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Identification of breath-prints for the COPD detection associated with smoking and household air pollution by electronic nose

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ABSTRACT

Purpose: The analysis of breath-print, has been proposed as an attractive alternative to investigate possible biomarkers of Chronic Obstructive Pulmonary Disease (COPD). The aim of the present study was to discriminate between healthy subjects, patients with COPD associated with smoking (COPD-S) and patients with COPD associated with household air pollution (COPD-HAP).

Methods: A cross-sectional study of 294 participants was conducted, 88 with smoking associated COPD, 28 associated with HAP and 178 healthy subjects. Breath-print analysis was performed by using the Cyranose 320 electronic nose. Group data were evaluated by Principal Component Analysis (PCA), Canonical Discriminant Analysis (CDA) and Support Vector Machine (SVM) and the test's diagnostic power by means of ROC (Receiver Operating Characteristic) curves.

Results: The results indicated that the breath-print of patients with COPD is different from the one of healthy subjects explaining a variability of 93.8% with a correct prediction of 97.8% and correct classification of 100%, also positive and negative predictive value of 96.5 and 100% respectively. Furthermore, the breath-print of exhaled breath from patients with COPD-S and COPD-HAP does not present any difference.

Conclusions: The breath-print of exhaled breath from patients with COPD-S and COPD-HAP does not present any difference, which demonstrates that the breath-print is related to the disease and not to causality. With these results, the analysis of the breath-print of COPD is proposed as an alternative for a screening method in future clinical applications.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of death worldwide. The World Health Organization estimates that more than 3.2 million deaths occur annually and 64 million people suffer from the disease [1]. In high-income countries, there is the available information on the prevalence, morbidity, and mortality of COPD, however even in these countries, reliable information on the epidemiology of the disease is limited due to the cost and difficulties in diagnosis [2,3]. In fact, it is recognized that 90% of the deaths associated with this disease are in low- and middle-income countries, the main risk factors for this disease being smoking, household air pollution (HAP) from solid fuels, including biomass burning, particles in occupational environments, ozone and passive smoking [4,5]. Prompt and adequate detection of COPD is essential for the prognosis of this chronic disease [6]. Lung function tests such as spirometry are essential in the diagnosis, however, none of the parameters derived from the test are as specific in detecting peripheral damage to small airways [7], furthermore, in a

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study of epidemiological surveys from 44 sites in 27 countries, it was associated that there is a high probability of under-diagnosis of COPD with male sex, young age, recent smoking or never having smoked, less education, lack of previous spirometry and any severe airflow limitation [8]. On the other hand, the study requires considerable time, physical effort from the patient and must be carried out by trained technicians according to international guidelines.

Some strategies have advocated the search for screening techniques to improve the rate of diagnosis more easily, cost-effectively and quickly. The role of Volatile Organic Compounds (VOCs) in exhaled breath has been widely studied for the evaluation of airway inflammation and screening for COPD, asthma, lung cancer, among other diseases [9–15]. These compounds are products of metabolic and inflammatory processes related to physiopathological changes that take place in the respiratory tract [16].

Thus, the analysis of VOCs in exhaled breath, called breath-print, has been proposed as an attractive alternative to investigate possible biomarkers of lung diseases, assuming that the traces of the compounds are specific to the disease and without the influence of causality. Therefore, the objective of the present study was to determine the discrimination in three population groups: i) healthy subjects, ii) patients with COPD associated with smoking, and iii) patients with COPD associated with household air pollution (HAP).

2. Materials and methods

2.1. Patients, healthy subjects and study design

A cross-sectional case-control study was conducted at the Ismael Cosío Villegas National Institute of Respiratory Diseases (INER), which belongs to the Mexican Ministry of Health, a referral center for respiratory diseases in Mexico City (2240 m above sea level), taking care mostly of uninsured patients. The study was reviewed and approved by the Ethics Committee of INER CONBIETICA-09-CEI-003-20160427 with number C54-18.

2.2. Lung function test

Spirometry (EasyOne® Plus Diagnostic portable spirometer) was performed to all study participants before and after the administration of 400 μg of Salbutamol, following the guidelines of the American Thoracic Society/European Respiratory Society (ATS/ERS) standards and by trained experienced technicians [17]. The reference values for spirometry were those established for the Mexican-American population from the National Health and Nutrition Examination Survey III study (NHANES III) [18]. The significant response to the bronchodilator was defined as an increase in Forced Expiratory Volume at the first second (FEV_1) in the post-bronchodilator test equal to or greater than 200 mL and 12%. The post-bronchodilator value of FEV_1, Forced Vital Capacity (FVC) expressed as percentage of predicted, and the ratio of FEV_1/FVC were determined [19].

Airflow obstruction was defined as a post-bronchodilator ratio of $FEV_1/FVC<0.7$, and,the severity was rated according to guide GOLD (Global Iniciative for Chronic Obstructive Lung Disease) stages: GOLD 1 ($FEV_1 \geq 80\%$ of predicted value), GOLD 2 ($50\% \leq FEV_1 < 80\%$ of predicted value), GOLD 3 ($30\% \leq FEV_1 < 50\%$ of predicted value), GOLD 4 ($FEV_1 < 30\%$ of predicted value) [19]. The group of patients diagnosed with COPD in addition to airflow obstruction (post-bronchodilator $FEV_1/FVC<0.7$), had a history of smoking or a history of exposure to smoke from biomass burning, presented chronic cough with sputum and/or dyspnea, but were clinically stable and without pulmonary exacerbation in the last four weeks according to GOLD 2019 guidelines [19].

Five comparisons between breath-prints were assessed: a) COPD vs. Healthy subjects; b) COPD-Smokers (COPD-S) vs. Healthy subjects; c) COPD-associated with HAP (COPD-HAP) vs Healthy subjects; d) COPD-S vs COPD-HAP; and e) Mild COPD (GOLD stage 1 and 2) vs moderate and severe COPD (GOLD stage 3 and 4) and were also compared.

Patients were required to suspend short-acting bronchodilators and inhaled corticosteroids for at least 12 h and long-acting bronchodilators for at least 24 h before the day of exhaled breath sampling. For all groups, participants with a history of upper or lower respiratory tract infection, asthma or other lung disease were excluded from the study.

Healthy subjects were considered if they lacked absence of chronic cough/sputum or dyspnea; had a normal chest physical examination, with post-bronchodilator FEV_1 values $> 80\%$ of those predicted, FEV_1/FVC ratio >0.7 with a reversibility of less than 12% in FEV_1 after administration of 400 μg salbutamol, as well as, absence of airway hyperreactivity. They also reported a history of smoking less than 5 pack-years and were not current smokers, and no use of wood or biomass for cooking.

2.3. Collection of exhaled breath

The sample collection was based on the European Respiratory Society guide [20]; relaxed participants underwent three deep inhalations and then exhaled deeply into Breath Collection Bags (BCB) which consisted of a 1.4 L metalized plastic ball previously purged twice with ultra-pure nitrogen [21]. The sample was collected in duplicate from healthy patients and subjects in fasting conditions, without smoking before the study, without oral hygiene and before taking the medications. The samples were transported at 4 °C and analyzed on the same day. Besides, an environmental control sample was taken to eliminate possible interference [22].

2.4. Analysis of exhaled breath

The Cyranose 320 (Sensigent ®, California, US) was employed to determine the breath-print of the study groups. This equipment is a portable electronic nose that has 32 carbon polymer composite chemoresistors incorporated into a matrix that adsorbs the VOCs from the exhaled breath causing an increase in the electrical resistance of each sensor.

Each chemoresistor possesses different properties in the adsorption of volatile organic compounds producing varying degrees of response due to their polymer composition (poly-vinyl butyral, polyvinyl acetate, polystyrene, and polyethylene oxide) and the conduction nanoparticles (black carbon and carbon nanotubes) they are comprised of.

For sample processing, the samples were incubated at 37 °C for 5 min before reading with the electronic nose. The configuration of the electronic nose consisted of a constant flow rate of 120 mL/min for 40 s of baseline recording with ultra-pure nitrogen and a 90-s sample analysis period, then increased to a flow rate of 180 mL/min of ultra-pure nitrogen for sample line purging and air intake, with a substrate temperature of 32 °C. During the analysis, the instrument recorded the increase in electrical resistance of each sensor as a result of the adsorption of VOCs onto the sensors. As an internal quality control, the resistance of the 32 sensors was recorded every day of the reading to evaluate the quality of the analysis (Table 1. Supplementary material).

2.5. Statistical analysis

The multivariate analyses were performed using the increase in resistance of the 32 sensors obtained from the fractional difference: $\Delta R/Ro = (Rmax-Ro)/Ro$ where Rmax is the maximum system response of each sensor, and Ro is the reference reading of each sensor (ultra-pure nitrogen).

Subsequently, a summation normalization was performed to reduce the environmental effect by dividing the response of each sensor by the sum of the absolute values of the response of each sensor: $(\Delta R/Ro)i = (\Delta R/Ro)i/\sum |\Delta R/Ro|j$.

Table 1
Clinical characteristics of patients with COPD.

Parameters	COPD-S (n = 88)	COPD-HAP (n = 28)	Healthy (n = 178)
Age (year)	71.5 ± 9.5	76.7 ± 7.0	45 ± 6.0
Sex (%)			
Men	77.3	7.1	15.0
Women	22.7	92.8	85.0
Height (m)	1.62 ± 0.08	1.44 ± 0.06	1.63 ± 0.05
Weight (Kg)	67.9 ± 15.9	55.4 ± 10.2	62.0 ± 7.0
BMI (Kg/m^2)	25.5 ± 5.3	26.6 ± 4.6	23.3 ± 2.8
Comorbidities (%)			
SAH	22.7	35.71	–
T2DM	10.2	14.3	–
Smoking (Pack-Years)	40.0 (7.5–134.0)	4.5 (1.0–7.4)	1.0 (0 ± 2.4)
Biomass exposure (hour-Years)	0	320.5 (120–840)	0
FEV_1 (%P)	54.9 ± 22.7	65.1 ± 23.3	–
FVC (%P)	81.9 ± 22.3	81.1 ± 23.8	–
FEV_1/FVC	0.48 ± 0.20	0.61 ± 0.1	–
GOLD (%)			
I	15.9	25	–
II	31.8	53.6	–
III	22.7	10.7	–
IV	29.5	10.7	–

HAP: Burning biomass fuel. Mean ± standard deviation; median (minimum-maximum). SAH: systemic arterial hypertension. T2DM: Type 2 Diabetes mellitus. FEV_1: Forced Expiratory Volume to the first second. FVC: Forced Vital Capacity, both expressed as percentage of predicted (%P).

In addition, a self-scaling was carried out to eliminate the effects of the magnitude of the sensor responses, by subtracting the average of the samples from the individual response of each sample and dividing it by the standard deviation of the samples.

To capture the greatest amount of variability in the data, Principal Component Analysis (PCA) was performed using the Chemometric Data Analysis software CDAnalysis (Sensigent®), thus reducing the data from the 32 sensors to three main components.

The sensors with a higher importance index were used to obtain the canonical discriminant analysis (CDA) and support vector machines (SVM) discrimination models through a cross-validation value (leaving one out of the procedure and thus predicting the group association and obtaining overall classification success rates) and the Mahalanobis distance between the group means in units of standard deviation.

Support vector machines (SVM) is a kernel-based (radial Gaussian) supervised learning classification method that determines the optimal boundaries (support vectors) that precisely separate groups [23]. By giving n training pairs (x_1,y_1) ,(x_2,y_2),...,(x_n,y_n), where x_i is an input vector and $y_i \in \{-1, +1\}$, the SVM solves the following main problem:

$$\min_{\beta,b} \frac{1}{2}\beta^T\beta + C\sum_{i-1}^{n}\xi_i$$

$$s.t.\ \ y_i\left\{\beta^T\varphi(x_i)+b\right\} \geq 1-\xi_i,$$

$$\xi_i \geq 0, \quad i = 1,2,...,n,$$

Where β is a unit vector (i.e., $||\beta|| = 1$), T denotes the transposition of the matrix to Kernel, C is the adjustment parameter denoting the compensation between the margin width and the training data error and $\xi_i \geq 0$ are stationary variables. For an unknown input pattern x, we have the decision function:

$$f\ (x) = \sum_{i=1}^{n}\alpha_i y_i K(x,x_i) + b$$

Where, {αi, i = 1,2, .., n; αi ≥ 0} are the Lagrange multipliers, K (x, xi) ≡ φ (xi) T φ (xi) is the Kernel function. The Gaussian radial base function is used as the kernel function $K(x,\ x_i) = \exp(-\gamma||x-x_i||^2)$. Where $\gamma > 0$ are fixed parameters, $\gamma\ ||x-x_i||^2 = < x-x_i, x-x_i > [14,\ 23]$.

The performance of the CAP model was evaluated by ROC analysis (Receiver Operating Characteristic) with 95% confidence interval and threshold value was selected with the highest specificity/sensitivity ratio. The analysis was performed using XLSTAT version 12.0 (Stat-Soft®, Tulsa, Oklahoma, USA) The sensitivity = [number of true positives/(number of true positives + number of false negatives)]. Specificity = [number of true negatives/(number of true negatives + number of false positives)]. The predictive positive value = [number of true positives/(number of true positives + number of false positives)]. The negative predictive value = [number of true negatives/(number of true negatives + number of false negatives)].

3. Results

The characteristics of patients with smoking-associated COPD and exposure to biomass burning are described in Table 1. The study included 294 subjects, 116 patients with COPD (88 patients with COPD-S and 28 patients with COPD-HAP) and 178 healthy subjects (Fig. 1).

The patients with COPD had grade 1 (mild) to grade 4 (very severe) airflow limitation; the predominant category according to the grades of GOLD obstruction was grade 2 > 1 > 3 > 4 for patients with COPD caused by exposure to biomass burning and for patients with COPD caused by smoking it was 2 > 4 > 3 > 1. No signs or symptoms of respiratory disease were detected in healthy subjects, as well as risk activities such as smoking or exposure to wood smoke.

Fig. 2 (a-e) shows the graphs of the PCAs that were carried out among the study groups. Evidently, a separation is observed in the model in Fig. 2a, 2b, and 2c, the percentage that explains the variability between the groups is 93.76%, 93.02%, and 93.48%, respectively. In the models in Fig. 2d and 2e, it is shown that there is no separation between the groups either by causality (**2d**) or by stages of the disease (**2e**).

Twenty-one sensors with the highest importance indexes were used to perform the canonical discriminant analysis (CDA) (Table 1 Supplementary material). Table 2 shows the percentage of correct classification of the proposed models, the results show a high correct classification in the model **a, b** and **c**, however, the models do not allow discrimination between causality (**model d**) and disease statification (**model e**) (Fig. 1 Supplementary material).

Table 3 shows the correct classification of the models performed through the Support Vector Machine Model. Correct classification of 100% is observed in models a, b and c, while in models d and e the correct classification decreases to 75.7% and 58.2%. (Fig. 2 Supplementary material).

Likewise, with the values created in the CDA score, the cut-off point of −0.04 was established, which provided a sensitivity of 100% and specificity of 97.8%, with a negative predictive value of 100%, a positive predictive value of 96.5% and an accuracy of 98.6% across to ROC curve (Fig. 3).

4. Discussion

In the present study, it was identified that the breath-print analysis is able to discriminate between healthy subjects and patients with COPD using the electronic nose.

The main contribution of our study is based on the analysis of well-characterized groups of patients with COPD caused by smoking and exposure to household air pollution, which does not achieve discrimination. This is important because it indicates that breath-print is associated with the disease and not with causality. According to the literature review, this is the first study to analyze the comparison between the main causalities of COPD.

De Vries and collaborators conducted a similar study among the discrimination of patients with COPD and healthy subjects with an

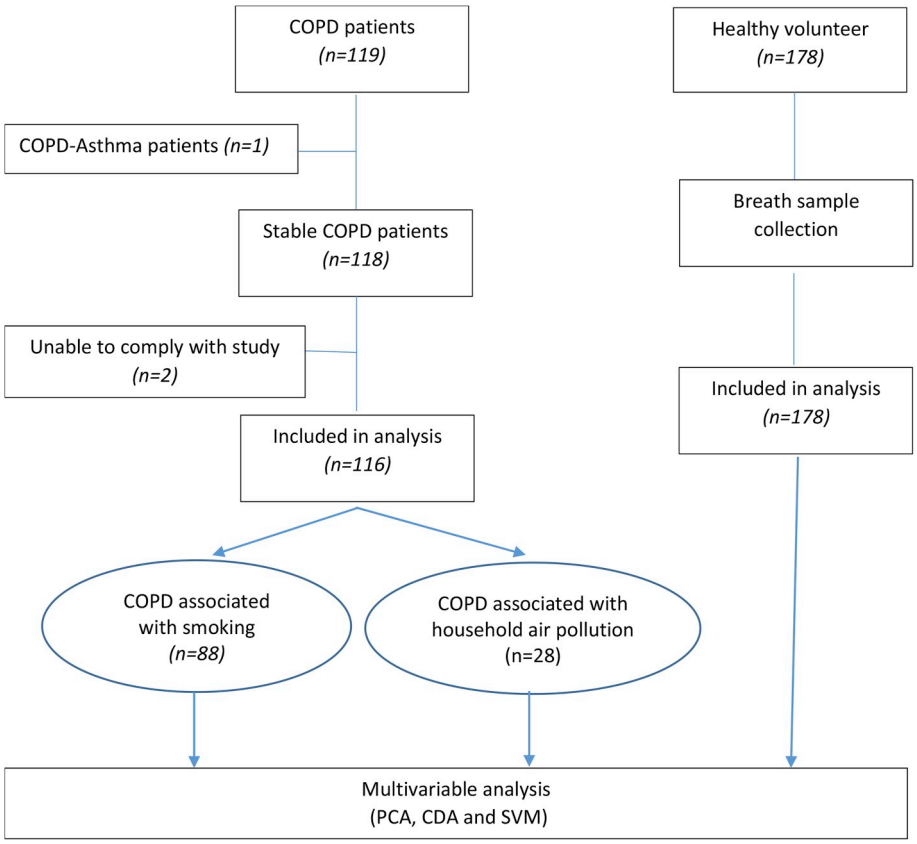

Fig. 1. Flow diagram shows COPD patients excluded for exhaled breath analysis by electronic nose.

electronic nose composed of five oxide semiconductor sensor arrays. The model they proposed was built with 31 patients with COPD and 45 healthy subjects and achieved an accuracy of 78% with a sensitivity of 80% [24].

On the other hand, other research has focused on the differences between COPD breath-print concerning other lung diseases. Studies with the Cyranose 320 reported that the patterns of VOCs in exhaled breath of patients with non-small cell lung cancer (n = 10) and patients with COPD (n = 10) are discriminated, pointing out that these differences are due to the physiopathology of the disease [9]. Tirzīte and collaborators managed to obtain discrimination patterns using SVM with a 100% correct classification among lung cancer with COPD (n = 79) with respect to healthy subjects (n = 78), however when the breath-print is analyzed among lung cancer with COPD with only COPD (n = 15) it reaches 33.3% of correct classification, this could be probably due to the small number of samples and a hypothesis proposed in this study is the presence of VOCs in lung cancer associated with oxidative events, which suggests a change in the pattern of breath-print and cannot differentiate between the groups [14]. This specificity is clinically relevant, we believe that the creation of disease-specific mathematical models can substantially improve population screening analyses and should be accompanied by clinically meaningful criteria such as smoking rate, or the number of wood hours in case of marginalized communities, as this indicates disease development. Our results indicate that the causal factors of COPD are not related to breath-print and provide an adequate differentiation between healthy subjects and patients so that this technique can be used in a point of care setting. Furthermore, there was no statistical difference between the age of the patients, but in the proportion of sexes, we observed that there are more women in the COPD-HAP group, due to cultural issues. This result shows that breath-print is not attributed to sex and age, confirming that VOCs are attributed to the disease and not to anthropometric parameters as indicated in other studies [10,25].

Although we obtained the ability to discriminate between predefined patients with COPD, diagnosed with gold tests (spirometry) and not sick as indicated by international guidelines [26], the model shows that the electronic nose is unable to identify the stages of the disease.

This result is consistent with the one performed by Incalzi and collaborators, where they demonstrated in 25 patients that there was no difference in VOCs in stages 1–3 and a difference was seen in stage 4 [27]. On the contrary, it has been shown that VOC profiles reflect different patterns associated with oxidative stress and inflammatory phenotype by eosinophils or neutrophils in COPD discriminating between stages 2 and 3 of stage 4 [28]. These results agree with Martinez-Lozano and collaborators, were using time of flight mass spectrometry they can discriminate between stage 1 and 2 with respect to stage 3 and 4 with a sensitivity of 92.3% and specificity of 83.3%. They also point out that the main metabolites that make the difference are acetone and indole [29]. The main advantage of mass spectrometry is that it allows the characterization of molecules, in addition to detection limits reaching parts per billion and parts per trillion, this is important because it contributes to the elucidation of altered metabolic pathways and possible therapeutic targets [30]. Nevertheless, this equipment cannot be used in screening methods due to the high cost, especially in low- and middle-income countries where, in parallel to smoking, the use of firewood increases the number of people at risk [13,31].

The limitations of our study include the fact that it was carried out in only one center, and although the number of patients with COPD and healthy subjects is relatively large, different lung diseases have been not included to evaluate the discrimination of COPD in the presence of comorbidities such as lung cancer. Therefore, a multicenter study is needed to contribute to the development of this technology so that it can be used as a population screening.

It is important to note that sensitivity and specificity are critical in test validation and that our results are very high at up to 100% and

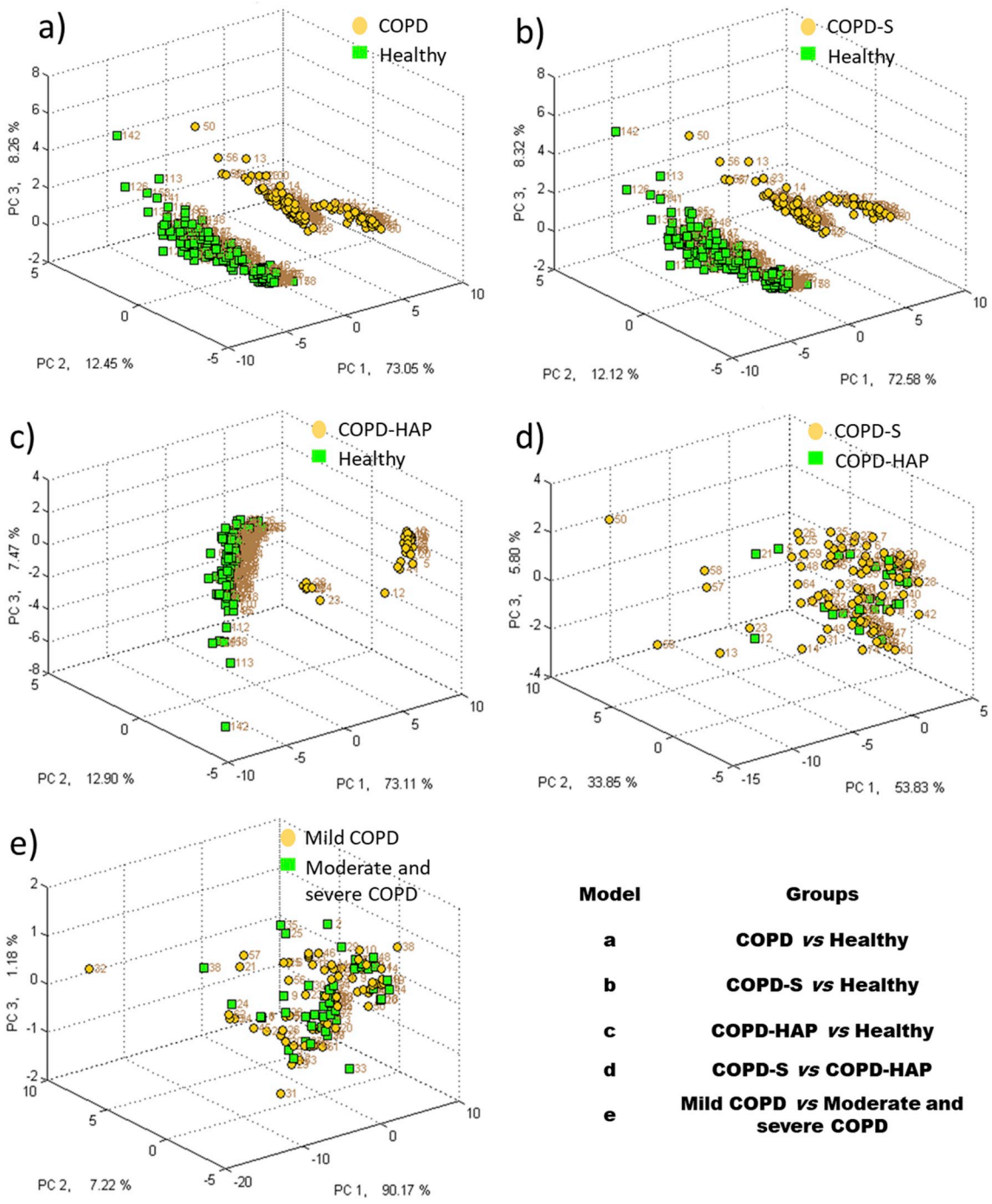

Fig. 2. Principal Component Analysis model of the breath-print of the proposed groups in the study. Fig. 2a) COPD vs Healthy subjects, **2b)** COPD-S vs Healthy subjects; **2c)** COPD-HAP vs Healthy subjects; **2d)** COPD-S vs COPDHAP; **2e)** stages of the disease.

Table 2
Percentage of correct prediction obtained in canonical discriminant analysis.

Canonical discriminant analysis model	Number of PCs	Percentage of Correct Prediction	*p*-value
a) COPD vs Healthy	3	97.8	<0.001
b) COPD-S vs Healthy	5	98.1	<0.001
c) COPD-HAP vs Healthy	7	97.5	<0.001
d) COPD-S vs COPD-HAP	10	2.5	>0.05
e) Mild COPD vs Moderate and severe COPD	5	1.6	>0.05

PC: Principal components.

Table 3
Cross-validation values for the models' discrimination.

Support Vector Machine Model	Cross-Validation Correct Rate (%)
a) COPD vs Healthy	100
b) COPD-S vs Healthy	100
c) COPD-HAP vs Healthy	100
d) COPD-S vs COPD-HAP	75.7
e) COPD (GOLD 1 and 2) vs COPD (GOLD 3 and 4)	58.2

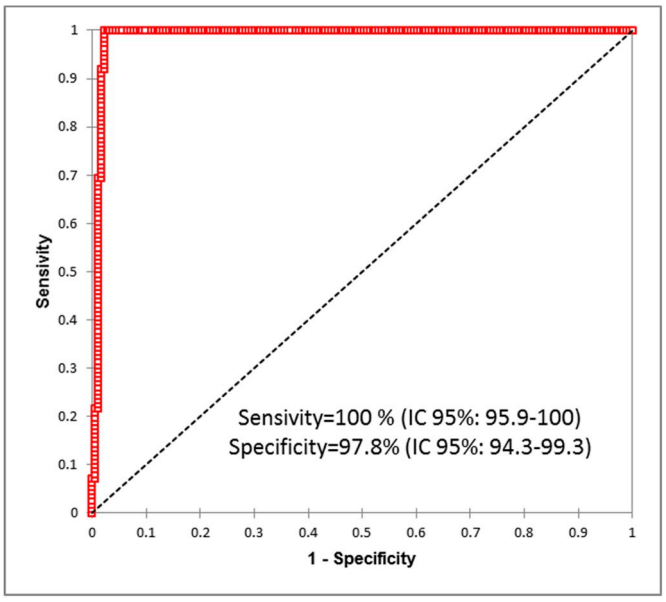

Fig. 3. ROC curve for the diagnosis of COPD when using the CAP_1 axis. An AUC of 0.989 was obtained when using a cut-off point of −0.04.

97.8%, respectively, which is consistent with other studies [32,33]. As we consider that by increasing the number of patients and healthy subjects, the positive and negative predictive values can be improved and thus the mathematical model of breath-print can be used as a method of screening for COPD in the general population.

5. Conclusion

It has been demonstrated that exhaled breath analysis technology can discriminate healthy subjects with respect to patients with COPD. This study was also included two clinically relevant groups of patients with COPD associated with the two main causes, smoking and the use of biomass as fuel, demonstrating that there are no differences between breath-prints.

This provides a simple, fast and non-invasive approach to testing for COPD without the need for patient effort, thus facilitating an excellent screening method for future clinical applications.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The author Omar Ornelas Rebolledo who is director of the company LABINNOVA which financed the study and the Cyranose 320 equipment.

CRediT authorship contribution statement

Maribel Rodríguez-Aguilar: Conceptualization, Methodology, Software, Validation. **Lorena Díaz de León-Martínez:** Methodology, Writing - original draft, Software, Validation. **Patricia Gorocica-Rosete:** Methodology, Project administration, Writing - original draft. **Rogelio Pérez Padilla:** Supervision, Writing - review & editing. **Ireri Thirión-Romero:** Investigation, Data curation. **Omar Ornelas-Rebolledo:** Writing - original draft, Funding acquisition. **Rogelio Flores-Ramírez:** Conceptualization, Writing - review & editing, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2020.105901.

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