data, medication, smoking habits and the last meal/drink schedule were recorded. All participants had a physical examination and a CXR, as well as a five-minute breath sampling with the eNose. Adverse events during measurement were documented. Participants presenting with an anamnesis (structured questionnaire was used) and/or CXR abnormalities (evaluated by the study physician and later checked by two pulmonologists) were asked to provide at least one early morning sputum sample (produced spontaneously or induced by nebulization of hypertonic saline). Sputum samples were stored for a maximum of two days in a refrigerator in the local health centre until transport to the capital (in an iced cool box, transportation time

Fig. 1 *Map of Paraguay. Green circle represents the study area (Alto Paraguay Chaco), located 650 km from the capital Asuncion.*

16–24 hours) where Ziehl–Neelsen (ZN) stain, GeneXpert MTB/RIF (Cepheid) and mycobacterial culture (Ogawa–Kudoh solid medium) were performed. The gold standard to establish TB diagnosis was a positive culture of *Mycobacterium tuberculosis* complex. In case mycobacterial cultures were contaminated, a new sputum sample was cultured extended with cultures for fungi and respiratory pathogens.

The analysis of the VOCs of individual 'smell prints' was done by the eNose Company. The methodology to train the artificial neural network (ANN) was described in detail before [6]. Basis for the analysis of the breath profiles in this study cohort was the calibration training dataset of a previous study [5] to which we added 47 participants from the actual study cohort and de-blinded their diagnosis (clinical status and microbiological outcomes) to the company to further optimize the ANN to perform the analysis of the remaining study participants. The reason to optimize the previous ANN by adding participants from the actual cohort is that VOCs from this indigenous population, having a different genetic background, living circumstances and diet, may not be similar to the hospitalized patients from the previous cohort that originated mainly from the capital. The manufacturer did not take part in study design and logistics and were kept blinded

for patient characteristics and sputum outcomes of the remaining participants. After the study, the investigators stayed in contact with the doctors from the village to follow up on the indigenous community for one year to see whether new cases with active TB were detected after the study was completed.

Results

Hundred and thirty-one adults (131/160; 82%) participated. Their characteristics are shown in Table 1. Twenty-seven participants (21%) had symptoms and/or CXR abnormalities. Three people provided one sputum sample, nine participants gave two, and 15 provided three. All sputum samples were ZN, GeneXpert MTB/RIF and culture-negative. Four participants had a contaminated culture in the first place but resulted negative on the second occasion. One person was currently receiving TB treatment (pulmonary TB, third month). Three subjects were on antibiotic treatment (2 with amoxicillin/1 azithromycin). Six subjects had just finished antibiotics from the penicillin group (ended > 24 hours before participating in the study). In the 12 months after the study was completed, no cases of active TB were diagnosed.

Table 1 *Patient characteristics*

Characteristics	Participants (N = 131)
Mean age years (SD)	37.3 (16.5)
Sex male/female (%)	45.8 / 54.2
Toxic habits N (%)	
Smoking (daily)	43 (32.8)
Alcohol (≥3 times per week)	46 (35.1)
Median time of food–beverage intake before test hours (range)	3 (0.1–23)
Comorbidity N (%)	
Arterial hypertension	4 (3.1)
Heart failure	2 (1.5)
Diabetes mellitus	2 (1.5)
Asthma	4 (3.1)
COPD	1 (0.8)
Treated for TB in the past N (%)	15 (11.5)
Years post-treatment (range)	(0.3–43)
Symptoms	
Asymptomatic	80 (61.1)
Cough	27 (20.6)
Sputum	27 (20.6)
Dyspnoea	1 (0.8)
Fever	4 (3.1)
Chest X-ray N (%)	
Normal	99 (75.6)
Abnormal[a]	32 (24.4)

[a]27 showed pulmonary lesions; five corresponded to a cardiovascular process and a former rib fracture.

Aeonose™ results

The new ANN 'training model' yielded an area under the curve (AUC) of 0.90, a sensitivity of 87% (95% CI: 71–95%) and a specificity of 92% (95% CI: 85–96%). Figure 2a shows the values from the training dataset (N = 153 (accuracy 91% with cut-off value 0.0)) using 'Leave-10%-Out' cross-validation. Participants with false-positive (N = 9) and false-negative results (N = 5) were analysed in depth. Two false positives had respiratory symptoms < 15 days: one of them with CXR with infiltrates and cavities and the other presented with overweight. The remaining seven false positives showed comorbidities varying from asthma, arterial hypertension and overweight. False negatives included two with culture-negative pleural TB (both used ceftriaxone before TB diagnosis was established) and 2 others had microbiologically proven pulmonary TB, also treated with antibiotics (penicillin group) for ≤ 3 days. The last one was obese.

After training, the ANN was fixed and the remaining 84 (N = 131–47 = 84) blind 'smell prints' were then classified ('blind prediction model'). The results are depicted in Fig. 2b. The specificity of the eNose was 99% (95% CI: 93–99%) and the NPV was 100%, as only one participant was falsely classified as positive; this participant had no respiratory symptoms or CXR abnormalities. As not a single community member in the blind prediction group had active TB, the sensitivity and positive predictive value of the eNose could not be calculated from this cohort. Figure 2c shows the ensemble model with 237 samples (153 training samples + 84 blind samples).

Discussion

We report pilot experience with a portable, 'point-of-care' electronic nose device to screen an

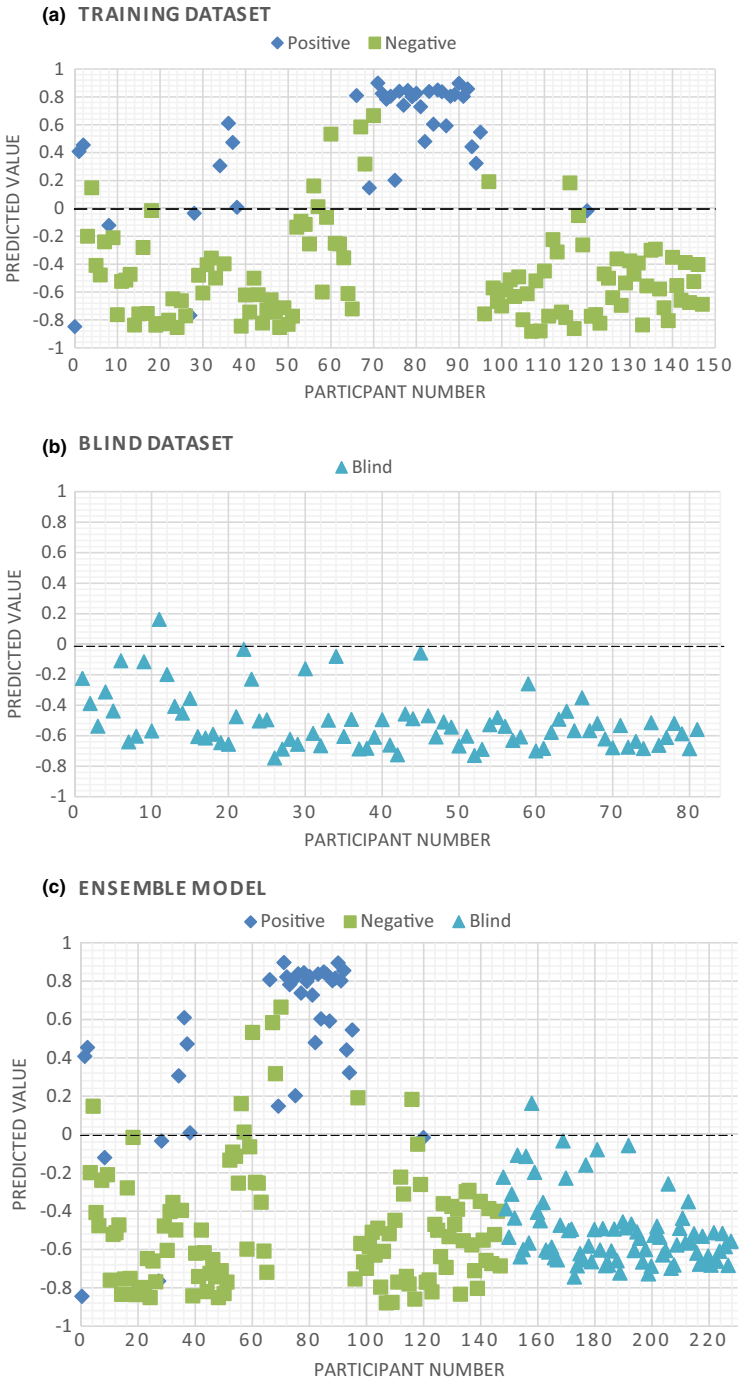

Fig. 2 *eNose predictions results. X-axis: (a) training cohort with participants of former study (N = 106) and actual study (N = 47), total: 153 participants. (b) Participants used for the blind prediction (N = 84). (c) Total of samples for training and blind prediction set (N = 237). Y-axis: Aeonose™ classification values; a high value predicts active TB (maximum + 1); a low value (minimum −1) predicts participants not suspected of TB. Dark blue diamonds represent participants with active, proven TB. Green squares represent participants not suspected of TB. Light blue triangles represent blind predictions. Above the cut-off value of 0.0, participants are classified as positive and below that value as negative.*

indigenous population for active TB. The eNose showed a high specificity and NPV, possibly making it a promising triage test to select patients for more specific microbiological test (GeneXpert or culture) to confirm or rule out TB diagnosis.

In this community, 21% of all participants presented symptoms and/or CXR abnormalities. The current WHO screening recommendations [7], using CXR or ZN staining of sputum in symptomatic individuals, would have been challenging and very unpractical in this remote community. For example, in our study we were able to collect only one sputum sample from some participants after nebulization with saline. The introduction of a triage test like the eNose can limit the use of more expensive tests and efficiently use the health economic resources [8]. A triage test is considered optimal if it delivers a result within 15 minutes [8]. In our study, participants breathe for 5 minutes via the Aeonose^{TM}; even the samples of three participants who tested shorter were analysed successfully. Shorter test time and immediate data analysis in a cloud-based database on a portable platform connected to the eNose would make it a real 'Point of Care' test.

In this study, some participants presented with chronic diseases and smoking habits that may have had an effect on breath prints [9–12]. In a previous study, we observed that the use of antibiotics might cause alterations in individual VOCs, especially when used for more than 5 days prior to TB diagnosis [5]. We must also emphasize that a diet from an indigenous person is not very similar to a non-native, as they mostly consume fruits and vegetables. This may exert some influence on the metabolism of the host or microbiome and might impact on VOCs as well [13–14]. Another possible important influencing factor is the living area of the participants. Despite not having excessive pollution by hydrocarbons as in large cities, the excessive indoor pollution of wood and coal smoke, as well as the use of more pesticides and different drinking water purification processes, may influence breath prints [15–17].

This study has some important limitations. First, there were no active TB cases detected in this small and isolated indigenous community, and therefore, the sensitivity and the positive predictive value of the eNose could not be established. Given the TB incidence of 245/100,000 inhabitants for indigenous communities (data of PNCT), we should have sampled at least a few thousand people to detect enough positive TB cases to evaluate its accuracy. Amplifying the sample size with indigenous people from other communities would have introduced a potential bias as we are not informed whether for example differences in genetics or food habits may influence a persons' breath signal. Second, we did not procure sputum specimens of all subjects to exclude active TB disease. As the sensitivity of mycobacterial sputum culture is very low in asymptomatic people [18–19] and also the fact that during the follow-up period of one year no new TB diagnoses were established, we assume that the negative predictive value of the eNose in this cohort was adequate.

In summary, the eNose holds promise as a portable TB rule-out test in a remote area. A study in a high incidence setting with a much larger sample size to assure enough confirmed TB cases is required to evaluate the sensitivity and PPV to assess the eNose as a point-of-care diagnostic test for TB.

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Conflict of interest

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

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