International Journal of COPD

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ORIGINAL RESEARCH

# Validation of exhaled volatile organic compounds analysis using electronic nose as index of COPD severity

This article was published in the following Dove Press journal:
International Journal of COPD

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**Aim:** Six-minute walking test distance (6MWD) and body mass index, obstruction, dyspnea and exercise (BODE) index are measures of functional status in COPD patients, but require space, time and patient's compliance. Exhaled volatile organic compounds (VOCs) analysis via electronic nose is a quick and easy method that has already been used to discriminate COPD phenotypes. The aim of this study is to evaluate whether VOCs analysis can predict functional status and its variation over time in COPD patients.

**Methods:** A monocentric prospective study with 1 year of follow-up was carried out. All patients underwent pulmonary function tests, arterial gas analysis, bioimpedance analysis, 6-minute walking test, and VOCs collection. Exhaled breath was collected with Pneumopipe® and analyzed using BIONOTE electronic nose. Outcomes prediction was performed by *k*-fold cross-validated partial least square discriminant analysis: accuracy, sensitivity and specificity as well as Cohen's kappa for agreement were calculated.

**Results:** We enrolled 63 patients, 60.3% men, with a mean age of 71 (SD: 8) years, median BODE index of 1 (interquartile range: 0–3) and mean 6MWD normalized by squared height (n6MWD) of 133.5 (SD: 42) m/m$^2$. The BIONOTE predicted baseline BODE score (dichotomized as BODE score $<3$ or $\geq 3$) with an accuracy of 86% and quartiles of n6MWD with an accuracy of 79%. n6MWD decline more than the median value after 1 year was predicted with an accuracy of 86% by BIONOTE, 52% by Global Initiative for Chronic Obstructive Lung Disease (GOLD) class and 78% by combined BIONOTE and GOLD class.

**Conclusion:** Exhaled VOCs analysis identifies classes of BODE and n6MWD quartiles, and outperforms GOLD classification in predicting n6MWD variation.

**Keywords:** volatile organic compounds, electronic nose, 6-minute walking test, COPD, functional status, BODE index

## Introduction

Since 2010, COPD has become the third leading cause of death, accounting for 2.8 million deaths worldwide.[1] Identifying patients with a steeper progression toward disability and death is warranted to implement monitoring and therapy.

Different systems have been proposed to categorize COPD severity and rate of functional status decline. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) proposed a severity classification based on reduction of forced expiratory volume in the first second ($FEV_1$) expressed as percent of predicted value,[2] and then updated the risk stratification adding clinical parameters[3] to take into account the burden of symptoms. Nonetheless, neither the first nor the second GOLD classification has demonstrated sufficient discrimination ability in stratifying COPD functional

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http://dx.doi.org/10.2147/COPD.S159684

impairment severity.[4–6] Compared with GOLD classifications, the 6-minute walking test (6MWT) has a better association with clinical outcomes, such as mortality,[7–9] either alone or when used together with other variables in the body mass index (BMI), obstruction, dyspnea and exercise (BODE) index.[10] The 6MWT should be performed in a corridor of at least 30 m length, which may not be available in some settings, and requires patient's compliance to obtain reliable measures;[11] therefore, new approaches that are less demanding in terms of space and patient's compliance may be helpful to rate functional impairment.

Exhaled volatile organic compounds (VOCs) analysis through electronic nose is a promising approach in the study of lung disease. This technique provides a "fingerprint" of the exhaled breath (dubbed "breathprint"), obtained by interaction occurring between VOCs and a sensor array, which has been shown to discriminate COPD patients from healthy controls, patients with asthma[12] and patients with heart failure,[13] and seems promising in COPD phenotyping.[14,15] Considering that factors affecting COPD patients' functional status (ie, systemic inflammation and hypoxemia[16]) are also modifiers of VOCs production,[17] this proof-of-concept study aims to evaluate whether exhaled fingerprint predicts the functional status in COPD patients and whether and to which extent it might improve the GOLD discrimination ability. Therefore, the objectives of this study are 1) to evaluate whether VOCs analysis of COPD patients using electronic nose discriminates quartiles of 6-minute walking test distance normalized by squared height (n6MWD) and classes of BODE index, also in comparison with GOLD classification; and 2) to evaluate whether VOCs analysis predicts changes in the n6MWD after 1 year.

# Patients and methods

## Study design

This was a 1-year monocentric and observational prospective study carried out at "Campus Bio-Medico" Hospital in Rome (Italy). The data used in the present study were collected from September 2015 to September 2016. The study protocol was approved by the Ethical Committee of Campus Bio-Medico di Roma University (protocol number: 30/15 PAR CMB). All the study participants provided written informed consent.

Inclusion criteria were diagnosis of COPD defined as the evidence of persistent airflow limitation at spirometry, which is defined as $FEV_1$/forced vital capacity $<0.7$ after administration of 400 μg of salbutamol,[18] and the absence of exacerbations and of changes in pharmacological therapy in the previous 3 months.

Exclusion criteria were inability to perform an acceptable spirometry following the American Thoracic Society and the European Respiratory Society (ATS/ERS) guidelines,[18] diagnosis of pulmonary cancer or pulmonary fibrosis, severe cognitive impairment as indicated by a mini-mental state examination (MMSE) score $<10$, inability to perform the 6MWT due to mobility limitations, heart failure of New York Heart Association (NYHA) class III or IV and, finally, refusal to provide informed consent.

## Clinical assessment

The study involved 63 stable COPD patients consecutively enrolled. Demographic and physiological characteristics, level of dyspnea (determined using the modified Medical Research Council [mMRC] dyspnea scale), number of exacerbations, pulmonary function tests (post-bronchodilator spirometry and lung volumes), 6MWT, arterial gas and bioimpedance analysis, multidimensional assessment results and comorbidities, which were identified based on patients' documentation, medical history, physical examination and routine blood analysis, were recorded during the visit, both at baseline and after 1 year.

Forced expiratory volumes were measured using a water-sealed bell spirometer (Biomedin, Padua, Italy) following the acceptability and reproducibility criteria proposed by the ATS/ERS.[18] Total lung capacity and residual volume were determined using the Helium-rebreathing technique.[19] Multidimensional assessment was made up by the following: functional ability, estimated using the Katz activities of daily living and the Lawton and Brody instrumental activities of daily living;[20,21] cognitive function, evaluated using MMSE;[22] and depressive symptoms, estimated using 15-item Italian version of the Geriatric Depression Scale.[23]

## Collection and analysis of VOCs

Exhaled breath was collected only at baseline, in the morning, early after awakening, with patients fasting, smoking free and having refrained from medication consumption for at least 8 hours. All patients performed breath collection in the same room and with the same procedure.

Patients were asked to breath tidally for 3 minutes into a mouthpiece connected with Pneumopipe® (European patent no 12425057.2; Rome, Italy), a device ensuring in a fixed time a noninvasive collection of mixed expiratory sample into an adsorbent Tenax GR cartridge (Supelco; Sigma-Aldrich, St Louis, MO, USA).[24] Cartridge content was then thermally desorped into the sensor chamber of our electronic nose, named BIONOTE, by a device uniformly heating the

cartridge at four different temperatures: 50°C, 100°C, 150°C and 200°C. BIONOTE is a seven quartz microbalance (QMB) sensor array. Sensors are covered with anthocyanins extracted from three different plant tissues and used as chemical interactive materials. Desorbed VOCs interact with the chemicals covering the sensor's surface via weak bonding forces, and interaction results in seven frequency shifts of each of the QMB with respect to their typical resonance frequency for each desorption temperature, so we finally achieve 28 responses (seven QMB tested per four different temperatures) in the analysis. This technology has been validated, and its performance has been evaluated in gases and vapors calibration experiments.[25]

## 6MWT and BODE index

Six-minute walking test is a clinical exercise test measuring the distance that a patient can quickly walk in a period of 6 minutes. It provides global information on the organs contributing to exercise capacity, but it is not able to disentangle the contribution of each system. The test was performed in the morning, after exhaled breath collection, according to the ATS guidelines.[11] In summary, participants were asked to walk in a flat, straight and 30 m hallway for 6 minutes as quickly as they could, under the supervision of a trained technician. $SpO_2$ was measured throughout the test using a pulse oximeter with a finger probe. Dyspnea was rated at the end of the test using Borg scale. The 6-minute walking test distance (6MWD), basal $SpO_2$, nadir $SpO_2$ and Borg score were recorded. Since anthropometric features affect 6MWD,[26–28] we used n6MWD to avoid their impact on the treaded distance as has been suggested for $FEV_1$.[29]

The BODE index is a scoring system used to predict all-cause mortality in patients with stable COPD, and it is based on four variables: BMI, $FEV_1$ (percent of predicted), dyspnea rated using COPD Assessment Tool or mMRC and 6MWD. Total score ranges from 0 to 10; the higher is the score, the lower is the estimated survival. A BODE score higher than 3 is associated with a poorer prognosis.[10]

## Statistical analysis

We reported the characteristics of our sample using descriptive statistics. Participants were divided into quartiles of n6MWD, which were compared using analysis of variance (ANOVA) and Kruskal–Wallis test for normally and non-normally distributed continuous variables, respectively, and $\chi^2$ test or Fisher's exact test for categorical variables, as appropriate.

Partial least square discriminant analysis (PLS-DA), applied on patients' BIONOTE sensor responses, previously centered and scaled, was used to predict the quartiles of n6MWD taking into account the individual's anthropometric features affecting 6MWD. The same method was used to predict BODE score, classified as "good prognosis" (BODE score $<3$) and "poor prognosis" (BODE score $\geq 3$),[30] which represented the "gold standard" for this analysis. To avoid overfitting, we used a "repeated *k*-fold cross validation". The overall effectiveness of classification was expressed as diagnostic accuracy, which is the proportion of subjects correctly classified by the PLS-DA among all subjects, sensitivity and specificity. Furthermore, we measured the agreement between the model based on BIONOTE responses and reference n6MWD quartiles and BODE classes using Cohen's kappa.[31] Then, we compared the quartiles of n6MWD ($m/m^2$) with 2014 GOLD classes, measuring overall effectiveness and agreement with the same procedure as done before, and analyzed the distribution of classes of BODE score across GOLD classes.

Finally, we evaluated whether VOC analysis could predict changes in the n6MWD over time. For this analysis, the "gold standard" was represented by the variation of walked distance which was dichotomized using the median ($-7.9$ $m/m^2$) as the cut-off value into "stable/improved" or "worsened". For this analysis, we compared predictions based on VOC only with predictions made on GOLD classes only and on the combination of the previous two parameters. Accuracy, sensitivity and specificity, as well as Cohen's kappa were calculated.

All the analyses were performed using R version 3.3.0 (The R Foundation for Statistical Computing, Vienna, Austria).

# Results

The characteristics of the participants at baseline are summarized in Table 1. Mean age of the participants was 71 (SD: 8) years, 38 (60.3%) were men, mean BMI was 28.1 (SD: 5.9) $kg/m^2$, mean $FEV_1$% was 69.6 (SD: 22.3), mean pack/year was 35.1 (SD: 26.6) and 25 (39.7%) were current smokers, while 32 (50.8%) were former smokers. Of the 63 participants, 35 (55.6%) were in GOLD A class, nine (14.5%) in GOLD B class, eight (12.4%) in GOLD C class and 11 (17.5%) in GOLD D class. Mean 6MWD was 366.8 (SD: 111.5) m, and mean n6MWD was 133.5 (SD: 42) $m/m^2$. Patients in the first quartile of n6MWD treaded from 166 $m/m^2$ to 215 $m/m^2$, those in the second from 146 $m/m^2$ to 165 $m/m^2$, those in the third from 112 $m/m^2$ to 145 $m/m^2$ and those in the fourth quartile from 22 $m/m^2$ to

**Table 1** Baseline characteristics of the population (N = 63) and quartiles of 6MWD normalized by squared height (m/m²)

| Characteristics | Population, N = 63 | 22–111 m/m², n = 16 | 112–145 m/m², n = 16 | 146–165 m/m², n = 16 | 166–215 m/m², n = 15 | *P*-value |
|---|---|---|---|---|---|---|
| Age | 71 (8) | 73.9 (6.9) | 71.6 (8.6) | 70.2 (6.8) | 68.2 (8.5) | 0.22 |
| Sex (M) | 38 (60.3) | 12 (75) | 11 (69) | 9 (56) | 6 (40) | 0.21 |
| Body mass index (kg/m²) | 28.1 (5.9) | 30.2 (7.1) | 31.1 (6.3) | 25.2 (2.8) | 25.8 (4.5) | 0.004 |
| mMRC | | | | | | |
| 0–1 | 43 (68) | 6 (37) | 13 (81) | 12 (75) | 12 (80) | 0.01 |
| ≥2 | 20 (32) | 10 (63) | 3 (19) | 4 (25) | 3 (20) | 0.01 |
| Exacerbation/year | | | | | | |
| 0–1 | 48 (76) | 13 (81) | 10 (63) | 12 (75) | 13 (87) | 0.46 |
| ≥2 | 15 (24) | 3 (19) | 6 (37) | 4 (25) | 2 (13) | 0.46 |
| Smoking habit | | | | | | |
| Current smokers | 25 (39.7) | 5 (31) | 7 (44) | 8 (50) | 5 (33) | 0.6 |
| Former smokers | 32 (50.8) | 9 (56) | 9 (56) | 7 (43) | 7 (47) | 0.6 |
| Pack/year | 35.1 (26.6) | 41.2 (30) | 40.9 (26.7) | 26.4 (18) | 31.7 (28) | 0.32 |
| GOLD classification 2014 | | | | | | |
| GOLD A | 35 (55.6) | 4 (25) | 9 (56) | 11 (69) | 11 (73) | 0.01 |
| GOLD B | 9 (14.5) | 5 (31) | 0 (0) | 2 (13) | 2 (13) | 0.01 |
| GOLD C | 8 (12.4) | 1 (6) | 3 (19) | 2 (13) | 2 (13) | 0.01 |
| GOLD D | 11 (17.5) | 6 (38) | 4 (25) | 1 (6) | 0 (0) | 0.01 |
| BODE index | 1 (0–3) | 3 (2–5) | 1 (0–3) | 0 (0–1) | 0 (0–1) | <0.001 |
| Spirometry | | | | | | |
| $FEV_1$/FVC | 63.2 (9.6) | 61.5 (12.6) | 61.5 (9.2) | 65 (8.4) | 65 (7.3) | 0.55 |
| $FEV_1$ (cm³) | 1.7 (0.7) | 1.5 (0.6) | 1.7 (0.5) | 2 (1) | 1.8 (0.5) | 0.2 |
| $FEV_1$% | 69.6 (22.3) | 57.9 (22) | 64.6 (20.7) | 78.6 (27) | 77.7 (9.4) | 0.02 |
| FVC (cm³) | 2.8 (1) | 2.5 (1.1) | 2.7 (0.6) | 3.1 (1.4) | 2.8 (0.9) | 0.45 |
| FVC% | 86.3 (22.6) | 72.4 (21.5) | 82.3 (23.1) | 94.7 (25.2) | 96.3 (9.8) | 0.006 |
| TLC (cm³) | 5.9 (1.6) | 5.9 (1.5) | 5.8 (1.2) | 6.6 (2.1) | 5.3 (1.4) | 0.17 |
| TLC% | 100.9 (24.7) | 97.2 (40.7) | 92.4 (13.7) | 114.1 (15) | 100.2 (17) | 0.1 |
| RV/TLC (cm³) | 52.5 (11.5) | 57.5 (10.9) | 49.4 (15.4) | 52 (9.5) | 51.5 (8.5) | 0.29 |
| RV/TLC% | 126.5 (26.5) | 132.4 (29.8) | 124.7 (31) | 125.4 (22) | 123.9 (23) | 0.84 |
| Arterial gas analysis | | | | | | |
| pH | 7.41 (0.03) | 7.41 (0.02) | 7.42 (0.03) | 7.42 (0.03) | 7.42 (0.04) | 0.13 |
| $pO_2$ (mmHg) | 77.6 (10.7) | 74.4 (11.4) | 74.5 (9.9) | 77.8 (11) | 84 (8.5) | 0.04 |
| $pCO_2$ (mmHg) | 39.3 (5) | 41 (6.3) | 40.2 (3) | 39.2 (5.5) | 36.5 (4) | 0.08 |
| $SO_2$ | 95.1 (1.7) | 94.5 (1.7) | 94.7 (1.7) | 95.2 (1.8) | 96.1 (0.9) | 0.04 |
| Multidimensional assessment | | | | | | |
| CIRS comorbidity index | 0 (0–1) | 0 (0–1) | 1 (0–2) | 0 (0–1) | 0 (0–1) | 0.2 |
| CIRS severity index | 0.7 (0.4) | 0.7 (0.4) | 0.8 (0.3) | 0.5 (0.2) | 0.7 (0.4) | 0.16 |
| MMSE | 27.9 (2.9) | 27.5 (2.4) | 28.9 (1.7) | 27.4 (3.5) | 27.7 (3.7) | 0.4 |
| Clock test | 11 (2.8) | 11.2 (2.8) | 11.2 (2) | 10.8 (3.5) | 10.9 (2.9) | 0.95 |
| ADL | 5.8 (0.5) | 5.8 (0.4) | 5.6 (0.8) | 5.9 (0.2) | 5.8 (0.4) | 0.4 |
| IADL | 7.7 (1) | 7.4 (1.5) | 7.6 (1) | 7.9 (0.5) | 7.9 (0.3) | 0.34 |
| GDS | 1.8 (2.7) | 2 (3.6) | 2.6 (3.2) | 1 (1.7) | 1.7 (1.6) | 0.4 |
| Blood analysis | | | | | | |
| WBC count (×10³/μL) | 7.1 (1.7) | 7.5 (1.5) | 6.8 (1.7) | 6.9 (1.9) | 7 (1.5) | 0.68 |
| Hb (g/dL) | 14.2 (1.4) | 13.9 (1.9) | 14.4 (1.4) | 14.3 (1) | 14.3 (1.5) | 0.81 |
| ESR (mm/h) | 25.9 (23.7) | 35.6 (36.1) | 22.8 (15.9) | 20.9 (18.7) | 25.9 (20.9) | 0.42 |
| CRP (mg/L) | 3.2 (5.1) | 3.7 (4) | 1.4 (2.4) | 4.9 (7.9) | 2.3 (3.2) | 0.32 |
| Creatinine (mg/dL) | 1 (0.3) | 1.1 (0.3) | 0.9 (0.3) | 0.8 (0.2) | 0.9 (0.3) | 0.06 |
| eGFR(CKD-EPI) | 74.2 (19.5) | 64.2 (18) | 76.3 (21) | 80.6 (18.3) | 74.4 (18.9) | 0.15 |
| Vitamin D (ng/mL) | 20.9 (10.6) | 23.9 (12.3) | 20.5 (8.1) | 18.8 (7.8) | 20.5 (14) | 0.67 |
| Vitamin B12 (pg/mL) | 368.1 (204.7) | 338.8 (122.2) | 272.5 (122) | 449.1 (304) | 383.7 (150) | 0.2 |
| Folic acid (ng/mL) | 7.5 (7.2) | 5.5 (3.1) | 5.7 (2.5) | 9.9 (11.1) | 8.3 (6.7) | 0.38 |
| Bioimpedance analysis | | | | | | |
| Total body water % | 52.1 (6) | 51.4 (6.7) | 50.9 (5.4) | 54.5 (6.4) | 51.7 (5.3) | 0.31 |
| Fat mass % | 30.1 (8.4) | 32.1 (9.3) | 32.9 (6.4) | 25.8 (8.8) | 29.7 (7.3) | 0.07 |
| Fat-free mass % | 69.9 (8.4) | 67.9 (9.3) | 67.1 (6.4) | 74.2 (8.8) | 70.3 (7.3) | 0.06 |

**Note:** Continuous variables are shown as mean (standard deviation) or median (interquartile range), as appropriate, while categorical variables are shown as n (%).

**Abbreviations:** 6MWD, 6-minute walking test distance; ADL, activities of daily living; BODE, body mass index, obstruction, dyspnea and exercise; CIRS, Cumulative Illness Rating Scale; CRP, C-reactive protein; eGFR(CKD-EPI), estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration); ESR, erythrocyte sedimentation rate; $FEV_1$, forced expiratory volume in the first second; FVC, forced vital capacity; GDS, Geriatric Depression Scale; GOLD, Global Initiative for Chronic Obstructive Lung Disease; Hb, hemoglobin; IADL, instrumental activities of daily living; mMRC, modified Medical Research Council; MMSE, mini-mental state examination; RV, residual volume; TLC, total leukocyte count; WBC, white blood cell.

**Table 2** Cross-validated partial least square discriminant analysis prediction of quartiles of n6MWD (upper) and classes of BODE (bottom)

| Prediction n6MWD | Reference n6MWD | | | |
|---|---|---|---|---|
| | 22–111 m/m² | 112–145 m/m² | 146–165 m/m² | 166–215 m/m² |
| 22–111 | 15 | 0 | 1 | 1 |
| 112–145 | 0 | 13 | 1 | 3 |
| 146–165 | 0 | 2 | 12 | 1 |
| >165 | 1 | 1 | 2 | 10 |
| Accuracy: 0.79 (95% CI 0.67–0.88, $P < 0.001$). Cohen's kappa: 0.72 (95% CI 0.59–0.85, $P < 0.001$) | | | | |
| **Prediction BODE classes** | **Reference BODE classes** | | | |
| | **Good prognosis (BODE < 3)** | | **Poor prognosis (BODE ≥ 3)** | |
| Good prognosis | 39 | | 6 | |
| Poor prognosis | 3 | | 15 | |
| Accuracy: 0.86 (95% CI 0.75–0.93, $P < 0.001$). Sensitivity: 0.71; specificity: 0.93. Cohen's kappa: 0.67 (95% CI 0.47–0.87, $P < 0.001$) | | | | |

**Abbreviations:** n6MWD, 6-minute walking test distance normalized by squared height; BODE, body mass index, obstruction, dyspnea, and exercise.

111 m/m². After 1 year of follow-up, 35 participants (55.6%) were still in GOLD A class, 10 (15.8%) were in GOLD B class, nine (14.3%) in GOLD C class and nine (14.3%) in GOLD D class; mean 6MWD was 342.8 (SD: 119) m, and mean n6MWD was 123.9 (SD: 42) m/m². Median decline in the 6MWD over the year was −23.8 m, and median decline in the n6MWD was −7.9 m/m².

Analyzing the baseline quartiles of n6MWD, we observed a progressive reduction in the mean $FEV_1$% (from 77.7 [SD: 9] to 57.9 [SD: 22], ANOVA $P = 0.02$) and in the mean $pO_2$ (from 84 mmHg [SD: 8.5] to 74.4 mmHg [SD: 11.4], ANOVA $P = 0.04$), as well as a reduction in the mean fat-free mass percent (FFM%), from the quartile with the highest treaded distance to that with the lowest, and an increase in mMRC score, BODE index and BMI. The PLS-DA based on electronic nose sensors was able to discriminate baseline quartiles of n6MWD, correctly allocating 50 out of 63 patients with an overall accuracy of 0.79 (95% CI 0.67–0.88, $P < 0.001$) and a Cohen's kappa of 0.72 (95% CI 0.59–0.85, $P < 0.001$). Moreover, 54 patients out of the 63 participants were correctly classified into the baseline classes based on BODE index ("good prognosis" vs "poor prognosis") with an accuracy of 0.86 (95% CI 0.75–0.93, $P < 0.001$), a sensitivity of 0.71, a specificity of 0.93 and a Cohen's kappa of 0.67 (95% CI 0.47–0.87, $P < 0.001$) (Table 2).

GOLD classes did not identify quartiles of n6MWD (m/m²), and we did not find a linear reduction of the walked distance across GOLD classes, obtaining an accuracy in predicting quartiles of walked meters per meter square of 0.35 (95% CI 0.23–0.48, $P = 0.05$) with a poor agreement: Cohen's kappa of 0.14 (95% CI –0.02 to 0.29, $P = 0.03$) (Table 3).

Prediction of the two classes ("stable/improved" vs "worsened") of n6MWD variation based on the electronic nose sensors responses correctly classified 54 out of the 63 COPD patients with an accuracy of 0.86 (95% CI 0.75–0.93,

**Table 3** Confusion matrix with the distribution per GOLD classes of the n6MWD (top) and classes of BODE (bottom)

| GOLD classes | Reference n6MWD | | | |
|---|---|---|---|---|
| | 22–111 m/m² | 112–145 m/m² | 146–165 m/m² | 166–215 m/m² |
| A | 4 | 9 | 11 | 11 |
| B | 5 | 0 | 2 | 2 |
| C | 1 | 3 | 2 | 2 |
| D | 6 | 4 | 1 | 0 |
| Accuracy: 0.35 (95% CI 0.23–0.48, $P = 0.05$). Cohen's kappa: 0.14 (95% CI –0.02 to 0.29, $P = 0.03$) | | | | |
| **GOLD classes** | **Reference BODE classes** | | | |
| | **Good prognosis (BODE < 3)** | | **Poor prognosis (BODE ≥ 3)** | |
| A | 34 | | 1 | |
| B | 3 | | 6 | |
| C | 4 | | 4 | |
| D | 1 | | 10 | |

**Abbreviations:** GOLD, Global Initiative for Chronic Obstructive Lung Disease; n6MWD, 6-minute walking test distance normalized by squared height; BODE, body mass index, obstruction, dyspnea, and exercise.

**Table 4** Cross-validated partial least squares discriminant analysis prediction of the n6MWD variation classes over the year of follow-up based only on BIONOTE (top), only on GOLD classification (center) and on BIONOTE + GOLD classification (bottom)

| **BIONOTE-based prediction of Δn6MWD classes** | **Reference Δn6MWD classes** | |
|---|---|---|
| | **Worsened** | **Stable/improved** |
| Worsened | 28 | 5 |
| Stable/improved | 4 | 26 |
| Accuracy: 0.86 (95% CI 0.75–0.93, $P < 0.001$). Sensitivity: 0.84; specificity: 0.88. Cohen's kappa: 0.71 (95% CI 0.54–0.88, $P < 0.001$) | | |
| **GOLD-based prediction of Δn6MWD classes** | **Reference Δn6MWD classes** | |
| | **Worsened** | **Stable/improved** |
| Worsened | 15 | 13 |
| Stable/improved | 17 | 18 |
| Accuracy: 0.52 (95% CI 0.39–0.65, $P = 0.45$). Sensitivity: 0.58; specificity: 0.47. Cohen's kappa: 0.05 (95% CI –0.20 to 0.29, $P = 0.35$) | | |
| **BIONOTE + GOLD-based prediction of Δn6MWD classes** | **Reference Δn6MWD classes** | |
| | **Worsened** | **Stable/improved** |
| Worsened | 25 | 6 |
| Stable/improved | 7 | 25 |
| Accuracy: 0.79 (95% CI 0.67–0.88, $P < 0.001$). Sensitivity: 0.81; specificity: 0.78. Cohen's kappa: 0.59 (95% CI 0.39 to 0.79, $P < 0.001$) | | |

**Abbreviations:** n6MWD, 6-minute walking test distance normalized by squared height; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

$P < 0.001$), a sensitivity of 0.84 and a specificity of 0.88, while the accuracy obtained using GOLD classes was 0.52 (95% CI 0.39–0.65, $P = 0.45$). Applying PLS-DA on GOLD classes and electronic nose responses together, 49 out of 63 patients were correctly classified with an accuracy of 0.79 (95% CI 0.67–0.88, $P < 0.001$), a sensitivity 0.81 and a specificity of 0.78. Cohen's kappa was 0.71 (95% CI 0.54–0.88, $P < 0.001$) for BIONOTE-based prediction, 0.05 (95% CI –0.20 to 0.29, $P = 0.35$) for GOLD-based prediction and 0.59 (95% CI –0.39 to 0.79, $P < 0.001$) for the model based on their combination. Data are summarized in Table 4.

## Discussion

Exhaled VOCs analysis using electronic nose discriminates baseline BODE classes and quartiles of n6MWD, and is able to predict n6MWD variation over 1 year of follow up, better than the GOLD classes.

Our data show that inclusion of dyspnea and exacerbation rate notwithstanding, the problem of GOLD classification may be that it remains mainly focused on respiratory parameters, ignoring that COPD is a disease with important systemic impact. Indeed, risk factors of COPD, that is, cigarette smoking, induce systemic inflammation and oxidative stress,[32] as well as changes in endothelial function favoring a prothrombotic state,[33] resulting in a higher prevalence of chronic comorbidities (eg, heart failure, pulmonary vascular diseases, etc).[34] Thus, COPD is seldom an isolated disorder, and usually comorbidity impacts health outcomes, particularly survival, in COPD patients. Indeed, COPD exacerbation leading to respiratory failure accounts only for 35% of COPD patients' death.[35] This evidence fosters the use of VOCs analysis. VOCs are low-weight molecules produced by cellular metabolism; therefore, bacterial infections, metabolic abnormalities, toxic ingestion, chronic diseases (eg, heart failure, liver cirrhosis) and cancer produce a qualitative and quantitative change in their production.[17] Hence, although COPD induces its particular change in exhaled VOCs, useful in the diagnosis of the disorder, patient's breathprint is the end result of the overall cellular metabolism disarray, also caused by comorbidities.[36] Indeed, analyzing the characteristics of patients divided per quartiles of n6MWD, we observed that VOCs are not just influenced by airflow limitation and dyspnea, also captured by GOLD classification, but besides they take into account body composition, as described by a statistically significant increase in BMI associated with a reduction in FFM%, a well-demonstrated factor affecting COPD functional status.[37] Furthermore, they are not affected by the number of exacerbations per year, which, although improving ability to predict hospital admission due to COPD exacerbation,[4] do not seem to be correlated with functional status. This is a possible explanation of why the model based on VOCs was able to discriminate better than GOLD classes both 6MWD and BODE index as well as to identify, in the longitudinal analysis, a worsening of patients' functional status.

Moreover, our results confirm the known limitations of the 2014 GOLD classification in the identification of COPD functional status. This new classification, despite considering dyspnea and exacerbation, did not outperform the 2007 GOLD classification based only on airflow limitation,[4,6] and it is characterized by a poor ability in COPD functional status

stratification: subgroup B has a poorer survival and a higher rate of hospital admission than subgroup C[4,5] and is characterized by a lower 6MWD and a higher BODE index than subgroup C.[38] In our analysis, GOLD classification showed a poor agreement both with baseline quartiles of n6MWD and classes of BODE and with longitudinal variation of n6MWD, as demonstrated by Cohen's kappa, significantly lower than that obtained by VOCs analysis. Furthermore, we observed that while GOLD A is clearly composed of subjects with a good functional status, and GOLD D of subjects with the worst, GOLD B and GOLD C have subjects with intermediate impairment and are rather similar, with GOLD B being slightly worse; hence, there is no linear progression of impairment confirming reports of previous studies.[38]

### Strengths and limitations

This study has some limitations. Firstly, using an electronic nose technology, we could not identify single components of exhaled breath, as is possible with other techniques (eg, gas chromatography–mass spectrometry). However, the purpose of this study was to develop a simple and inexpensive metabolic marker: electronic nose analysis costs are about €10, whereas analytic methods of breath samples are costly and not suitable for routine clinical use, and comparable, or even cheaper, than that of 6MWT (about €50). Secondly, VOCs may be influenced by room conditions, smoke, drugs and food, but we minimized the potential bias of these factors, asking patients not to smoke, eat, or drink and not to consume medication for at least 8 hours, and performing collection always in the same room. Furthermore, to date, there does not exist an electronic nose considered as the "gold standard" in VOCs analysis; thus, BIONOTE has never been compared with other electronic nose devices. Finally, we acknowledge that the choice to normalize 6MWD per patients' squared height to minimize the impact of anthropometric features is arbitrary, although conventionally used.

The study also has some strengths. We performed a multidimensional assessment of the whole population, not limited to respiratory parameters. Furthermore, the longitudinal design of the study allowed us to analyze the correlation between exhaled VOCs analysis and the variation of the 6MWD.

## Conclusion

Exhaled VOCs analysis using electronic nose may be used for a better characterization of COPD patients' functional status. Considering that exhaled breath collection and analysis has a very low cost, does not require a long and straight corridor and is suitable for all patients, even for those who are unable to perform a 6MWT or a spirometry, it represents a promising technique in the COPD functional status assessment.

## Acknowledgments

The authors would like to thank Dr Federica Sabato, Dr Veronica Adiletta and Dr Leonardo Grisafi for their help in data collection. This study was funded by an unrestricted grant from Fondazione Roma, Italy.

## Author contributions

PF, CP and RAI participated in planning the study. PF and CP performed data analyses. All authors participated in data collection, revised the final version of the manuscript and gave their consent to publication.

## Disclosure

The authors GP, MS and RAI are holders of the Pneumopipe® patent. The other authors report no conflicts of interests in this work.

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