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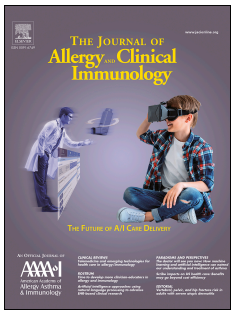

eNose breathprints as a surrogate biomarker for classifying asthma patients by atopy

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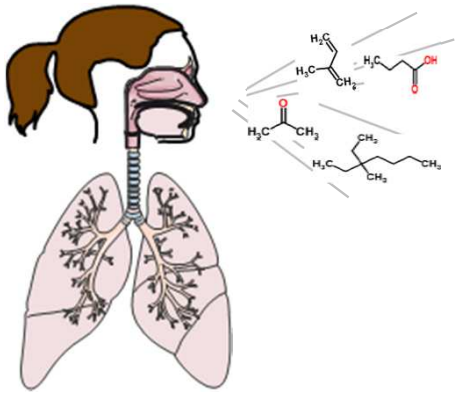
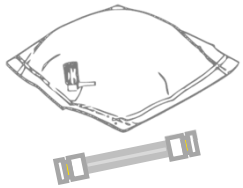
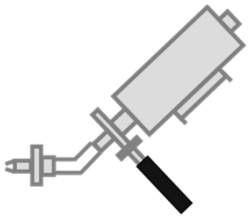




Offline eNose platform



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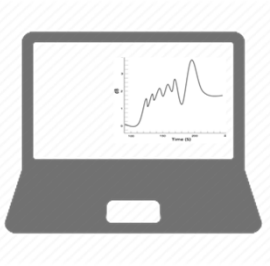

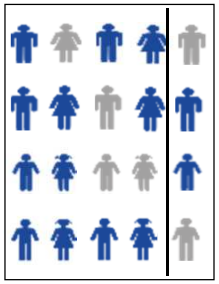

4 asthma cohorts

- BreathCloud
- U-BIOPRED adults
- U-BIOPRED paediatrics
- PACMAN2

Measure patterns of volatile organic compounds mixtures in exhaled air

eNose technology

Machine learning (2 training + 2 validation sets)

eNose accurately classifies atopy

eNose breathprints as a surrogate biomarker for classifying asthma patients by atopy

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Conflicts of interest:

MIA, PB, SJHV, AHN, SH, YWFD, AB, GH, IMA and ADK have no conflicts of interest to disclose. RdV reports personal fees and other from Breathomix, during the conduct of the study. JHR is employed by and owns shares in GlaxoSmithKline. KFC has received honoraria for participating in advisory board meetings of GSK, AstraZeneca, Novartis, Merck, Boehringer Ingelheim and TEVA regarding treatments for asthma and chronic obstructive pulmonary disease and has also been remunerated for speaking engagements. RD reports receiving fees for lectures at symposia organised by Novartis, AstraZeneca and TEVA, consultation for TEVA and Novartis as member of advisory boards, and participation in a scientific discussion about asthma organised by GlaxoSmithKline. RD is a co-founder and current consultant, and has shares in Synairgen, a University of Southampton spin out company. LJF reports grants from Asthma UK and fees for expert consultation and speakers fees from AstraZeneca, GlaxoSmithKline, Novartis, Teva, Bohringer Ingelheim, Respiri and Sanofi paid direct to her institution and outside of the submitted work. CM reports personal fees and other from GSK, personal fees from Novartis, personal fees from Thermo Fisher, other from Boehringer Ingelheim, outside the submitted work. UF reports grants from Swiss National Science Foundation, during the conduct of the study. FS reports personal fees from Vertex, personal fees from Novartis, outside the submitted work. GR reports grants from EU IMI, during the conduct of the study. SED reports personal fees from AstraZeneca, GlaxoSmithKline, Merck & Co, Novartis, Regeneron, Sanofi & Teva, outside the submitted work. SJF reports personal fees and non-financial support from AstraZeneca, grants and personal fees from Boehringer Ingelheim, personal fees from Novartis, personal fees from Teva, personal fees from Chiesi, outside the submitted work. KK is employee of Danone Nutricia Research. PJS reports grants from Public-private Innovative Medicines Initiative (IMI) covered by the European Union (EU) and the European Federation of Pharmaceutical Industries and Associations (EFPIA), during the conduct of the study; other from being scientific advisor and having a formally inconsiderable interest in a start-up company Breathomix BV, outside the submitted work. AHM reports grants and personal fees from Boehringer Ingelheim, grants from

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This article has an online data supplement.

Abstract

Background

Electronic noses (eNose) are emerging point-of-care tools that may help in the subphenotyping of chronic respiratory diseases, such as asthma.

Objective

We aimed to investigate whether eNoses can classify atopy in paediatric and adult asthma patients.

Methods

Asthmatic/wheezing participants from 4 independent cohorts were included; BreathCloud (n=429), U-BIOPRED adults (n=96), U-BIOPRED paediatrics (n=100), and PACMAN2 (n=30). Atopy was defined as a positive skin prick test (⩾3mm) and/or a positive specific IgE (⩾0.35kU/L) for common allergens. Exhaled breath profiles were measured using either an integrated eNose platform or the SpiroNose. Data were divided into 2 training and 2 validation sets according to the technology used. Supervised data analysis involved the use of 3 different machine learning algorithms to classify atopic versus non-atopic patients with reporting area under receiver operating characteristic curves (AUCs-ROC) as a measure of model performance. In addition, an unsupervised approach was performed using Bayesian network (BN) to reveal data-driven relationships between eNose volatile organic compounds (VOCs) profiles and asthma characteristics.

Results

Breath profiles of 655 participants (n=601 asthmatics and 54 pre-school wheezing children, 68.2% atopic) were included in this study. Machine learning models utilizing VOCs profiles discriminated between atopic vs non-atopic participants with AUCs-ROC of at least 0.84 and 0.72 in the training and validation sets respectively. The unsupervised approach revealed that breath profiles classifying atopy are not confounded by other patient characteristics.

Conclusion

eNoses accurately detect atopy in asthmatic and wheezing patients in cohorts with different age groups, and could be used in asthma phenotyping.

Key messages:

- Signals of exhaled volatile organic compounds (VOCs) mixtures, measured by electronic noses (eNoses), can adequately classify asthma patients by atopy. This was supported by data from 4 independent asthma cohorts.
- The eNose may serve as a quick non-invasive tool for asthma phenotyping.

Capsule summary:

Signals of exhaled volatile organic compounds (VOCs) mixtures, measured by electronic noses (eNoses), discriminate between atopic and non-atopic asthma patients in cohorts with different age groups. Therefore, eNoses could be used in asthma phenotyping.

Keywords:

VOCs; eNose; Asthma; Atopy; Discrimination; Machine learning;

List of abbreviations:

ACQ5: 5-item asthma control questionnaire,
ALASSO: Adaptive Least Absolute Shrinkage and Selection Operator
AQLQ: asthma quality of life questionnaire
AUC-ROC: area under the Receiver Operating Characteristics curve
BIC: Bayesian Information Criterion
BMI: body mass index
BN: Bayesian network
DAG: directed acyclic graph
eNose: electronic nose
FE_{NO}: Fractional exhaled nitric oxide
FEV_1: forced expiratory volume in 1 second
FVC: forced vital capacity
GBM: Gradient Boosting Machine
ICS: inhaled corticosteroids
IgE: immunoglobulin E
IMS: ion mobility spectrometry
IQR: interquartile range
LABA: long acting beta agonist
MOS: metal oxide semiconductor
OCS: oral corticosteroids
PACMAN2: Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects 2
ppb: part per billion
QMBs: quartz crystal microbalances
SABA: short acting beta agonist
SCIT: subcutaneous immunotherapy
SLIT: sublingual immunotherapy
sPLS-DA: sparse Partial Least Squares Discriminant Analysis
SPT: skin prick test
STARD: standards for Reporting Diagnostic Accuracy
U-BIOPRED: Unbiased BIOmarkers in PREDiction of respiratory disease outcomes
VOCs: volatile organic compounds

Introduction

Describing asthma as extrinsic (atopic) and intrinsic (non-atopic) was an early attempt to classify the disease into subgroups based on sensitization to common aero-allergens.[1] However, asthma is now recognized as a complex, heterogeneous, chronic inflammatory disease comprising different clinical and biological mechanisms other than allergic sensitization (atopy). Nonetheless atopy is one of the most consistent characteristics associated with certain asthma phenotypes using data-driven methods.[2-6] This suggests that atopy and its underlying biological pathway may be a driving factor of certain asthma-associated phenotypes and play an important role in the pathophysiology of the disease process. Therefore, atopy (or its associated phenotypes) must be characterized as a marker of disease severity and/or a treatable trait in asthma precision medicine.

The diagnosis of atopy is based on allergen-specific IgE measurement and/or skin prick test (SPT) with predefined allergens.[7] While SPT is faster to perform (usually within 15-20 minutes) than *in vitro* measurement of allergen-specific IgEs,[8] it is relatively invasive and may lead to redness, swelling, itching, bleeding, as well as delayed allergic skin reaction and, in some rare circumstance, anaphylactic reactions.[8-10] Also, it is of limited use in patients with severe dermatological conditions or those taking antihistamine (and several other) medications[8] which is common in asthmatics.[11] Conversely, allergen-specific IgE measurements are more costly, and require venepuncture and results are usually not immediately available.[8]

Electronic noses (eNose) are emerging point-of-care tools for diagnosing and phenotyping different respiratory diseases including asthma.[12-14] They are cheap, easy to use and the samples can be collected quickly. They can be used during the doctor's visit with immediate results, which could be beneficial in the clinical decision-making process. eNoses consist of multiple cross-reactive sensors that enable pattern recognition of the complete mixture of volatile organic compounds (VOCs) without identifying their molecular entities,[15, 16] creating unique breath profile for individual subjects. Clustering techniques on eNose breath profiles of both adult and paediatric asthmatics have identified asthma phenotypes with differences in atopy, inflammatory biomarkers and other characteristics.[12, 13, 17]

In this study, we aimed to investigate whether exhaled breath profiles generated by eNose platforms can discriminate between atopic and non-atopic asthmatics, following the Standards for Reporting Diagnostic Accuracy (STARD) guidelines.[18] We hypothesized that assessment of VOCs in exhaled breath offers a fast and non-invasive diagnostic biomarker for atopic asthma, offering a precision medicine tool by characterizing atopy associated treatable traits in asthma and, hence, directing treatment decisions to the needs of individual patients.

Methods

Subjects:

Adult and paediatric participants from 4 independent asthma cohorts were included in this analysis: Unbiased BIOmarkers in PREDiction of respiratory disease outcomes (U-BIOPRED) adult and paediatric cohorts,[19, 20] BreathCloud asthma cohort[12] and Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects 2 (PACMAN2) cohort.[21] Most participants had mild-to-moderate or severe asthma, except a subset within the paediatric U-BIOPRED cohort with preschool wheezing (n=54). For a brief summary of the included cohorts, see Table E1. The flow diagram for patient inclusion is shown in Figure 1.

Outcome definition:

Atopy was defined as a positive SPT defined by a wheal diameter ⩾3mm and/or a positive allergen-specific IgE ⩾0.35kU/L to a pre- specified allergen listed in Table E2. This list represents the most common allergens encountered at the study recruiting centers. Table E3 shows the main allergen category and the type of the test used to diagnose atopy in each included cohort.

Non-invasive exhaled breath measurements:

Exhaled VOCs

Offline eNose technology was used to measure exhaled breath VOCs in U-BIOPRED and PACMAN2 cohorts, while real-time eNose technology (SpiroNose) was used to measure exhaled VOCs in BreathCloud as described previously in details.[12-14] We have followed standard operating procedures for breath collection using validated instruments.[12-14, 22, 23] The exhaled breath measurement is depicted schematically in Figure 2 and described in more detail in the online supplement.

Fractional exhaled nitric oxide (FE_{NO})

Fractional exhaled nitric oxide (FE_{NO}) values in parts per billion (ppb) were measured at a constant flow rate of 50 mL/s with a portable analyzer (NIOX Mino System; Aerocrine, Solna, Sweden), according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.[24] FE_{NO} measurements were available in a subset of patients in each cohort.

Data analysis

The general overview of the used data analysis approach is summarized in Figure E1.

Supervised analysis with machine learning models

In order to investigate the discriminative potential of the eNoses sensors to classify atopic vs non-atopic subjects, we applied a supervised machine learning approach using different machine learning models.[25] The classification performance (high vs low) of the same machine learning model has been reported to vary by dataset/cohort.[26, 27] Building conclusions on a single classification model may be biased and reduce potential validation, hence hindering biomarker discovery. As proof of concept, three different and powerful machine learning techniques modelled on eNose signals were used: sparse Partial Least Squares Discriminant Analysis (sPLS-DA),[28] adaptive least absolute shrinkage and selection operator (Adaptive LASSO) [29] and Gradient Boosting Machine (GBM)[30] previously used in metabolomics research.[25-27] This is to provide an estimate of the robustness of statistical performance across different models and to evaluate eNose accuracy in classifying atopic asthma patients without bias from "single model selection". The three methods were selected based on the merit of feature reduction (selection) which avoids the risk of model overfitting. For more details see online supplement.

Individual cohort classification with internal cross validation

For each cohort, atopic and non-atopic participants were classified using the eNose-driven models (sPLS-DA, Adaptive LASSO and GBM). For internal validation, 10-fold cross validation (50 repeats for model tuning and prediction estimation) was implemented in each model as recommended.[31]

Classification using training and validation sets in the pooled cohort and the BreathCloud cohort

Datasets from the 3 independent cohorts (U-BIOPRED adults, U-BIOPRED paediatrics and PACMAN2) that used the same eNoses platform for offline breath analysis were combined (pooled cohorts) because internal validation alone cannot assess the robustness of the eNoses sensors to detect atopy in independent validation sets. ComBat batch correction[32] was applied on the combined sensors data and normal distributions were assessed using histograms. The pooled dataset was then randomly divided into a training and a validation set using an approximate ratio of 0.75:0.25 as recommend.[12, 31] The latter step was also applied to the BreathCloud cohort using the SpiroNose for real-time breath analysis, resulting in two training sets and two validation sets (Figure 1). The sample size calculation of the training and validation sets, based on the measure of area under the Receiver Operating Characteristics curve (AUC-ROC), is explained in the online supplement. The training datasets were used to train the 3

models using a 10-fold internal cross validation (50 repeats for model tuning and prediction estimation). The predictive potential of the fitted models was assessed in the validation sets.

The performance of the obtained classification/predictive model was evaluated by computing the AUC-ROC and associated model accuracies, specificities and sensitivities. In order to calculate 95% confidence intervals, error estimates were obtained by performing 2000 non-parametric stratified bootstrapped replicates using random sampling with replacement and with the percentile method.[33, 34] Differences in performance between the applied machine learning models were estimated by performing pairwise comparison of the obtained AUCs-ROC using the Venkatraman method[35] (1000 permutations).

Sensitivity analysis after exclusion of non-aeroallergens

A sensitivity analysis was performed to check whether the discrimination using eNoses would change if patients sensitized with non-aeroallergens (e.g. food, latex, etc.) were excluded. Pairwise comparisons of the AUCs-ROC obtained before and after exclusion of patients with non-aeroallergens sensitization were estimated using the Venkatraman method[35] (1000 permutations).

Unsupervised investigation of atopy and data-driven potential confounders

As exhaled breath is an emerging research field, there is no adequate information in the literature on the possible confounding factors that should be considered or adjusted for. Unsupervised data-driven approaches such as Bayesian Networks (BNs) may provide an opportunity to reveal or select hidden confounders[36] in metabolomics breath research. The BreathCloud dataset was used in this analysis being the largest breath cohort with available information on smoking, diet intake, and their time of food/drink consumption and environmental factors (related to ambient conditions), as well as demographics, spirometry, inflammatory parameters, and asthma medications.

Bayesian Network (BN)

Unsupervised learning of the BN was performed on the complete BreathCloud dataset. More details are provided in the online supplement. The final network was depicted using Cytoscape[37] version 3.7.1 showing the probabilistic relationship between different variables in the form of directed acyclic graph (DAG).

Discriminative ability of FE_{NO} to classify atopic and non-atopic asthma

A ROC curve was generated to assess the accuracy of FE_{NO} to discriminate atopic from non-atopic asthmatics. Continuous FE_{NO} values (in ppb) pooled from all cohorts (BreathCloud, PACMAN2 and U-BIOPRED cohorts) were used in this analysis. Subsequently, different clinically utilised cut-off values (>20, >35 and >50 ppb) were used to categorize high and low FE_{NO}, to investigate whether these cut-offs would provide better insight than the uncategorized continuous values. These cut-offs were chosen based on previous recommendations.[38, 39] AUCs-ROC for classifying atopy with bootstrapped (2000 non-parametric stratified replicates) confidence intervals[33, 34] were constructed using each measure (continuous and categorized).

All analyses were performed using R studio (version 1.2.1335) with R software (version 3.6.1) supported with the following packages; mixOmics, glmnet, gbm, caret, pROC, boot, wiseR, bnlearn.

Results:

eNose data from a total of 655 participants (98 school-aged asthmatic children, 54 preschool wheezing children and 503 asthmatic adults) were included in this study (Table 1). The prevalence of atopy was ranging from 63% to 83% within the different cohorts. Most atopic patients (412 out of 447 patients, 92.2%) had sensitization to at least one aeroallergen.

Individual cohort classification using exhaled breath profiles with internal cross validation

Atopic versus non-atopic asthma patients were classified in the 4 independent cohorts using 3 different machine learning models (sPLS-DA, Adaptive LASSO, and GBM) with the representative AUCs-ROC shown in Figure 3. For each individual cohort, the obtained AUCs-ROC were at least 0.85.

Classification using exhaled breath profiles with training and validation sets in the pooled cohort and the BreathCloud cohort

The predictive performance of the trained machine learning models was evaluated in two validation sets showing relatively high AUCs-ROC of at least 0.72 and 0.91 in the pooled cohorts and BreathCloud, respectively as shown in Figure 4 (pooled cohorts) and Figure 5 (BreathCloud). The associated accuracies, specificities and sensitivities and their 95% confidence intervals are also shown (Figure 4, Figure 5 and Table E4). In the pooled cohort, most discriminative signals were derived from the Owlstone Lonestar, while in the BreathCloud cohort, the sensor peak/breath hold ratios (Figure E2) contributed most to the discriminative signal (data not shown). The GBM model performance in the training sets (pooled cohort and BreathCloud) was better than either sPLS-DA or adaptive LASSO models as estimated by higher AUCs-ROC using the Venkatraman's test (Table E5). However, this outperformance was eliminated when model predictions were applied on the validation sets.

Sensitivity analysis after exclusion of non-aeroallergens

The AUCs-ROC obtained after exclusion of patients sensitized with non-aeroallergens were relatively similar to the previous findings in both the training and validation sets (Table E6). Venkatraman test showed no significant differences in the AUCs-ROC before and after exclusion of these patients.

Data-driven Bayesian Network (BN)

Figure E3 shows a DAG of BN that reveals probabilistic associations between SpiroNose sensor signals against different patient characteristics (including atopy) and other factors. The data-driven BN shows no edges connecting the atopy-associated VOCs sensor signals and other

340 measured characteristics. Other VOCs signals showed connection with eosinophils counts and
341 oral corticosteroid (OCS) use.

342 **FE_{NO} in discriminating atopic and non-atopic asthma**

343 FE_{NO} was available in a subset of patients within the included cohorts (n=357). Figure E4 shows
344 that FE_{NO} did not accurately discriminate between atopic and non-atopic asthmatics using either
345 the continuous values (AUC-ROC = 0.51) or the categorized estimates (AUCs-ROC=0.52 for FE_{NO}
346 >20 ppb, 0.49 for FE_{NO} >35 ppb and 0.50 for FE_{NO} >50 ppb).

Discussion:

To our knowledge, this is the first study to investigate the ability of eNose technology to detect atopy in asthmatic patients across different age groups. Using a composite of benchmarking supervised and unsupervised analysis techniques on different eNose platforms from 4 independent cohorts, we have shown that different eNoses can appropriately discriminate between atopic versus non-atopic asthmatics. Importantly, we show that observations made using offline eNose technology are replicated with the real-time SpiroNose technology where sensors are place in line with standard equipment for spirometry.

By testing the eNose technology separately in individual cohorts, a highly discriminative signal between atopic versus non-atopic asthma patients was observed as indicated by the different cross-validated machine learning models showing an AUCs-ROC ≥0.85. The eNose VOCs profiles distinguished both groups when the data of three cohorts using the same eNoses platform for exhaled breath analyses (U-BIOPRED adult, U-BIOPRED paediatric and PACMAN2 cohorts) were pooled, which suggests generalizability of eNoses that probably capture certain distinct VOCs associated with atopy. However, there was a slight decrease in performance of the models in both the training and validation sets of the pooled cohorts as compared to the performance in the individual cohorts, with AUCs-ROC of at least 0.84 and 0.72, respectively. This may be related to differences in study populations in respect of age and asthma-associated characteristics and, possibly, different eNoses batch versions within subjects of the pooled cohorts, which may introduce more diverse VOCs patterns (more noise). However, discrimination was possible despite these variations. These factors may also explain, in part, why the BreathCloud cohort had higher performance of the models compared with the pooled cohorts in both the training and validation sets with AUCs-ROC of at least 0.93 and 0.91, respectively. Another important factor that may explain the higher performance within BreathCloud, is the real-time capability to capture the VOCs compared with the offline approach of the eNoses platform within the pooled cohort. The latter might be subject to some contaminant VOCs and/or VOCs loss during transportation from the Tedlar bags and Tenax tubes.[40] Furthermore, the larger sample size in the BreathCloud study (twice as big as the pooled cohorts) meant more statistical power and, therefore, better training of the machine learning models.

The GBM model outperformed both sPLS-DA and adaptive LASSO in the training sets which might indicate an overfitting issue of the GBM model; however, no difference in performance was observed in the validation sets, suggesting comparable statistical performance across different machine learning models. This may indicate robustness of eNose-technology in classifying atopic asthma patients despite using different machine learning models.

Studies investigating the relationship between exhaled VOCs and atopic asthma are scarce. In a previous study, VOCs profile captured by Cyranose C320 showed only a trend (p=.07) for classification between atopic versus non-atopic childhood asthmatics.[41] This study only included a small sample size of children with asthma (n=31), which may have led to lack of statistical power. In addition, in our study the Owlstone Lonestar is a major driver of the signal that is responsible for discrimination in contrast to the the Cyranose C320 which was also included in our study.

The atopy discriminating VOCs signals in this study were probably driven by aeroallergens as most included atopic patients were sensitized by at least one aeroallergen. This was supported by a sensitivity analysis in which non-aeroallergens sensitized patients were excluded, showing similar findings. Yet, this warrants further investigation to explore whether the VOCs signals detected are related to the underlying pathophysiology locally (in the lung) or systemically or both.

FE_{NO} >20 ppb has been reported to be associated with atopy in 1199 children aged 13 to 15 years from Peru, showing an AUC-ROC of 0.65 in non-asthmatics (n=1110) and an AUC-ROC of 0.82 in asthmatics (n=89).[38] Our analysis showed that the eNose technology provided higher AUCs-ROC in the included paediatric cohorts (≥0.85 in the U-BIOPRED paediatric and ≥0.97 in PACMAN2 cohorts) provides better accuracy in detecting atopy. In addition, in our study, FE_{NO} did not accurately discriminate between atopic and not-atopic asthmatics (AUC-ROC=0.52), indicating a limited accuracy of FE_{NO} in detecting atopy as compared to eNose technology in a broad age-range multinational cohort.

The Bayesian Network (BN) revealed the unsupervised data-driven relationship between the SpiroNose data points and all other measured characteristics. From the DAG network, we identified no clear relationship between exhaled breath profiles associated with atopy and other measured variables, suggesting the profiles predicting atopy are not be confounded by other measured factors in the BreathCloud cohort. In addition, the BN also showed relationships between VOCs signals (from different sensors) and eosinophil counts and OCS use, as previously reported findings.[12, 13, 42] However, BNs do not prove causality between variables, and can only be used for probabilistic reasoning. Therefore, the association between atopy and exhaled breath sensors signals is a probabilistic estimation and further research to investigate possible confounders is still required.

This study has multiple strengths. First, we used 4 independent asthma cohorts to validate the findings that showed a relative steadiness of the atopy classification potential. Second, the utilization of different eNoses platforms covering both offline and real time measurement of exhaled breath may serve as a sign of the generalizability of the eNose technology to detect atopy in asthma patients. Third, the followed benchmarking analysis strategy in this study is

418 coupling both supervised and unsupervised approaches to further support findings. Finally, we
419 used 3 different powerful machine learning techniques to provide an indication of the
420 robustness of statistical performance across the tested models.

421 However, this study has also limitations. First, eNose technology in general does not allow for
422 identification of individual VOCs but are based on cross-reactive sensor arrays that allow for
423 powerful VOCs signal pattern recognition which does not hamper the clinical application of
424 eNose technology. Identifying individual discriminating VOCs in future studies, by e.g. mass
425 spectrometry techniques, will help to get more insight on the underlying pathophysiological
426 pathways. Second, we only investigated exhaled breath profiles and its relation to atopy at a
427 single time point. In one study, diurnal variations in the VOCs profiles of atopic moderate
428 asthmatics were observed.[43] Therefore, assessment of the temporal stability of atopy-
429 discriminative signature is required. Third, a large percentage of the included subjects were
430 white Caucasians, hence, this demands broader investigation in other ethnicities to assess
431 generalisability. Fourth, validation should be ideally performed by training a model on a cohort
432 and then validate the findings on a different cohort. This was not feasible in the current study as
433 we used two different eNose technologies (offline versus real-time measurement), which make
434 direct validation of the findings impossible due to different sets of sensors from the two
435 technologies. In addition, due to the limited sample sizes in datasets of offline eNoses platform,
436 pooling the data was essential to achieve the statistical power necessary for appropriate
437 training and validation of the models. Fifth, this study was not meant to provide a
438 comprehensive comparison on the performance of different machine learning models, but
439 rather to provide an estimation of statistical robustness as a proof of concept. Whether other
440 models will provide different outcomes merits further investigation. Finally, we did not
441 investigate differences in the allergen specific VOCs signals and whether it would differ
442 according to the numbers of sensitized allergens, time course of atopy and/or levels of specific
443 IgE or wheal size. A previous study have shown that these atopy-associated outcome measures
444 could identify subphenotypes within children.[44]

445 SPT or allergen-specific IgE measurements remain the gold standard for diagnosing atopy and
446 offer details regarding the allergen to which the patient is sensitized. The eNose at this time
447 point cannot be considered as alternative, however, it may offer a very quick (in minutes) non-
448 invasive screening tool in situations where the standard methods cannot be used, such as in
449 patients with dermatological conditions, taking interfering medications and/or with low
450 acceptability to skin testing or blood withdrawal (e.g. children).

451 By detecting atopy, in addition to its potential to perceive changes in inflammatory biomarkers
452 such as eosinophils and neutrophils or oral corticosteroid use,[12, 13] the eNose may help to
453 identify asthma phenotypes and asthma-associated treatable traits.[45] Therefore, it can serve as

a tool in asthma precision medicine approaches. Managing atopic asthma may require different steps from environmental avoidance of allergens[46] to symptomatic control with add-on therapies such as antihistamines or antihistamine/leukotriene antagonists combinations.[47] In addition, more targeted therapies, such as allergen-specific subcutaneous immunotherapy (SCIT) in patients with stable asthma,[48] sublingual immunotherapy (SLIT) and anti-IgE monoclonal antibody omalizumab,[49] can be directed to the atopic asthma patients. The use of eNose may, thus, help the non-invasive and quick tailoring of these interventions to the need of each individual patient.

In conclusion, the eNose technology can accurately and robustly classify asthma patients by atopic status. Coupling supervised and unsupervised machine learning approaches reveals that the associations between exhaled breath and atopy and our results were generalizable. The present findings suggest that exhaled breath analysis by eNose allows meaningful phenotyping of asthma patients and may, therefore, be used in personalized asthma clinical decisions.

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588 **Tables:**

589 **Table 1:** Participant baseline characteristics.

Characteristics	U-BIOPRED adults (n=96)	BreathCloud (n=429)	U-BIOPRED paediatrics (n=100)	PACMAN2 (n=30)
Age (years), median (IQR)	55.0 (43.0-62.0)	50.0 (34.0-62.0)	5.0 (4.0-12.8)	11.5 (9.8-13.6)
Age group • Adults (≥18 years) • Children (<18 years)	 96 (100%) NA	 407 (94.9%) 22 (5.1%)	 NA 100 (100%)	 NA 30 (100%)
Females (%)	54 (56.3%)	252 (58.7%)	36 (35%)	16 (53.3%)
BMI (kg/m^2), median (IQR)	26.9 (23.9-32.5)	26.9 (23.5-30.6)	16.6 (15.4-19.7)	18.3 (17.3-19.7)
BMI (kg/m^2) z-score, median (IQR)	1.3 (0.4-2.5)	1.3 (0.4-2.1)	0.3 (-0.4-1.1)	0.1 (-0.6-1.1)
Race (white Caucasian, %)	90 (93.8%)	366 (85.3%)	75 (75%)	30 (100%)
Atopy (%) • Aeroallergen • Non-aeroallergen	**70 (72.9%)** 63 (65.6%) 7 (7.3%)	**289 (67.4%)** 266 (62%) 23 (5.4%)	**63 (63%)** 59 (59%) 4 (4%)	**25 (83.3%)** 24 (80%) 1 (3.3%)
Current smokers (%)	26 (27.1%)	45 (10.5%)	NA	NA
Patient class • **Asthmatic** • **Pre-school wheezing**	 96 (100%) NA	 429 (100%) NA	 46 (46%) 54 (54%)	 30 (100%) NA
FEV_1 % predicted pre salbutamol, median (IQR)	67.8 (54.8-87.6)	86.0 (74.0-100.5)	93.9 (82.5-106.3)	86.5 (78.0-96.5)
FEV_1 % predicted post salbutamol, median (IQR)*	81.3 (64.1-100.0)	92.0 (80.0-101.8)	101.5 (91.8-112.1)	95.0 (83.0-101.0)
FEV_1/FVC % predicted pre salbutamol, median (IQR)	75.4 (76.1-88.2)	87.0 (75.0-97.0)	92.1 (82.6-100.9)	94.5 (88.5-100.3)
FEV_1/FVC % predicted post salbutamol, median (IQR)*	80.6 (72.3-94.8)	89.0 (78.0-100.0)	97.9 (89.3-103.4)	98.0 (92.0-104.0)
FE_{NO} in ppb, median (IQR)[¶#]	28.0 (14.0-49.0) (n=94)	21.0 (13.0-39.0) (n= 192)	30.0 (13.0-59.0) (n=43)	28.0 (11.0-64.0) (n=28)
FE_{NO} in ppb, median (IQR)[¶#] • Atopic subjects • Non-atopic subjects	 25.0 (13.0-41.5) (n=69) 34.0 (22.0-77.5) (n=25)	 22.0 (12.5-37.0) (n=133) 20.0 (13.0-41.0) (n=59)	 35.5 (14.0-65.78) (n=38) 12.00 (8.50-21.0) (n=5)	 35.0 (14.0-68.0) (n=23) 11.0 (5.0-17.50) (n=5)
ACQ5 score average, median (IQR)	1.8 (0.8-2.8)	1.4 (0.7-2.3)	NA	NA
ACT score average, median (IQR)	NA	NA	18.00 (13.8-21.0)	18 (9.8-21.3)
P(AQLQ) score average, median (IQR)	5.2 (3.8-5.9)	NA	5.5 (4.0-6.8)	6.0 (5.1-6.5)

Current asthma medication used (%):				
• **ICS**	96 (100%)	357 (83.2%)	86 (86%)	30 (100%)
• **SABA**	61 (63.5%)	198 (46.2%)	92 (92%)	24 (80%)
• **LABA**	78 (81.3%)	307 (71.6%)	52 (52%)	16 (53.3%)
• **OCS**	38 (39.6%)	58 (13.5%)	6 (6%)	0 (0%)
• **Short-acting Anticholinergics**	2 (4.2%)	33 (7.7%)	1 (1%)	0 (0%)
• **Long-acting Anticholinergics**	24 (25%)	79 (18.4%)	0 (0%)	0 (0%)
• **Leukotriene antagonists**	28 (29.2%)	78 (18.2%)	57 (57%)	2 (6.7%)
• **Theophylline**	13 (13.5%)	2 (0.5%)	2 (2%)	0 (0%)
• **Antihistamine**	9 (9.4%)	96 (22.4%)	9 (9%)	NA

Variables described as n (% of n) unless specified, IQR: interquartile range, BMI: body mass index, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, FE_{NO}: fraction of exhaled nitric oxide, ACQ5: 5-item asthma control questionnaire, P: paediatric, AQLQ: asthma quality of life questionnaire, ICS: inhaled corticosteroids, SABA: short acting beta agonist, LABA: long acting beta agonist, OCS: oral corticosteroids, NA: not applicable which corresponds to either data not measured in this cohort or not applicable regarding the cohort criteria. *In BreathCloud, the personal best measurements were reported for post-bronchodilator forced expiratory volume in 1 second (FEV_1; % predicted) from data of routine clinical practice collected <12 months prior to the study visit. ¶In BreathCloud, FE_{NO} measurements were reported as corresponding to the latest recorded values in routine clinical practice. #Data were not available for the complete study participant.

Figure legends:

Graphical abstract

Figure 1: Flowchart for the included participants from 4 independent asthma cohorts. *eNoses platform is a composite of 4 differently developed eNoses (Cyranose C320, Tor Vergata, Comon Invent, and Owlstone Lonestar). ¶Inclusion criteria violator: participants that were excluded from the analysis due to violation of the inclusion criteria defined by the U-BIOPRED. #Uncertain atopy diagnosis: patients who do not have confirmed diagnoses for atopy by either skin prick test or allergen-specific IgE.

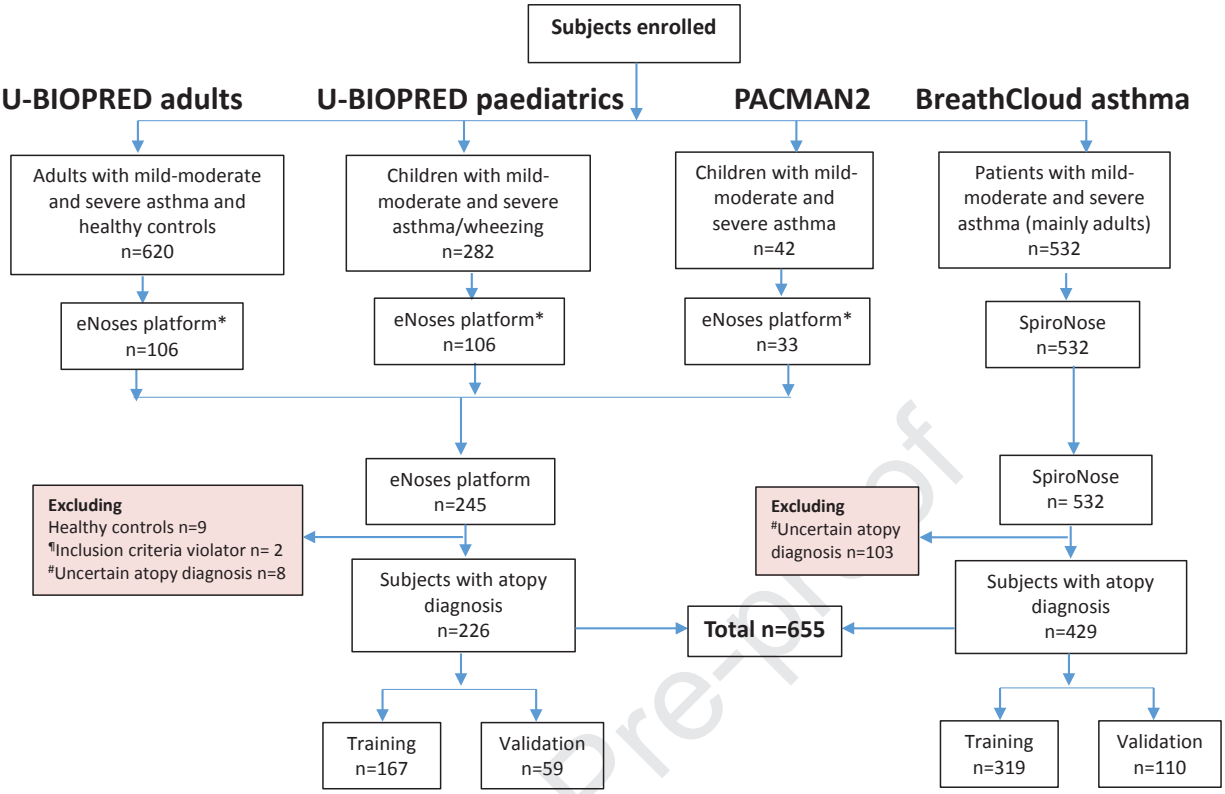
Figure 2: Exhaled breath measurement using the eNoses platform (U-BIOPRED adults, U-BIOPRED paediatrics and PACMAN2) and the SpiroNose (BreathCloud). Upper panel, breath collection and measurement using the eNoses platform in U-BIOPRED adults, U-BIOPRED paediatrics, and PACMAN2 cohorts. Subjects exhaled a single vital capacity volume in a 10 litre Tedlar bag, followed by sampling air on a Tenax thermal desorption tube to capture volatile organic compounds (VOCs). A thermal desorption oven was used for desorption of the VOCs from the tubes and then transferred into a Tedlar bag using a carrier nitrogen gas. Subsequent detection of the signal pattern of VOCs mixture was performed through multiple cross-reactive sensors in a composite eNoses platform. Lower panel, real time collection of the exhaled breath using the SpiroNose in the BreathCloud cohort. Patients were instructed to perform 5 tidal breaths, followed by inspiration of a single vital capacity volume, a breath hold for 5 seconds and then expiration towards residual volume. The sensors' signals are recorded in real time by the SpiroNose and transferred into a cloud environment for further automated analysis. QMBs: quartz crystal microbalances, MOS: metal oxide semiconductor, IMS: ion mobility spectrometry.

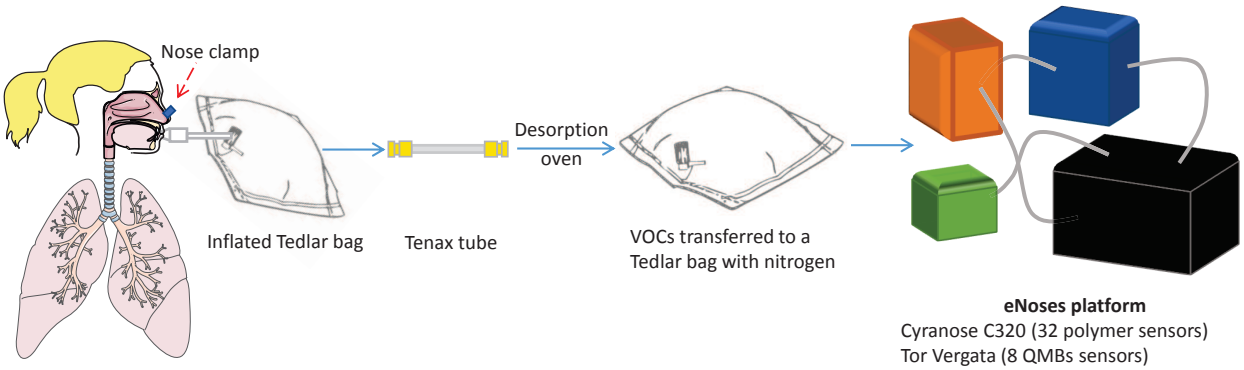
Figure 3: Area under Receiver Operating Characteristics curves (AUCs-ROC) with 95% bootstrapped confidence intervals for 3 different machine learning models classifying atopic versus non-atopic asthma in U-BIOPRED adult, BreathCloud, U-BIOPRED paediatric and PACMAN2 cohorts. GBM: Gradient Boosting Machine, sPLS-DA: sparse Partial Least Squares Discriminant Analysis, ALASSO: is denoting Adaptive LASSO.

Figure 4: Upper panel, the Area under Receiver Operating Characteristics curves (AUCs-ROC) with 95% bootstrapped confidence intervals for 3 different machine learning models on eNose VOCs breath profiles from the training subset (≈ 75%) of the pooled cohorts with their associated accuracies, specificities and sensitivities (95% confidence interval) are shown. Lower panel, the predictive potential of the trained model was evaluated using a validation subset (≈ 25%) from the pooled cohorts showing relatively high AUCs-ROC with depiction of their associated accuracies, specificities and sensitivities (95% confidence interval). GBM: Gradient Boosting Machine, sPLS-DA: sparse Partial Least Square-discriminant analysis, ALASSO: is denoting Adaptive LASSO.

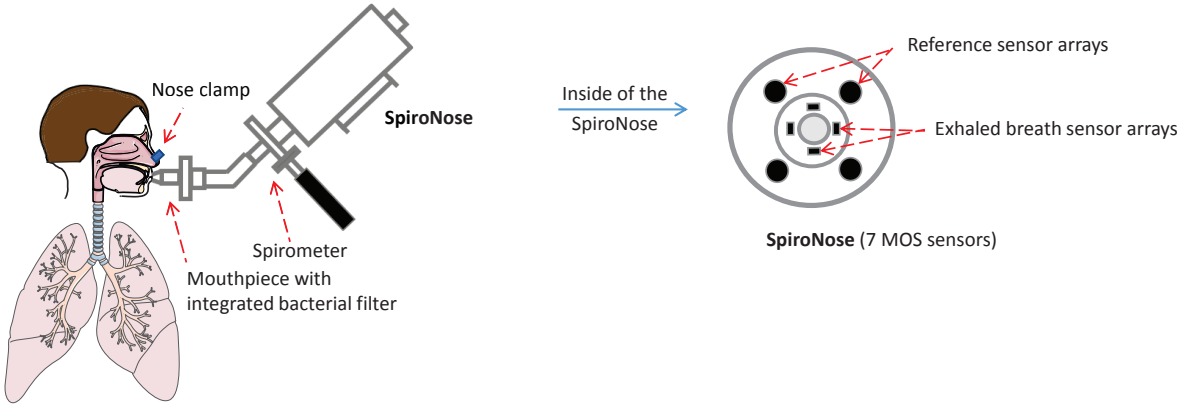
Figure 5: Upper panel, the Area under Receiver Operating Characteristics curves (AUCs-ROC) with 95% bootstrapped confidence intervals for 3 different machine learning models on SpiroNose VOCs breath profiles from the training subset (≈ 75%) of the BreathCloud cohort with their associated accuracies, specificities and sensitivities (95% confidence interval) are shown. Lower panel, the predictive potential of the trained model was evaluated using a validation subset (≈ 25%) from the BreathCloud cohort

639 showing relatively high AUCs-ROC with depiction of their associated accuracies, specificities and
640 sensitivities (95% confidence interval). GBM: Gradient Boosting Machine, sPLS-DA: sparse Partial Least
641 Square-discriminant analysis, ALASSO: is denoting Adaptive ALASSO.

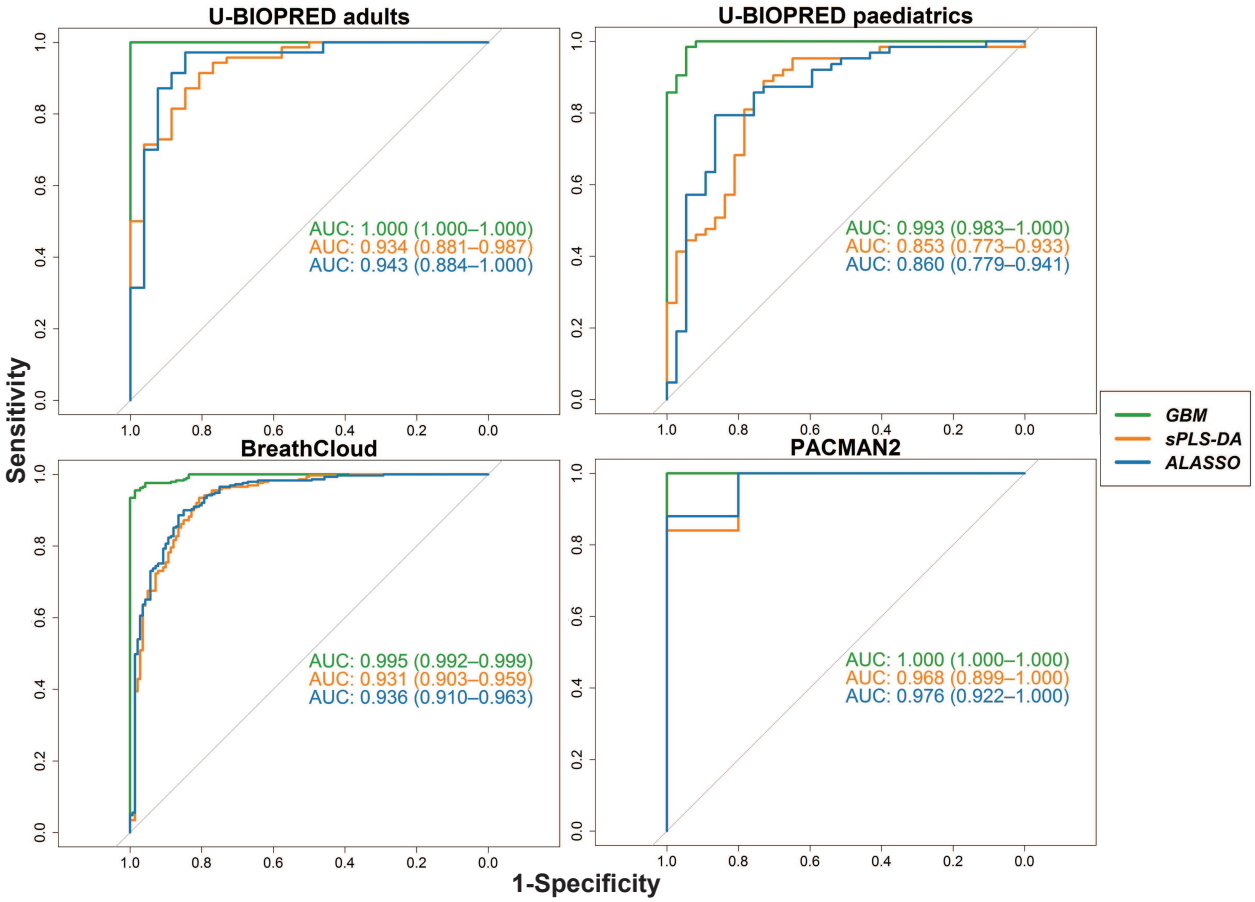


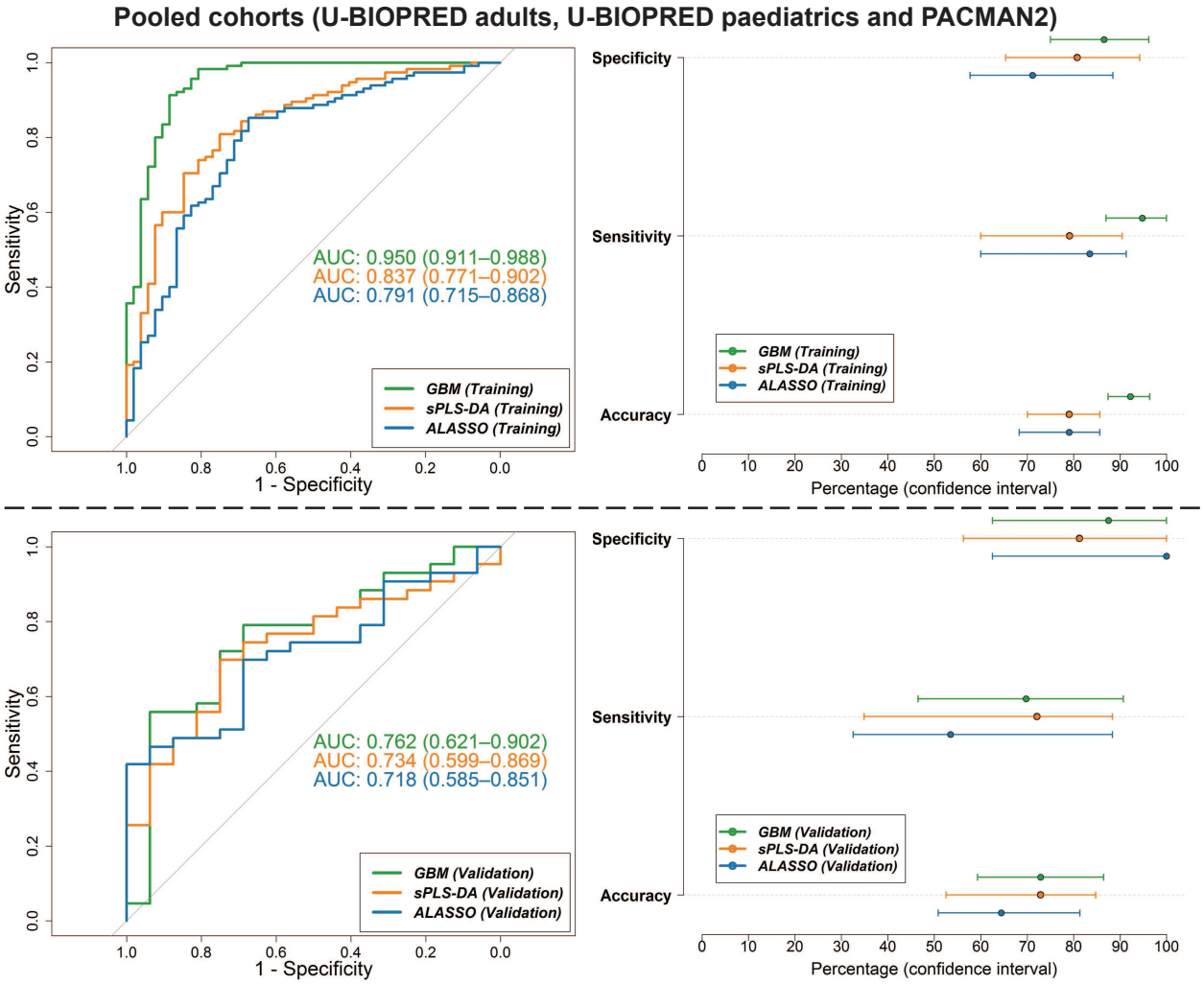


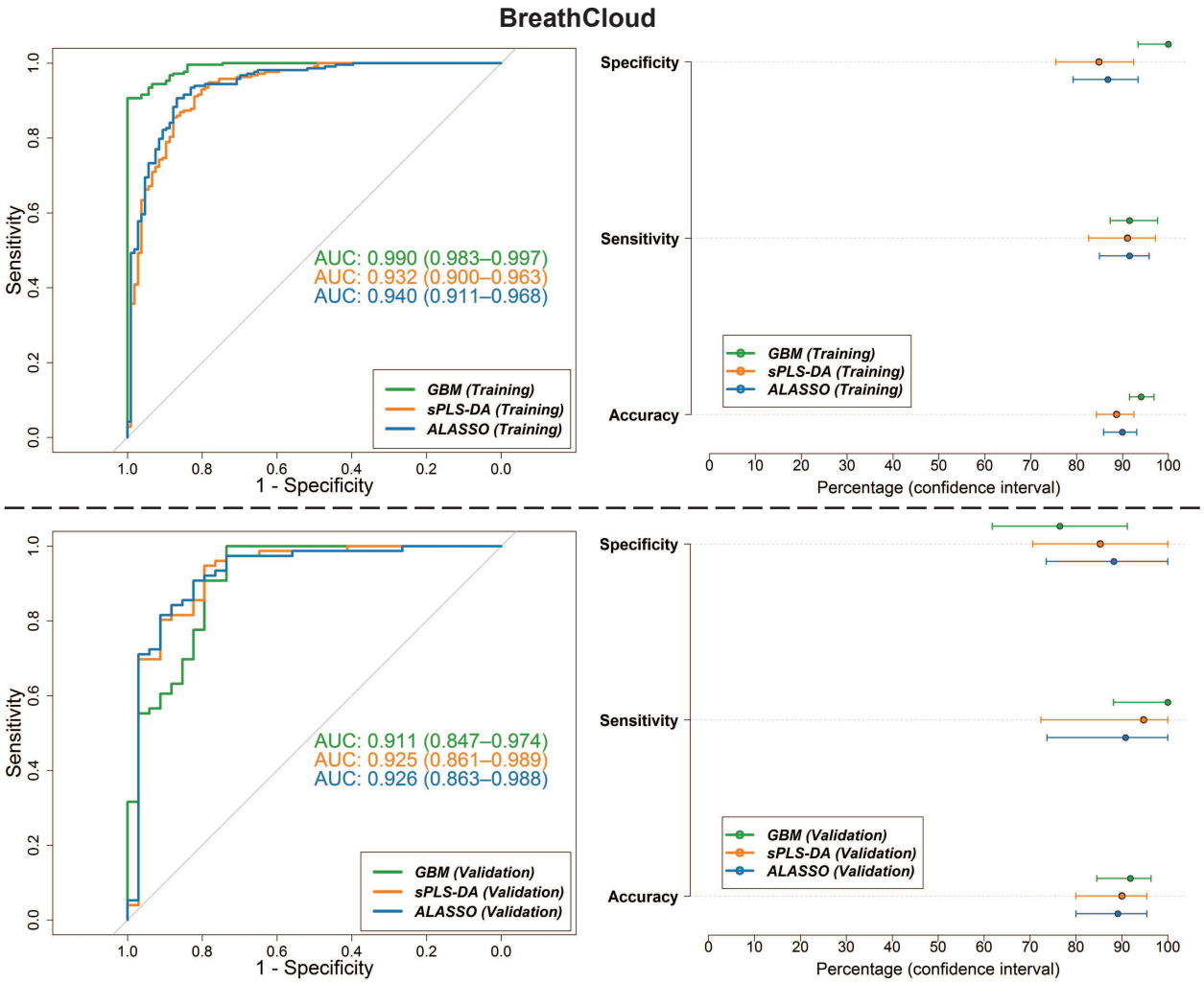
eNoses platform
Cyranose C320 (32 polymer sensors)
Tor Vergata (8 QMBs sensors)
Comon Invent (8 MOS sensors)
Owlstone Lonestar (110 IMS)



SpiroNose (7 MOS sensors)


U-BIOPRED adults
AUC: 1.000 (1.000–1.000)
AUC: 0.934 (0.881–0.987)
AUC: 0.943 (0.884–1.000)
U-BIOPRED paediatrics
AUC: 0.993 (0.983–1.000)
AUC: 0.853 (0.773–0.933)
AUC: 0.860 (0.779–0.941)
BreathCloud
AUC: 0.995 (0.992–0.999)
AUC: 0.931 (0.903–0.959)
AUC: 0.936 (0.910–0.963)
PACMAN2
AUC: 1.000 (1.000–1.000)
AUC: 0.968 (0.899–1.000)
AUC: 0.976 (0.922–1.000)
Sensitivity
1-Specificity
GBM
sPLS-DA
ALASSO


Pooled cohorts (U-BIOPRED adults, U-BIOPRED paediatrics and PACMAN2)
Sensitivity
1 - Specificity
AUC: 0.950 (0.911–0.988)
AUC: 0.837 (0.771–0.902)
AUC: 0.791 (0.715–0.868)
GBM (Training)
sPLS-DA (Training)
ALASSO (Training)
Specificity
Sensitivity
Accuracy
Percentage (confidence interval)
AUC: 0.762 (0.621–0.902)
AUC: 0.734 (0.599–0.869)
AUC: 0.718 (0.585–0.851)
GBM (Validation)
sPLS-DA (Validation)
ALASSO (Validation)


BreathCloud
Sensitivity
1 - Specificity
AUC: 0.990 (0.983–0.997)
AUC: 0.932 (0.900–0.963)
AUC: 0.940 (0.911–0.968)
GBM (Training)
sPLS-DA (Training)
ALASSO (Training)
Specificity
Sensitivity
Accuracy
Percentage (confidence interval)
AUC: 0.911 (0.847–0.974)
AUC: 0.925 (0.861–0.989)
AUC: 0.926 (0.863–0.988)
GBM (Validation)
sPLS-DA (Validation)
ALASSO (Validation)