

In conclusion, PRM adds significantly to the diagnostic value of current CT quantification methods in assessing the presence of COPD. It could be helpful in identifying smoking-related changes and might contribute to the effect of lung cancer screening. Therefore, this novel quantification technique deserves further investigation. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Electronic Nose Identifies Bronchoalveolar Lavage Fluid Eosinophils in Asthma

To the Editor:

Asthma can be categorized into different inflammatory endotypes based on eosinophilic and/or neutrophilic airway inflammation (1). The type of airway inflammation can predict treatment response to inhaled corticosteroids and biological treatments (1, 2). The gold standard for assessing inflammation in the lower respiratory tract is bronchoscopy and bronchoalveolar lavage (BAL) (3), but this method is invasive and not always well tolerated. Sputum induction and processing is a less invasive alternative, but it is time consuming and the results are not readily available.

Recently, others and ourselves have shown that sputum cell counts are related to exhaled molecular profiles, as measured by gas chromatography-mass spectrometry and by electronic noses (eNoses) (4, 5).

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Therefore, we investigated whether eNose, as a noninvasive alternative to the above-mentioned procedures, captures eosinophilia in BAL fluid (BALF) in mild allergic asthma.

Data were derived from baseline assessments from patients with mild allergic asthma participating in a larger project (6). Characteristics of subjects and methods are described elsewhere in more detail (6, 7).

All subjects were current nonsmokers (<5 pack-years) and steroid-naïve. Asthma was defined by Global Initiative for Asthma criteria (<http://www.ginasthma.org/>) and the presence of airway hyperresponsiveness (provocative concentration of methacholine causing a 20% fall in FEV_1 < 8 mg/ml) on inclusion. For subject characteristics, see Table E1 in the online supplement.

BALF was collected by standardized methods (3), and differential cell counts were determined from cytospins. Fractional exhaled nitric oxide (FE_{NO} ; Niox Aerocrine AB, Solna, Sweden) was measured. Samples of exhaled breath were analyzed by Cyranose 320 eNose (Intelligent Optical Systems, Pasadena, CA) (7). eNose sensor data were reduced by principal component (PC) analysis to four PCs (PC1–4) based on an Eigenvalue greater than 1 (see the online supplement). Inflammatory cell counts were log transformed. Multivariable regression analysis was performed to test the relationship of eNose PC1–4 with BALF eosinophil counts. As a rule of thumb, 10 subjects per variable are needed to ensure proper sample size in a regression analysis. In total, 13 patients with mild allergic asthma (seven males, six females) were enrolled. See Table E1 for subject characteristics.

Multivariate regression analysis showed that the eNose breathprint was significantly related to BALF percentage eosinophils ($r = 0.76$; $r^2 = 0.58$; $P = 0.002$; Figure 1). PC2, PC3, and PC4 contributed independently to the prediction model, whereas PC1 did not contribute. For factor (PC) loadings constituting the breathprint, see Table E2. No relation was found for breathprints and BALF neutrophils, macrophages, and lymphocytes. FE_{NO} was not associated with any BALF inflammatory marker.

This study reports, for the first time, the correlation between exhaled breathprints assessed by an eNose and BALF eosinophil counts in mild allergic asthma. The findings in this study are based on a rather small sample size and need to be validated in an independent cohort of patients with asthma. However, they may indicate the possibility to assess inflammation in the lower airways by means of noninvasive breath testing.

Although other studies have already shown a good correlation of sputum inflammatory cell counts with individual volatile organic compounds (4, 5), this study demonstrates a similar correlation for BALF eosinophils with eNose exhaled breathprints. The significance of this advance is that these results hint toward a link between exhaled markers and lower airway inflammation, of which BALF cell counts are considered the gold standard measurement.

In this study, we failed to demonstrate a correlation for FE_{NO} with BALF eosinophils. Several studies have examined the association between FE_{NO} and eosinophils in BAL, but the observed strength of this relationship varies widely (8–11). This may not be unexpected, as FE_{NO} and eosinophilic inflammation are partly driven by entirely distinct pathogenetic pathways.

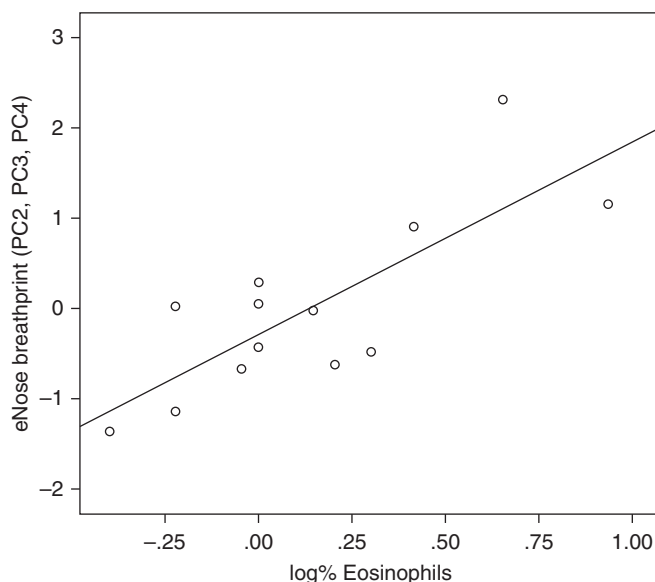


Figure 1. Correlation of electronic nose (eNose) breathprint (model based on principal component [PC] 2, PC3, and PC4) with bronchoalveolar lavage fluid log% eosinophils in patients with asthma.

The present findings indicate that the presence of a complex inflammatory process is better associated with multiple gaseous molecules, warranting a composite assessment of exhaled markers (breathomics). Indeed, breath analysis in a single measurement by (handheld) eNose can identify patients with asthma, and can be used to predict the clinical response to corticosteroids with greater accuracy than FE_{NO} , lung function, or sputum eosinophils (12, 13).

The sample size of the current study was limited for ethical reasons because of the bronchoscopy included in the study procedures. Validation studies in larger cohorts backed up with gas chromatography–mass spectrometry analysis for identification of individual compounds are needed to confirm our results and to gain insight into specific underlying pathophysiological mechanisms.

We carefully selected patients with mild allergic asthma, as representatives for the most common asthma phenotype. Moreover, we selected steroid-naïve patients to avoid any suppressive influence by steroids on the inflammatory profile. Therefore, the results of this study cannot simply be translated to those patients already treated with (inhaled) corticosteroids, which requires subsequent studies in patients with asthma treated with various antiinflammatory therapies. It seems likely that local airway inflammation is driving the signal because the association between exhaled breathprints and eosinophil count was only observed in BALF and not in blood (data not shown).

The clinical implication of this study lies in the monitoring of and tailoring therapy for patients with specific inflammatory phenotypes of asthma (14). Peripheral airway function seems to be highly relevant in asthma (15), but sampling peripheral airway inflammation is not a clinical option. The present study shows that exhaled molecular profiling can help to identify patients with asthma with peripheral eosinophilic inflammation, which may

widen the potential of targeted therapy by steroids or newly developed biologicals.

In conclusion, lower airway eosinophilic inflammation in mild allergic asthma can be adequately assessed by eNose measurements of exhaled breath. This brings us a step closer to being able to phenotype and identify patients with asthma eligible for antiinflammatory therapies in the doctor's office ■

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Imaging Neutrophil Extracellular Traps in the Alveolar Space and Pulmonary Capillaries of a Murine Sepsis Model by Multiphoton Microscopy

To the Editor:

We read with great interest the article by Sayah and colleagues regarding neutrophil extracellular traps being pathogenic in primary graft dysfunction after lung transplantation (1).

The authors demonstrated that neutrophil extracellular traps (NETs) were increased in the bronchoalveolar lavage fluid of experimental murine models of primary graft dysfunction (PGD), and platelets were also increased in the lung. Furthermore, the authors showed that both disruption of NETs by intrabronchial DNaseI administration and platelet inhibition reduced NET formation and lung injury, and subsequently improved oxygenation. They concluded that disruption of NETs may be a promising therapeutic strategy to prevent or treat PGD.

The work of Sayah and colleagues is worthy of praise as it is. However, several issues should be resolved to draw their conclusion: Are the primary therapeutic targets NETs in the alveolar space (bronchoalveolar lavage fluid), those in pulmonary capillaries (circulating blood), or those in the pulmonary interstitial space?

We recently investigated the morphological characterization of NETs in various organs of a murine sepsis model by multiphoton microscopy (2). We clearly visualized two forms of intravascular NETs: circulating cell-free NETs that were released away from neutrophils, and anchored NETs that were anchored to neutrophils *in vivo* in real time. Circulating cell-free NETs interacted with platelets or vascular endothelium. Cell-free NETs appeared to be associated with platelet thrombus formation or the formation of platelet aggregates or leukocyte-platelet aggregates in both arterioles and venules. These

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