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

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MESH KEYWORDS
COPD; Bacterial colonization; Inflammation; Volatile organic compounds

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, FEV1 41 ± 10% ref.) and 13 healthy controls (62.8 ± 5.2 yrs, FEV1 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
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 ≥10⁵ colony forming units (CFU)/ml [23]. Specific microorganisms were identified according to standard methods and classified as PPM (Pseudomonas aeruginosa and Streptococcus pneumoniae, Moraxella catarrhalis, Gram negative-bacilli, and Hemophilus influenzae).
Staphylococcus aureus) or non-PPM (Streptococcus viridans, Candida spp, Corynebacterium spp, and Staphylococcus epidermidis) for analysis [24].

Exhaled VOCs measurement by e-nose

To assess VOC profiles by e-nose, exhaled gas was collected as previously described [15,16]. Exhaled gas specimens were obtained before the bronchoscopic procedures in COPD patients. In short, exhaled breath was collected in 10-L Tedlar bags after 3 min of tidal breathing through a Hans Rudolph valve with an inspiratory filter and an expiratory silica reservoir exposed to dry air. All participants stopped their inhaled medications, stop smoking and food-drink intake at least 12 h before the study. The e-nose device (Cyanose 320; Smith Detections, Pasadena, CA) was then connected to the Tedlar bag for 5 min and changes in the electrical resistance of its 32 organic polymeric composite sensor array generated a breath-print VOC profile in each participant. The measurement is based on a resistance variation in each sensor when exposed to a VOC mixture. The differential responses across the array (resistance shifts) are presented as patterns [13–16].

Data analysis

Breath-print data from COPD patients (colonized and non-colonized) and healthy controls were compared with each other using a pattern-recognition application built in the MATLAB software (v.R2012a). They were represented by logarithmic regression as mono- or bidimensional graphics following previously published algorithms [15,16]. Raw data were first reduced by principal component analysis (PCA) to three principal factors. These PCA factors entered a univariate ANOVA followed by post-hoc least significant difference test. Patient classification, based on these PCA factors, was performed using a linear canonical discriminant analysis, calculated as the one that obtains the better percentage of correctly classified patients. The discriminant function is trained with all minus one subject samples. Then the remaining subject samples are tested. If three or four of those samples are addressed to the true class, we consider the subject as "well classified". That process is repeated for all the available subjects and the percentage of correctly classified patients built. That method is known as "leave-one-out" method [16]. A p value of less than 0.05 for the trained discriminant function is considered statistically significant. A Receiver Operating Characteristics (ROC) was generated using the results of the discriminant function and combining all the samples of one subject. The

| Table 1 | Demographic, clinical and functional characteristics of the three groups of subjects studied. |
|-----------------|-----------------------------------------------|-----------------------------------------------|
|                | Colonized COPD patients (n = 10) | Non-colonized COPD patients (n = 27) | Healthy controls (n = 13) |
| Age (years)    | 68.1 ± 10.9                     | 67.8 ± 6.9                             | 62.8 ± 5.2                     |
| Gender (M/F)   | 8/2                            | 20/7                                     | 9/4                              |
| BMI (Kg/m²)    | 26.1 ± 4.0                     | 24.1 ± 5.5                              | 26.9 ± 3.1                     |
| FEV₁ postbronchodilator (L) | 1.04 ± 0.37               | 1.27 ± 0.32                             | 2.67 ± 0.54                     |
| FEV₁ postbronchodilator (%) pred | 38 ± 8                   | 42 ± 8                                  | 99 ± 10                         |
| FEV₁/FVC       | 0.39 ± 0.09                    | 0.41 ± 0.08                             | 0.76 ± 0.04                     |
| GOLD-grade (II/III/IV) | 0/8/2                   | 5/18/4                                  | NA                              |
| Current/ex-/never-smoker | 4/6/0                    | 9/18/0                                  | 5/5/0                           |
| Pack-years     | 43.0 ± 8.3                     | 48.9 ± 13.8                             | 16.5 ± 15.2                     |
| ICS use        | 9 (90%)                        | 27 (100%)                               | NA                              |
| LABA use       | 10 (100%)                      | 27 (100%)                               | NA                              |
| LAMA use       | 9 (90%)                        | 25 (92%)                                | NA                              |
| Roflumilast use | 1 (10%)                        | 2 (7%)                                  | NA                              |
| ≥2 exacerbations last year | 3 (30%)             | 4 (15%)                                 | NA                              |
| Weeks from last exacerbation | 15.0 ± 6.2      | 20.7 ± 14.5                             | NA                              |

Values are mean ± standard deviation. BMI: body mass index; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; LABA: long acting beta agonists; LAMA: long acting muscarinic agonists; ICS: inhaled corticosteroids; NA: not applicable.

Figure 1 Two-dimensional principal component analyses (PCA) plot showing the discrimination of breath-prints in colonized COPD patients and non-colonized COPD patients (accuracy 89%, p < 0.0001). Four samples for each subject are displayed and connected to the class center identifier with scatter lines to improve readability.
Area Under Receiver Operating Characteristics (AUROC) curve was calculated using multiple logistic regression.

Results

Participant characterization

Thirty-seven patients with stable moderate-severe COPD and 13 healthy controls were included in the study. PSB cultures were positive for PPM in 10 (27%) and negative in 27 (73%) COPD patients; the former were considered colonized and the latter non-colonized. *H. influenzae* was isolated in PSB cultures in 5 patients (50%). *M. catarrhalis* in 2 patients (20%) and *S. pneumoniae*, *E. coli* and *N. meningitidis* in 1 patient (10%).

Table 1 presents the principal characteristics of all participants. Age, sex, Body Mass Index (BMI) and proportion of current smokers was similar in the three groups. Likewise, in patients with COPD, the severity of airflow limitation (GOLD grade), current treatment and time from last exacerbation were similar in colonized and non-colonized individuals, although there was a non statistically significant trend towards lower FEV₁ values (1.04 L ± 0.37 vs. 1.27 ± 0.37, *p* = 0.075) and higher proportion of frequent exacerbators (≥2 exacerbations in the previous year) (30% vs. 15%, *p* = 0.6) in colonized patients.

Breath-print analysis

Breath-prints from colonized vs. non-colonized COPD patients were clearly distinct on visual assessment (Fig. 1). Canonical discriminant analysis showed a cross-validated accuracy of 89% (*p* < 0.001). AUROC curve was 0.92, with a sensitivity of 82% and a specificity of 96% (Table 2, Fig. 2).

Breath-prints corresponding to colonized COPD patients vs. healthy controls and non-colonized COPD patients vs. healthy controls were also fairly distinguishable (Fig. 3), with a cross-validated accuracy of 88% (*p* < 0.004), AUROC of 0.98, sensitivity of 80% and specificity of 93% for the former, and a cross-validated accuracy of 83% (*p* < 0.001), AUROC of 0.93, sensitivity of 81% and specificity of 86% for the latter (Table 2).

When all COPD patients were considered as a single group, irrespective of the presence of bacterial colonization (*n* = 37), COPD breath-prints could also be discriminated from healthy controls with a cross-validated accuracy of 79% (*p* < 0.0001), AUROC of 0.89, sensitivity of 83% and specificity of 76%.

Discussion

The main finding of this study is that an electronic nose can identify accurately the presence of bacterial airway colonization by PPM in clinically stable COPD patients.

Previous studies

It is well established that a proportion of clinically stable patients with COPD have bacterial colonization of their lower airways and that this is not innocuous since it is associated with greater levels of inflammation, increased frequency of exacerbations and an accelerate decline of lung function [3–5,25,26]. The diagnosis of airway

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**Table 2** Receiver operating characteristics analyses of breath-prints between colonized COPD patients, non-colonized COPD patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Colonized vs. non-colonized COPD patients</th>
<th>Colonized COPD patients vs. healthy controls</th>
<th>Non-colonized COPD patients vs. healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-validation accuracy</td>
<td>89%</td>
<td>88%</td>
<td>83%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.82</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.96</td>
<td>0.93</td>
<td>0.86</td>
</tr>
<tr>
<td>AUROC</td>
<td>0.922</td>
<td>0.986</td>
<td>0.937</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.87</td>
<td>0.89</td>
<td>0.92</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.92</td>
<td>0.87</td>
<td>0.72</td>
</tr>
</tbody>
</table>

AUROC: area under the receiver operating characteristic.

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**Figure 2** Receiver operating characteristic (ROC) curve of the model predicting the presence of bacterial airway colonization in COPD patients.
bacterial colonization in clinical practice often relies on sputum culture. This method, however, has well known limitations, including the need to produce a valid sputum sample (not a given in many patients), the need for sample processing and the fact that sputum largely reflects processes occurring in the trachea and major bronchi [4,27,28]. Alternatively, the fact that a quantitative culture of PSB, the gold standard for the diagnosis of distal airway infections [9,10], requires bronchoscopy limits its use in the clinic. Hence, a non-invasive, easy to use, cheap and reliable method for the diagnosis of airway colonization by PPM in COPD may be a valuable asset for the management of these patients. Our results suggest that the e-nose may fulfill these requirements.

A few previous studies have used an e-nose to identify micro-organisms causing respiratory tract infections in a variety of different experimental and clinical conditions. Lai et al. demonstrated that the e-nose could distinguish the VOCs patterns of various common respiratory pathogens in culture, including H. influenza, S. pneumoniae and P. aeruginosa, from control swabs [17]. Thaler et al. showed that the e-nose identified correctly the diagnosis of bacterial sinusitis in 72% of the patients [18]. Finally, Hanson et al. reported that the e-nose could effectively identify patients with ventilator-associated pneumonia [19]. Collectively, therefore, these previous observations indicate that the presence of bacteria, both in the upper and lower respiratory tracts, produce a specific breath-print that e-nose may detect. To our knowledge, though, our study is the first to explore its potential utility in identifying the presence of bacterial airway colonization in clinically stable COPD patients.

**Interpretation of findings**

COPD is associated with an enhanced inflammatory response in the airways and lung parenchyma [20,29] that persist despite smoking cessation [30]. This chronic airway inflammation can cause a specific VOCs breath-print. Supporting this possibility, two previous studies using the same e-nose we used here were able to discriminate COPD from both asthma (accuracy 96%) and smoker controls (accuracy 66%) [16] as well as COPD from patients with non-small cell lung cancer (accuracy 85%) [13]. Our results confirmed that COPD breath-prints are distinct from healthy controls. More recently Fens et al. reported that exhaled molecular profiles in COPD are closely associated with the types of inflammatory cells present, as well as with their activation status, and suggested that breath analyses may be a novel alternative for the assessment and monitoring of airway inflammation in COPD [31]. Our results provide further support to this possibility since it is well established that the presence of bacterial colonization in some COPD patients is associated with increased airway inflammation [3,4] and our results indicate that the VOCs pattern of COPD patients with bacterial colonization are also different and identifiable using an e-nose. Whether the treatment of these patients with antibiotics influence their clinical course is outside the scope of this study but it clearly is an important area of future research [8].

**Strengths and limitations**

The major strength of our study is that it tests a novel and non-invasive diagnostic tool (e-nose), using PSB quantitative cultures as the gold standard method for the diagnosis of distal airway infection [9,10], in a population of patients never studied before. Our results confirm, therefore, that e-noses have the potential to be used in respiratory medicine because of their easy and noninvasive use and rapid results [32]. We acknowledge, however, that it has limitations. First, since it was a pilot study that sought to explore the feasibility and potential validity of our working hypothesis, we investigated a relatively small number of patients in a single center so, despite the statistically significant differences between groups observed here, we think that further investigations in larger cohorts of COPD patients in, likely, multicenter studies are warranted to firmly confirm these promising results. Similarly, the ability of the e-nose to identify specific bacterial species will
require further studies. Second, we did not investigate the reproducibility of results. Yet, previous studies using the same methodology and e-nose device showed good reproducibility [14,16,31]. Third, we did not identify the molecular correspondence of the different VOCs [31,33], since this requires the use of gas chromatography and mass spectrometry (GC–MS). It would be of great interest to identify which compounds are characteristic of each group in order to confirm these results and detect specific VOCs related to colonized and non-colonized COPD patients. Finally, our data analysis was limited to a discriminant analysis approach. Future studies may use more advanced data analysis techniques, such as Support Vector Machines [34] or Back Propagation Neural Networks [35].

Conclusions

This pilot study shows that the e-nose may be a simple, easy and non-invasive alternative to identify bacterial colonization in COPD patients in clinical practice.

Summary to take home

An electronic nose can identify the presence of airway bacterial colonization in clinically stable patients with COPD.

Conflict of interest

All authors have no conflicts of interest.

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