Identification of recent exacerbations in COPD patients by eNose

-SUPPLEMENTARY MATERIALS-

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Methods

Subjects

The study subjects have been enrolled from December 2015 until May 2017 across 6 different sites. Physician-reported COPD diagnosis has been assessed by local researchers. All patients had either a clinical diagnosis for COPD (n=354, of which 1 patient with α_1-antitrypsin deficiency) or for COPD and Asthma (n=10).

Measurements

Clinical data (including patient characteristics, spirometry, and laboratory measurements) and assessment of COPD control by the clinical COPD questionnaire (CCQ)[1, 2], were recorded during inclusion. The personal best FEV1 as % of predicted within 12 months prior to inclusion was retrieved from routine clinical data. The same applies for absolute blood eosinophil- and neutrophil counts prior to inclusion, when this data was available.

Signal Processing

Analysis of raw sensor data was performed using Matlab (MathWorks, Natick, MA, USA). Signal processing was performed as described previously[3, 4] (Online supplement), before detection of highest sensor peaks and calculation of the ratio between sensor peak and breath-hold point. Signals were normalized to the most stable sensor (sensor 2) and corrected for ambient air VOCs. Sensor stability was monthly verified using a test gas (Lindegas) as quality control. The sensor peak value and peak/breath hold ratio were selected as parameters for statistical analysis.

Statistical Analysis

The metal oxide sensors that are used by the SpiroNose are cross-reactive, i.e. they have an overlapping sensitivity for different VOCs. This means that a high degree of multicollinearity exists between the different sensor values, in its turn making a linear discriminant analysis at high risk of overfitting data [5]. A dimension reduction in principal

components solves this multicollinearity problem by constructing orthogonal components from the multidimensional dataset. A comprehensive review of different analytical approaches for eNose data by Leopold *et al.* found the combination of PC reduction and LDA as a well-established approach in case-control studies [6]. Sensor loadings and explained variance of the constructed PCs are shown in s-Table 4, colour coding of the loading factors shows multicollinearity between sensors for the different principal components.

Sensitivity Analysis

For further sensitivity analyses, the total population was divided into quartiles based on pack years, on average vital capacity during the two manoeuvres they had to perform during measurement and on recorded CCQ-scores. The discriminatory ability of the eNose sensors between exacerbation and no exacerbation did not change between these groups of patients. Pack years, CCQ-score and average vital capacity did not show any significant correlation with PC1-4 in the total population (s-Table 4). Smoking status and ICS use were evenly distributed across both training and validation set and the eNose sensors did not show any ability to discriminate between current/ex-smokers or use of ICS. The results of these sensitivity analysis can be found in s-Figures 1-3.

Results

s-Table 1. Patient characteristics of validation set.

	Total Validation	**Exacerbation**	**No Exacerbation**	**P=**
Number of patients (n)	110	13	97	
Age, years (SD)	65.9 (8.7)	73.0 (7.5)	64.9 (8.5)	**0.00**
Female, n (%)	59 (53.6)	6 (46.2)	53 (54.6)	0.78
Blood eosinophils, median (IQR)	0.12 (0.055-0.20)	0.16 (0.11-0.21)	0.11 (0.04-0.20)	0.40
Blood neutrophils, median (IQR)	6.46 (4.95-8.77)	5.83 (5.08-7.59)	6.50 (4.84-9.13)	0.77
BMI, kg/m^2, median (IQR)	26.2 (22.8-30.0)	30.3 (24.8-34.3)	26.2 (22.6-29.4)	0.06
Pre-BD FEV$_1$, % pred (SD)	59.7 (19.2)	61.9 (24.6)	59.4 (18.4)	0.72
FEV$_1$/FVC-ratio, mean (SD)	0.47 (0.13)	0.51 (0.13)	0.47 (0.13)	0.33
Pts with exa, n (%)				
- total		13 (11.8)		
- OCS in the past 3 months		4		
- AB in the past 3 months		3		
- AB+OCS in the past 3 months		4		
- Currently using AB		0		
- Currently using OCS		2		
- Currently using AB+OCS		0		
Pts with ICS, n (%)	72 (65.5)	8 (61.5)	64 (66.0)	1.00
CCQ score, mean (SD)	2.2 (1.1)	2.7 (1.1)	2.1 (1.1)	0.10
CCQ-score > 3, n (%)	30 (28.8)	6 (46.2)	24 (24.7)	0.17
Smoking history				0.08
- Never, n (%)	1 (0.9)	0 (0)	1 (1.0)	
- Ex, n (%)	71 (64.5)	12 (92.3)	59 (60.8)	
- Current, n (%)	38 (34.5)	1 (7.7)	37 (38.1)	
Pack years, median (IQR)	38 (26.5-54.5)	48 (40-55)	38 (25-54)	0.17

Exa: Exacerbation, SD: Standard Deviation, IQR: Interquartile Range, Quartile 1 – Quartile 3, BMI: Body Mass Index, OCS: Oral Corticosteroids, AB: Antibiotics, CCQ: Clinical COPD Questionnaire.

s-Table 2. Patient characteristics of training set versus validation set.

	Training	Validation	p
Number of patients (n)	254	110	
Age, years (SD)	66.7 (9.3)	65.9 (8.7)	0.401
Female, n (%)	121 (47.6)	59 (53.6)	0.349
Blood eosinophils, median (IQR)	0.12 (0.065-0.23)	0.12 (0.055-0.20)	0.476
Blood neutrophils, median (IQR)	5.83 (4.32-8.26)	6.46 (4.95-8.77)	0.176
BMI, kg/m^2, median (IQR)	26.4 (23.4-30.4)	26.2 (22.8-30.0)	0.574
Pre-BD FEV_1, % pred (SD)	61.2 (20.9)	59.7 (19.2)	0.516
FEV_1/FVC-ratio, mean (SD)	0.49 (0.14)	0.47 (0.13)	0.393
Patients with exa, n (%)			
- **total**	37 (14.6)	13 (11.8)	0.594
- **OCS in the past 3 months**	7	4	
- **AB in the past 3 months**	9	3	
- **OCS+AB in the past 3 months**	12	4	
- **Currently using AB**	2	0	
- **Currently using OCS**	6	2	
- **Currently using OCS+AB**	1	0	
Patients with ICS, n (%)	157 (61.8)	72 (65.5)	0.587
CCQ score, mean (SD)	2.1 (1.1)	2.2 (1.1)	0.378
CCQ-score > 3, n (%)	55 (22.6)	30 (28.8)	0.273
Smoking history			0.471
- **Never, n (%)**	7 (2.8)	1 (0.9)	
- **Ex, n (%)**	168 (66.1)	71 (64.5)	
- **Current, n (%)**	79 (31.1)	38 (34.5)	
Pack years, median (IQR)	36 (20-49)	38 (26.5-54.5)	0.0504

Exa: Exacerbation, SD: Standard Deviation, IQR: Interquartile Range, Quartile 1 – Quartile 3, BMI: Body Mass Index, OCS: Oral Corticosteroids, AB: Antibiotics, CCQ: Clinical COPD Questionnaire.

s-Table 3. Accuracy measures without current OCS and/or Antibiotics users.

Data	ROC-AUC (95%-CI)	Accuracy value (95%-CI)	No information rate	Sensitivity	Specificity	PPV	NPV
Training-set	0.99 (0.98-1.00)	0.96 (0.93-0.98)	0.89**	0.68	1.00	0.95	0.96
Training-set LOO-CV	0.99 (0.98-1.00)	0.96 (0.93-0.98)	0.89**	0.68	1.00	0.95	0.96
Validation set	0.98 (0.94-1.00)	0.95 (0.90-0.99)	0.90*	0.64	0.99	0.88	0.96

OCS: oral corticosteroids, ROC: Receiver-operator characteristic, AUC: area under the curve, LOO-CV: leave-one-out cross-validation, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value. *AUC > NIR: $p<0.05$, **AUC > NIR: $p<0.0001$.

s-Table 4. Composition of and variance explained by the principal components used for further analysis.

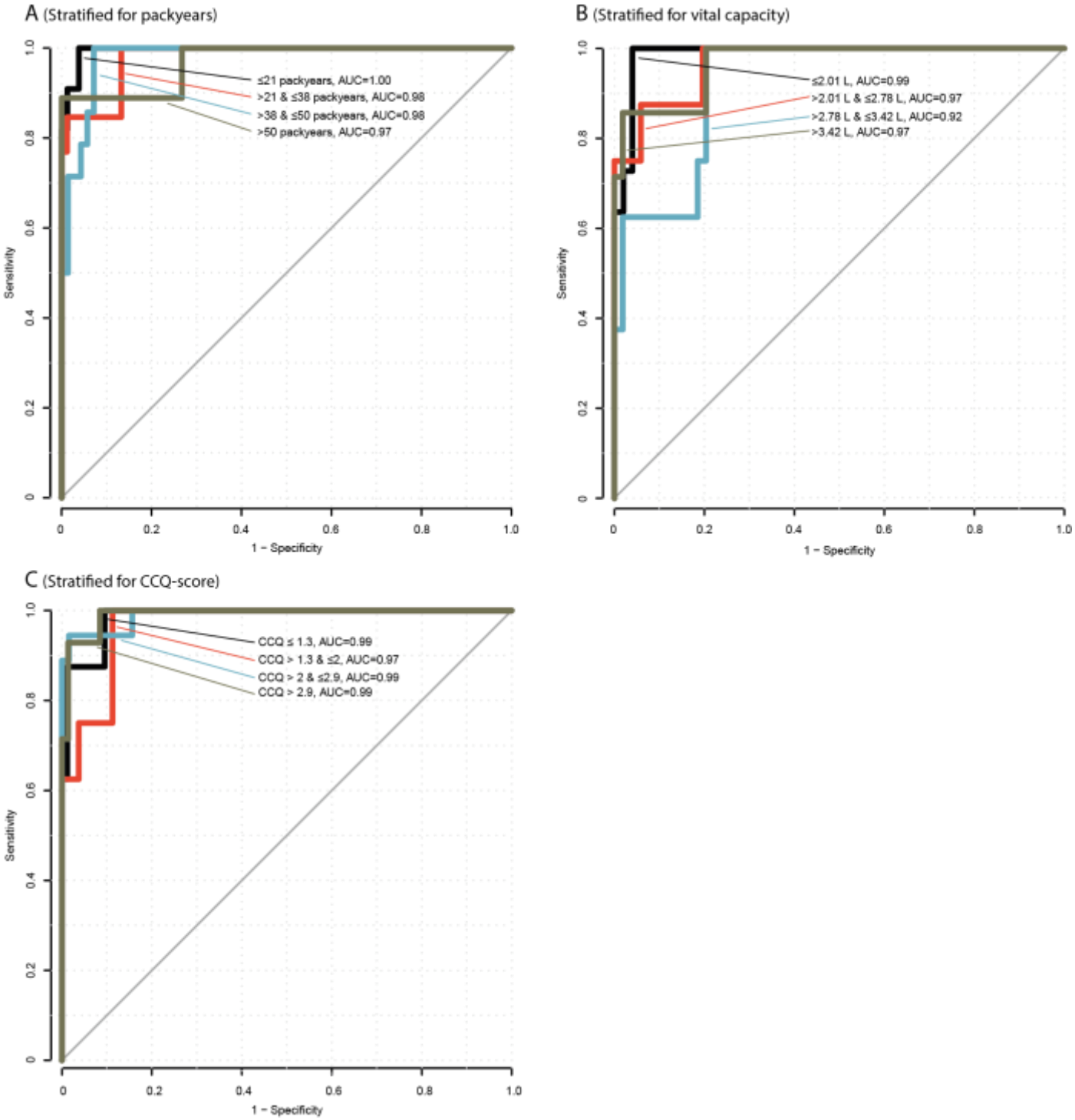
		PC1	PC2	PC3	PC4
Explained variance		0,344	0,154	0,131	0,089
- **Cumulative proportion**		0,344	0,498	0,629	0,717
Sensor signal Loadings	**S1 Peak**	-0,055	0,533	-0,356	0,093
	S3 Peak	0,202	0,135	-0,545	0,139
	S4 Peak	-0,248	0,095	0,329	0,565
	S5 Peak	-0,172	0,533	0,091	0,204
	S6 Peak	-0,220	0,323	0,462	-0,014
	S7 Peak	0,098	-0,445	0,084	0,535
	S1 ratio	0,389	0,247	0,135	0,072
	S3 ratio	0,403	0,159	0,041	-0,039
	S4 ratio	0,419	0,121	0,226	-0,052
	S5 ratio	0,251	0,003	0,374	-0,203
	S6 ratio	0,440	0,040	0,121	0,065
	S7 ratio	0,248	0,008	-0,119	0,520

PC: principal component, S: Sensor. Colour coding: Darker red means more negative contribution of the sensor value towards the principal component, darker green means a more positive contribution.

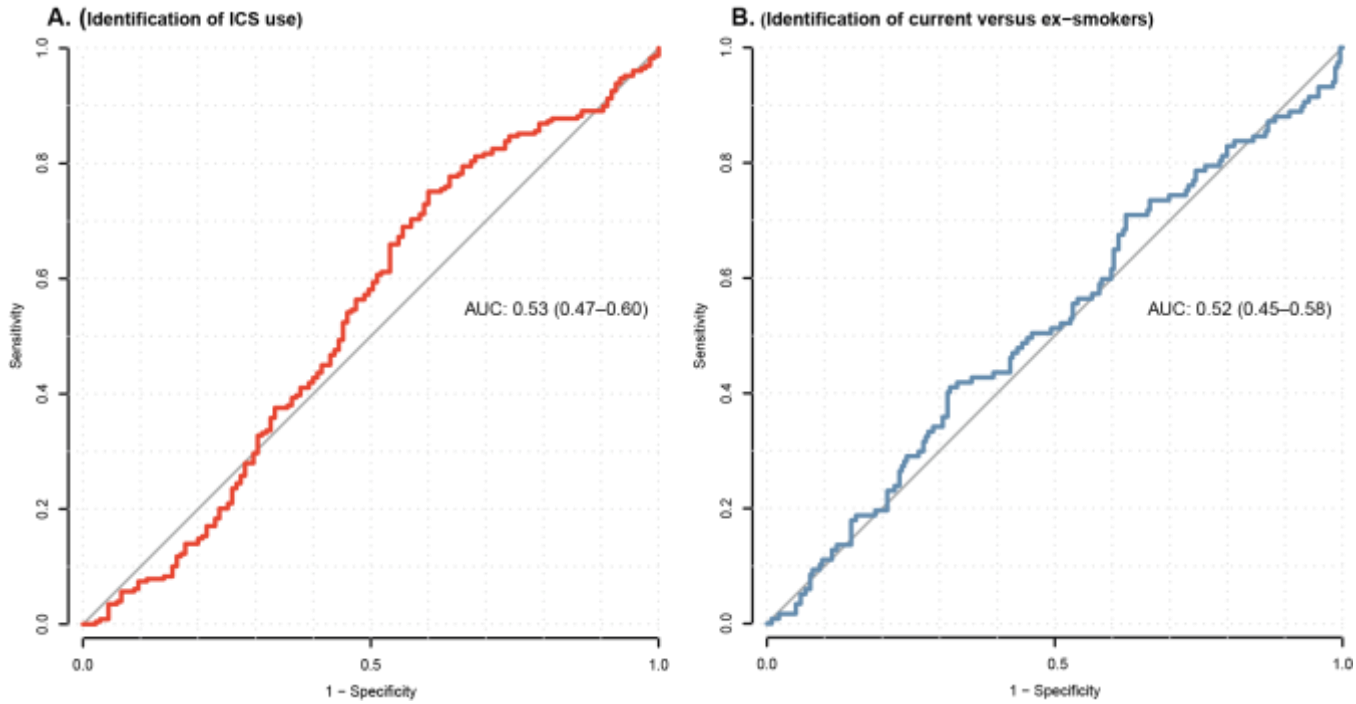
s-Table 5. Correlations between PCs 1-4 and possible confounding variables.

	Pack-years		**Average VC**		**CCQ**	
	r	**p**	**r**	**p**	**r**	**p**
PC 1	-0.030	0.580	-0.053	0.409	-0.002	0.962
PC 2	-0.039	0.470	-0.014	0.827	-0.037	0.493
PC 3	0.028	0.601	-0.026	0.687	-0.064	0.234
PC 4	0.089	0.098	-0.0466	0.473	0.167	**0.002**

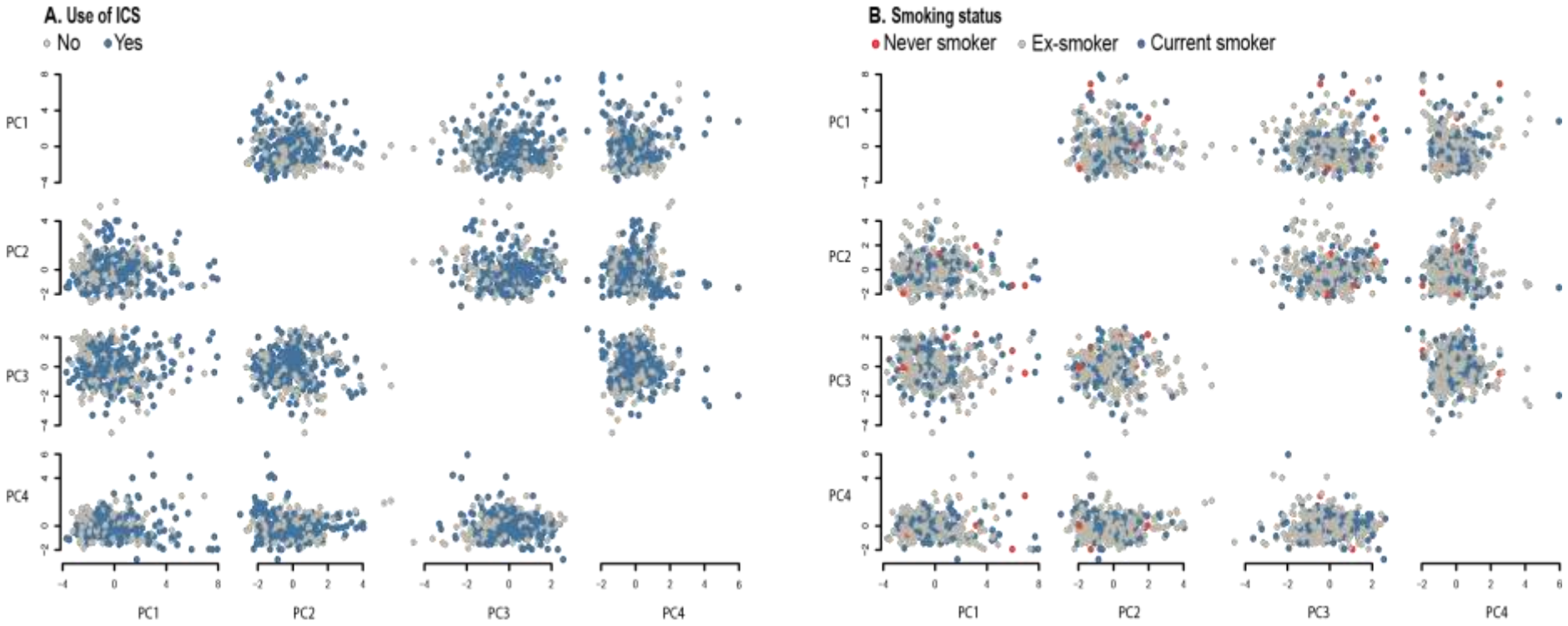
PC: principal component, VC: vital capacity during measurement, CCQ: Clinical COPD Questionnaire, r = Pearson's correlation coefficient.



s-Figure 1. ROC analyses showing the accuracy of the linear discriminant model based on principal component reduction in the total COPD set to detect exacerbations with the population stratified for **A**: pack-years, quartiles 1-4 **B:** average vital capacity during measurement, quartiles 1-4. **C:** CCQ-score, quartiles 1-4.



s-Figure 2. A: The ability of the eNose to discriminate between patients who use inhaled corticosteroids (ICS) and patients who do not. **B:** The ability of the eNose to discriminate between current smokers and patients that are ex-smokers.



s-Figure 3. A. Scatter matrices of smoking status of the total population of COPD patients over the different principal components. **B.** Scatter matrices of ICS use of the total population of COPD patients over the different principal components. ICS: Inhaled Corticosteroids, PC: Principal Component.

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