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Identification of airway bacterial colonization by an electronic nose in Chronic Obstructive Pulmonary Disease

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Summary
Background: Airway bacterial colonization by potentially pathogenic microorganisms occurs in a proportion of patients with Chronic Obstructive Pulmonary Disease (COPD). It increases airway inflammation and influences outcomes negatively. Yet, its diagnosis in clinical practice is not straightforward. The electronic nose is a new non-invasive technology capable of distinguishing volatile organic compound (VOC) breath-prints in exhaled breath. We aim to explore if an electronic nose can reliably discriminate COPD patients with and without airway bacterial colonization.
Methods: We studied 37 clinically stable COPD patients (67.8 ± 5.2 yrs, FEV_1 41 ± 10% ref.) and 13 healthy controls (62.8 ± 5.2 yrs, FEV_1 99 ± 10% ref.). The presence of potentially pathogenic microorganisms in the airways of COPD patients (n = 10, 27%) was determined using quantitative bacterial cultures of protected specimen brush. VOCs breath-prints were analyzed by

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discriminant analysis on principal component reduction, resulting in cross-validated accuracy values. Area Under Receiver Operating Characteristics (AUROC) was calculated using multiple logistic regression.
Results: Demographic, functional and clinical characteristics were similar in colonized and non-colonized COPD patients but their VOC breath-prints were different (accuracy 89%, AUROC 0.92, $p > 0.0001$). Likewise, VOCs breath-prints from colonized (accuracy 88%, AUROC 0.98, $p < 0.0001$) and non-colonized COPD patients (accuracy 83%, AUROC 0.93, $p < 0.0001$) were also different from controls.
Conclusions: An electronic nose can identify the presence of airway bacterial colonization in clinically stable patients with COPD.

Introduction

In about 20—50% of patients with clinically stable Chronic Obstructive Pulmonary Disease (COPD), potentially pathogenic microorganisms (PPM) can be isolated from their airway secretions [1,2]. This bacterial colonization is associated with enhanced airway inflammation [3,4] and more frequent and severe episodes of exacerbation [5], both of which can impact the clinical course of the disease negatively and increase mortality [6,7]. A proper identification of these patients may, therefore, be clinically relevant [8].

Sputum culture has well-known limitations to identify the presence of bacterial airway colonization in COPD [4,9]. The gold standard for the diagnosis of distal airway infections is the quantitative culture of protected specimen brush (PSB) [9,10], but its invasiveness limits its use in routine clinical practice. The electronic nose (e-nose) is an emerging non-invasive technology that detects volatile organic compounds (VOCs) in the exhaled gas [11]. It uses an array of sensors that react with different VOCs and generate a specific "breath-print" for each individual. The exhaled gas contains a complex mix of VOCs that are derived from various metabolic and inflammatory pathways in the lung [11,12]. Specific breath-prints of some respiratory diseases have been successfully used for diagnostic screening of lung cancer, malignant pleural mesothelioma, asthma and chronic obstructive pulmonary disease [13—16]. In addition, other studies have demonstrated that the e-nose is also able to identify specific upper respiratory bacterial pathogens from *in vitro* cultures [17], as well as in patients with bacterial sinusitis [18] and ventilator-associated pneumonia [19]. To date, however, no previous study has explored the potential utility of the e-nose to identify bacterial airway colonization in clinically stable COPD patients, but it is conceivable that those with PPM in their airways may have a distinct breath-print profile than those without bacterial colonization. Accordingly, we hypothesized that the use of an e-nose in clinically stable COPD patients will allow identification of patients with PPM in their airways. This pilot study sought to explore this hypothesis.

Methods

Study design and ethics

This is a cross-sectional, descriptive and controlled study that included COPD patients with and without airway bacterial colonization ($n = 10$ and $n = 27$, respectively), as well as healthy controls ($n = 13$). This sample size is similar to that of previous studies that, using the same e-nose device and methodology used here [13—15], identified significant differences between groups. The study protocol was approved by the institutional review board (IIBSP-ENO-2009-21), and all subjects signed their informed consent. ClinicalTrials.Gov identifier: NCT01976117.

Participants

The diagnosis of COPD was established according to the GOLD recommendations [20] and the presence airway colonization in COPD patients by PSB (see below). All of them were clinically stable as defined by the absence of an exacerbation that required antibiotic or steroid treatment within 30 days prior to inclusion. Patients receiving treatment with oral steroids or other immunosuppressive agents were excluded. Healthy controls were recruited by advertisements in the hospital.

Clinical and functional characterization

Demographic data, level of current symptoms, number of exacerbations in the previous year, time from last exacerbation, relevant comorbid conditions and current treatments were recorded at inclusion using standardized questionnaires. Spirometry (Datospir-500, Sibelmed SA, Barcelona, Spain) was performed according to the Spanish Respiratory Society (SEPAR) guidelines [21], using the predicted values for Mediterranean populations [22].

Microbiological evaluation

PSB samples were obtained from right medium lobe using a bronchoscope and a sterile disposable microbiological brush (ConMed, New York, NY) in all COPD patients and processed using standard methodology [9]. In short, PSB samples were serially diluted (1:10, 1:100, 1:1000). All microbiological specimens were plated on blood, chocolate, Wilkins-Chalgren and Sabouraud's agar. The cultures were evaluated for growth after 72 h. Bacterial load was considered significant when $\geq 10^2$ colony forming units (CFU)/ml [23]. Specific microorganims were identified according to standard methods and classified as PPM (*Hemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, Gram negative-bacili, *Pseudomonas aeruginosa* and