

ORIGINAL ARTICLE

External validation of exhaled breath profiling using an electronic nose in the discrimination of asthma with fixed airways obstruction and chronic obstructive pulmonary disease

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Summary

Background Fixed airflow limitation can be found both in asthma and chronic obstructive pulmonary disease (COPD), posing a day-to-day diagnostic challenge.

Objective We aimed to determine the external validity of metabolomic analysis of exhaled air by electronic nose for distinguishing asthma and COPD in patients with fixed airways obstruction.

Methods One hundred patients were included in a cross-sectional design: 60 asthma patients: 21 with fixed airways obstruction (fixed asthma), 39 with reversible airways obstruction (classic asthma) and 40 COPD patients (GOLD stages II–III). Standardized sampling of exhaled breath was performed and volatile organic compounds were captured using an electronic nose resulting in breathprints. External validity in newly recruited patients (validation sets) was tested using a previous and independent training set. Breathprints were analysed by principal component and canonical discriminant analysis and area under the curve (AUC) of receiver operating characteristic curves.

Results External validity of breathprints showed 88% accuracy for distinguishing fixed asthma from COPD (AUC 0.95, 95% CI 0.84–1.00, sensitivity 85%, specificity 90%) and 83% for classic asthma (AUC 0.93, 95% CI 0.87–1.00, sensitivity 91%, specificity 90%) (both $P < 0.001$). Discriminative accuracy was not confounded by current smoking.

Conclusions and Clinical Relevance External validation of exhaled breath molecular profiling shows high accuracy in distinguishing asthma and COPD in newly recruited patients with fixed airways obstruction. Exhaled air analysis may therefore reduce misdiagnosis in obstructive airways diseases, potentially leading to more appropriate management.

Keywords asthma, COPD, diagnostic accuracy, electronic nose, exhaled breath analysis, fixed airways obstruction, metabolomics, persistent airflow limitation, volatile organic compounds

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Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are common respiratory diseases characterized by airways obstruction. Typically, the airways obstruction in asthma is reversible and variable, whereas in COPD such reversibility is absent or incomplete and the obstruction is often progressive [1, 2]. However, a proportion of asthma patients develop fixed airflow limitation and similar symptoms as COPD [3–7]. This poses a regular diagnostic dilemma, especially in elderly patients [4, 5, 8, 9]. More

importantly, following adequate diagnostics, the therapeutic strategies in these 'overlap patients' should be tailored towards the underlying disease (asthma vs. COPD) and inflammatory characteristics [4, 6, 10, 11].

The inflammatory profile of asthma patients with fixed airways obstruction is different from that of COPD patients, but is similar to that of asthmatics with reversible airways obstruction despite the functional resemblance to COPD [7, 12, 13]. The discrimination between asthma and COPD by using bronchial biopsies and induced sputum is burdensome, time consuming and expensive and cannot

be performed in a primary care setting. Thus, a simple and non-invasive approach providing immediate and integrative results about the phenotypic profile is needed.

Exhaled air molecular profiling may serve this purpose by performing integrative assessment of biomarker profile in patients with asthma and COPD [14–17]. Exhaled breath contains thousands of volatile organic compounds (VOCs) that are generated in conjunction with metabolic and inflammatory processes systemically and locally in the airways [18–21].

In an earlier proof of concept study, we showed that exhaled breath molecular profiling by electronic nose (e-nose) can discriminate well-defined training sets of 'classical' (reversible) asthma and COPD in an *internal* cross-validation analysis [15]. An e-nose consists of an array of sensors that can identify a mixture of VOCs, translating this into a breath fingerprint (breathprint) [22]. The next essential step in assessing the diagnostic accuracy of this non-invasive tool is the *external* validation in clinical practice: to assess the diagnostic accuracy of the test in new groups of patients (validation sets), following the STARD guidelines [23]. The eventual clinical value of the diagnostic test should be assessed in difficult to diagnose groups of patients, such as asthma and COPD patients with similar degrees of fixed airways obstruction [3–7].

Therefore, we hypothesized that exhaled breath molecular profiling by e-nose can correctly discriminate between asthma and COPD even in the presence of similar degrees of airflow limitation. The primary aim of the study was to test this hypothesis in a validation set of newly recruited asthmatics with fixed airways obstruction and COPD patients. The secondary aim was to compare asthmatics with classical disease and COPD patients as the gold standard external validation. Additionally, we explored whether the outcome was influenced by current smoking and whether fixed and classic asthma could also be discriminated based on exhaled breathprints.

Methods

Subjects

The study comprised a total of 100 patients with an established diagnosis of asthma or COPD, who were recruited in Pulmonology outpatient clinics of the three participating centres between August 2007 and March 2010. This included 21 asthma patients with fixed airways obstruction (fixed asthma), 39 asthma patients with reversible airways obstruction (classic asthma) [1], and 40 COPD patients GOLD stages II–III [2].

Fixed asthma was defined by: episodic chest symptoms, smoking history <10 pack-years, a doctor's diagnosis of asthma based on reversibility in forced expiratory volume in 1 s (FEV₁) of $\geq 12\%$ pred or 200 mL after 400 µg salbutamol or airway hyperresponsiveness (PC₂₀

methacholine or histamine <8 mg/mL) in the past, and postbronchodilator (400 µg salbutamol) FEV₁/forced vital capacity (FVC) ratio <0.70 regardless of FEV₁ changes after short-acting β_2 -agonists. Classic asthma was defined by: episodic chest symptoms, smoking history <10 pack-years, reversibility in FEV₁ of $\geq 12\%$ pred or 200 mL after 400 µg salbutamol, airway hyperresponsiveness (PC₂₀ methacholine or histamine <8 mg/mL) and postbronchodilator FEV₁/FVC ratio ≥ 0.70 [1]. Inclusion criteria for COPD were: symptoms of dyspnoea, chronic cough and/or sputum production, smoking history ≥ 15 pack-years, postbronchodilator FEV₁ between 30% and 80% of predicted, and postbronchodilator FEV₁/FVC ratio <0.70 [2].

Patients were seen in a stable condition and were excluded in case of any other pulmonary disease than asthma or COPD, current exacerbation, current cancer, cardiovascular disease, systemic infection and pregnancy.

The patients used for the *validation sets* were newly recruited from a different hospital than those in the *training set*, who were derived from a fully independent study [15] according to STARD guidelines [23]. Twenty of 30 COPD patients from the training set in the previous study were selected for the training set in this study based on matching of FEV₁, FEV₁/FVC, age and gender with the fixed asthma validation set. All 20 patients with classic asthma from the previous study were used for the training set in this study.

The study was approved by the Medical Ethical Committee of the participating centres, and all patients gave their written informed consent. The study was registered in the Netherlands Trial Register, www.trialregister.nl under NTR 1282. The study was conducted according to the declaration of Helsinki.

Design

The study had a cross-sectional design. After screening for in- and exclusion criteria, the measurements were performed at a single visit. Before e-nose measurement, patients were asked to refrain from eating, drinking and smoking for at least 2 h and to stop any inhalation medication and oral corticosteroids, antihistamines, and leukotriene receptor antagonists for at least 12 h to avoid interference on exhaled breath molecular profiles. After 5–10 min of resting, exhaled breath was collected and sampled in duplicate by an electronic nose, followed by the other lung function measurements. Patients in the training set were all measured in a different hospital than patients in the validation sets. Asthma and COPD patients were recruited in random order, and all patients were measured in the same rooms at the three study sites.

Measurements

Symptoms. Symptoms of asthma and COPD were measured by a validated questionnaire [24].

Lung function. Spirometry (MasterscreenPneumo, Jaeger, Würzburg, Germany) was performed by trained lung function technicians according to the latest ERS recommendations [25]. FEV₁ and FVC were measured before and after 400 µg of inhaled salbutamol. Airway hyperresponsiveness was assessed by histamine or methacholine challenge testing using the standardized tidal breathing method [26]. Diffusion capacity for carbon monoxide was measured using the single breath method according to the recommendations, corrected for haemoglobin level [27].

Allergy testing. Allergy testing was performed using a skin prick test to 12 common airborne allergens (ALK-Abello, Hørsholm, Denmark) or RAST. Atopy was indicated as a > 3 mm weal to one or more allergens, or positive RAST.

Breath collection. Exhaled breath analysis was performed by a validated method as described previously [15, 16]. Patients breathed normally for 5 min through a three-way non-re-breathing valve with a VOC filter (A2, North Safety, Middelburg, the Netherlands) at the inspiratory port and a silica filter at the expiratory port. After a maximal deep inspiration, patients exhaled a single vital capacity volume into a 10 L Tedlar bag connected to the expiratory port and silica reservoir.

Electronic nose. We used the Cyranose 320 electronic nose (Smiths Detection, Pasadena, CA, USA). The Tedlar bag was connected to the e-nose and sampled within 10 min. Sampling of the exhaled air was done in parallel to a second Tedlar bag filled with VOC-filtered room air for comparison for the duration of 1 min. The changes in electrical resistance of the 32 polymer sensors on binding to the VOCs present in exhaled breath constitute the raw data [28], and were used for further analysis with offline pattern recognition software [29]. Breath samples were measured in duplicate, and every very first measurement in each session was excluded from analysis as recommended by the e-nose manufacturer due to deviant sensor deflections (first sniff effect).

Statistical analysis

SPSS (version 18.0) and Graphpad Prism (version 5.01) were used for data analysis.

External validity was assessed (1) to discriminate fixed asthma and COPD, (2) to discriminate classic asthma and COPD, (3) to discriminate asthma and COPD in current non-smokers only and (4) to discriminate fixed and classic asthma. E-nose raw data (change in resistance of sensors) were restructured by principal component analysis from the 32 sensors to four principal components (PCs) that captured 98% of the variance in the dataset. PCs were constructed for all subjects, based on data from subjects in the training set from the previous independent study [15].

Next, the three PCs that discriminated between asthma and COPD were used for further pattern recognition analysis and are hereafter referred to as 'the breathprint'. Linear canonical discriminant analysis was performed on the PCs for classification of subjects in a categorical way, resulting in accuracy values (percent of correctly classified patients) for discrimination between asthma and COPD in the validation sets (external validity). The training set consisted of patients with COPD and patients with classic asthma, and the independent validation sets consisted of (1) patients with fixed asthma and COPD and (2) patients with classic asthma and COPD. Subsequently, the discriminant functions resulting from the linear canonical discriminant analysis were used to construct receiver operating characteristic (ROC) curves. Optimal sensitivity and specificity were determined, and positive (LR+) and negative likelihood ratios (LR-) were calculated. This procedure was repeated on current non-smokers only, to eliminate possible influence of current smoking on the breathprints.

The sample size was based on our aim to limit the standard error of the estimated diagnostic measures (sensitivity, specificity) to 6% at most. In our pilot project, we found that the diagnostic measures were all >85% accurate and assuming such values in the present project as well, a sample size of 20 patients per group sufficed.

A 10-fold bootstrapping procedure combined with cross-validation was performed to test the reproducibility of the model (see supporting information). Additional analyses were performed to assess possible influence of different types of inhalation medication on the diagnostic accuracies (see supporting information).

Results

A total of 100 subjects with asthma or COPD, aged 18–87 years, were included in the study. The subject characteristics are described in Table 1. As expected, asthma patients with reversible airways obstruction were younger, had higher pre- and postbronchodilator FEV₁ (L and %pred) and a higher FEV₁/FVC ratio than patients with COPD and asthma with fixed airways obstruction ($P < 0.05$). No significant differences were found for lung function parameters between COPD and asthma with fixed airways obstruction, except for diffusion capacity (KCO) that was lower in COPD.

External validity for fixed asthma vs. chronic obstructive pulmonary disease

Newly recruited patients with fixed asthma and COPD could be adequately discriminated, when using the previous training set model based on three breathprint PCs (PC1, 2 and 4) from patients with classic disease, with an accuracy of 88% ($P < 0.001$) (Fig. 1a). The AUC for

Table 1. Subject characteristics

	Asthma, fixed (<i>n</i> = 21)	Asthma, reversible (<i>n</i> = 39)	COPD (<i>n</i> = 40)
Male [<i>N</i> (%)]	12 (57%)*	12 (31%)	21 (52%)
Age [years (range)]	64 (43–76)	35 (18–68)*	63 (49–87)
FEV ₁ , prebronchodilator (L)	1.60 (0.51)	3.23 (0.79)*	1.35 (0.59)
FEV ₁ , prebronchodilator (%pred)	56 (13)	92 (17)*	49 (16)
FEV ₁ , postbronchodilator (L)	1.72 (0.57)	3.51 (0.79)*	1.62 (0.66)
FEV ₁ , postbronchodilator (%pred)	60 (14)	100 (15)*	56 (15)
FEV ₁ /FVC (%)	0.47 (0.10)	0.78 (0.08)*	0.43 (0.11)
KCO (%pred)	99 (17)	98 (11)	68 (21)*
Smoking history [pack-years (range)]	1.5 (0–8)	0.5 (0–5)	42 (15–89)*
Current smoking, yes/no (%)	5 (24%)/16 (76%)	0 (0%)/39 [†] (100%)*	13 (33%)/27 [‡] (67%)
ICS, yes/no (%)	21 (100%)/0 (0%)*	30 (77%)/9 (23%)*	21 (52%)/19 (48%)*
OCS, yes/no (%)	5 (24%)/16 (76%)*	0 (0%)/39 (100%)	0 (0%)/40 (100%)
Atopy, yes/no (%)	15 (72%)/6 (28%)	31 (79%)/8 (21%)	6 (15%)/34 (85%)*

Data are expressed as mean (SD).

*Significant difference between groups.

[†]Four ex-smokers.

[‡]All ex-smokers.

FEV₁, forced expiratory volume in 1 s; %pred, percentage of predicted; FVC, forced vital capacity; KCO, corrected carbon monoxide transfer coefficient; ICS, inhaled corticosteroids; OCS, oral corticosteroids.

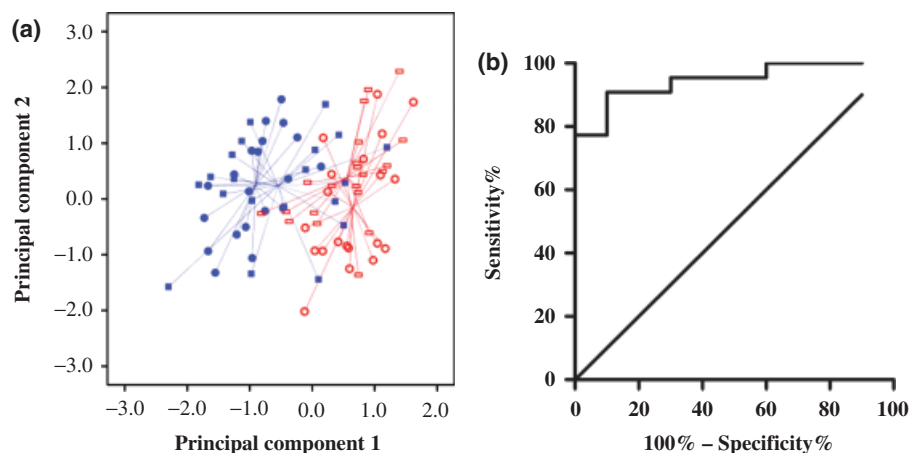


Fig. 1. External validity for asthma vs. chronic obstructive pulmonary disease (COPD): fixed airways obstruction. (a) Two-dimensional principal component plot showing the discrimination of breathprints between patients with fixed asthma (open markers) and COPD (closed markers) along two out of three discriminative composite principal components. Circles represent training set, rectangles validation set. Accuracy 88%, $P < 0.001$. (b) Receiver operator characteristic (ROC) curve with line of identity of the breathprint discriminant function (representing PC1, 2 and 4), predictive for the differential diagnosis of asthma and COPD in the validation set of asthma and COPD patients with a similar degree of fixed airways obstruction (AUC 0.95). AUC, area under the curve.

external validity reached 0.95 (95% CI 0.87–1.00) (Fig. 1b), with sensitivity 91%, specificity 90%, LR+ 9.1 and LR– 0.1. Results of all validity tests are summarized in Table 2. See supporting information for results of all bootstrapping procedures and the influence of inhalation medication on exhaled breathprints.

External validity for classic asthma vs. chronic obstructive pulmonary disease

Newly recruited patients with classic asthma and COPD could also be adequately discriminated using the training

set model, with an accuracy of 83% ($P = 0.002$) (Fig. 2a). ROC analysis resulted in an AUC for external validity of 0.93 (95% CI 0.84–1.00) (Fig. 2b) with optimal sensitivity of 85%, specificity 90%, LR+ 8.5 and LR– 0.2.

External validity for asthma vs. chronic obstructive pulmonary disease in current non-smokers only

In non-smokers, a 90% ($P < 0.001$) accuracy was reached for the discrimination between asthma and COPD breathprints, regardless the presence of fixed airways obstruction. ROC

Table 2. Validation results for fixed vs. classic asthma vs. COPD

Set	Acc (%)	P-value	AUC	95% CI	Sens (%)	Spec (%)	LR+	LR–	PPV	NPV
Fixed asthma vs. COPD	88	<0.001	0.95	0.87–1.00	91	90	9.1	0.1	0.9	0.9
Fixed asthma vs. classic asthma	58	0.225	0.68	0.50–0.85	60	67	1.8	0.6	0.6	0.6
Classic asthma vs. COPD	83	0.002	0.93	0.84–1.00	85	90	8.5	0.2	0.9	0.9
Ex-/non-smoking asthma vs. COPD	90	<0.001	0.96	0.90–1.00	85	100	∞	0.002	1.0	0.9

Validation results of diagnosing fixed and classic asthma and COPD by breath analysis using the e-nose in the external validation (fixed asthma vs. COPD; fixed asthma vs. classic asthma; classic asthma vs. COPD; ex-/non-smoking asthma vs. COPD).

COPD, chronic obstructive pulmonary disease; Acc, accuracy; AUC, area under the curve; 95% CI, 95% confidence interval; Sens, sensitivity; Spec, specificity; LR+, positive likelihood ratio; LR–, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

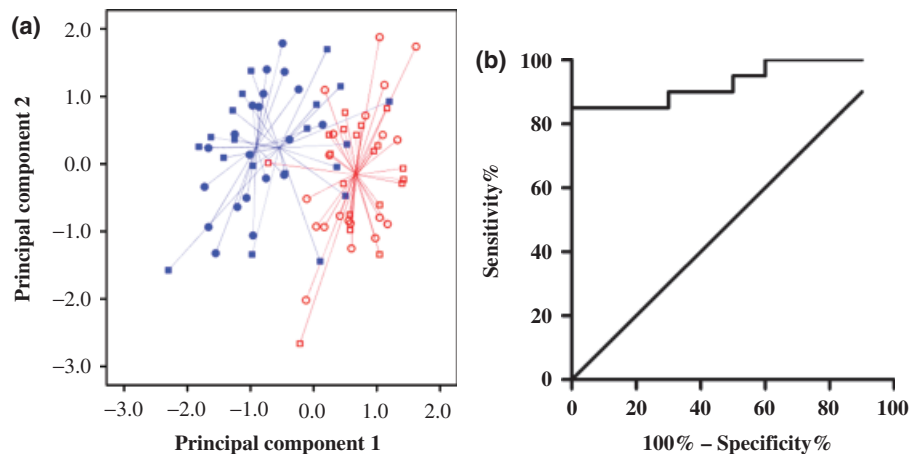


Fig. 2. External validity for asthma and chronic obstructive pulmonary disease (COPD): classic asthma vs. COPD. (a) Two-dimensional principal component plot showing the discrimination of breathprints between patients with asthma (open markers) with reversible airways obstruction (classic asthma) (circles; training set and squares; validation set) and COPD (closed markers; circles; training set and squares; validation set) along two out of three discriminative composite principal components. Accuracy 83%, $P = 0.002$. (b) Receiver operator characteristic (ROC) curve with line of identity of the breathprint discriminant function (representing PC1, 2 and 4), predictive for the differential diagnosis of asthma and COPD in the validation set of classic asthma and COPD patients (AUC 0.93). AUC, area under the curve.

analysis showed an AUC of 0.96 (95% CI 0.90–1.00), with sensitivity 85% and specificity 100%.

External validity for fixed asthma vs. classic asthma

Patients with fixed asthma could not be distinguished from patients with classic asthma. The accuracy for discrimination between fixed and classic asthma reached 58% ($P = 0.225$). The AUC of the ROC curve reached 0.68 (95% CI 0.50–0.85) with optimal sensitivity of 60%, specificity 67%, LR+ 1.8 and LR– 0.6.

Discussion

This external validation study shows that patients with fixed asthma and COPD can be adequately discriminated using exhaled breath molecular patterns based on a previous training set. Not only could fixed asthma be distinguished from COPD patients with a similar degree of fixed airways obstruction, but also newly recruited patients

with classic asthma and COPD could be discriminated as the gold standard external validation of diagnostic tests. Notably, the discrimination between the groups was manifest regardless of current smoking and not influenced by the use of inhalation medication. Our previous findings indicated that exhaled molecular patterns differ between the two obstructive airways diseases. This is now extended by external validation involving newly recruited patients, including groups with overlapping spirometry. Our results may therefore facilitate future diagnostic classification and therapeutic decisions.

This is the first study to assess the external validity of breath testing in patients with a diagnosis of asthma and COPD, including asthmatics with persistent airflow limitation that pose a regular diagnostic dilemma in day-to-day clinical care. Notably, the breath test was accurate in distinguishing asthma patients from COPD patients irrespective of spirometric values.

Previously, breathprint analysis has been shown to successfully discriminate asthmatics from controls and

from patients with COPD in three independent studies based on internal validation only [15–17]. The present study makes the mandatory next steps, by demonstrating the external validity as required by STARD guidelines for the assessment of diagnostic accuracy including clinically challenging groups such as patients with fixed asthma [23].

Asthma and COPD are inflammatory airways diseases, but differ in inflammatory profile as assessed by induced sputum and bronchial biopsies [7, 12, 13, 30]. In asthma, airways inflammation is characterized by eosinophils, mast cells and CD4⁺ T cells and an increase in Th2 cytokines [31]. Airways inflammation in COPD is predominated by CD8⁺ T cells, neutrophils and macrophages [32]. VOCs are produced as a result of metabolic- or disease-associated processes [19, 33]. Recent data from our group show an association between mediators of eosinophilic and neutrophilic inflammation in sputum of COPD patients [34]. Therefore, the exhaled breathprint is likely to reflect the inflammatory profile in the airways, providing different fingerprints for both diseases.

E-nose technology is not suitable for determining individual VOCs and their concentration [22]. It can rather be described as a high-throughput 'omics' technique such as metabolomics. The present study was not designed to specify which VOCs are distinguishing between asthma and COPD, but to address the differential diagnostic problem in a standard and clinically applicable way. For detailed analysis on the VOCs driving the signal, gas chromatography and mass spectrometry (GC–MS) analysis should be applied [14, 18, 35]. These studies show that multiple VOCs contribute to the discriminatory signals between airway diseases and controls. The clinical advantage of e-nose technology is that it is non-invasive and real-time [21]. It uses a composite objective measure of metabolic/inflammatory activity by assessing the whole spectrum of VOCs in breath. This is combined with fast pattern recognition in a hand-held device potentially making future on-the-spot diagnosis a realistic option [21].

Asthma and COPD require distinct therapeutic approaches [1, 2]. Nevertheless, clinicians are regularly facing patients who feature symptoms of asthma together with fixed airways obstruction. Such overlap between asthma and COPD occurs at all levels of medical care, actually representing the majority of patients with airways obstruction at elderly age [4, 5]. Traditional assessment by symptoms and lung function measurements cannot adequately diagnose these patients [4, 6]. Even though inflammatory phenotyping can certainly assist a correct diagnosis [7, 12] and management of asthma or COPD [10, 11] the progression to fixed airways obstruction appears to share a common inflammatory profile in induced sputum [36]. Therefore, it is not only mandatory to validate novel procedures for establishing the traditional diagnoses of asthma and COPD, but also to identify the accompanying

inflammatory and metabolomic profiles. This will eventually allow appropriate treatment regimens in patients with overlapping features of asthma and COPD. Our findings indicate that high-dimensional, molecular pattern recognition in exhaled air may be used as a (differential) diagnostic procedure. It remains to be established whether this also holds for further subphenotyping, and whether similar accuracies can be reached in diagnosing patients with an 'intention to diagnose' [37] in daily clinical practice.

Several measures were taken for quality control of the data. First, exhaled breath testing by e-nose depends critically on the method of breath collection and sampling. We used a validated method with filtering of inspiratory air, drying of exhaled air and minimizing influence of environmental VOCs, contamination of material and changes in VOC pattern by expiratory flow rate and volume [16]. Furthermore, e-nose measurements are highly reproducible and show good inter-device and inter-day compatibility in controls [15]. Nevertheless, the unmet need of mapping between individual e-noses is still a limitation of these devices [38]. Omics-based diagnostic techniques such as e-nose require extensive external validation of the diagnostic profile as opposed to traditional validation procedures for single markers based on determination of ranges in normals and disease [29]. Reassuringly, e-nose breathprints showed no influence of age and smoking history in healthy controls [15, 16]. This can facilitate future application of this technique.

Second, all of our subjects were well characterized according to international guidelines [1, 2]. Nevertheless, there were some differences between the groups with respect to age and lung function parameters, possibly introducing bias by measuring an age- and obstruction-dependent difference in breathprints. As the classic phenotypes of asthma and COPD differ regarding these parameters, this was not unexpected. Again, in previous studies we observed that age and FEV₁ *per se* did not influence the exhaled breathprint [15, 16, 39]. This is confirmed by the present data demonstrating that relatively older asthmatics with fixed airways obstruction were classified similar to (young) classic asthmatics and not as (older) COPD patients. Current smoking and the use of inhalation medication may have influenced the observed VOC profiles, despite stopping smoking at least 2 h and inhalation medication 12 h before testing. However, in the current and our previous study we showed that the discriminatory power of the e-nose was maintained when correcting for current smoking and the use of ICS and tiotropium (see supporting information) [15]. This suggests that the observed differences in breathprints are disease- and not smoking- or medication-dependent. Finally, breathprints did not differ between asthma patients with and without persistent airways obstruction. This suggests that the metabolomic signal in exhaled air is rather specific for the disease.

Third, we performed bootstrapping cross-validation analyses using separate independent training and validation sets (see supporting information) in order to assess the reproducibility of the validation procedure. This showed highly reproducible accuracies for discriminating asthma from COPD, both in fixed and classic disease. As asthma with fixed airways obstruction can be especially difficult to distinguish from COPD, this group was not added to any training set to ensure genuine and real life testing of the model for asthma in these patients [23, 37]. Notably, our patients were selected on not having a mixed phenotype (both asthma and COPD). Therefore, it remains to be established whether exhaled markers can be used to (sub)phenotype this clinically relevant group.

In conclusion, we have shown that external validation of exhaled breath profiling leads to adequate discrimination between asthma with persistent airflow limitation and COPD. Furthermore, patients with classic asthma were

correctly separated from those with COPD (which was not confounded by current smoking), showing the gold standard external validity of this diagnostic test. Exhaled molecular profiling may therefore decrease misdiagnosis among obstructive airway diseases, thereby facilitating decisions towards the right therapy for the right patient.

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All authors declare that there are no competing interests.

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Supporting Information

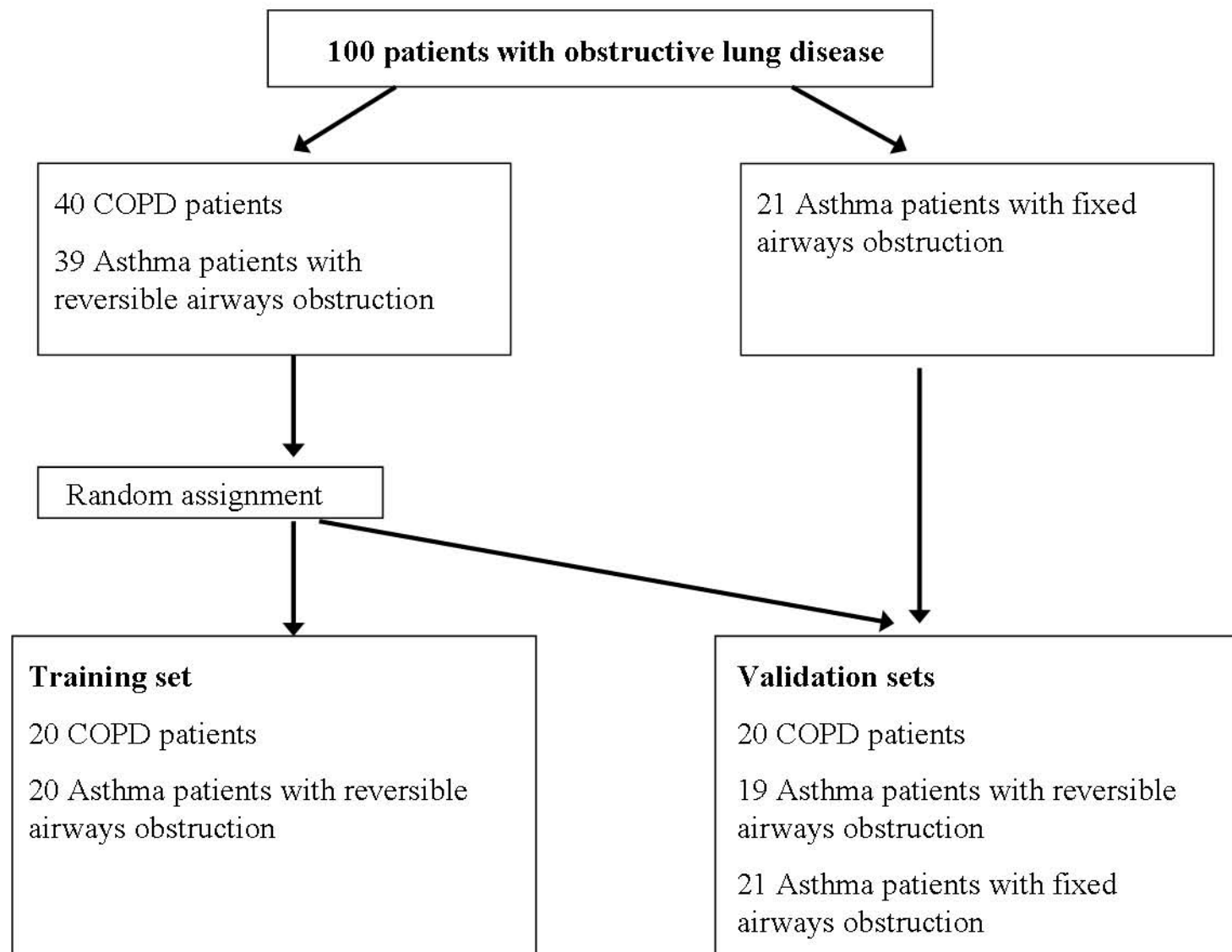
Additional Supporting Information may be found in the online version of this article:

Figure S1. Bootstrapping procedure.

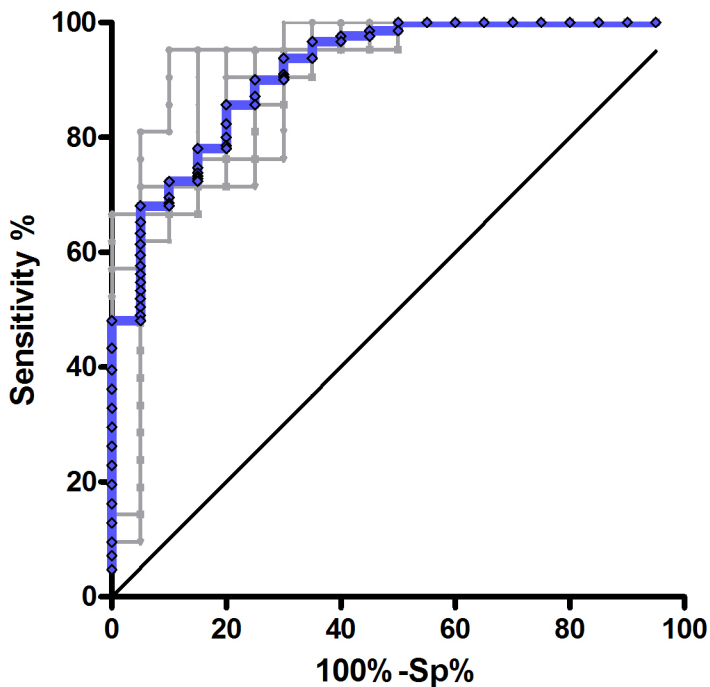
Figure S2. Reproducibility for fixed airways obstruction.

Figure S3. Reproducibility for classic asthma and COPD.

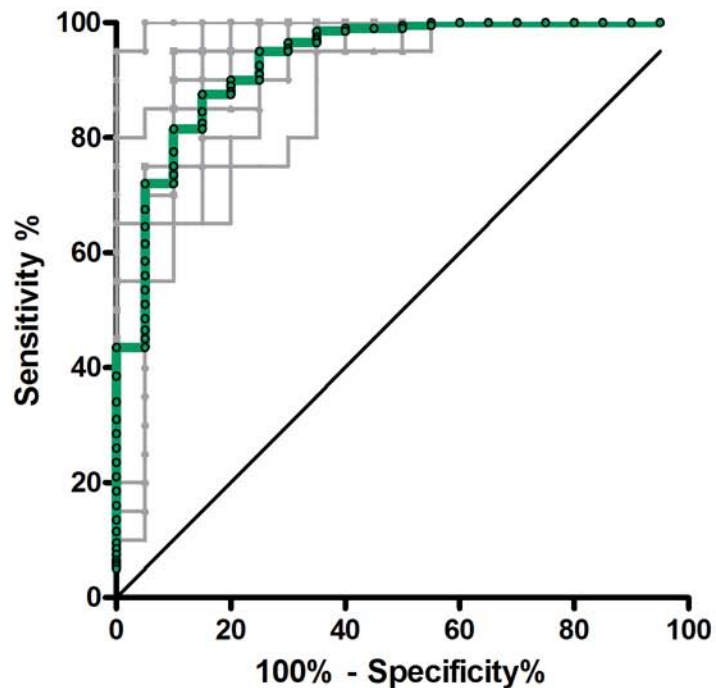
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Fixed airways obstruction



a. Internal validity: training sets



b. External validity: validation sets

