Exhaled breath analysis by use of eNose technology: a novel diagnostic tool for interstitial lung disease

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ABSTRACT

Background

Early and accurate diagnosis of interstitial lung diseases (ILDs) remains a major challenge. Better non-invasive diagnostic tools are highly needed. We aimed to assess the accuracy of exhaled breath analysis using eNose technology to discriminate between ILD patients and healthy controls, and to distinguish ILD subgroups.

Methods

In this cross-sectional study, exhaled breath of consecutive ILD patients and healthy controls (HC) was analyzed using eNose technology (SpiroNose). Statistical analyses were done using Partial Least Square Discriminant Analysis (PLS-DA) and Receiver Operating Characteristic (ROC) analysis. An independent training and validation set (2:1) was used in larger subgroups.

Results

A total of 322 ILD patients and 48 HCs were included; sarcoidosis (n=141), idiopathic pulmonary fibrosis (n=85), ILD associated with connective tissue disease (n=33), chronic hypersensitivity pneumonitis (n=25), idiopathic NSIP (n=10) and interstitial pneumonia with autoimmune features (n=11), and other ILDs (n=11). eNose sensors fully accurately discriminated between ILD and HCs, with an AUC of 1.0 in the training and validation set. Comparison of patients with IPF and patients with other ILDs yielded an AUC of 0.91 (95% CI 0.85-0.96) in the training set, and an AUC of 0.87 (95% CI 0.77-0.96) in the validation set. The eNose reliably distinguished between individual diseases, with AUCs ranging from 0.85 to 0.99.

Conclusion

eNose technology can completely distinguish ILD patients from healthy controls, and can accurately discriminate between different ILD subgroups. Hence, exhaled breath analysis using eNose technology could be a novel new biomarker in ILD, enabling timely diagnosis in the future.

BACKGROUND

Interstitial lung diseases (ILDs) encompass a diverse group of more than 200 different disorders, which are associated with substantial morbidity and mortality (1, 2). Idiopathic pulmonary fibrosis (IPF) is the most common ILD and has the worst prognosis (3). The disease course of ILDs is very heterogeneous; some ILDs are reversible and may be self-limiting, others remain stable, and a subgroup of patients has a progressive phenotype (1). Moreover, a substantial minority of patients (around 10%) have unclassifiable ILD (4, 5). Establishing an accurate diagnosis can be challenging, because symptoms as cough and dyspnea are non-specific (6). Many patients receive one or more misdiagnoses, and often undergo invasive diagnostic procedures, before the diagnosis of ILD is confirmed (7). A study in IPF showed that 55% of patients consulted three or more physicians before receiving a final diagnosis. Furthermore, the majority of patients reported a treatment delay of more than one year between initial presentation and confirmed diagnosis (8). Currently, a multidisciplinary team (MDT) discussion is considered as the "gold standard" for diagnosis of ILD (9).

With the availability of different treatment options, it has become increasingly important to achieve an early diagnosis (10, 11). For IPF, two antifibrotic drugs (nintedanib and pirfenidone) are available that slow down disease progression. More recently, antifibrotic drugs have also shown efficacy in other progressive fibrotic ILDs, which will change the treatment landscape for these patients (12). Patients with fibrotic ILDs other than IPF can have a predominantly inflammatory phenotype, a more fibrotic phenotype, or a combination of both (3). This emphasizes the importance of accurate phenotyping of patients, to decide which treatment should be given (i.e. immunosuppressive medication, antifibrotic medication, or a combination). Currently, no biomarkers are available to reliably make this distinction. Thus, there is a major need for non-invasive, widely available, inexpensive tools for diagnosis and monitoring of disease course, especially in the current era with advancing treatment options.

An emerging non-invasive diagnostic technique is the analysis of volatile organic compounds (VOCs) in exhaled breath. The body creates volatile organic compounds (VOCs) during metabolic and pathological processes; thousands of these VOCs can be found in exhaled breath (13). The composition of VOCs in exhaled breath could serve as biomarker in a wide range of diseases (14, 15). One method to analyze VOCs is by using electronic nose (eNose) technology. ENoses have several cross-reactive gas sensors, which react to multiple compounds in the VOC mixture. This results in a unique pattern of sensor responses: the breathprint. Individual cases can be classified in disease groups by use of pattern recognition algorithms. The field of breathomics is rapidly evolving,

and relatively high diagnostic accuracies have been published in other (lung) diseases (16-19). Hence, eNose technology has been proposed as a diagnostic tool, for clinical and inflammatory phenotyping, and to predict response to therapy (16, 17). In ILD, studies on breathomics are scarce. One study in sarcoidosis showed that the breathprint of patients with untreated pulmonary sarcoidosis could be distinguished from healthy controls. Treated sarcoidosis patients could not be discriminated from healthy controls (20). Another recently published study evaluated the ability of eNose technology to identify different ILD subgroups, and to compare ILD with healthy controls and COPD patients (21). This study also showed adequate distinction between patients with ILD from healthy controls and COPD patients. However, different ILD subgroups could not be accurately separated from each other.

In this study, we aimed to investigate the reliability of exhaled breath analysis using eNose technology to discriminate between ILD patients and healthy controls, and to distinguish ILD subgroups.

METHODS

Study design and population

This was a single-center, cross-sectional study in the Erasmus Medical Center, Rotterdam, the Netherlands. This study was approved by the medical ethics committee (MEC-2019-0230). Between July 2019 and February 2020, consecutive outpatients with a diagnosis of ILD, according to the ATS/ERS criteria (1, 9), or a diagnosis of sarcoidosis, according to the WASOG criteria (22) were eligible to participate. We also included patients with interstitial pneumonia with autoimmune features (IPAF), for which we used the proposed classification criteria by Fischer et al (23). All patients signed written informed consent before inclusion in the study. The healthy controls (HCs) were hospital staff without a history of lung diseases, who gave informed consent.

Measurements

Measurements were performed using a cloud-connected eNose; SpiroNose (Breathomix, Leiden, the Netherlands). The SpiroNose is an integration between eNose technology and routine spirometry, and has been technically and clinically validated (24, 25). The SpiroNose has seven different types of cross-reactive metal-oxide semiconductor sensors. These sensors are present in duplicate in sensor arrays on both the inside (to measure VOCs in exhaled breath) and on the outside of the SpiroNose (to measure VOCs in ambient air). A SpiroNose measurement consists of five tidal breaths, followed by an inspiratory capacity maneuver to total lung capacity, a five second breath hold, and sub-

sequently a slow expiration (flow <0.4L/s) to residual volume. All eNose measurements were performed in duplicate. The sensor readings were sent in real time via a gateway to the online analysis platform, BreathBase, which includes the secured online database of Breathomix (ISO27001 and NEN7510 certified). A more detailed description of the methods and set up can be found in a previous publication by de Vries et al. (25).

Data collection

Participants completed a short survey about factors relevant for the measurement, such as smoking history and food intake in the last two hours. Data about medication use, lung function tests, pathology results, radiology, and recent laboratory parameters were collected from the medical records. Patients were labeled as having pulmonary fibrosis in case of reticulations with traction bronchiectasis, and/or honeycombing on the most recent CT scan. Data about forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide were collected if available.

Data analysis

The eNose sensor signals were processed, and corrected for ambient VOCs as previously published (24, 26). The peak value of each sensor for exhaled breath was determined, and normalized to the most stable sensor (sensor 2). Sensor-to-sensor ratios were used to reduce inter-array differences. Lastly, the ratio between peak sensor values and sensor values during breath hold were calculated. Both the normalized sensor peaks and the ratio between peak sensor values and breath hold values were used in data analysis. Statistical analysis was done using Partial Least Square Discriminant Analysis (PLS-DA) and receiver operating characteristic (ROC) analysis. In the ROC analysis, the areas under the curve (AUCs) and corresponding 95% confidence intervals (CIs) were determined. Sensitivity, specificity and accuracy were calculated. For larger subgroups (ILD vs HC, IPF vs non-IPF ILD, pulmonary fibrosis vs no pulmonary fibrosis), a training and validation set by split analysis (2:1) were used, as recommended for metabolomics experiments (27). During the validation step, the PLS-DA model derived from the training set was tested on the validation set (Figure S1). We compared the training and validation set based on the AUC of the ROC curves of PLS-DA components 1 and 2. For comparisons between individual diagnoses we did not use a training and validation set, because of the small sample sizes. For these groups only PLS-DA component 1 was used for ROC analysis. We focused on comparing diagnoses that often cause diagnostic dilemmas in clinical practice because of their similarities in clinical presentation and imaging. Descriptive statistics were used to analyze baseline data. Between-group comparisons were done using independent sample t-tests, ANOVA, chi square tests, Kruskal-Wallis tests, and Fisher's exact tests. Analyses were done using R version 3.6.2 (using the mixOmics package, Version 6.1.1) and SPSS (IBM SPSS statistics for Windows, Version 25).

RESULTS

In total, 322 consecutive patients with ILD and 48 healthy controls were included in this study. The overall mean age for ILD patients was 61.6 years (SD 13.3), 59.9% of patients were male and 5.3% of patients were current smokers. Mean FVC (n=316) was 3.23L (SD 1.1), or 82.4% of predicted (SD 19.1), and mean DLCO (n=305) was 60.6% of predicted (SD 21.9). Mean age and percentage of males were significantly higher in the ILD group, compared with healthy controls (**Table 1**). Furthermore, ILD patients had significantly more pack years than healthy controls, but there was no difference in the percentage of current smokers.

ILD patients were categorized in seven groups: idiopathic pulmonary fibrosis (IPF), sarcoidosis, connective tissue disease-associated interstitial lung diseases (CTD-ILD), chronic hypersensitivity pneumonitis (CHP), interstitial pneumonia with autoimmune features (IPAF), idiopathic non-specific interstitial pneumonia (iNSIP) and other ILDs. Diagnoses with less than ten included patients (cryptogenic organizing pneumonia, respiratory bronchiolitis-interstitial lung disease, asbestosis, drug-induced ILD, granulomatosis with polyangiitis and unclassifiable ILD) were classified as 'other ILDs'. Baseline characteristics of these individual groups can be found in supplementary table 1. There were significant differences in age, gender, pack years, FVC and DLCO between ILD subgroups, but no difference in the percentage of current smokers.

ILD versus healthy controls

The breathprint of 322 ILD patients and 48 healthy controls were compared; groups were divided in a training and a validation set. The training set consisted of 215 ILD patients and 32 healthy controls, the validation set of 107 ILD patients and 15 healthy controls. The results of PLS-DA for the training set, accompanied by the corresponding ROC curve are shown in **figure 1**. The eNose perfectly discriminated between ILD patients and healthy controls with an AUC of 1.00, for both the training and the validation set. Accordingly, the sensitivity, specificity and accuracy of the model were 100% (**Table 2**).

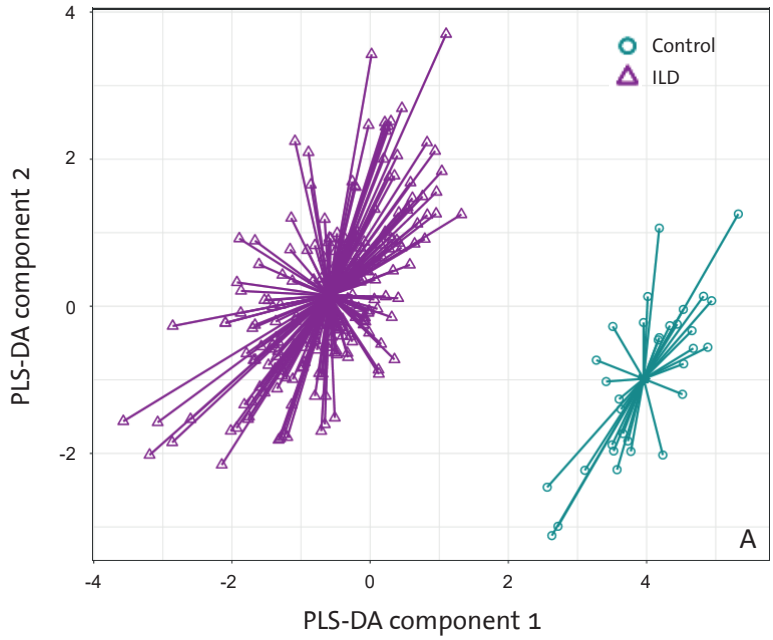
ILD subgroups

Patients with ILD were divided in IPF (n=85) and non-IPF ILDs (n=237), and separated in a training and validation set. The training set consisted of 57 IPF patients and 158 patients with non-IPF ILDs, the validation set consisted of 28 IPF patients and 79 patients with non-IPF ILDs. The results of PLS-DA for the training set are shown in **figure 2**, together with the corresponding ROC curve. In the training set, the AUC was 0.91 (95% CI 0.85-0.96), in the validation set the AUC reached 0.87 (95% CI 0.77-0.96). In the validation set the sensitivity was 95%, the specificity was 79% and the accuracy of the model was 91%. Results of the training set are shown in table 2.

Table 1. Baseline characteristics of ILD patients and healthy controls

	Sarcoidosis	IPF	CTD-ILD	CHP	IPAF	iNSIP	Other ILDs	Healthy controls
	n = 141	n = 85	n = 33	n =25	n = 11	n = 10	n = 17	n = 48
Mean age	53 ± 11	74 ± 7	57 ± 13	67 ± 8	61 ± 13	68 ± 9	66 ± 16	37 ± 12
Males (%)	72 (51.1)	77 (90.6)	11 (33.3)	13 (52.0)	0 (0.0)	6 (60.0)	14 (82.4)	15 (31.3)
Smoking history								
never	82 (58.2%)	10 (11.8%)	15 (45.5%)	12 (48.0%)	5 (45.5%)	3 (30.0%)	3 (17.6%)	37 (77.1%)
stopped	47 (33.3%)	73 (85.9%)	17 (51.5%)	13 (52.0%)	6 (54.5%)	7 (70.0%)	12 (70.6%)	7 (14.6%)
current	12 (8.5%)	2 (2.4%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (11.8%)	4 (8.3%)
Median pack years (IQR)	0.0 (0.0-3.9)	20.0 (6.8-37.9)	0.5 (0.0-20.0)	0.0 (0.0-15.0)	0.0 (0.0-16.9)	8.8 (0.0-33.0)	9.5 (2.3-27.3)	0.0 (0.0-0.0)
Mean FVC (L)	3.56 ± 1.06	3.17 ± 0.94	2.68 ± 0.76	3.03 ± 1.02	1.97 ± 0.86	2.54 ± 0.98	3.41 ± 1.11	NA
Mean FVC (% predicted)	87 ± 16	81 ± 20	73 ± 19	83 ± 19	65 ± 23	67 ± 11	85 ± 20	NA
Mean DLCO (% predicted)	76 ± 18	43 ± 13	54 ± 15	53 ± 14	46 ± 20	51 ± 15	57 ± 23	NA

± standard deviation, ILD: interstitial lung disease, IPF: idiopathic pulmonary fibrosis, CTD-ILD: connective tissue disease-associated interstitial lung disease, CHP: chronic hypersensitivity pneumonitis, IPAF: interstitial pneumonia with autoimmune features, iNSIP: idiopathic non-specific interstitial pneumonia, other ILDs: other interstitial lung diseases, IQR: interquartile range, FVC: forced vital capacity, DLCO: diffusing capacity of the lungs for carbon monoxide



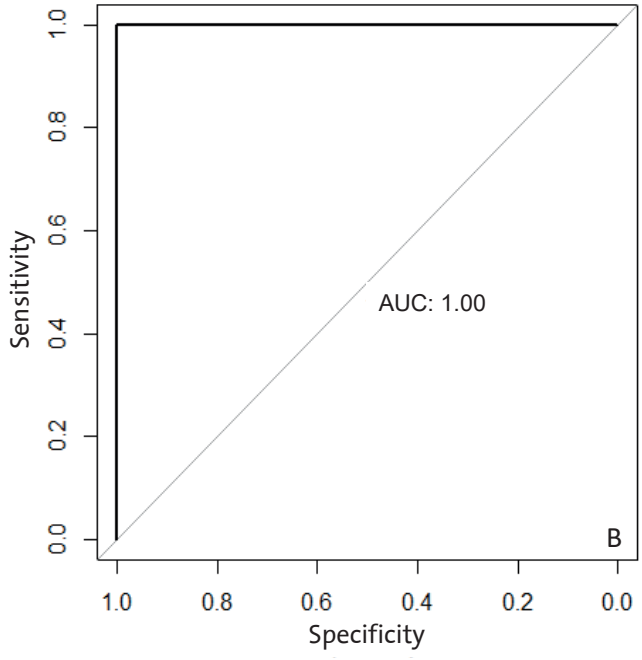


Figure 1A: Training set of breathprints from patients with interstitial lung disease (n=215) compared to breathprints from healthy controls (n=32)
Figure 1B: ROC curve of PLS-DA components 1&2 for training set

Table 2. Diagnostic performance of training and validation sets

Groups	AUC	95% CI	Sensitivity (%)	Specificity (%)	Accuracy (%)
ILD vs HC - training	1	-	100	100	100
ILD vs HC - validation	1	-	100	100	100
IPF vs non-IPF ILD - training	0.91	0.85-0.96	92	88	91
IPF vs non-IPF ILD - validation	0.87	0.77-0.96	95	79	91
Fibrosis vs no fibrosis - training	0.83	0.77-0.89	84	77	80
Fibrosis vs no fibrosis - validation	0.78	0.69-0.87	74	81	78

AUC = area under the curve, CI = confidence interval, ILD = interstitial lung disease, HC = healthy controls, IPF = idiopathic pulmonary fibrosis

The breathprints of ILD patients with pulmonary fibrosis (n=194) were compared to patients without pulmonary fibrosis (n=128). This group was split in a training set of 130 patients with fibrosis and 86 patients without fibrosis, and a validation set of 64 patients with fibrosis and 42 patients without fibrosis. The ROC curve reached an AUC of 0.83 (0.77-0.89) in the training set (**Figure 3**) and 0.78 (0.69-0.87) in the validation set. In the validation set the sensitivity of the model was 74%, the specificity 81% and the accuracy 78% (for training set see table 2).

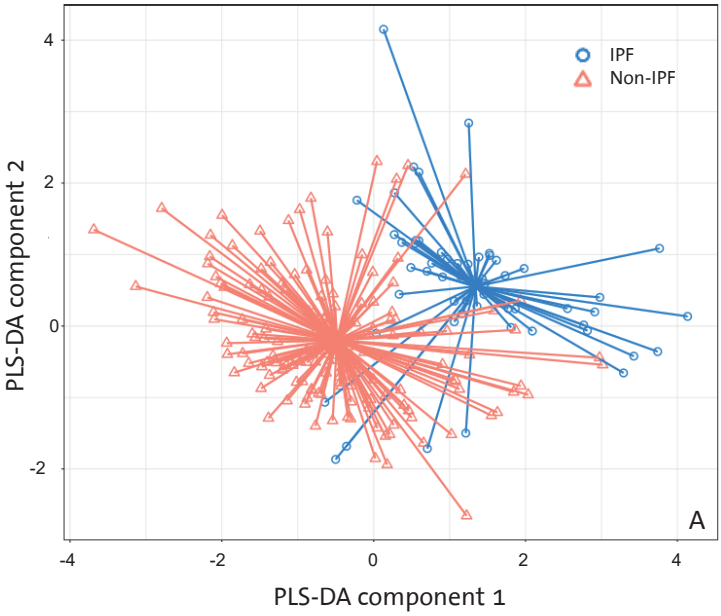
Individual ILDs

Subsequently, breathprints of individual diagnoses were compared with each other. The diagnostic performances of the models for comparison between different ILDs are presented in **table 3**, all figures can be found in the supplementary material (Figure S2). As we included a group of patients that did not have a classifying ILD diagnosis but fulfilled the criteria of IPAF, we did an exploratory analysis comparing these patient with both IPF and CTD-ILD, as these are the most common clinical differential diagnoses in IPAF (**Figure 4**).

Table 3 . Models for direct comparison between individual diagnoses

Groups	AUC	95% CI	Sensitivity (%)	Specificity (%)	Accuracy (%)
IPF vs CHP	0.85	0.76-0.94	75	84	77
IPF vs CTD-ILD	0.96	0.93-1.00	98	85	94
IPF vs iNSIP	0.94	0.86-1.00	92	90	92
IPF vs IPAF	0.94	0.90-0.99	87	100	89
CTD-ILD vs IPAF	0.99	0.80-1.00	100	67	75
CTD-ILD vs iNSIP	0.93	0.79-1.00	90	100	98
CHP vs sarcoidosis	0.89	0.80-0.98	94	72	90

AUC = area under the curve, CI = confidence interval, IPF = idiopathic pulmonary fibrosis, CHP = chronic hypersensitivity pneumonitis, CTD-ILD = connective tissue disease – interstitial lung disease, iNSIP = idiopathic non-specific interstitial pneumonia, IPAF = interstitial pneumonia with autoimmune features



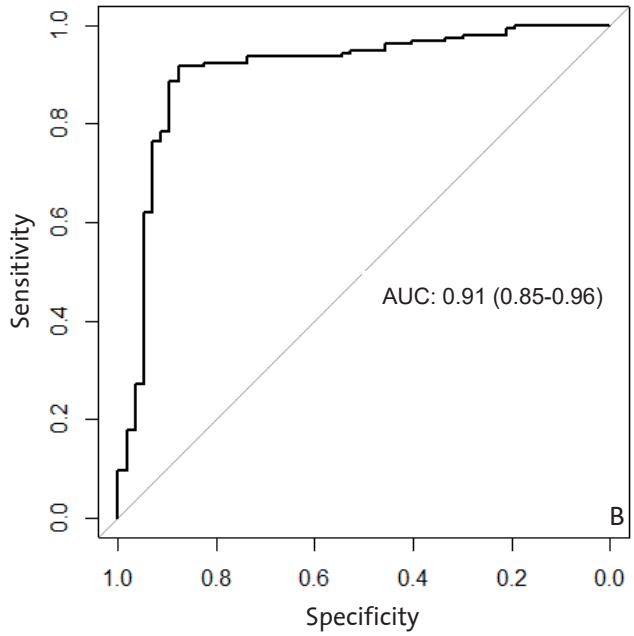
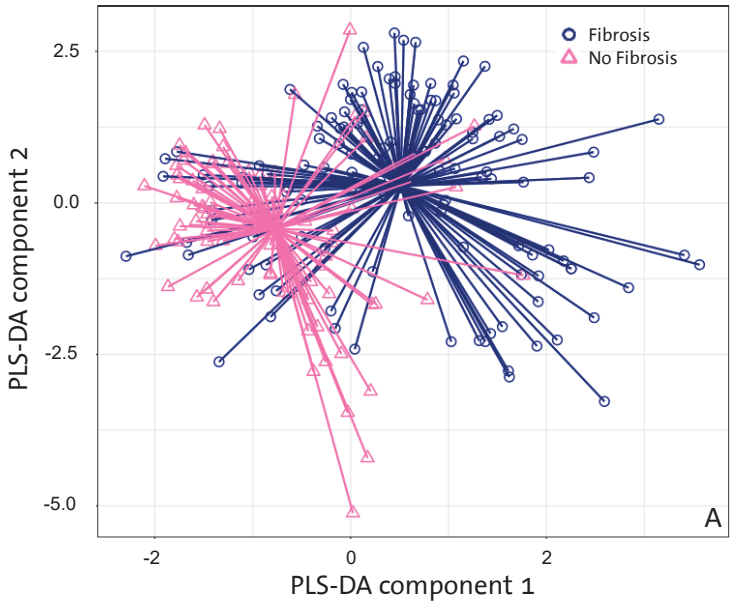


Figure 2A: Training set of breathprints from IPF patients (n=57) compared to breathprints from patients with non-IPF ILDs (n=158)
Figure 2B: ROC curve of PLS-DA components 1&2 for training set



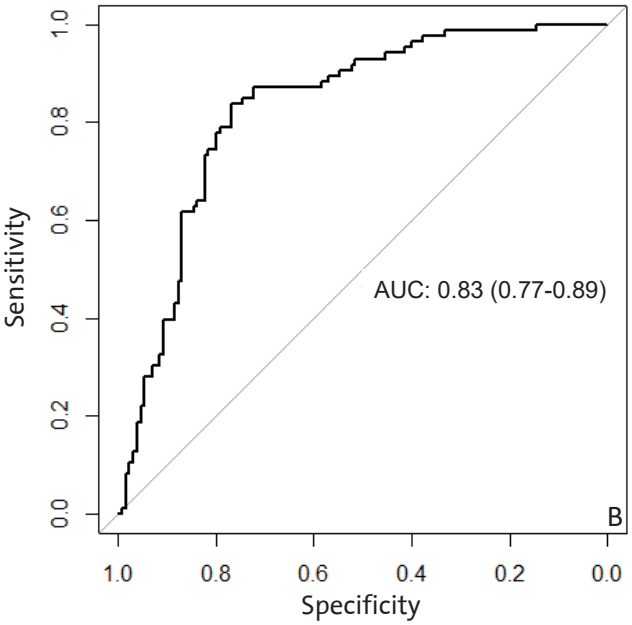


Figure 3A: Training set of breathprints from patients with fibrosis (n=130) compared to breathprints of patients without fibrosis (n=86)

Figure 3B: ROC curve of PLS-DA components 1&2 for training set

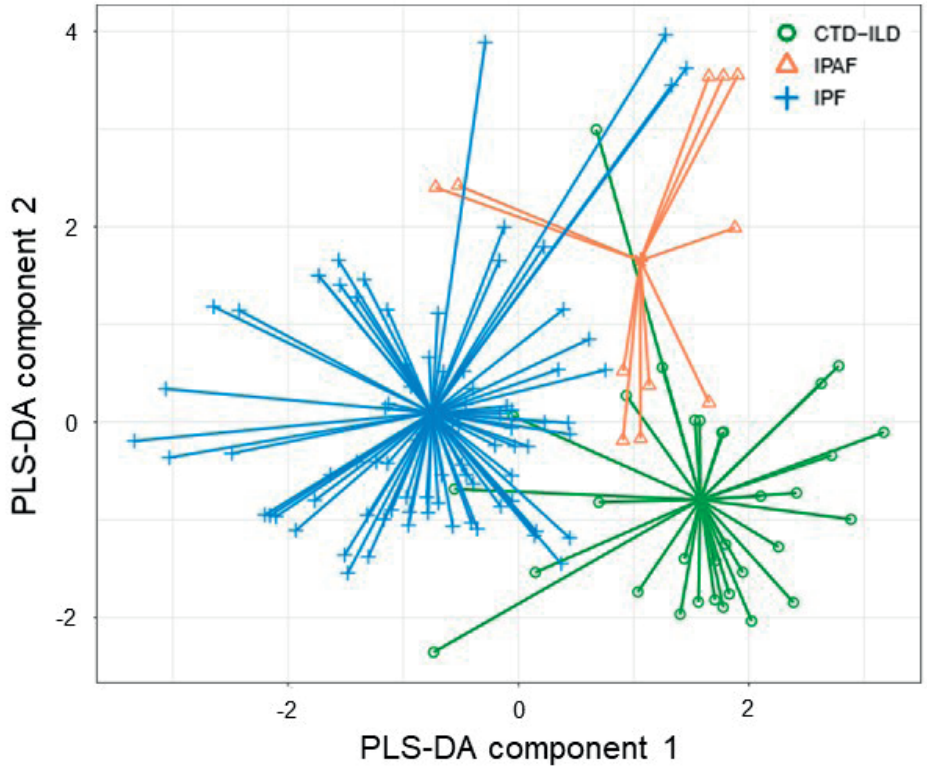


Figure 4: PLS-DA results of IPF, CTD-ILD and IPAF. IPF = idiopathic pulmonary fibrosis, CTD-ILD = connective tissue disease-ILD, IPAF = interstitial pneumonia with autoimmune features.

DISCUSSION

In this study, we aimed to evaluate the reliability of exhaled breath analysis using eNose technology to discriminate between ILD patients and healthy controls, and to distinguish ILD subgroups. The eNose fully accurately discriminated between ILD patients and healthy controls, both in the training and validation set. Moreover, the eNose adequately discriminated between individual ILDs, IPF and non-IPF ILDs, and patients with pulmonary fibrosis versus patients without pulmonary fibrosis.

Until now, one other pilot study in ILD investigated the ability of eNose technology to recognize ILDs (21). In line with our results, healthy controls could be distinguished from ILDs (IPF, CTD-ILD and cryptogenic organizing pneumonia). In the present study, we have confirmed and extended this finding in an independent training and validation cohort. The eNose in the current study could discriminate between individual diagnoses with a high sensitivity, specificity, and accuracy. This was not shown by Krauss et al. pre-

sumably due to a smaller number of ILD patients in their study (n=174) compared with the current study (n=322), which could have led to insufficient training of the device (21). The encouraging results in our study warrant further confirmation and external validation in larger (multi-center) cohorts. A larger cohort for the individual ILDs coming from different MDTs, will further increase the accuracy of eNose technology to detect ILD and distinguish between different diagnoses, making this a potentially new tool for rapid, non-invasive diagnosis of ILD.

The comparison of patients with and without pulmonary fibrosis yielded an acceptable accuracy, but the area under the curve was slightly lower than in other subgroups. Data of HRCT scans were collected from medical records, and most scans were not made at the same outpatient clinic visit as the eNose measurements. We only determined presence of pulmonary fibrosis and did not look at signs of inflammation, as this may have changed over time. Inflammatory processes change the VOC mixture in exhaled breath (16). Inflammation may have been present both in patients with pulmonary fibrosis, as well as patients without pulmonary fibrosis. Hence, it could be speculated that inflammation dominates the breathprint, leading to an overlap in breathprints of patients who have an inflammatory phenotype, irrespective of the presence of fibrosis. Future studies should further elucidate whether inflammatory and fibrotic phenotypes can be reliably distinguished, and whether specific HRCT patterns could be discriminated by exhaled breath analysis.

Surprisingly, patients with IPF, IPAF and CTD-ILD had a distinctive breathprint, and could be discriminated with a high accuracy. This raises the question whether IPAF could be a separate disease entity. Until now, the term IPAF is primarily used as a research concept, but not as a clinical diagnosis. IPAF is thought to have a significant overlap with IPF and CTD-ILD, and is often considered as undifferentiated CTD-ILD (23, 28). Moreover, IPAF is a very heterogeneous concept, as the classification criteria are based on a combination of features from three different domains (a clinical, serological, and morphological domain). This makes the clear discrimination between IPAF, CTD-ILD and IPF in the current study even more interesting. eNose technology could potentially be used to determine whether distinct phenotypic clusters can be identified within the patient group currently classified as IPAF. Obviously, these results need to be confirmed in larger studies. Nevertheless, our results highlight the importance of refining the classification criteria for IPAF in the coming years. (23, 28).

A potential limitation in this study is the fact that the group of healthy controls was younger, had less pack years, and consisted of a significantly lower percentage of males. A previously published study showed that age and gender do not affect the breathprint

(29). Hence, we believe that these differences in demographics have not impacted our results. A possible obstacle for the further development of eNose technology towards a point-of-care tool in ILD, is the lack of gold standard for the diagnosis of ILD. Diagnoses are based on multidisciplinary team meetings, and a substantial part of ILD remains unclassifiable (1, 4, 5, 9). Because there is no real gold standard, it is highly likely that a minority of patients has been incorrectly diagnosed. This means that the pattern recognition algorithms receive wrong so-called 'gold-standard' information, and the algorithm is trained based on partly incorrect information. This same limitation has been mentioned by Walsh et al., in a recent study where a deep learning algorithm learned to classify fibrotic lung disease on HRCT (30). A larger training dataset would result in a better performing algorithm, as the percentage of incorrectly labelled patients will be relatively smaller. This further emphasizes the need of future research on eNose technology in ILD, preferably as a multicenter effort, to increase the size of the available datasets and account for difference in diagnoses between MDTs (31).

Based on our data, we believe that exhaled breath analysis has the potential to enable early and accurate diagnosis of ILD in the future. Results of eNose measurements could give guidance during multidisciplinary team meetings and enhance diagnostic certainty in clinical practice. Furthermore, unsupervised cluster analysis can be performed to cluster patients based on their breathprint, irrespective of the underlying diagnosis, similar to a recent study among patients with asthma or COPD (26). This data-driven approach could potentially distinguish different disease phenotypes which have not yet been clinically identified. Further studies should reveal whether patients with distinct eNose-based phenotypes have a different disease behavior and/or response to therapy. If so, this may be a novel technology to predict disease progression and prognosis in ILD.

In conclusion, eNose technology has the potential to become a novel diagnostic tool for ILDs. ENose measurements can hopefully be used in the future to increase diagnostic confidence, and allow for point-of-care diagnostics in the doctor's office, thereby reducing diagnostic delays and improving care and treatment for patients with ILD.

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Supplementary table 1. Baseline characteristics of individual groups classified as 'other ILDs'

	Asbestosis	RB-ILD	(C)OP	Drug-induced ILD	Unclassifiable ILD	GPA
	n = 1	n =1	n = 6	n = 2	n = 4	n = 3
Mean age	81	54	68 ± 9	67 ± 12	75 ± 2	46 ± 26
Males (%)	1 (100)	1 (100)	4 (66.7)	2 (100)	3 (75)	3 (100)
Smoking history						
never	1 (100)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (25.0%)	0 (0.0%)
stopped	0 (0.0%)	0 (0.0%)	5 (83.3%)	2 (100%)	3 (75.0%)	2 (66.7%)
current	0 (0.0%)	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)
Median pack years (IQR)	0.0	25.0	6.3 (2.3-17.2)	41.7	10.0 (1.0-20.5)	NA
Mean FVC (L)	3.16	4.80	3.67 ± 0.97	3.45 ± 1.01	2.21 ± 0.51	4.56 ± 1.20
Mean FVC (% predicted)	92	103	93 ± 25	71 ± 25	71 ± 11	87 ± 6
Mean DLCO (% predicted)	30	41	75 ± 19	46 ± 21	43 ± 19	76 ± 4

± standard deviation, RB-ILD: respiratory bronchiolitis-interstitial lung disease, (C)OP: (cryptogenic) organizing pneumonia, drug-induced ILD: drug-induced interstitial lung disease, unclassifiable ILD: unclassifiable interstitial lung disease, GPA: granulomatosis with polyangiits, IQR: interquartile range, FVC: forced vital capacity, DLCO: diffusing capacity of the lungs for carbon monoxide

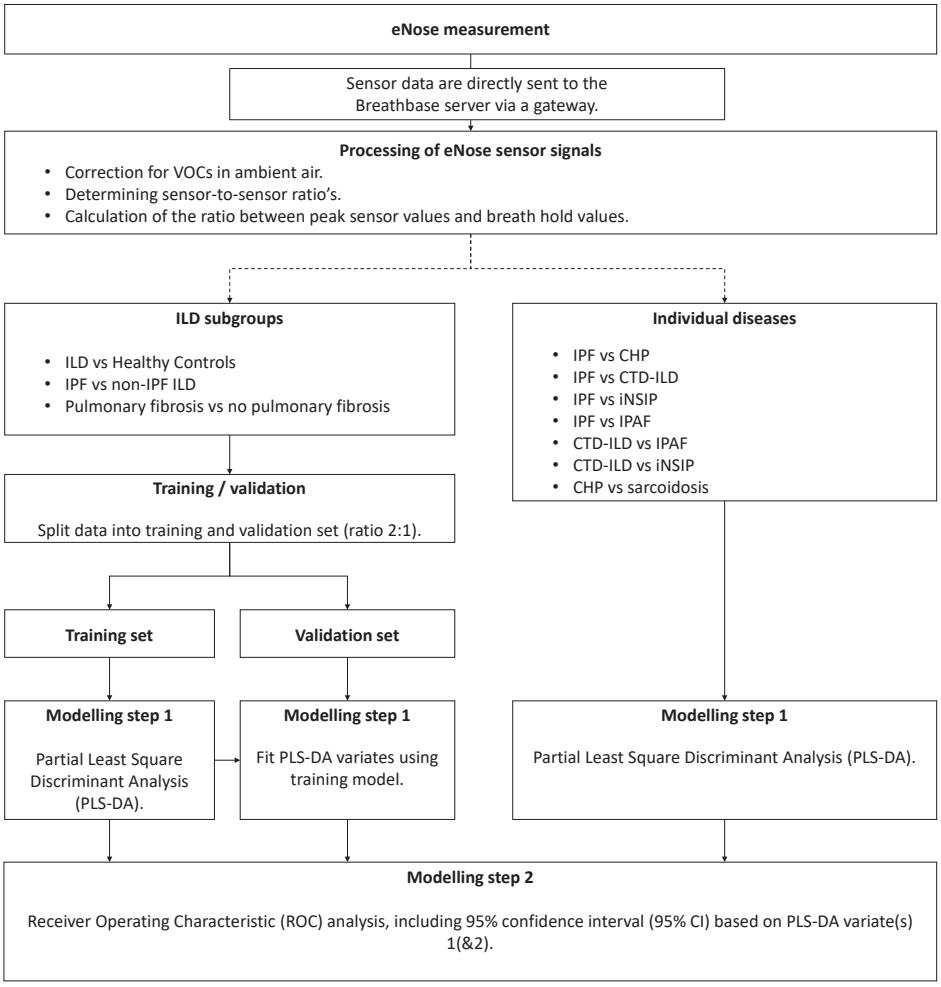

Figure S1: Data analysis flow chart

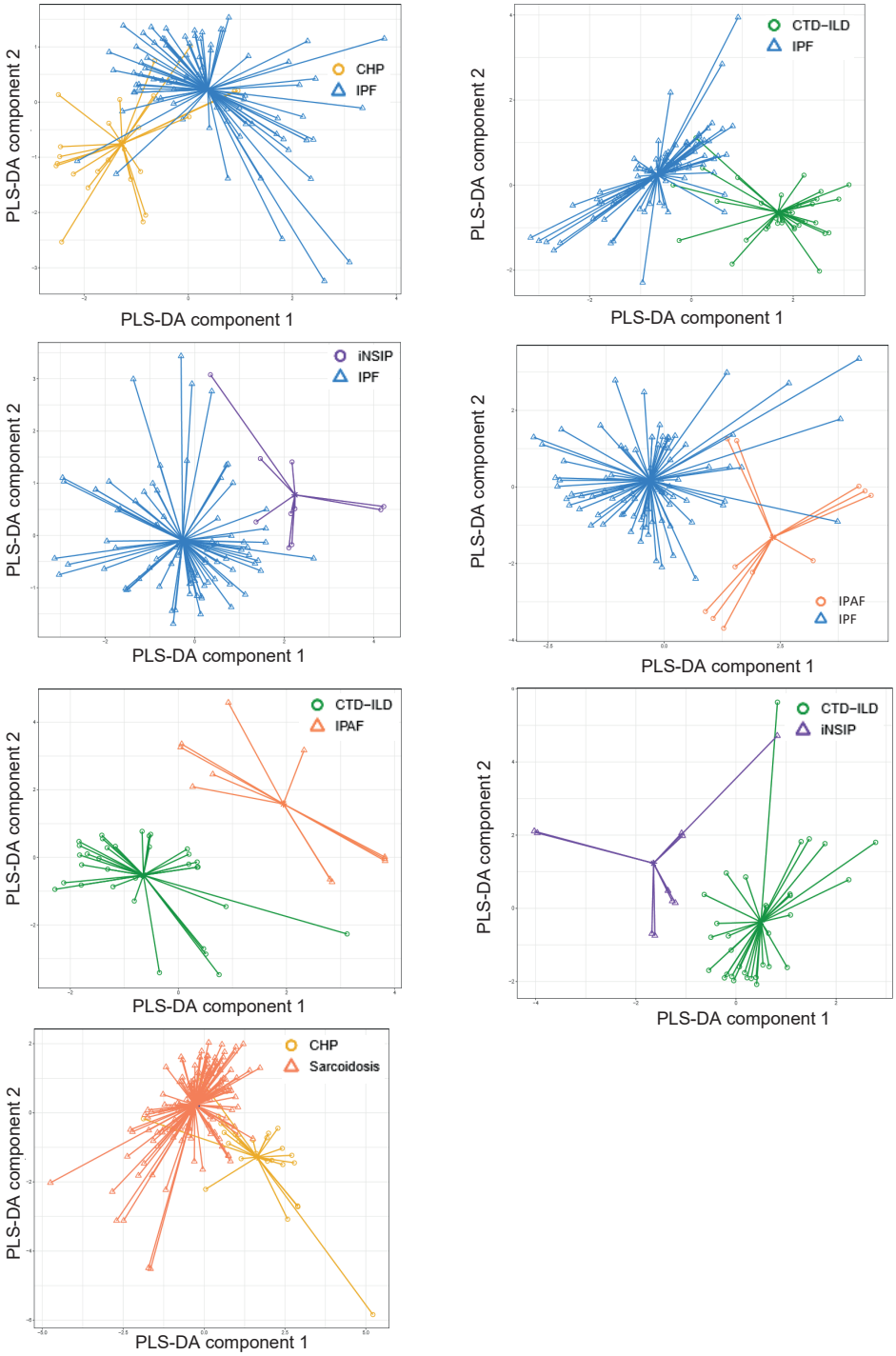

Figure S2: Results of the PLS-DA analysis for comparison of individual diseases