Biomarker discovery for asthma phenotyping: From gene expression to the clinic

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CHAPTER 6

Predicting eosinophilic airway inflammation in asthma using exhaled breath profiling


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ABSTRACT

Background
Management of asthma based on inflammatory profiling improves clinical outcomes. However, there is a need for surrogate markers of airway eosinophilia for daily practice. Exhaled air metabolomics by gas-chromatography and mass-spectrometry enables identification of eosinophilic inflammation. The electronic nose (eNose) offers a low-cost, rapid, point of care alternative for breath profiling.
Objectives: To validate breathprints obtained by a composite eNose platform in the prediction of sputum eosinophilia in asthma.

Methods
This multicenter study included 104 patient visits (58 baseline visits and 46 longitudinal visits) of the U-BIOPRED project. Induced sputum and blood eosinophils were measured. Breath samples were collected locally and analysed centrally by 84 eNose sensors in parallel (based on four different technologies). Discriminant accuracy of eNose sensors and blood eosinophil counts for sputum eosinophilia was obtained by receiver operating characteristics (ROC) analysis in the baseline cohort and validated in the longitudinal cohort.

Results
Models derived from eNose sensors classified patients based on sputum eosinophilia ≥3% with an area under the ROC curve (AUC) of 73%. Validation in the longitudinal cohort, 57% of whom had not provided breath samples at baseline resulted in an AUC of 78%. Discriminant analysis using blood eosinophil counts produced an AUC of 69%.

Conclusions
eNose breathprints discriminate eosinophilic from non-eosinophilic airway inflammation in asthma, which was validated at a second study visit in a largely independent cohort of patients. Blood eosinophil counts showed similar accuracies. This suggests that eNoses have potential to capture eosinophilic airway inflammation in a quick way, thereby facilitating personalized asthma management.
INTRODUCTION

Classification of asthma phenotypes based on sputum differential cell counts characterises patients with eosinophilic and/or neutrophilic airway inflammation (1). Inflammatory phenotyping of asthma has been shown to be of clinical importance, since eosinophilic asthma responds well to corticosteroid treatment, whereas non-eosinophilic asthma responds poorly (2;3). By selecting patients based on sputum eosinophil count specific antibodies against IL5 and IL4/IL13 have been shown to be effective (4-6). Several studies have consistently demonstrated that targeting sputum eosinophils by tailoring inhaled steroid therapy reduces asthma exacerbations by 60% (7-9). Alternatively, azithromycin is suggested to reduce asthma exacerbations in patients with non-eosinophilic asthma (10). These studies show that personalizing therapy by identification of asthma inflammatory subtypes lead to a better disease outcome then indiscriminate treatment (9).

Sputum induction by hypertonic saline is considered a safe and non-invasive method to identify airway inflammation in patients with asthma (11). However, processing of sputum is technically demanding and time-consuming. Notably, about 25% of patients fail to produce an adequate sputum sample, and in patients with uncontrolled asthma induction can cause significant airway narrowing (12). Therefore, there is a need for adequate surrogate markers of airway inflammation in asthma. Blood differential cell counts and fractional exhaled nitric oxide (FE NO) have been considered as alternative biomarkers to diagnose asthma inflammatory subtypes. Recently, we have shown that blood eosinophils represent an accurate biomarker for sputum eosinophils in two independent cohorts of patients with mild to severe asthma (13), even though a recent meta-analysis was still inconclusive (14). Notably, FE NO and sputum eosinophils are only weakly to moderately correlated (15-17). Therefore, it may not be surprising that guiding steroid therapy based on FE NO is not effective on asthma outcomes (9), although using an improved algorithm did show reduced exacerbations by FE NO-guided therapy (18) and a recent study in primary care demonstrated a positive result (19).

Exhaled air contains volatile organic compounds (VOCs) that may qualify as non-invasive biomarkers of disease (20). Assessment of the profile of these volatiles by gas-chromatography and mass-spectrometry (GC-MS) and sensors of various types of electronic noses (eNoses) allows capturing disease-related molecular patterns (21;22). The latter approach combines the non-invasiveness of measuring exhaled breath with real-time analysis of a metabolomic fingerprint (breathprint) (23;24). It has been shown that electronic noses are able to discriminate exhaled breath between well-characterized subjects with asthma, COPD and controls (25;26). Interestingly, recent studies demonstrated that GC-MS of exhaled air can identify eosinophilic and neutrophilic inflammation in asthma (27) and COPD (28;29). Furthermore, the eNose was successfully used in predicting steroid responsiveness in a small cohort of patients with asthma (30) and...
very recently exhaled molecular profiles have shown potential in asthma clustering (31). This raises the question whether exhaled molecular profiling by eNoses can recognize inflammatory phenotypes in asthma since this could provide a low-cost, rapid, point of care alternative for breath profiling.

In this study, we hypothesized that breathprints analysed by eNoses can be used as surrogate markers of eosinophilic airway inflammation in asthma. We aimed to test this hypothesis by examining the relationship of breathprints analysed by a composite eNose platform with eosinophil counts in induced sputum in a wide spectrum of patients with mild to severe asthma and to validate this relationship in a second cohort. Subsequently, we aimed to compare the resulting accuracies by the eNose with those by blood eosinophil counts.

METHODS

Subjects
We included patients with mild to severe asthma (aged ≥18y), recruited by six clinical centres in Europe (Amsterdam, Netherlands; Budapest, Hungary; Catania, Italy; Manchester, United Kingdom; Rome, Italy; Southampton, United Kingdom). These patients were amongst those recruited for the Unbiased BIOmarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED)-study within the framework of the Innovative Medicines Initiative (IMI). The first cohort included patients as part of the baseline visit (baseline cohort). A second cohort to validate discriminant models included patients as part of the longitudinal visit (longitudinal cohort).

The diagnosis of asthma was defined by a physician's diagnosis of asthma, including a history of wheeze, together with reversibility in FEV1 of at least 12% and 200mL and/or airway hyperresponsiveness (PC20 methacholine < 8 mg/mL) and/or diurnal variation in peak flow and/or a decrease in FEV1 of at least 12% and 200 mL within four weeks after tapering of treatment. Patients with mild-moderate asthma were on low to moderate dose of inhaled corticosteroids (ICS) (≤500mcg fluticasone propionate (FP) or equivalent) and were controlled or partly controlled according to GINA guidelines (32). Patients with severe asthma were diagnosed according to the IMI-criteria (33): patients used high dose ICS (≥1000mcg FP or equivalent) plus at least one other controller medication and were uncontrolled according to GINA guidelines (32) and/or had frequent severe exacerbations (≥2 per year) and/or required prescription of daily or alternate day oral corticosteroids (OCS) to achieve asthma control. Patients were excluded if they had had a severe asthma exacerbation in the previous month prior to the study visits. Furthermore, smokers or ex-smokers with a smoking history ≥5 pack years were excluded.
among the patients with mild asthma only. The clinical methods and entire adult cohort of the U-BIOPRED study have recently been published (34).

The study was approved at all local Medical Ethics Committees and all patients gave their written informed consent. The study was registered on ClinicalTrials.gov, Identifier: NCT01982162.

Design

During this multicenter study, all patients visited the hospital for a screening visit and a baseline study visit. First, inclusion and exclusion criteria were examined. Next, exhaled breath was collected for eNose analysis, lung function was performed, peripheral blood was collected and sputum was induced by hypertonic saline. The longitudinal visit was planned 12-18 months after the baseline visit and included identical procedures as the baseline visit.

Measurements

Lung function and blood eosinophil counts
Spirometry was performed according to ATS/ERS recommendations (35). Peripheral blood eosinophil counts were obtained from standard complete blood counts done at each centre locally.

Exhaled breath collection
Exhaled breath was collected locally at each site as previously described (25;36). In short, patients breathed normally for five minutes through a mouthpiece connected to a three-way non re-breathing valve and an inspiratory VOC-filter (A2, North Safety, NL). Next, the patient exhaled one vital capacity volume into a 10 L Tedlar bag (SKC Inc, Eighty Four, PA, USA). The content of the Tedlar bag was drawn through stainless steel desorption tubes packed with Tenax (Tenax GR SS 6mm x 7” (Gerstel), SS compression cap (Swagelok)) by a peristaltic pump. Tubes were sent to Amsterdam for desorption of VOCs using a thermal desorption oven (Gerstel TDS 3) and transferred into a Tedlar bag with nitrogen as carrier gas.

Subsequent analysis by a composite eNose platform was carried out. Storage of VOCs has been shown to preserve the eNose signal (37). The eNose platform consists of five eNoses from four different brands, using distinct measurement technologies (38): 1) two Cyranoses C320 (Smiths Detection Inc., Pasadena, CA, USA) using carbon-polymer sensors (39), 2) one Tor Vergata TEN (University of Tor Vergata, Rome, Italy) using quartz microbalance metalloporphyrins sensors (40), 3) one Common Invent eNose (Common Invent B.V., Delft, The Netherlands) using metal oxide semiconductor sensors (41), and 4) one Lonestar (Owlstone Nanotech Ltd., Cambridge, UK) based on field asymmetric
ion mobility spectrometry (42). Preliminary within-sample repeatability data showed a relative percentage difference in sensor deflection of 6.21% (43).

**Inflammatory status**

Induced sputum was collected according to a standardized protocol (44). Selected plugs were processed with 0.1% DTT and differential cell counts were expressed as percentage of non-squamous cells. Cells were counted centrally (AMC, Amsterdam, The Netherlands) by the same certified technician, and 10% of the cell counts were validated by a second independent technician. According to previous literature, we used a sputum eosinophil count of 3% as cut-point to define eosinophilic or non-eosinophilic airway inflammation for our primary analysis (7). Since others have reported different cut-points, additional discriminate analysis was done using a sputum eosinophil count of 2% as cut-off (8).

**Statistical analysis**

SPSS (V.22.0; IBM Corp, Armonk, NY, USA) and R (V.3.01; R Foundation for Statistical Computing, Vienna, Austria) (45) were used for data analysis. One of the Cyranoses had missing values from three subjects because of technical problems. Since the eNose platform includes two Cyranoses, we decided to exclude data from the Cyranose with missing values. Owlstone Lonestar sensors were included if more than 10% of the subjects had ion currents three times the noise level, which is considered 0.02. Batch effects were adjusted for using empirical Bayes methods (46). The eNose sensor data was normalized to the same scale with a mean of 0 and standard deviation of 1. Data were transformed in case of non-normal distribution. The relationship between sputum eosinophil percentages and blood eosinophil counts were analysed using Pearson's correlation coefficient. Subsequently, a t-test was used to test for significant relationships between sensors and eosinophilic or non-eosinophilic airway inflammation. The significantly associated sensors (unadjusted \( p \)-value<0.05) were further analysed for variable selection and model fitting using sparse partial least squares regression of which tuning parameters were chosen by 10-fold cross-validation (47). The discrimination performance of the retrieved model was determined by receiver operating characteristic (ROC) curve analysis and externally validated in the longitudinal cohort (48). Furthermore, similar analysis was done for blood eosinophil counts and for the combination of eNose and blood eosinophil counts.

**RESULTS**

Complete data were available for 104 study visits. This included 58 patients in the baseline cohort, and 46 in the longitudinal validation cohort, of which 20 patients were
included in both cohorts. Baseline characteristics are presented in Table 1. Patients featuring the presence or absence of eosinophilic airway inflammation from the baseline cohort did not differ with regard to clinical characteristics, except for oral steroid usage (see Table 1).

**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Baseline Cohort</th>
<th></th>
<th>Longitudinal Cohort</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N=58</td>
<td>EO ≥3% N=32</td>
<td>EO &lt;3% N=26</td>
<td>EO ≥3% N=19</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 (13.8)</td>
<td>56.1 (13.3)</td>
<td>51.4 (14.2)</td>
<td>58.6 (10.9)</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>39.7</td>
<td>31.3</td>
<td>50.0</td>
<td>47.8</td>
</tr>
<tr>
<td>OCS use (%)</td>
<td>31.0</td>
<td>43.8</td>
<td>15.4</td>
<td>60.9</td>
</tr>
<tr>
<td>Severe asthma (%)</td>
<td>85</td>
<td>88</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>Pb predicted FEV₁ (%)</td>
<td>80.3 (21.1)</td>
<td>79.7 (21)</td>
<td>81.0 (21.6)</td>
<td>70.1 (21.1)</td>
</tr>
<tr>
<td>Pb FEV₁/FVC (%)</td>
<td>79.7 (13.6)</td>
<td>76.8 (13.5)</td>
<td>83.2 (13.2)</td>
<td>73.3 (15.1)</td>
</tr>
<tr>
<td>FENO (ppb)</td>
<td>29.0</td>
<td>37.5</td>
<td>20.0</td>
<td>29</td>
</tr>
<tr>
<td>Blood eosinophils (×10⁹/L)</td>
<td>0.30</td>
<td>0.41</td>
<td>0.19</td>
<td>0.30</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>5.0</td>
<td>15.7</td>
<td>1.13</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Mean (SD); † Median (IQR). *t-test p<0.05; **t-test p<0.001. EO, sputum eosinophils; Pb, post-bronchodilator; OCS, oral corticosteroids

**Prediction of eosinophilic airway inflammation using the eNose platform**

Using 3% sputum eosinophils as criterion for the diagnosis of eosinophilic and non-eosinophilic asthma, the t-test identified 13 out of 84 eNose sensors as potential biomarker predictors that were analysed for model fitting. Subsequently, the diagnostic accuracy of this model described as area under the receiver operating characteristic curve (AUC), was 73% (95% CI 0.60 to 0.87) in the baseline cohort, (see Figure 1). Validation of this model in the longitudinal cohort produced an AUC of 78% (95% CI 0.64 to 0.92) (see Figure 1). Additionally, using 2% sputum eosinophils as criterion, 14 eNose sensors were identified as potential predictors, producing an AUC of 73% (95% CI 0.58 to 0.88). External validation of this model in the longitudinal cohort resulted in an AUC of 68% (95% CI 0.52 to 0.84) (see Figure 1).

The sensitivity, specificity, positive predictive values and negative predictive values using the eNose to predict eosinophilic airway inflammation are presented in Table 2. Overall, there were nine identical eNose sensors included as predictors in both models.
Prediction of eosinophilic airway inflammation using blood eosinophil counts

Sputum eosinophil differential cell counts were positively associated with blood eosinophil counts (r=0.54, p<0.001). The accuracies of blood eosinophil counts to differentiate sputum eosinophilia in the baseline and longitudinal cohorts at 3% and 2% sputum eosinophils exhibited similar ranges as those observed for eNose (Table 2). The best combination of sensitivity, specificity, positive predictive values and negative predictive values using a blood eosinophil count cut-off of 0.22×10^9 cells/L are also shown in Table 2.

Combining eNose and blood eosinophils

When eNose and blood eosinophils were combined in the same model using the baseline cohort, the prediction of sputum eosinophilia slightly improved towards an AUC of 83% (95% CI 0.71 to 0.94) and 81% (95% CI 0.69 to 0.94) for sputum eosinophil counts of 3% and 2%, respectively (Figure 2).

Figure 1A. ROC curve analyses
ROC curve analyses of eNose sensors for the assessment of eosinophilic airway inflammation in sputum, using 3% sputum eosinophils as threshold, as obtained in the baseline cohort and validated in the longitudinal cohort. AUC, area under the receiver operating curve.

Figure 1B. ROC curve analyses
ROC curve analyses of eNose sensors for the assessment of eosinophilic airway inflammation in sputum, using 2% sputum eosinophils as threshold, as obtained in the baseline cohort and validated in the longitudinal cohort. AUC, area under the receiver operating curve.
The results of this study show that VOCs analysed by eNoses have diagnostic potential for detecting eosinophilic airway inflammation in asthma. The eNose platform was able to discriminate between eosinophilic and non-eosinophilic asthma which was validated at the second study visit one year after. These findings suggest that eNoses could be used for the non-invasive assessment of the eosinophilic profile of asthma, which can improve and facilitate the guidance of individualized asthma treatment.

**Table 2.** ROC parameters

<table>
<thead>
<tr>
<th>Threshold sputum eosinophils</th>
<th>eNose platform</th>
<th>Blood eosinophil cut-off: 0.22×10^9 cells/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Baseline AUC</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Baseline sensitivity</td>
<td>88</td>
<td>82</td>
</tr>
<tr>
<td>Baseline specificity</td>
<td>58</td>
<td>63</td>
</tr>
<tr>
<td>Baseline PPV</td>
<td>72</td>
<td>82</td>
</tr>
<tr>
<td>Baseline NPV</td>
<td>79</td>
<td>63</td>
</tr>
<tr>
<td>Longitudinal AUC</td>
<td>78</td>
<td>68</td>
</tr>
<tr>
<td>Longitudinal sensitivity</td>
<td>74</td>
<td>60</td>
</tr>
<tr>
<td>Longitudinal specificity</td>
<td>70</td>
<td>71</td>
</tr>
<tr>
<td>Longitudinal PPV</td>
<td>64</td>
<td>71</td>
</tr>
<tr>
<td>Longitudinal NPV</td>
<td>79</td>
<td>60</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value

**Figure 2.** ROC curve analyses

ROC curve analyses of the combination of eNose sensors and blood eosinophil count for the assessment of eosinophilic airway inflammation in sputum as obtained in the baseline cohort. Sputum eos, sputum eosinophils.

**DISCUSSION**

The results of this study show that VOCs analysed by eNoses have diagnostic potential for detecting eosinophilic airway inflammation in asthma. The eNose platform was able to discriminate between eosinophilic and non-eosinophilic asthma which was validated at the second study visit one year after. These findings suggest that eNoses could be used for the non-invasive assessment of the eosinophilic profile of asthma, which can improve and facilitate the guidance of individualized asthma treatment.
To our knowledge, this is the first multicentre study analysing breathprints by different types of eNoses to find surrogate markers for sputum eosinophils in patients with asthma. Our results support and extend recent studies that were able to identify eosinophilic inflammation by GC-MS breath analysis in asthma (27) and COPD (28;29). Even though these GC-MS results merit subsequent validation in other larger cohorts, they can be regarded as independent confirmation that exhaled VOCs capture eosinophilic airway inflammation. The eNose appeared to have similar accuracies as blood eosinophil cell counts in the prediction of sputum eosinophil percentages varying between 68-78% depending on the threshold of sputum eosinophils. This confirms previous data showing modest to high diagnostic accuracies of blood eosinophils to predict sputum eosinophil percentages (2;13;14;17;49;50). Notably, the accuracies slightly improved when combining eNose and blood eosinophils in the same model. This suggests that eNose and blood eosinophils represent partly complementary information with regard to the presence or absence of sputum eosinophilia.

We believe the strength of this study is that we validated our results by using two visits separated by 12-18 months and by partially including different patients. Therefore, these two study visits were regarded as being sufficiently distinct, in order to pursue independent validation. Moreover, reanalysing our data using only unique samples (n=26) for a true external validation resulted in an AUC of 78% (95% CI 0.59 to 0.97) and 65% (95% CI 0.41 to 0.89) using sputum eosinophil counts of 3% and 2%, respectively. Another potential strength is the inclusion of patients with a wide range of asthma severity in six different centres from four different countries, thereby reducing potentially confounding effects of geographical locations on VOCs (51). Furthermore, the eNose platform enabled centralised analysis of breathprints by multiple sensor systems in parallel (from 4 different types of eNoses), which took the benefit of combining distinct sensor technologies. This maximized the differential sensor distribution of exhaled VOCs and thereby the potential of successful pattern recognition. In addition, all centres were trained to carry out sputum induction, sputum processing, and the collection and transport of exhaled air in the same way in order to minimize external influences on the sputum and breathprints. Finally, sputum differential cells were counted and reproduced centrally by the same experienced analysts.

A total of 13 and 14 amongst 84 eNose sensors were significantly different between patients with high and low sputum eosinophils depending on the sputum eosinophils cut-off values. The fact that nine identical eNose sensors were significantly different using both cut-offs and that external validation reproduced similar accuracies substantiates our findings. The most discriminative sensors comprised metal oxide semiconductor sensors and the field asymmetric ion mobility spectrometer. This indicates that various technologies may still be required to obtain discriminative eNose data in the clinical setting. In order to optimize eNose performance it is warranted to tailor the various sensors
Exhaled Breath Profiling in Asthma

Towards the VOCs of interest, in this case those VOCs that are associated with eosinophilic airways inflammation. Based on an earlier GC-MS study in asthma this comprises for instance alkanes (27). Specific sensor development for such individual components is challenging, but not unrealistic (52).

The predictive accuracy for sputum eosinophilia by the eNose platform showed moderate results with areas under the ROC curve of 68-78%. These values are definitely suboptimal, however they serve as impetus for further studies of increased sample size. Furthermore, these accuracies are similar to those obtained by FENO in asthma (15-17), which has led to the usage of FENO as surrogate marker of sputum eosinophils in recent studies with novel targeted treatments in asthma (53). Similarly, blood eosinophils are increasingly used for the phenotyping of patients in clinical trials (53;54) based on comparable levels of accuracy for sputum eosinophilia (14). The present data show that the combination of eNose and blood eosinophil data leads to somewhat improved predictive accuracy. This suggests that easily available biomarkers such as exhaled air and peripheral blood together provide adequate information for replacing sputum induction in the phenotyping of eosinophilic asthma. Notably, in the present study we have reported the best combination of sensitivity, specificity, positive predictive values and negative predictive values. However, the model could be tailored to a clinical application where a higher positive predictive value is preferable, for example in a study design for novel therapies (49).

Although previous studies on the diagnostic accuracy of eNoses for asthma and COPD were unlikely to be largely influenced by the use of inhaled corticosteroids or long-acting bronchodilators (25;55), it cannot be excluded that such treatment may have affected the exhaled profiles of patients. In the present study, significantly more patients with eosinophilic asthma used daily or alternate day oral corticosteroids as compared to non-eosinophilic asthma (see Table 1). Nevertheless, 56% of patients with high sputum eosinophilia did not require oral steroids. Therefore, it is unlikely that the diagnostic accuracy of the eNose sensors to differentiate eosinophilic and non-eosinophilic inflammation was driven by oral steroids usage.

The present results suggest that the exhaled breath of patients with asthma contain metabolites that are directly related to, or are a product of eosinophilic airway inflammation. The presence of sputum eosinophils in asthma reflects underlying airway pathology, and it predicts treatment response and exacerbation rate (2-9;56). Therefore, it is likely that different inflammatory processes in the airways of patients with varying subtypes of asthma generate partially distinct volatile metabolites. The individual VOCs found previously by GC-MS to be associated with sputum eosinophilia differed between asthma and COPD (27;28), thereby suggesting that eosinophil-related VOCs are disease-specific as well. Apparently, the eNose platform sensors are capturing these biomarkers, but they are principally unable to identify the individual VOC. The latter is not required...
for medical decision making per se, but is certainly needed when investigating the underlying pathophysiological mechanisms. Specific analysis by GC-MS will then be necessary in order to unravel the identity of the combination of VOCs that are driving the prediction of eosinophilic inflammation.

The discriminant accuracies of VOCs to assess eosinophilic airway inflammation can have diagnostic implications. The analysis of exhaled air by eNoses is a relatively easy, non-invasive technique with prompt results in comparison with sputum induction and processing. Thus, it could provide a surrogate for sputum eosinophils in the phenotyping and monitoring of asthma patients (9), particularly with regard to excluding sputum eosinophilia of 3% or more, since the sensitivity of the eNose for this was 88%. Interestingly, a recent proof of concept trial indicated that eNose breathprints can indeed predict clinical efficacy by oral steroids in asthma, being even more accurate than sputum eosinophils or exhaled nitric oxide in patients (30). In addition, exhaled molecular profiles have shown potential in the clustering of asthma phenotypes (31). This suggests that composite biomarker fingerprints will become a powerful alternative to single cell or single molecule approaches in the management of asthma.

In conclusion, we demonstrated that VOCs measured by eNoses can discriminate eosinophilic from non-eosinophilic airway inflammation, which was validated in an independent data set. These data warrant further development of tailored eNose sensors and validation in larger cohorts in order to substantiate the accuracy of eNoses in establishing the eosinophilic phenotype amongst patients with asthma. Taken together, our data suggest that eNose breathprints have potential to provide a quick and simple alternative to the assessment of sputum eosinophilia in asthma, thereby facilitating phenotyping and personalized asthma treatment.
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