# eNose breath prints as a surrogate biomarker for classifying patients with asthma by atopy



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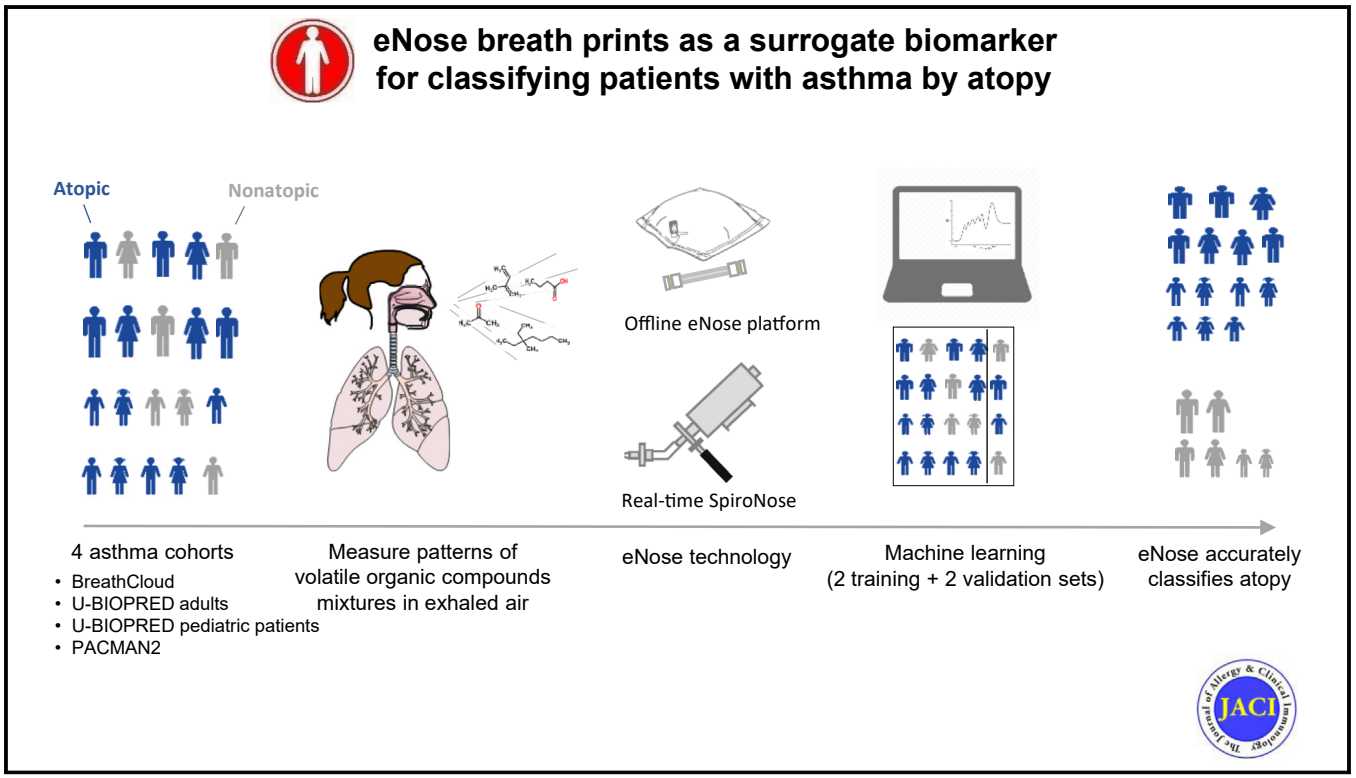
## GRAPHICAL ABSTRACT

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U-BIOPRED has received funding from the Innovative Medicines Initiative Joint Undertaking (under grant agreement no. 115010), the resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007-2013) and in-kind contributions of companies belonging to the European Federation of Pharmaceutical Industries and Associations (www.imi.europa.eu). The PACMAN study was funded by an unrestricted GlaxoSmithKline grant. BreathCloud was sponsored by the public charity Dutch Vriendenloterij. The salary of M.I.A. was sponsored by Egyptian Government PhD Research Scholarships.

**Background: Electronic noses (eNoses) 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 pediatric and adult patients with asthma.**
**Methods: Participants with asthma and/or wheezing from 4 independent cohorts were included; BreathCloud participants (n = 429), Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes adults (n = 96), Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes pediatric participants (n = 100), and Pharmacogenetics of Asthma Medication in Children: Medication with Anti-Inflammatory Effects 2 participants (n = 30). Atopy was defined as a positive skin prick test result (≥3 mm) and/or a positive specific IgE level (≥0.35 kU/L) for common allergens. Exhaled breath profiles were measured by 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 patients with atopic versus nonatopic asthma with reporting of areas under the receiver operating characteristic curves as a measure of model performance. In addition, an unsupervised approach was performed by using a bayesian network to reveal data-driven relationships between eNose volatile organic compound profiles and asthma characteristics.**
**Results: Breath profiles of 655 participants (n = 601 adults and school-aged children with asthma and 54 preschool children with wheezing [68.2% of whom were atopic]) were included in this study. Machine learning models utilizing volatile organic compound profiles discriminated between atopic and nonatopic participants with areas under the receiver operating characteristic curves 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 individuals with asthma and wheezing in cohorts with different age groups and could be used in asthma phenotyping. (J Allergy Clin Immunol 2020;146:1045-55.)**

***Key words:*** *VOCs, eNose, asthma, atopy, discrimination, machine learning*

Describing asthma as extrinsic (atopic) and intrinsic (nonatopic) was an early attempt to classify the disease into subgroups based on sensitization to common aeroallergens.[1] However, asthma is now recognized as a complex, heterogeneous, chronic inflammatory disease comprising different clinical and biologic mechanisms other than allergic sensitization (atopy). Nonetheless, atopy is among the most consistent characteristics associated with certain asthma phenotypes when data-driven methods are used.[2-6] This suggests that atopy and its underlying biologic pathway may be a driving factor of certain asthma-associated phenotypes and may thus 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) results with predefined allergens.[7] Although an 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, and bleeding, as well as to delayed allergic skin reaction and, in some rare circumstances, anaphylactic reactions.[8-10] Also, it is of limited use in patients with severe dermatologic conditions or those taking antihistamine (and several other) medications[8] which is common in patients with asthma.[11] Conversely, allergen-specific IgE measurements are more costly, they require venepuncture, and their results are usually not immediately available.[8]

Electronic noses (eNoses) are emerging point-of-care tools for diagnosing and phenotyping different respiratory diseases, including asthma.[12-14] They are cheap and easy to use, and the samples can be collected quickly. They can be used during a doctor's visit with immediate results, which could be beneficial in the

Disclosure of potential conflict of interest: R. de Vries reports personal fees and grants from Breathomix during the conduct of the study. J. H. Riley is employed by and owns shares in GlaxoSmithKline. K. F. Chung has received honoraria for participating in advisory board meetings of GlaxoSmithKline, AstraZeneca, Novartis, Merck, Boehringer Ingelheim, and TEVA regarding treatments for asthma and chronic obstructive pulmonary disease and has also been remunerated for speaking engagements. R. Djukanovic reports receiving fees for lectures at symposia organized by Novartis, AstraZeneca, and TEVA, as well as consultation fees from TEVA and Novartis for service as a member of advisory boards and participation in a scientific discussion about asthma organized by GlaxoSmithKline; in addition, R. Djukanovic is a cofounder and current consultant of and has shares in Synairgen, a University of Southampton spinout company. L. J. Fleming reports grants from Asthma UK and fees for expert consultation and speakers fees from AstraZeneca, GlaxoSmithKline, Novartis, Teva, Boehringer Ingelheim, Respiri, and Sanofi that were paid directly to her institution and outside of the submitted work. C. Murray reports personal fees and grants from GlaxoSmithKline, personal fees from Novartis and Thermo Fisher, and grants from Boehringer Ingelheim outside the submitted work. U. Frey reports grants from the Swiss National Science Foundation during the conduct of the study. F. Singer reports personal fees from Vertex and Novartis outside the submitted work. G. Roberts reports grants from the European Union Innovative Medicines Initiative during the conduct of the study. S.-E. Dahlén reports personal fees from AstraZeneca, GlaxoSmithKline, Merck & Co, Novartis, Regeneron, Sanofi, and Teva outside the submitted work. S. J. Fowler reports personal fees and nonfinancial support from AstraZeneca; grants and personal fees from Boehringer Ingelheim; and personal fees from Novartis, Teva, and Chiesi outside the submitted work. K. Knipping is an employee of Danone Nutricia Research. P. J. Sterk reports grants from the Public-Private Innovative Medicines Initiative covered by the European Union and the European Federation of Pharmaceutical Industries and Associations during the conduct of the study, as well as grants from being a scientific advisor and having a formally inconsiderable interest in the start-up company Breathomix BV outside the submitted work. A. H. Maitland-van der Zee reports grants and personal fees from Boehringer Ingelheim, grants from Chiesi, personal fees from AstraZeneca, grants from Vertex, and grants and personal fees from GlaxoSmithKline outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 15, 2020; revised April 30, 2020; accepted for publication May 5, 2020.

Available online June 10, 2020.

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0091-6749



https://doi.org/10.1016/j.jaci.2020.05.038

*Abbreviations used*

AUROCC: Area under the receiver operating characteristic curve
BN: Bayesian network
DAG: Directed acyclic graph
eNose: Electronic nose
FENO: Fractional exhaled nitric oxide
GBM: Gradient boosting machine
LASSO: Least absolute shrinkage and selection operator
OCS: Oral corticosteroid
PACMAN2: Pharmacogenetics of Asthma Medication in Children: Medication with Anti-Inflammatory Effects 2
ppb: Parts per billion
sPLS-DA: Sparse partial least squares discriminant analysis
SPT: Skin prick test
U-BIOPRED: Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes
VOC: Volatile organic compound

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 profiles for individual subjects. Clustering techniques on eNose breath profiles of both adult and pediatric patients with asthma 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 patients with atopic asthma and patients with nonatopic asthma, following the Standards for Reporting Diagnostic Accuracy guidelines.[18] We hypothesized that assessment of VOCs in exhaled breath offers a fast and noninvasive 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 pediatric participants from 4 independent asthma cohorts were included in this analysis: the Unbiased BIOmarkers in PREDiction of respiratory disease outcomes (U-BIOPRED) adult and pediatric cohorts,[19,20] the BreathCloud asthma cohort,[12] and the Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects 2 (PACMAN2) cohort.[21] Most participants had mild-to-moderate or severe asthma; the only exception was a subset of preschoolers within the U-BIOPRED pediatric cohort with wheezing (n = 54). For a brief summary of the included cohorts, see Table E1 (in this article's Online Repository at www.jacionline.org). The flow diagram for patient inclusion is shown in Fig 1.

### Outcome definition

Atopy was defined as a positive SPT result, defined by a wheal diameter of 3 mm or more and/or a positive allergen-specific IgE level of at least 0.35 kU/L to a prespecified allergen listed in Table E2 (in this article's Online Repository at www.jacionline.org). This list represents the most common allergens encountered at the study recruiting centers. Table E3 (in this article's Online Repository at www.jacionline.org) shows the main allergen category and the type of the test used to diagnose atopy in each included cohort.

### Noninvasive exhaled breath measurements

**Exhaled VOCs.** Offline eNose technology was used to measure exhaled breath VOCs in U-BIOPRED and PACMAN2 cohorts, whereas real-time eNose technology (SpiroNose, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands) was used to measure exhaled VOCs in BreathCloud, as described previously in detail.[12-14] We followed standard operating procedures for breath collection by using validated instruments.[12-14,22,23] The exhaled breath measurement is depicted schematically in Fig 2 and described in more detail in this article's Online Repository at www.jacionline.org. The highest sensitivities of the SpiroNose sensors to different mixtures of volatile organic compounds (VOCs)/gases are shown in Table E4.

**FENO value.** Fractional exhaled nitric oxide (FENO) values in parts per billion (ppb) were measured at a constant flow rate of 50 mL/s by using a portable analyzer (NIOX Mino System, Aerocrine, Solna, Sweden) according to the American Thoracic Society/European Respiratory Society recommendations.[24] FENO measurements were available for a subset of patients in each cohort.

### Data analysis

A general overview of the data analysis approach used is provided in Fig E1 (in this article's Online Repository at www.jacionline.org).

**Supervised analysis with machine learning models.** To investigate the discriminative potential of the eNose sensors to classify atopic versus nonatopic 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 data set and/or 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, we used the following 3 different and powerful machine learning techniques modeled on eNose signals that were previously used in metabolomics research[25-27]: sparse partial least squares discriminant analysis (sPLS-DA),[28] adaptive least absolute shrinkage and selection operator (LASSO),[29] and gradient boosting machine (GBM).[30] This was to provide an estimate of the robustness of statistical performance across different models and to evaluate eNose accuracy in classifying patients with atopic asthma without bias from "single-model selection." The 3 methods were selected on the basis of merit of feature reduction (selection), which avoids the risk of model overfitting. For more details, see the Online Repository.

**Individual cohort classification with internal cross-validation.** For each cohort, atopic and nonatopic participants were classified by 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, see details in Table E5 in this article's Online Repository at www.jacionline.org) was implemented in each model as recommended.[31]

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

Data sets from the 3 independent cohorts (U-BIOPRED adults, U-BIOPRED pediatric patients, and PACMAN2) that used the same eNose platform for offline breath analysis were combined (pooled cohorts) because internal validation alone cannot assess the robustness of the eNose sensors to detect atopy in independent validation sets. ComBat batch correction[32] was applied to the combined sensor data, and normal distributions were assessed by using histograms. The pooled data set was then randomly divided into a training set and a validation set by using an approximate ratio of 0.75:0.25 as recommended.[12,31] The latter step was also applied to the BreathCloud cohort by using the SpiroNose for real-time breath analysis, resulting in 2 training sets and 2 validation sets (Fig 1). The calculation of sample size of the training and validation sets on the basis of the measure of area under the receiver operating characteristic curve (AUROCC) is explained in the Online Repository. The training data sets

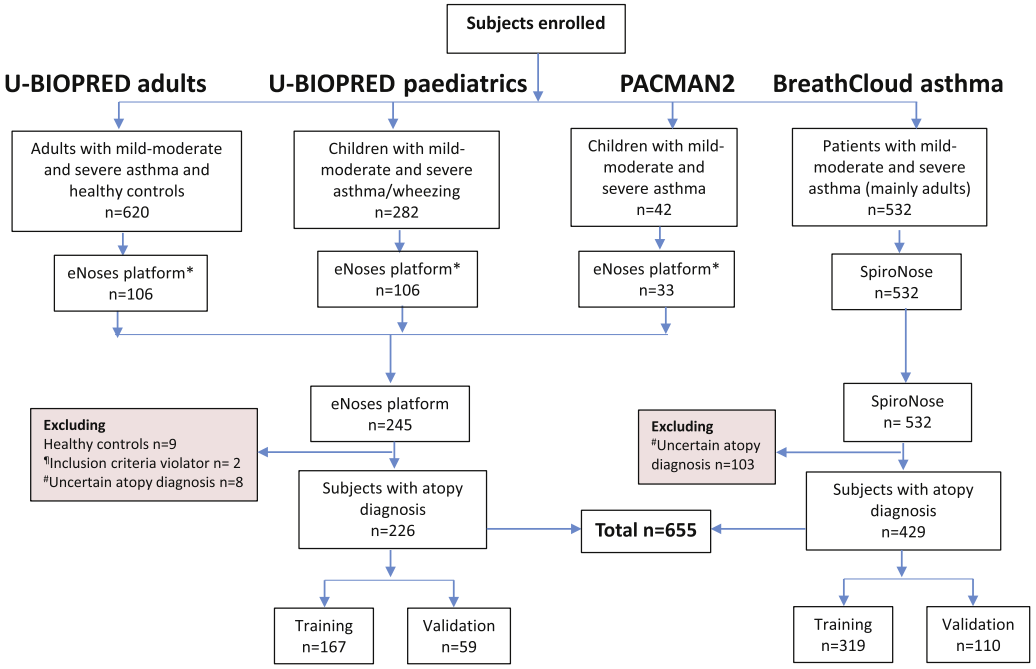


**FIG 1.** Flowchart for the included participants from 4 independent asthma cohorts. *The eNose platform is a composite of 4 differently developed eNoses (Cyranose C320, Tor Vergata, Comon Invent, and Owlstone Lonestar). ¶The term *inclusion criteria violator* refers to participants excluded from the analysis because of violation of the inclusion criteria defined by the U-BIOPRED. # The term *uncertain atopy diagnosis* refers to patients who do not have confirmed diagnoses for atopy by either SPT result or allergen-specific IgE level.

were used to train the 3 models by using a 10-fold internal cross-validation procedure (50 repeats for model tuning and prediction estimation, see details in Table E5). 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 AUROCCs and associated model accuracies, specificities, and sensitivities. To calculate 95% CIs, error estimates were obtained by performing 2000 nonparametric 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 AUROCCs using the Venkatraman method[35] (1000 permutations).

**Sensitivity analysis after exclusion of nonaeroallergens.** A sensitivity analysis was performed to check whether the discrimination using eNoses would change if patients sensitized with nonaeroallergens (eg, food, latex) were excluded. Pairwise comparisons of the AUROCCs obtained before and after exclusion of patients with nonaeroallergen sensitization were estimated by 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 data set was used in this analysis as it was the largest breath cohort with available information on smoking, dietary intake and time of food/drink consumption, and environmental factors (related to ambient conditions), as well as information on demographics, spirometry results, inflammatory parameters, and asthma medications.

**BNs.** Unsupervised learning of the BN was performed on the complete BreathCloud data set (Table E6). More details are provided in the Online Repository. The final network was depicted by using Cytoscape,[37] version 3.7.1, showing the probabilistic relationship between different variables in the form of a directed acyclic graph (DAG).

**Discriminative ability of FENO value to classify atopic versus nonatopic asthma.** A receiver operating characteristic curve was generated to assess the accuracy of FENO value in discrimination of patients with atopic asthma from those with nonatopic asthma. Continuous FENO values (in ppb) pooled from all cohorts (the BreathCloud, PACMAN2, and U-BIOPRED cohorts) were used in this analysis. Subsequently, different clinically utilized cutoff values (>20, >35, and >50 ppb) were used to categorize high and low FENO values to investigate whether these cutoffs would provide better insight than the uncategorized continuous values. These cutoffs were chosen on the basis of previous recommendations.[38,39] AUROCCs for classifying atopy with bootstrapped (2000 nonparametric stratified replicates) CIs[33,34] were constructed by using each measure (continuous and categorized).

All analyses were performed by 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, and bnlearn.

## RESULTS

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

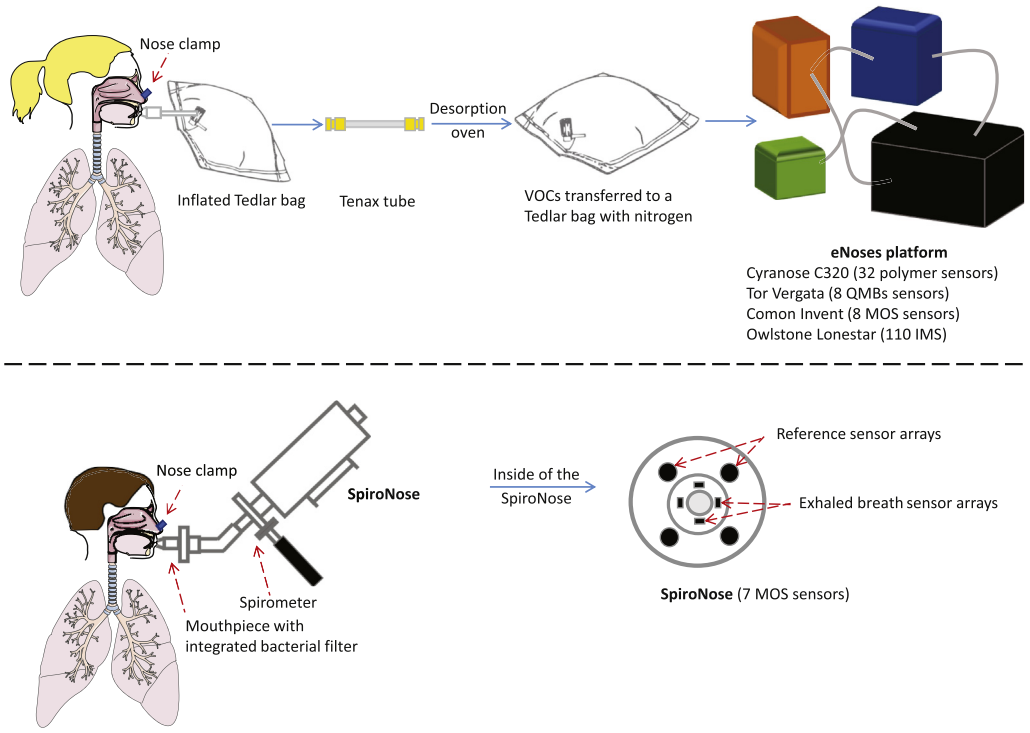


**FIG 2.** Exhaled breath measurement using the eNose platform (U-BIOPRED adults, U-BIOPRED pediatric, and PACMAN2 cohorts) and the SpiroNose (BreathCloud). (*Upper panel*) Breath collection and measurement using the eNoses platform in U-BIOPRED adult, U-BIOPRED pediatric, and PACMAN2 cohorts. Subjects exhaled a single vital capacity volume into a 10-L Tedlar bag (SKC, Eighty Four, Pa), followed by sampling of air on a Tenax thermal desorption tube (Tenax GR SS 6 mm 3 7 in; Gerstel, Mülheim an der Ruhr, Germany) to capture VOCs. A thermal desorption oven was used for desorption of the VOCs from the tubes, which were then transferred to a Tedlar bag by using a carrier nitrogen gas. Subsequent detection of the signal pattern of the VOC mixture was performed via 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 toward residual volume. The sensors' signals are recorded in real-time by the SpiroNose and transferred to a cloud environment for further automated analysis. *IMS*, Ion mobility spectrometry; *MOS*, metal oxide semiconductor; *QMB*, quartz crystal microbalance.

## Individual cohort classification using exhaled breath profiles with internal cross-validation

Patients with atopic versus nonatopic asthma were classified into the 4 independent cohorts by using 3 different machine learning models (sPLS-DA, adaptive LASSO, and GBM), with the representative AUROCCs shown in Fig 3. For each individual cohort, the obtained AUROCCs were at least 0.85.

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

The predictive performance of the trained machine learning models was evaluated in 2 validation sets showing relatively high AUROCCs of at least 0.72 and 0.91 in the pooled cohorts and BreathCloud, respectively, as shown in Fig 4 (pooled cohorts) and Fig 5 (BreathCloud). The associated accuracies, specificities, and sensitivities and their 95% CIs are also shown (Figs 4 and 5 and see Table E7 in this article's Online Repository at www.jacionline.org). In the pooled cohorts, most discriminative signals were derived from the Owlstone Lonestar system (Owlstone Medical, Cambridge, UK), whereas in the BreathCloud cohort, the sensor peak–to–breath hold ratios (see Fig E2 in this article's Online Repository at www.jacionline.org) contributed most to the discriminative signal (data not shown). The GBM model performance in the training sets (pooled cohorts and BreathCloud) was better than either the sPLS-DA or adaptive LASSO models, as estimated by higher AUROCCs with use of the Venkatraman's test (see Table E8 in this article's Online Repository at www.jacionline.org). However, this outperformance was eliminated when model predictions were applied to the validation sets.

## Sensitivity analysis after exclusion of nonaeroallergens

The AUROCCs obtained after exclusion of patients sensitized with nonaeroallergens were relatively similar to the previous findings in both the training and validation sets (see Table E9 in this article's Online Repository at www.jacionline.org). The

**TABLE I.** Participant baseline characteristics

| Characteristic | U-BIOPRED adults (n = 96) | BreathCloud (n = 429) | U-BIOPRED pediatric patients (n = 100) | PACMAN2 (n = 30) |
|---|---|---|---|---|
| Age (y), 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, no. (%) | | | | |
| Adults (≥18 y) | 96 (100%) | 407 (94.9%) | NA | NA |
| Children (<18 y) | NA | 22 (5.1%) | 100 (100%) | 30 (100%) |
| Females, no. (%) | 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) |
| White race, no. (%) | 90 (93.8%) | 366 (85.3%) | 75 (75%) | 30 (100%) |
| Atopy, no. (%) | 70 (72.9%) | 289 (67.4%) | 63 (63%) | 25 (83.3%) |
| Aeroallergen | 63 (65.6%) | 266 (62%) | 59 (59%) | 24 (80%) |
| Nonaeroallergen | 7 (7.3%) | 23 (5.4%) | 4 (4%) | 1 (3.3%) |
| Current smokers, no. (%) | 26 (27.1%) | 45 (10.5%) | NA | NA |
| Patient class | | | | |
| With asthma, no. (%) | 96 (100%) | 429 (100%) | 46 (46%) | 30 (100%) |
| With preschool wheezing | NA | NA | 54 (54%) | NA |
| $FEV_1$ % predicted before 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 after 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 before 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 after 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) |
| FENO value 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) |
| FENO value in ppb, median (IQR)†‡ | | | | |
| Atopic subjects | 25.0 (13.0-41.5) (n = 69) | 22.0 (12.5-37.0) (n = 133) | 35.5 (14.0-65.78) (n =38) | 35.0 (14.0-68.0) (n = 23) |
| Nonatopic subjects | 34.0 (22.0-77.5) (n = 25) | 20.0 (13.0-41.0) (n = 59) | 12.00 (8.50-21.0) (n = 5) | 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, no. (%) | | | | |
| 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 anticholinergic | 2 (4.2%) | 33 (7.7%) | 1 (1%) | 0 (0%) |
| Long-acting anticholinergic | 24 (25%) | 79 (18.4%) | 0 (0%) | 0 (0%) |
| Leukotriene antagonist | 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 |

*ACQ5*, 5-Item Asthma Control Questionnaire; *AQLQ*, Asthma Quality of Life Questionnaire; *BMI,* body mass index; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroid; *IQR*, interquartile range; *LABA*, long-acting β-agonist; *NA*, not applicable (which corresponds to either data not measured in this cohort or not applicable regarding the cohort criteria); *P(AQLQ)*, Pediatric Asthma Quality of Life Questionnaire; *SABA*, short-acting β-agonist.

*In BreathCloud, the personal best measurements were reported for postbronchodilator $FEV_1$ % predicted from data on routine clinical practice that were collected less than 12 months before the study visit.

†In BreathCloud, FENO measurements were reported as corresponding to the latest recorded values in routine clinical practice.

‡Data were not available for the complete set of study participants.

Venkatraman test showed no significant differences in the AUROCCs before and after exclusion of these patients.

## Data-driven BN

Fig E3 (in this article's Online Repository at www.jacionline.org) shows the DAG of a 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 VOC sensor signals and other measured characteristics. Other VOC signals showed connection with eosinophils counts and oral corticosteroid (OCS) use.

## FENO value in discriminating atopic and nonatopic asthma

FENO values were available for a subset of patients within the included cohorts (n = 357). Fig E4 (in this article's Online Repository at www.jacionline.org) shows that FENO value did not accurately discriminate between patients with atopic versus nonatopic asthma when either the continuous values (AUROCC = 0.51) or the categorized estimates (AUROCC = 0.52 for FENO values >20 ppb, 0.49 for FENO values >35 ppb, and 0.50 for FENO values >50 ppb) were used.

## DISCUSSION

To our knowledge, this is the first study to investigate the ability of eNose technology to detect atopy in patients with asthma

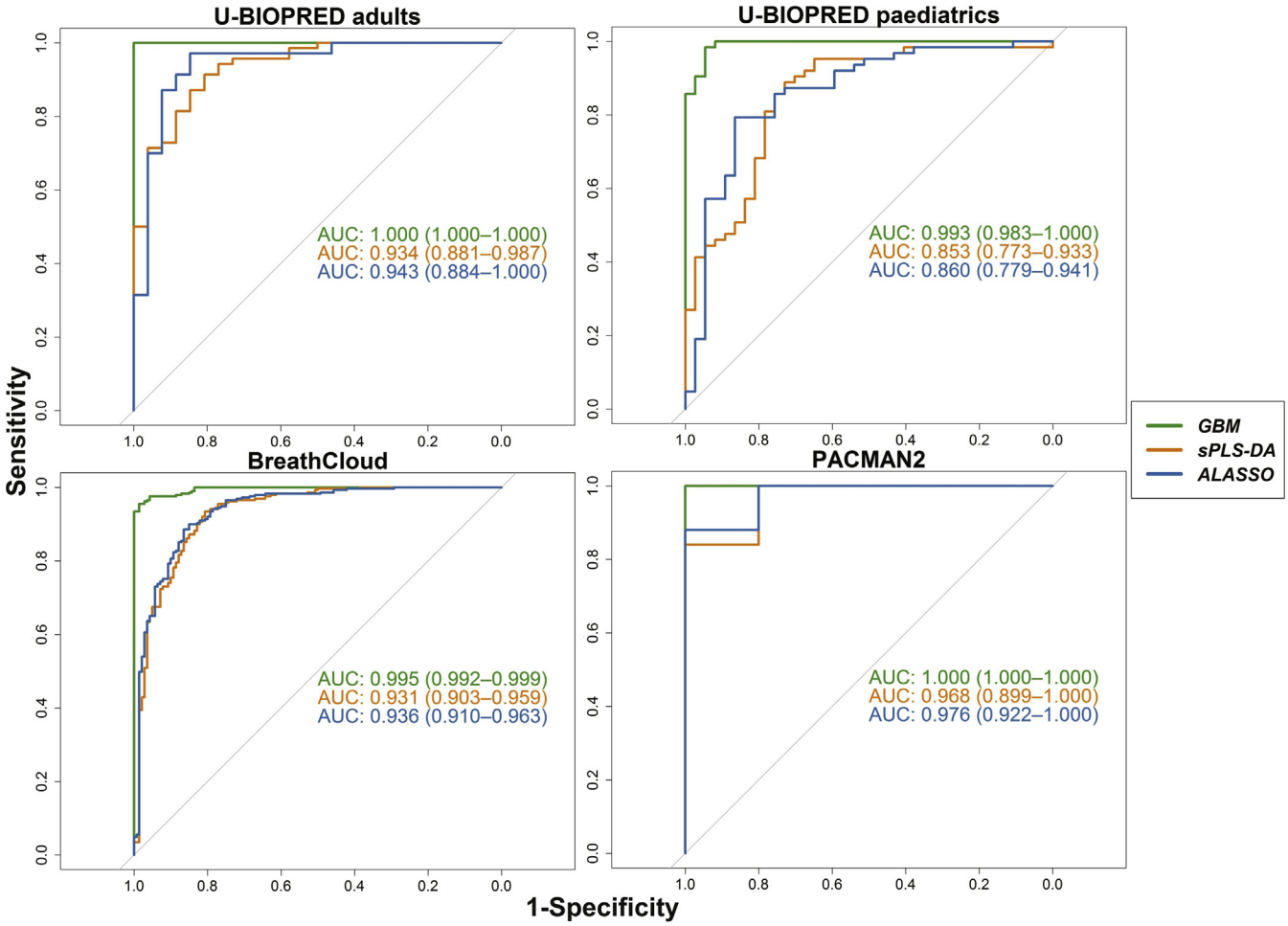


**FIG 3.** AUROCCs with bootstrapped 95% CIs for 3 different machine learning models classifying atopic versus nonatopic asthma in the U-BIOPRED adult, BreathCloud, U-BIOPRED pediatric, and PACMAN2 cohorts. *ALASSO*, Adaptive LASSO; *AUC,* area under the curve.

across different age groups. By using a composite of benchmarking supervised and unsupervised analysis techniques to analyze data on different eNose platforms used with 4 independent cohorts, we have shown that different eNoses can appropriately discriminate between individuals with atopic versus nonatopic asthma. Importantly, we have shown that observations made by using offline eNose technology are replicated with the real-time SpiroNose technology where sensors are placed in line with standard equipment for spirometry.

By testing the eNose technology separately in individual cohorts, a highly discriminative signal between patients with atopic versus nonatopic asthma was observed, as indicated by the different cross-validated machine learning models showing an AUROCC of at least 0.85. The eNose VOC profiles distinguished both groups when the data on the 3 cohorts obtained by using the same eNose platform for exhaled breath analyses (U-BIOPRED adult, U-BIOPRED pediatric, 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 with the performance in the individual cohorts, with AUROCCs of at least 0.84 and 0.72, respectively. This decrease may be related to differences in study populations with respect to age and asthma-associated characteristics and, possibly, to different eNose batch versions within subjects of the pooled cohorts, which may introduce more diverse VOC patterns (more noise). However, discrimination was possible despite these variations. These factors may also explain, in part, why the BreathCloud cohort was characterized by higher performance of the models than the pooled cohorts in both the training and validation sets (with AUROCCs 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 of capturing the VOCs compared with that of the offline approach of using the eNose platform within the pooled cohorts. The latter might be subject to some contaminant VOCs and/or VOC loss during transportation from the Tedlar bags (SKC, Eighty Four, Pa) and Tenax tubes (Tenax GR SS 6 mm 3 7 in; Gerstel, Mülheim an der Ruhr, Germany).[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

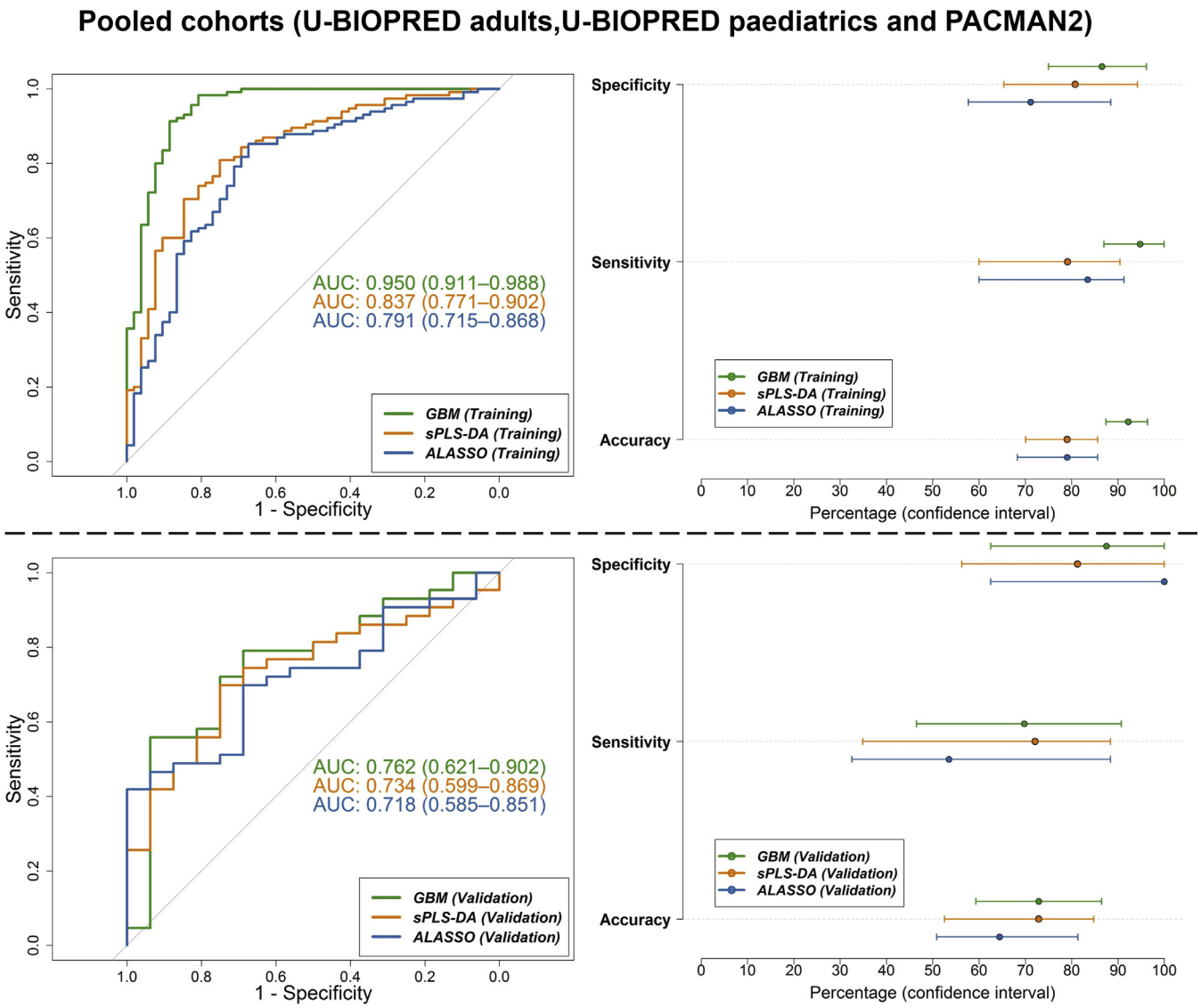


**FIG 4.** (*Upper panel*) AUROCCs with bootstrapped 95% CIs for 3 different machine learning models on eNose VOC breath profiles from the training subset (~75%) of the pooled cohorts with their associated accuracies, specificities, and sensitivities (95% CIs) are shown. (*Lower panel*) The predictive potential of the trained model was evaluated by using a validation subset (~25%) from the pooled cohorts showing relatively high AUROCCs with depiction of their associated accuracies, specificities, and sensitivities (95% CIs). *ALASSO,* Adaptive LASSO.

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 patients with atopic asthma despite the use of different machine learning models.

Studies investigating the relationship between exhaled VOCs and atopic asthma are scarce. In a previous study, VOC profile captured by Cyranose C320 showed only a trend ($P = .07$) for classification between participants with atopic versus nonatopic childhood asthma.[41] This study included only 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 system is a major driver of the signal that is responsible for discrimination, in contrast to the Cyranose C320 (Sensigent, Baldwin Park, Calif), which was also included in our study.

The atopy-discriminating VOC signals in this study were probably driven by aeroallergens, as most of the atopic patients included were sensitized by at least 1 aeroallergen. This was supported by a sensitivity analysis in which non–aeroallergen-sensitized patients were excluded, showing similar findings. Yet, this warrants further investigation to explore whether the VOC signals detected are related to the underlying pathophysiology locally (in the lung) or systemically or both.

FENO values greater than 20 ppb have been reported to be associated with atopy in 1199 children from Peru who were aged 13 to 15 years, showing an AUROCC of 0.65 in those without asthma (n = 1110) and an AUROCC of 0.82 in those

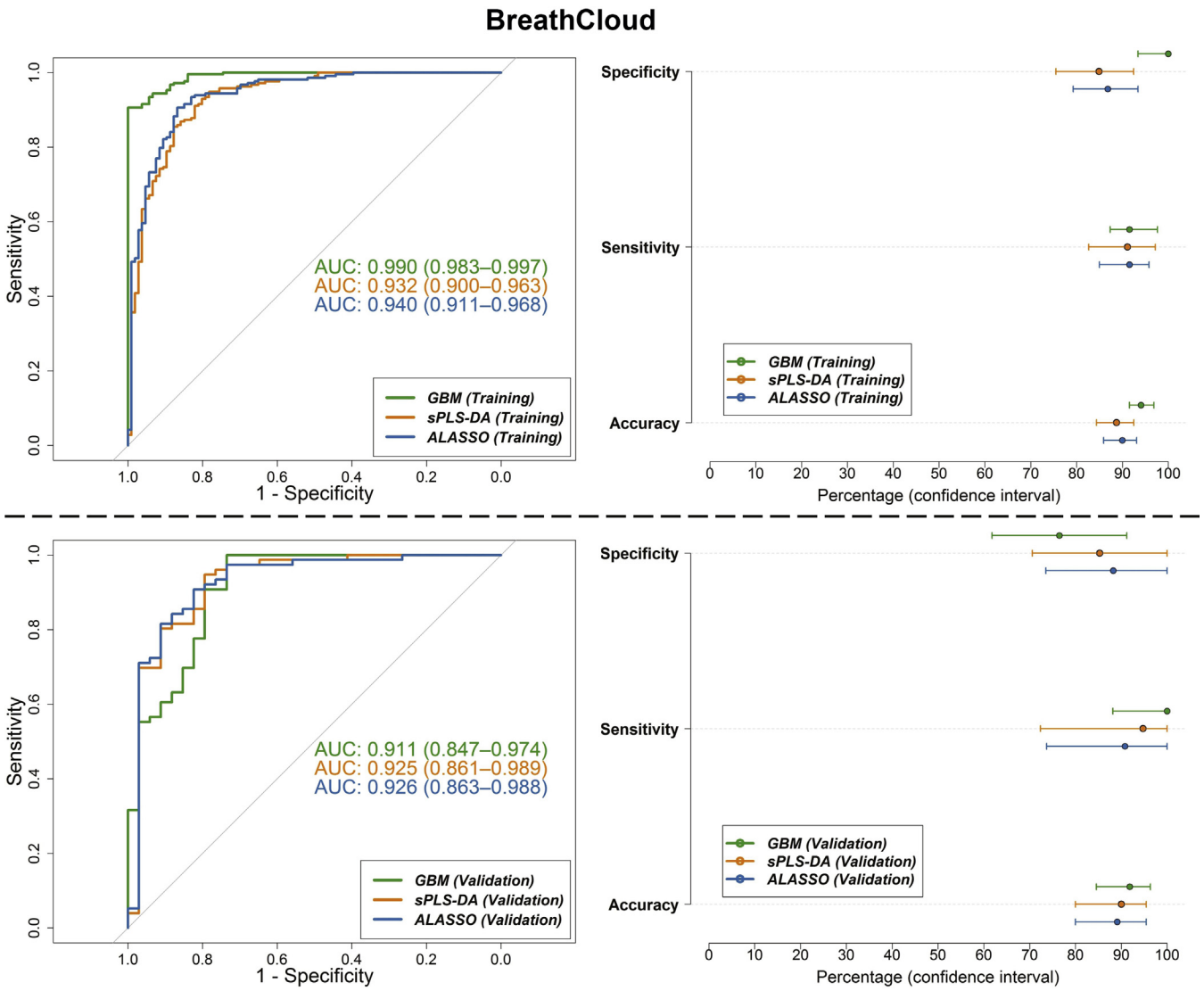


**FIG 5.** (*Upper panel*) The AUROCCs with bootstrapped 95% CIs for 3 different machine learning models on SpiroNose VOC breath profiles from the training subset (~75%) of the BreathCloud cohort with their associated accuracies, specificities, and sensitivities (95% CI) are shown. (*Lower panel*) The predictive potential of the trained model was evaluated by using a validation subset (~25%) from the BreathCloud cohort showing relatively high AUROCCs with depiction of their associated accuracies, specificities, and sensitivities (95% CI). *ALASSO*, Adaptive LASSO.

with asthma (n = 89).[38] Our analysis showed that the eNose technology provided higher AUROCCs in the included pediatric cohorts (≥0.85 in the U-BIOPRED pediatric cohort and ≥0.97 in the PACMAN2 cohort) and thus provides better accuracy in detecting atopy. In addition, in our study FENO value did not accurately discriminate between individuals with atopic and nonatopic asthma (AUROCC = 0.52), indicating a limited accuracy of FENO value in detecting atopy as compared with the accuracy provided by eNose technology in a broad age-range multinational cohort.

The 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 that the profiles predicting atopy are not be confounded by other measured factors in the BreathCloud cohort. In addition, the BN also showed relationships between VOC 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 they can be used only for probabilistic reasoning. Therefore, the association between atopy and exhaled breath sensor 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, which showed a relative steadiness of the atopy classification potential. Second, the utilization of different eNose platforms covering both offline and real-time measurement of exhaled breath may serve as a sign of generalizability of the eNose technology in detecting atopy in

patients with asthma. Third, the benchmarking analysis strategy that was followed in this study coupled both supervised and unsupervised approaches to further support findings. Finally, we used 3 different powerful machine learning techniques to provide an indication of the robustness of statistical performance across the models tested.

However, this study also has limitations. First, eNose technology in general does not allow for identification of individual VOCs but is based on cross-reactive sensor arrays that allow for powerful VOC signal pattern recognition, which does not hamper the clinical application of eNose technology. Identifying individual discriminating VOCs in future studies (eg, by mass spectrometry techniques) will help in getting more insight on the underlying pathophysiologic pathways. Second, we investigated only exhaled breath profiles and their relation to atopy at a single time point. In 1 study, diurnal variations in the VOC profiles of individuals with moderate atopic asthma were observed.[43] Therefore, assessment of the temporal stability of atopy-discriminative signature is required. Third, a large percentage of the included subjects were white, which demands broader investigation in other ethnicities to assess generalizability. Fourth, validation should ideally be performed by training a model on a cohort and then validating the findings on a different cohort. This was not feasible in the current study, as we used 2 different eNose technologies (offline versus real-time measurement), which made direct validation of the findings impossible because of the different sets of sensors from the 2 technologies. In addition, because of the limited sample sizes in the data sets of the offline eNose platform, pooling of the data was essential to achieve the statistical power necessary for appropriate training and validation of the models. Fifth, this study was not meant to provide a comprehensive comparison of the performance of different machine learning models, but rather to provide an estimate of statistical robustness as a proof of concept. Whether other models will provide different outcomes merits further investigation. Finally, we did not investigate differences in the allergen-specific VOC signals and whether such signals would differ according to the numbers of sensitized allergens, time course of atopy, and/or levels of specific IgE or wheal size. A previous study has shown that these atopy-associated outcome measures could identify subphenotypes within children.[44]

SPT or allergen-specific IgE measurements remain the criterion standard for diagnosing atopy and offer details regarding the allergen to which the patient is sensitized. At this time point, the eNose cannot be considered an alternative; however, it may offer a very quick (within minutes) noninvasive screening tool in situations in which the standard methods cannot be used, such as in situations involving patients with dermatologic conditions, patients taking interfering medications, and/or patients whose acceptance of skin testing or blood withdrawal is low (eg, children).

By detecting atopy, in addition to its potential to perceive changes in inflammatory biomarkers such as eosinophils and neutrophils or OCS use,[12,13] the eNose may help to identify asthma phenotypes and asthma-associated treatable traits.[45] Therefore, it can serve as a tool in precision medicine approaches to asthma management. Managing atopic asthma may require different steps ranging from environmental avoidance of allergens[46] to symptomatic control with add-on therapies such as antihistamines or antihistamine–leukotriene antagonist combinations.[47] In addition, more targeted therapies, such as allergen-specific subcutaneous immunotherapy in patients with stable asthma,[48] sublingual immunotherapy, and the anti-IgE mAb omalizumab,[49] can be directed to those patients with atopic asthma. The use of eNose technology may thus facilitate noninvasive and quick tailoring of these interventions to the needs of each individual patient.

In conclusion, e-Nose technology can accurately and robustly classify patients with asthma by atopic status. Coupling supervised and unsupervised machine learning approaches revealed that the associations between exhaled breath and atopy and our results are generalizable. The findings presented here suggest that exhaled breath analysis by eNose allows meaningful phenotyping of patients with asthma and may therefore be used in personalized clinical decisions related to asthma.

We would like to acknowledge the help of biostatistician Aruna Bansal, PhD.

**Key messages**

- **The assertion that signals of exhaled volatile organic compounds, mixtures measured by electronic noses (eNoses), can adequately classify patients with asthma by atopy is supported by data from 4 independent asthma cohorts.**
- **The eNose may serve as a quick, noninvasive tool for asthma phenotyping.**

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