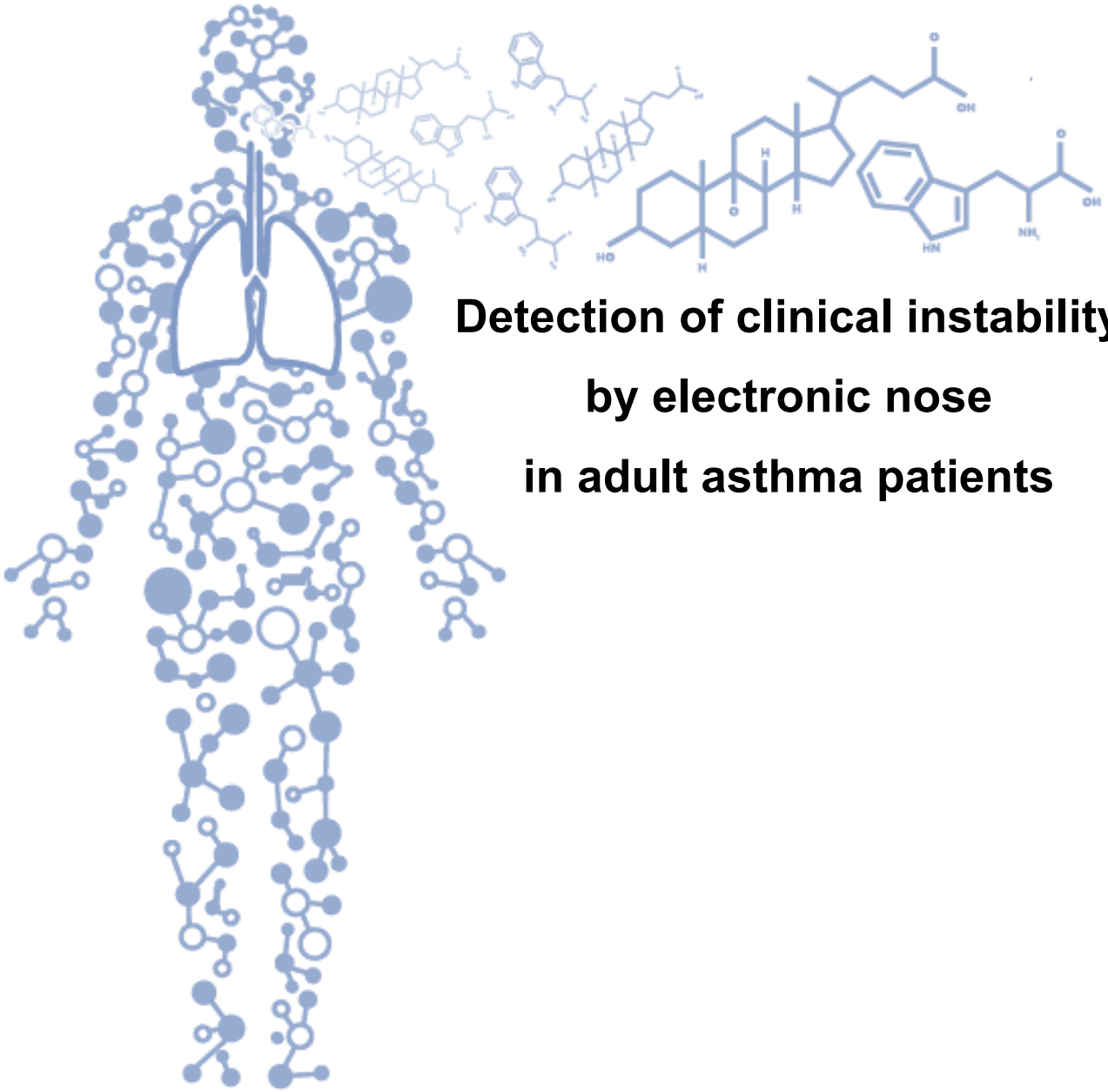

Detection of clinical instability by electronic nose in adult asthma patients

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LIST OF ABBREVATIONS

ACQ-6	6-item Asthma Control Questionnaire
ANOVA	analysis of variance
AUC	area under the curve
AUROC	area under the receiving operating characteristic
BMI	body mass index
COPD	chronic obstructive pulmonary disease
eNose	electronic nose
FeNo	fraction of exhaled nitric oxide
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GC-MS	gas chromatography – mass spectrometry
GINA	Global Initiative for Asthma
ICS	inhaled corticosteroid(s)
IL	interleukin
IQR	interquartile range
LABA	long-acting beta-2 agonist
LDA	linear discriminant analysis
MOS	metal oxide semiconductor
OCS	oral corticosteroid(s)
pb	post-bronchodilator
PCA	principal component analysis
PC	principal component
PEF	peak expiratory flow
ROC	receiver operating characteristic
SD	standard deviation
SABA	short-acting beta-2 agonist
VOC	volatile organic compound

ABSTRACT

English version

Asthma is a chronic respiratory disease characterized by reversible bronchial obstruction and symptoms including dyspnoea, wheeze, and coughing. Asthma management aims to achieve good asthma control; not only by minimalizing the symptoms, but also the risk of exacerbations. Measuring exhaled breath using an electronic nose (eNose) could be a possible tool for monitoring asthma and predicting the occurrence of exacerbations. This research aimed to evaluate whether an eNose could discriminate between clinically stable and clinically unstable adult asthma patients. The distinction in (in)stability was based on the presence of a recent exacerbation (less than three months ago), the level of asthma control, and the level of treatment. In addition, it was determined if the eNose signals could be used to cluster asthma patients based on their asthma control. 362 patients were included, that were randomly divided in a training (n = 260) and a validation (n = 102) set. The eNose output was analysed by means of principal component analysis (PCA). The first three principal components were subsequently used for linear discriminant analysis (LDA). This showed that asthma patients could be discriminated based on the recent occurrence of an exacerbation (area under the receiving operating characteristic curve [AUROC] = 0.687 for the training set; AUROC = 0.656 for the validation set). Additionally, through hierarchical clustering two clusters could be described, that differed in the occurrence of recent exacerbations ($P<0.001$), and the treatment of these exacerbations ($P<0.001$ for treatment with oral corticosteroids, and $P=0.02$ for treatment with antibiotics). This research showed that measurement of exhaled air with an eNose can make a distinction between clinically stable and clinically unstable asthma patients. This conclusion supports the use of eNose technology in clinical practice, for monitoring asthma and possibly preventing asthma exacerbations.

Dutch version

Astma is een chronische longaandoening die gekenmerkt wordt door reversibele bronchusobstructie en symptomen als benauwdheid, piepen, en hoesten. Het doel van de behandeling is het bereiken van goede astmacontrole. Hieronder valt niet alleen het minimaliseren van klachten, maar ook het risico op exacerbaties. Het meten van uitgeademde lucht met behulp van een *electronic nose* (eNose) zou een mogelijk instrument kunnen zijn om astma te monitoren, en het optreden van exacerbaties te kunnen voorspellen. Dit onderzoek had als doel om te bepalen of een eNose onderscheid kan tussen klinisch stabiele en klinisch instabiele volwassen astmapatiënten. Dit onderscheid in (in)stabiliteit werd gemaakt op basis van de aanwezigheid van een recente exacerbatie (minder dan drie maanden geleden), de mate van astmacontrole, en de medicamenteuze behandeling. Daarnaast werd bepaald of de eNose-signalen gebruikt konden worden om de astmapatiënten te clusteren op basis van hun astmacontrole. Er werden 362 patiënten geïncludeerd, die willekeurig werden verdeeld in een trainingset (n = 260) en een validatieset (n = 102). De output van de eNose werd geanalyseerd door middel van *principal*

component analysis (PCA). De eerste drie *principal components* werden vervolgens gebruikt voor *linear discriminant analysis* (LDA). Hieruit bleek dat er onderscheid gemaakt kon worden tussen astmapatiënten die wel of niet recent een exacerbatie hadden gehad (*area under the receiving operating characteristic curve* [AUROC] = 0.687 voor de trainingset; AUROC = 0.656 voor de validatieset). Daarnaast konden door middel van *hierarchical clustering* twee clusters worden beschreven, die verschilden in het optreden van exacerbaties ($P<0.001$), en de behandeling van de exacerbaties ($P<0.001$ voor behandeling met orale corticosteroïden, en $P=0.02$ voor behandeling met antibiotica). Dit onderzoek toonde aan dat meting van uitgeademde lucht met een eNose onderscheid kan maken tussen klinisch stabiele en klinisch instabiele astmapatiënten. Deze conclusie ondersteunt het gebruik van eNose-technologie in de klinische praktijk voor het monitoren van astma en het mogelijk kunnen voorkomen van exacerbaties.

INTRODUCTION

Asthma is a chronic disease characterised by variable expiratory airflow limitation and respiratory symptoms. These symptoms include wheeze, dyspnoea, chest tightness and cough [1]. International guidelines established two aims for management of asthma: (1) achieving good symptom control and (2) minimizing future risks [2]. Regarding pharmacological management, asthma is treated by a combination of two categories of drugs, namely controller and reliever medications. All asthma patients should be in possession of reliever, an asthma inhaler used for quick symptom relief. This includes a short-acting β_2 agonist (SABA) inhaler (commonly salbutamol), or an inhaler containing an ICS (inhaled corticosteroid) and formoterol [3]. Maintenance therapy usually consists of ICS, that can be combined with a long-acting β_2 agonist (LABA) in case of persistent moderate and severe asthma [4]. In addition, in recent years advances have been made in treatment of severe asthma with biological therapies [5].

One of the major possible future risks is the occurrence of exacerbations. These flare-ups are episodes of a progressive increase in symptoms and a progressive decrease in lung function [1]. Treatment usually consists of a short course of a systemic corticosteroid (OCS), and/or antibiotics when a respiratory infection is suspected [6]. Asthma exacerbations account for significant disease morbidity and health care costs [7]. Furthermore, patients with frequent exacerbations have a higher annual decline in lung function than non-frequent exacerbators [8]. Preventing exacerbations can be tried by identifying patients at risk. Risk factors for asthma exacerbations include poor symptom control, inadequate use of ICS, current smoking, obesity, and at least one severe exacerbation in the previous year [6,9–12].

Asthma is a heterogeneous condition: the clinical presentation differs between patients with respect to underlying pathogenic mechanism, treatment response, and quality of life [4]. For this reason, asthma is often classified in different phenotypes by using biomarkers [13]. However, because of the complexity of asthma, singular markers are probably not sufficient to be associated with a certain phenotype. This had led to the development of metabolics in exhaled air, or ‘breathomics’ [14]. This method is non-invasive, which provides an advantage over asthma biomarkers currently used in clinical practice, that need the be collected from blood, sputum, or biopsies, or via bronchoalveolar lavage [15].

Breathomics focuses on collecting, identifying and quantifying volatile organic compounds (VOCs) in exhaled air [16]. VOCs are organic molecules derived from exogenous and endogenous sources. The aim of breathomics is to find patterns of these compounds that are related to pathologic metabolic processes. There are two common approaches for analysis of VOCs [17]. Gas chromatography coupled to mass spectrometry (GC-MS) aims to identify individual VOCs. This makes the method particularly useful for pathophysiologic research [18]. Electronic noses (eNoses) on the other hand, measure the whole spectrum of VOCs in a sample, using multiple cross-reactive sensor arrays. The output of an eNose is called a ‘breathprint’ [19]. Exhaled breath analysis via

eNose has been applied to several respiratory diseases, including asthma, chronic obstructive respiratory disease (COPD), and lung cancer [20].

In 2015, De Vries et al. [21] presented the SpiroNose®, in which eNose technology is integrated with routine measurement of lung function (spirometry). The SpiroNose® could adequately distinguish asthma patients from patients with COPD, patients with lung cancer, and healthy controls. In a second study, it was shown that phenotyping a combined sample of asthma and COPD patients using the SpiroNose® provided validated clusters that are determined by clinical and inflammatory characteristics. Measurements of exhaled breath with the SpiroNose® in these subjects have led to the development of a breathprint database, called BreathBase [22].

Currently, blood eosinophils and interleukin 6 (IL-6) seem to be predictive biomarkers for an exacerbation-prone asthma phenotype [23]. However, obtaining these biomarkers is invasive. Breathomics as a non-invasive biomarker could play a significant role in identification of patients at risk of an asthma exacerbation [24]. In COPD patients, it has been demonstrated that eNoses can identify exacerbations with good accuracy [25–27]. The study of Van Bragt et al [27] demonstrated an area under the curve (AUC) of 0.98, both in the training and validation set. Because of the similarities between asthma and COPD, we hypothesized that recent asthma exacerbations could likewise be identified with an eNose. The objective of this study was to examine if a breathprint made with the SpiroNose® can distinguish between clinically unstable and clinically stable asthma patients, where stability is defined on basis of (1) the presence/absence of exacerbations in the past three months as a primary objective, and based on the (2) level of asthma control and (3) level of asthma treatment as secondary objectives. In addition, cluster analysis was applied on the sensor values to explore if different phenotypes of clinical stability could be identified.

METHODS

Study population and design

For this observation cross-sectional study, demographic, clinical, and exhaled breath data from adult asthma patients included in the BreathCloud database were used. BreathCloud was a multicentre cross-sectional study across six different Dutch sites that included patients with a physician-diagnosed respiratory disease (asthma, COPD, and/or lung cancer) and healthy controls. Patients were recruited from general practices, and regional and academic hospitals between December 2015 and May 2017 during routine visits. The study data were collected in the BreathCloud database. The medical ethical review board of all participating sites provided a waiver for ethical approval given the non-invasive nature of the study. BreathCloud excluded patients in case of insulin-depended diabetes, respiratory infection, and pulmonary diseases other than asthma, COPD, and/or lung cancer. For the current study, patients younger than 18 years, patients with respiratory co-morbidities (COPD and/or lung cancer), and (ex-)smokers with ten or more pack-years were excluded from analysis.

Exhaled breath analysis

Breath analysis was performed with the SpiroNose® (figure 1a). This eNose contains seven unique metal oxide semiconductor (MOS) sensors. Each sensor is present four times, which gives a total of 28 sensors in the SpiroNose®. The sensors are grouped in eight sensor arrays. Four arrays are used as reference to monitor ambient VOCs air, and four arrays are used to monitor VOCs in exhaled breath. Before measurement, patients were asked to rinse their mouth three times with water. The set-up includes a viral and bacterial filter with a soft bite mouthpiece attached to de SpiroNose®, and patients wear a nose clamp during the measurement. The measurement was then started by performing five tidal breaths, followed by a single inspiratory capacity manoeuvre, a breath hold of five seconds, and slow maximal expiration towards residual volume (figure 1b) [21,22].

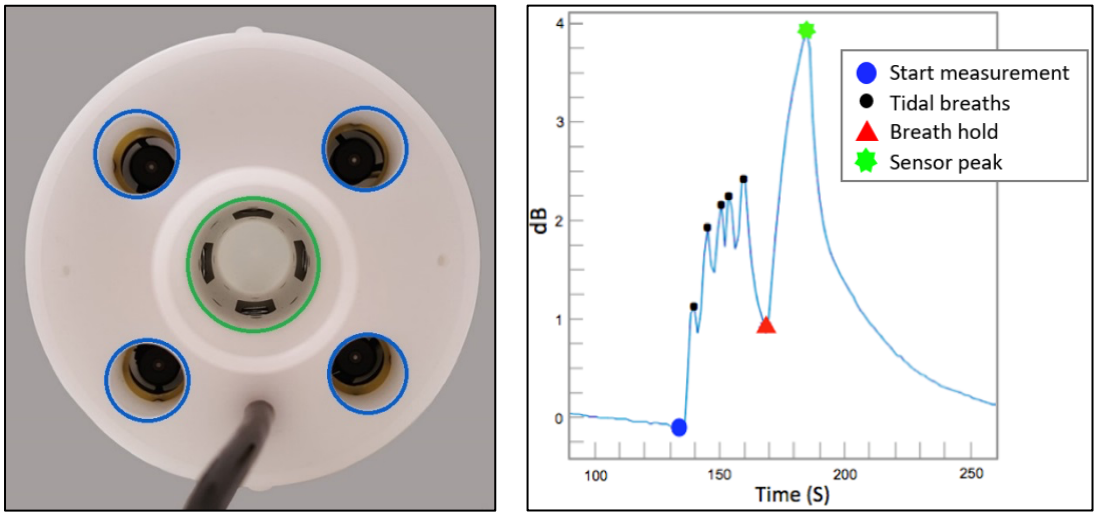

FIGURE 1. a) Front view of the SpiroNose®. Four central sensor arrays (green) monitor exhaled breath; four reference sensor arrays (blue) monitor environmental air. b) Example of processed data obtained during exhaled breath measurement. The manoeuvre consists of five tidal breaths (black dots), a single inspiratory capacity manoeuvre (between last black dot and red triangle), five seconds of holding breath (red triangle), and slow maximal expiration (between red triangle and green star). Adapted from [22].

Variables

Demographic variables included age, body mass index (BMI), and sex. Spirometry values (forced expiratory volume in 1 second [FEV_1], forced vital capacity [FVC], peak expiratory flow [PEF]), both pre- and post-bronchodilator, blood counts (eosinophils, neutrophils), and fraction of exhaled nitric oxide were obtained from (historical) data collected for routine clinical practice. Medication history was obtained from medical records as well. Clinical assessment of asthma control was made using the 6-item Asthma Control Questionnaire (ACQ-6). This validated questionnaire contains six questions about asthma symptoms (night-time waking, symptoms on waking, activity limitation, shortness of breath, wheeze, and use of rescue medication) over the past week that are scored by the patient. It is used to determine the efficacy of interventions on asthma control outcomes. A score lower or equal of 0.75 corresponds to controlled asthma, a score higher than 1.5 corresponds to uncontrolled asthma, and a score in between means the asthma is poorly controlled [28].

Patients were asked about their smoking habits. Finally, the presence of exacerbations in the preceding three months was explored.

Outcomes

For the primary outcome, clinical instability was determined by the occurrence of at least one asthma exacerbation in the preceding three months. An exacerbation was defined as an acute worsening of respiratory symptoms requiring a change in treatment (i.e. a short course of systemic corticosteroids and/or antibiotics) [29]. For the secondary outcomes, patients were categorised according to their level of treatment and level of symptom control. The level of therapy was defined according to the five treatment steps as described in the Global Initiative for Asthma (GINA) 2018 guideline [6]. Uncontrolled asthma is defined as an ACQ score >1.5 [30].

Data processing

An in-depth description of the pre-processing of the sensor data is published elsewhere [21]. In short, the highest peak of each sensor signal (figure 1b; green star) was selected and normalized with respect to sensor 2, the most stable sensor. Furthermore, the ratio between the peak value and the breath hold point (figure 1b; red triangle) was calculated for each sensor. Thus, for each subject 13 variables were available: six normalized peak values and seven ratios.

Statistical analysis

For comparison of baseline characteristics between groups, independent t-test, Mann-Whitney U-test, and Pearson's chi-squared test were used as appropriate. Statistical significance was predetermined as $P<0.05$. All available data were included in the analysis; no outliers were observed or excluded. For all patients, sensor data were available. However, for the other measurements, data was not always available for each patient.

Pre-processed sensor data were randomly split into approximately 70% for model building (training set) and 30% for validation (validation set). Data were restructured by principal component analysis (PCA) from the 13 variables to a non-predefined number of principal components (PCs) using the Kaiser criterion [31]. In the training set, unpaired t-test and ANOVA were used to select the PCs that are discriminative between groups. The selected PCs were included in a linear discriminant analysis (LDA) to categorise patients. This classification model was then repeated on the validation set. The discriminant functions were used to construct receiver operating characteristic (ROC) curves, of which the area under the curve (AUC) was determined, as a measure of the performance of the model.

Furthermore, eNose-driven hierarchical clustering was performed on all sensor data. As metric, squared Euclidian distance was used, in combination with Ward's minimum variance method as linkage criterion. To determine the number of clusters, the R package 'NbClust' was used [32].

Between-cluster comparisons were performed using independent t-test, Mann-Whitney U test, and Pearson's chi-squared test.

Statistical analysis was performed in SPSS (version 24.0), and RStudio (version 3.5.1) supported by the following packages: dendextend, factoextra, MASS, NbClust, prediction, and ROCR.

RESULTS

Baseline characteristics

In total, 362 asthma patients were included in the analysis, of which 61 (17%) had an asthma exacerbation in the three months prior to inclusion. Table 1 shows the baseline characteristics of the patients with versus without an asthma exacerbation. These two groups differed statistically significant in post-bronchodilator (pb) FEV_1, blood neutrophils, ACQ score, and treatment level. The asthma patients with a recent exacerbation had a lower $pbFEV_1$, higher blood neutrophils, a higher ACQ score, and more patients were treated according to a severe treatment level.

TABLE 1. Baseline characteristics of the asthma patients with and without an exacerbation in the three months prior to inclusion. Data are presented as %, n (%), mean±SD or median [IQR]. Between-group comparisons were performed using independent t-test, Mann-Whitney U test, and Pearson's chi-squared test. The significance level was set on P<0.05. Statistically significant P-values are printed in bold.

	Patients without an asthma exacerbation	***Patients with an asthma exacerbation***	***P-value***
Subjects, n	301	61	-
Age (years)	47.7±17.6	50.5±17.2	0.264
Male sex	37.2	27.9	0.165
BMI (kg/m^2)	27.7±6.5	27.6±5.9	0.896
$pbFEV_1$ (% predicted)	94 [81-105] n=220	87 [73-96] n=47	**0.039**
Smoking status: never / ex / current	71.1 / 21.3 / 7.6	72.1 / 27.9 / 0.0	0.059
Pack years	5.1±3.2 n=85	4.7±3.6 n=16	0.581
Blood eosinophils ($10^9/L$)	0.21 [0.08-0.41] n=199	0.21 [0.10-0.47] n=53	0.482
Blood neutrophils ($10^9/L$)	4.51 [3.48-6.14] n=194	5.41 [3.96-7.49] n=52	**0.044**
FeNO (ppb)	25 [13-42] n=152	25 [17-39] n=33	0.739
ACQ score	1.5 [0.9-2.4]	1.7 [0.6-2.6]	**0.040**
Uncontrolled asthma (ACQ score >1.5)	49.5	59.0	0.175
Treatment level: mild / moderate / severe	18.0 / 26.8 / 55.2 n = 261	6.7 / 16.7 / 76.7 n = 60	**0.008**

ACQ: Asthma Control Questionnaire; BMI: body mass index; FeNO: fraction of exhaled nitric oxide; $pbFEV_1$: post-bronchodilator forced expiratory volume in 1 s.

Patients were divided in a training set (n = 260) and a validation set (n = 102). Baseline characteristics of the two sets are shown in Table 2. No statistically significant differences existed between the sets regarding these patient characteristics.

TABLE 2. Baseline characteristics of the training and validation sets. Data are presented as %, mean±SD, or median [IQR. Between-group comparisons were performed using independent t-test, Mann-Whitney U test, and Pearson's chi-squared test. The significance level was set on P<0.05. Statistically significant P-values are printed in bold.

	Training set	***Validation set***	***P-value***
Subjects, n	260	102	-
Exacerbation ≤3 months, n	46 (17.7)	15 (14.7)	0.495
Age (years)	48.2±17.6	48.2±17.3	0.978
Male sex	37.7	30.4	0.192
BMI (kg/m^2)	27.6±6.4	27.8±6.2	0.738
pbFEV$_1$ (% predicted)	90 [79-104]	96 [85-105]	0.135
	n=197	n=70	
Smoking status: never / ex / current	71.5 / 21.2 / 7.3	70.6 / 25.5 / 3.9	0.378
Pack years	5.0±3.1	5.3±3.7	0.671
	n=73	n=28	
Blood eosinophils (10^9/L)	0.21 [0.09-0.43]	0.12 [0.06-0.32]	0.083
	n=90	n=34	
Blood neutrophils (10^9/L)	4.73 [3.50-6.70]	5.09 [3.18-7.18]	0.871
	n=110	n=47	
FeNO (ppb)	28 [16-40]	22 [15-48]	0.651
	n=83	n=30	
ACQ score	1.6 [0.9-2.4]	1.7 [0.7-2.6]	0.494
Uncontrolled asthma (ACQ score >1.5)	48.8	47.1	0.760
Treatment level: mild / moderate / severe	14.7 / 24.7 / 60.6	18.9 / 25.6 / 55.6	0.604
	n=231	n=90	

ACQ: Asthma Control Questionnaire; BMI: body mass index; FeNO: fraction of exhaled nitric oxide; pbFEV$_1$: post-bronchodilator forced expiratory volume in 1 s.

Classification model

PCA resulted in three PCs with an eigenvalue >1 (figure 2). Together, these PCs explained 58.7% of the variance in the dataset (PC1 36.4%, PC2 13.7%, PC3 8.6%). With the occurrence of an exacerbation as grouping variable, the first two PCs showed a statistically significant difference (P_{PC1} = 0.004, P_{PC2} = 0.016; cumulative variance = 50.1%). Scatterplots of PC1 and PC2 (training and validation sets) are depicted in figure 3. Dividing patients according to treatment level or asthma control did not result in significant different PCs.

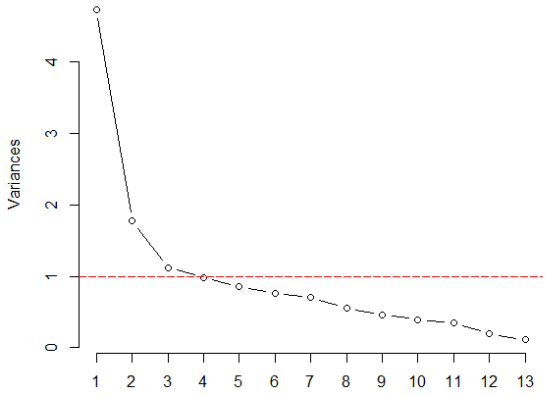

FIGURE 2. Screeplot of the principal component analysis on the sensor data. The plot shows the eigenvalue (y axis) for each principal component (x axis). The red dotted line shows the Kaiser criterion (eigenvalue >1). The first three principal components meet this criterion. Together they captured 58.7% of the variance in the dataset.

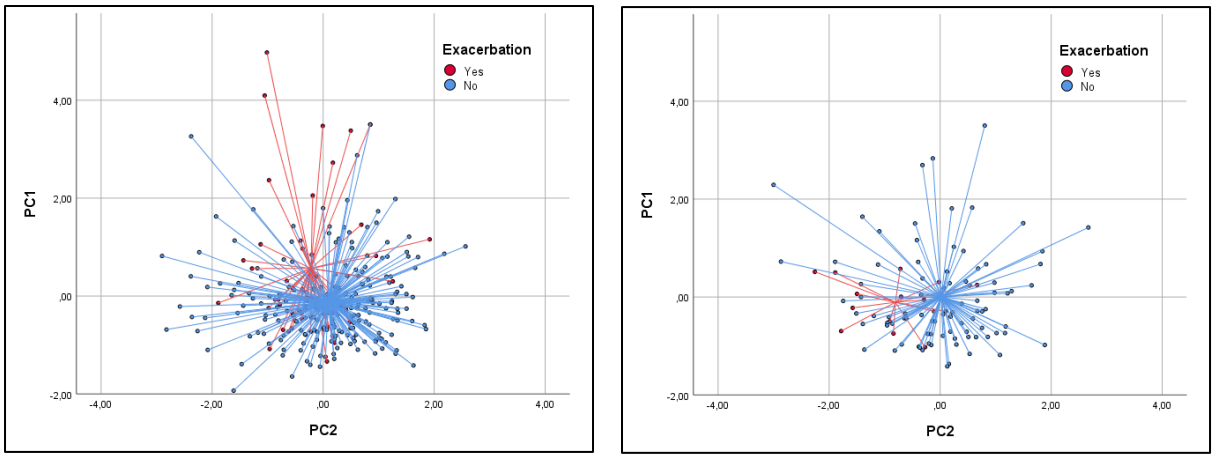

FIGURE 3. Scatterplots showing the discrimination of breathprints between patients with an exacerbation (red) and without an exacerbation (blue) in the three months prior to the SpiroNose® measurement, in the training (left) and validation (right) sets. Principal component (PC) 1 is plotted against PC2.

The performance of the classification model is shown in ROC curves (figure 4). The AUROC was 0.687 for the training set and 0.656 for the validation set.

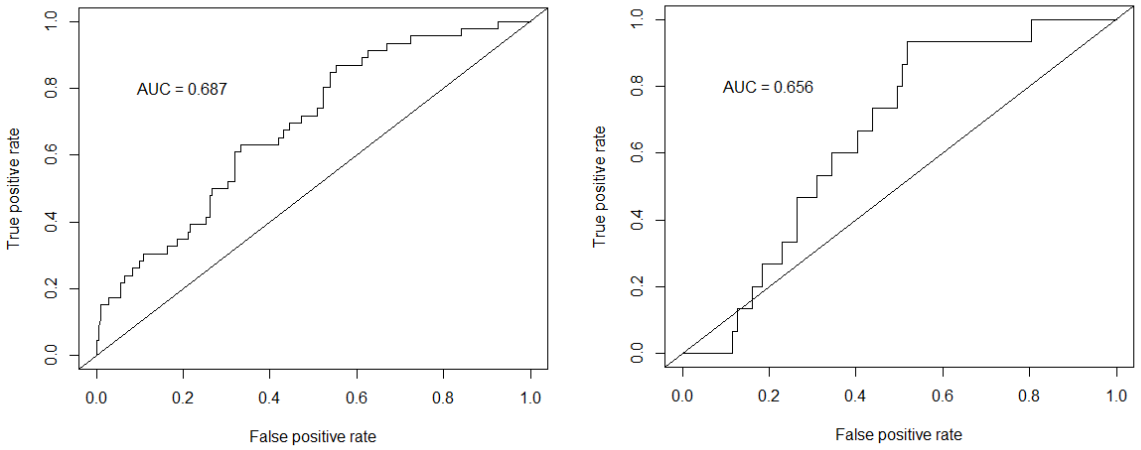

FIGURE 4. Receiver operating characteristic (ROC) curves showing the performance of the classification model. The true positive rate (or sensitivity) is plotted against the false positive rate (1 – specificity). The area under the curve (AUC) is 0.687 for the training set (left), and 0.656 for the validation set (right).

Hierarchical clustering

Clustering resulted in two significantly different eNose-driven clusters that differed regarding exacerbations in the past three months (P<0.001) (table 3). Furthermore, they differed statistically significant in treatment of the exacerbations with OCS (P<0.001) and antibiotics (P=0.02). Cluster 1 contained asthma patients with a high exacerbation rate: 24.3% had an exacerbation in the past three months. Subsequently, the number of patients treated for exacerbations was high as well: 21.7% was treated with OCS, and 10.6% with antibiotics. In cluster 2, only 4.4% had an exacerbation in the three months prior to measurement. These exacerbations were treated with OCS (2.2%) and/or antibiotics (3.7%).

TABLE 3. Comparison of the two clusters. Data are presented as %, n (%), mean±SD or median [IQR]. Between-group comparisons were performed using independent t-test, Mann-Whitney U test, and Pearson's chi-squared test.

	Cluster 1	***Cluster 2***	***P-value***
Subjects, n	226	136	-
Age (years)	48.5±17.6	47.7±17.4	0.68
Male sex	33.6	39.0	0.30
BMI (kg/m^2)	27.9±5.9	27.3±7.1	0.42
Primary care	12.4	19.9	0.06
Allergy	70.4	71.3	0.84
pbFEV$_1$ (% predicted)	91 [79-105]	93 [81-104]	0.69
	n=159	n=108	
Smoking status: never / ex / current	72.6 / 23.0 / 4.4	69.1 / 21.3 / 3.6	0.15
Pack years	4.8±3.4	5.4±3.0	0.31
	n=60	n=41	
Blood eosinophils (10^9/L)	0.17 [0.08-0.33]	0.23 [0.08-0.48]	0.15
	n=78	n=46	
Blood neutrophils (10^9/L)	5.09 [3.63-7.04]	4.23 [3.35-5.51]	0.08
	n=105	n=52	
FeNO (ppb)	25 [15-39]	30 [17-50]	0.44
	n=68	n=45	
ACQ score	1.7 [0.8-2.4]	1.4 [0.9-2.4]	0.70
Uncontrolled asthma (ACQ score >1.5)	54.5	47.1	0.17
Exacerbation <3 months	55 (24.3)	6 (4.4)	**<0.001**
Exacerbation treated with OCS	49 (21.7)	3 (2.2)	**<0.001**
Exacerbation treated with antibiotics	24 (10.6)	5 (3.7)	**0.02**
Treatment level: mild / moderate / severe	15.2 / 21.8 / 62.9	16.9 / 29.8 / 53.2	0.19
	n=197	n=124	

ACQ: Asthma Control Questionnaire; BMI: body mass index; FeNO: fraction of exhaled nitric oxide; OCS: oral corticosteroids; pbFEV$_1$: post-bronchodilator forced expiratory volume in 1 s.

DISCUSSION

This study shows that breathomics can be used to distinguish clinically stable from clinically unstable asthma patients. Exhaled breath analysis with the SpiroNose® provided moderate discrimination between asthma patients with and without exacerbations in the past three months. This classification model was confirmed in a validation set. In addition, eNose-driven phenotyping of asthma patients provided two clusters that differed regarding clinical stability.

Comparison with literature

To our knowledge, this is the first study trying to discriminate adult asthma patients based on the occurrence of real-life exacerbations using output from an eNose. A similar approach has previously been applied on patients with COPD (n = 363), of which 13.8% had an exacerbation in the previous three months [27]. The same definition was used for exacerbations. Instead of randomly dividing patients in a training and validation set, the cohort was separated based on ICS use. ROC curves showed AUCs of 0.996 (COPD patients using ICS) and 0.942 (patients not using

ICS). Given the comparable approach, the difference in AUC between discriminating exacerbations in asthma and in COPD is quite high. This would suggest a difference in VOCs generated by asthma exacerbation in comparison with COPD exacerbations. Another possible explanation could be that COPD exacerbations are underdiagnosed, leading to less false positives in the exacerbation group. In essence, both these respiratory diseases overlap in many ways, but also differ in pathophysiology, and this might lead to different signals that are captured.

Brinkman et al. [33] used a composite eNose platform to discriminate between clinically stable and unstable episodes in asthma patients (n = 23). Loss of control was induced by prompt and complete discontinuation of ICS and, if applicable, LABA. When loss of control was reached, patients were treated with a course of OCS, and treatment with ICS (and LABA) was restored. PCA was used to merge the sensor signals into PCs. PC2 could statistically significant discriminate between baseline and loss of control ($P<0.01$; accuracy = 95%), and between loss of control and recovery ($P<0.01$, accuracy = 86%). No significant differences were found for PC1. Real-life exacerbations are mostly caused by other factors than a reduction in ICS. Although our study was not longitudinal, it showed that breathomics can also discriminate patients based on real-life exacerbations, albeit with less accuracy.

eNose-driven phenotyping in has previously been performed in asthma patients [22,34]. Both studies performed PCA before applying cluster analysis on the sensor values, in contrast to this study, which used raw sensor values for clustering. De Vries et al. [22] applied cluster analysis on a combined sample of asthma and COPD patients. The cohort was divided in a training (n = 321) and a validation set (n = 114). Clustering in the training set resulted in five clusters, which differed statistically significant regarding BMI, ethnicity, FeNO, atopy, eosinophil and neutrophil blood counts, and exacerbation rate per person in the past three months ($P<0.01$). Applying the same algorithm on the validation set, resulted in similar clusters. Patients in cluster 4 had the highest exacerbation rate (0.43±0.3 exacerbations per person). The lowest exacerbation rate was found in cluster 3 (0.04±0.2 exacerbations per person). Brinkman et al. [34] identified three clusters in a sample of 78 patients with severe asthma. These clusters differed statistically significant regarding OCS use, blood eosinophils, and blood neutrophils, but not regarding the number of exacerbations per year ($P=0.785$).

Strengths and limitations

This study has a few limitations. First, it is possible that the definition used for exacerbations (treatment with OCS and/or antibiotics for deterioration of asthma) did not identify all patients with exacerbations. The American Thoracic Society and European Respiratory Society know two more criteria: (1) an increase in symptoms and/or use of SABAs for ≥2 days, and/or (2) the need for hospital admission [2]. This possibility is supported by a study of Pont et al. [35], who determined the reliability of identifying asthma exacerbation episodes from prescription data in general practices in the Netherlands. When using treatment with short-course OCS or antibiotics as

definition for an exacerbation, only 55% of the exacerbations could be identified. Broadening the definition for exacerbations may lead to less false negatives, resulting in better discrimination using breathomics. Second, the collection of information could be a limitation. The occurrence of exacerbations was a patient-reported outcome. Therefore, it could have been subjected to recall bias. However, especially in the participating hospitals (secondary care), patient claims were verified as much as possible by reviewing the medical records. Additionally, recall bias is likely limited because of the short time frame of a maximum of three months. A third weakness is lack of validation in the cluster analysis. However, using the training and validation set for this classification approach as well, would result in sample sizes too small for adequate clustering. External validation could further confirm the results, both from the classification model as the cluster analysis.

Regardless of these limitations, this study has important strengths. This is one of the first studies to employ an eNose for analysing breath samples of asthma patients with recent exacerbations. This could be the first step to further research into the prevention of asthma attacks. Secondly, this study used a validation set for building the classification model. The validation set confirmed the results from the training set: the AUROCs were comparable. None of the baseline characteristics differed statistically significant between the training and validation sets. Another strength is the multi-centre design of the primary study (BreathCloud). Asthma patients from both primary and secondary care performed a SpiroNose® measurement, which means the included patients reflect the general asthma population.

Interpretation, clinical relevance, and future research

Regarding the other two definitions of clinical stability, namely symptom control and treatment level, no significant PCs could be found. ACQ scores are used for monitoring the level of asthma symptom control within a patient. Because of the subjectivity of the tool, it can be difficult to use ACQ scores for comparing patients. Longitudinal eNose measurements in patients could be used to link breathprints to the level of symptom control.

In this study, assessing the treatment level was limited by the collection of the medication history. Prescription of drugs is often not equal to the actual usage by the patient. It would be interesting to examine if the medication has an influence on the breathprint. Not only the rescue and maintenance therapy, but also the medication used to treat the exacerbation. In this study we did not take into account when the exacerbation occurred; this could vary from three months to a few days ago. Further research would be needed to rule out what is being measured: the exacerbation itself or the medication that is used to treat the asthma or the asthma exacerbation.

The occurrence of exacerbations in the previous year is an important predictor for exacerbations in the future [36]. Thus, detecting past exacerbations could help to identify patients at risk for future exacerbations. Considering the moderate accuracy, an eNose measurement could be used in addition to standard risk assessment. However, past exacerbations are not the only risk factor for poor outcomes in the future. Real-life monitoring in asthma patients with an eNose could show if

the breathprint of a patient changes just before an exacerbation is going to occur. In that case, subsequent adjustments in treatment could possibly prevent the exacerbation. Additionally, clustering asthma patients could determine if a patient has an exacerbation-prone phenotype. This could warrant extra monitoring or instructions for the patient to prevent an asthma attack.

Overall, these findings suggest that eNoses are a promising tool for monitoring asthma patients and predicting the disease course. The fact that the measurement is fast and non-invasive, further supports a role for breath analysis in clinical practice.

Conclusion

In conclusion, breathomics enables discrimination between clinically stable and clinically unstable asthma patients, when defining stability as the occurrence of exacerbations in the past three months. This conclusion supports bringing eNose technology to clinical practice for monitoring and prevention of future risks in asthma patients.

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