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Modern Inflammatory Phenotyping of Asthma
Breathomics Is Here to Stay

The key progress in the management of asthma has been the recognition that individual phenotyping of patients, rather than standard assessment of the diagnosis and asthma severity, is a prerequisite for optimal disease outcome (1). This is predominantly based on the concept that inflammatory profiling enables us to improve the efficacy of steroids and modern biologicals targeting type 2 mechanisms (2). Hence, sputum eosinophils, and in some circumstances circulating eosinophils, have definitively shown their clinical value in the stratification of patients for interventions aimed to at least suppress asthma exacerbations.

This paradigm shift has been around for at least a decade, but is it catching on in the clinic? Remarkably, it is not. The main reason is not any dispute on the (highest) levels of evidence for this, but most likely the understandable hesitation to embark on complex (sputum) or invasive (blood) procedures for repeated assessment during the monitoring of patients with asthma.

Modern medicine is showing three developments that are changing the scene. First, capturing information on operational biological networks can now be done far beyond the mere presence of inflammatory cells. For instance, sputum eosinophilia or neutrophilia in asthma appear to be each associated with multiple, distinct molecular networks, as shown by high-throughput molecular analysis using transcriptomics (3) or proteomics (4) in sputum. Second, the information technology to accurately validate these "big data" for clinical precision medicine has been shaped (5). Third, it is now feasible to identify molecular fingerprints by noninvasive technologies, using metabolomics in exhaled air (6). This so-called "breathomics" is widely being validated and can also be used for inflammatory phenotyping of patients with asthma.

In this issue of the *Journal*, Schleich and colleagues (pp. 444–453) present a very solid study on the inflammatory phenotyping of patients with asthma by identification of exhaled volatile organic compounds (VOCs), using two different versions of well-standardized gas chromatography and mass spectrometry with varying resolutions (gas chromatography–mass spectrometry (GC-MS) and two-dimensional gas chromatography coupled with high-resolution time-of-flight mass spectrometry) (7). The authors recruited more than 500 patients from two separate cohorts (discovery and replication), each split into training and test sets, thereby establishing the largest GC-MS study in asthma that is meeting the international Standards for Reporting of Diagnostic Accuracy (STARD). This is exactly what is needed in the field, as sufficient statistical power and external validation are essential for establishing exhaled biomarker profiles for asthma. The authors used the presence or absence of sputum eosinophilia and/or neutrophilia as the gold standard of the inflammatory profile of the patients, and applied appropriate statistics for examining the discriminatory power and classification accuracies, as obtained by the VOCs.

The results show that eosinophilia and neutrophilia in sputum can be captured and discriminated by using various combinations of merely two to four exhaled VOCs. This included compounds such as hexane, 2-hexanone, and an unknown VOC for identification of eosinophilia, and 3-tetradecene with pentadecane for establishing neutrophilia. The two inflammatory profiles could be mutually discriminated by using 3,7-dimethylnonane, nonanal, and 1-propanol. In general, the areas under the receiver operating characteristic curves were excellent in the discovery study (0.85–0.99), whereas these areas under the curve reached more modest values in the replication study (0.68–0.73). Such loss in accuracy is generally to be expected, particularly in this project, as the discovery and replication studies were performed using different technologies. However, this has also a meaningful up side; namely, that the authors are providing true validation by using independent cohorts and methodologies.

Interestingly, the VOCs reached similar accuracies against sputum eosinophilia as exhaled nitric oxide (F_{ENO}) and blood eosinophil counts, whereas the combination of these three biomarkers performed best by far. Even though combining three biomarkers systems may not be appealing for clinical practice, this observation suggests that VOCs, F_{ENO}, and circulating eosinophil counts provide complementary information in relation to estimating sputum eosinophilia. One could even question whether sputum eosinophilia remains the appropriate gold standard for validating exhaled VOCs. The biological complexity of asthma is likely to be more comprehensively captured by composite molecular fingerprints than the mere presence of a granulocyte in sputum. Therefore, the limited accuracies of VOCs against sputum eosinophilia is not discouraging at all, as it may well be that the VOCs themselves will eventually do a better job in stratifying patients than cell counts.

Will GC-MS qualify for application in the clinic? Not at the moment, so the present study should be regarded as proof of principle. The value of the study is represented by the identification of particular VOCs in relation to inflammatory profiles. It should be emphasized that the present selections of discriminative VOCs were data-driven. Hence, the present high-throughput GC-MS platforms represent breathomics, not being based on *a priori* selection. This approach is empirical and hypothesis generating, providing a set of VOCs apparently being reproducibly discriminative for eosinophilia

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and neutrophilia. This is competence without comprehension, which is entirely justifiable, as it represents powerful, data-driven medicine. Would the identification of particular metabolites or their fragments by GC-MS in exhaled air by itself qualify for unraveling the critical pathobiological pathways in relation to eosinophilic or neutrophilic inflammation? This is unlikely, as the "big brothers" of this technology, such as transcriptomics and proteomics, are much closer to the mechanistic networks and are delivering at present (3, 4).

The prospect of using GC-MS in this context is therefore twofold. First, when associating the present VOCs with accompanying RNA and protein profiles, it might become feasible to indirectly establish any transcriptomic or proteomic fingerprints of asthma by noninvasive analysis of exhaled VOCs. Then breathomics is used for rapidly recognizing elaborate biological phenotypes, as has been done by Brinkman and colleagues for electronic nose (eNose) profiles against sputum transcriptomics profiles (8) (data in online supplement). Second, GC-MS data from the present study can also be used for tailoring cross-reactive sensors for eNoses toward their most discriminative and evidence-based combination for establishing eosinophilic or neutrophilic inflammation (9). Notably, eNoses have also been trained and validated in discriminating eosinophilic and neutrophilic inflammation with high accuracy, regardless of the clinical diagnosis of asthma or chronic obstructive pulmonary disease (8, 10). The advantage of the latter technology is that it can be linked to big online databases and cloud computing, providing real-time results in the doctor's office (www.breathbase.org).

The article by Schleich and colleagues (7) presents a landmark study on the validation of exhaled VOCs in the inflammatory profiling of asthma. This is needed for type 2 and increasingly also for non–type 2 asthma phenotypes. Ongoing clinical tailoring of breathomics is definitely bringing data-driven, precision medicine to the point of care. ■

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Seeing the Forest for the (Arterial) Tree: Vascular Pruning and the Chronic Obstructive Pulmonary Disease Pulmonary Vascular Phenotype

The clinical consequences of pulmonary hypertension (PH) in patients with chronic obstructive pulmonary disease (COPD) and the effect of right ventricular (RV) failure on prognosis has long been recognized. Mild to moderate PH is common in patients with severe COPD; however, severe PH (mean pulmonary arterial pressure [mPAP] $\geqslant$35 mm Hg or a mPAP $\geqslant$25 mm Hg with a cardiac index $<$2.0 $L \cdot min^{-1} \cdot m^{-2}$) is less frequent and, when present, is often associated with comorbid conditions such as left heart disease or chronic thromboembolic disease (1–3). More recently, however, it has been recognized that the pulmonary vasculature may be significantly compromised in some patients with mild to moderate COPD and minimal emphysema, the so-called "pulmonary vascular phenotype" (4). Often referred to as

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