Breath Diagnostics

By Dr Marc van der Schee and Dr Theodore W Wilson at Owlstone Medical

Exhaled breath contains a vast untapped resource of chemical information represented by hundreds of volatile organic compounds. As products of underlying metabolic activity, they directly reflect the state of cells and tissues and are fast becoming recognised as valuable disease biomarkers

The metabolic information provided by volatile organic compound (VOC) biomarker analysis offers possibilities beyond those generated by genomic data, which may provide a blueprint for disease predisposition but can lack detail on actual disease activity. Coupled with the non-invasive nature of breath sampling, using this method to perform a 'breath biopsy' is a promising approach for a wide range of diseases.

A growing body of evidence shows the potential of VOCs for directing treatment of disease. Their clinical application has now been accelerated by the development of new technologies for the reliable collection of patient breath samples and advances in VOC analysis technology – both of which have the potential to bring breath biopsy to point-of-care use when combined.

VOC Biomarkers in Breath

Breath is particularly valuable as a source of disease biomarkers for respiratory diseases, because it contains VOCs originating from local airways tissues. Biomarkers in breath are by no means limited to pulmonary conditions – breath also contains systemic VOCs from the circulatory system providing information about the rest of the body, as well as VOCs from exogenous sources (see Figure 1). Hence, VOC biomarkers in exhaled breath represent a rapid and non-invasive means to gain a detailed insight into the functioning of the whole body.

Keywords

Asthma
Biomarkers
Breath
Volatile organic compounds

Using the composition of exhaled breath to make clinical decisions is not a new idea – knowledge of distinctive breath odours related to specific diseases stretches back to Hippocrates. Despite this historical pedigree and the obvious promise held by breath analysis, very few breath-based medical tests have found a place in doctor's toolboxes. This is largely due to a lack of standardised breath sampling technology that has resulted in poor repeatability between studies looking for disease-specific VOC biomarkers (2,3).

Breath analysis research usually employs high-end analytical instrumentation such as gas chromatography mass spectrometry (GC-MS) and selected-ion flow-tube MS. These instruments are the gold standard, but are also expensive, bulky and require expertise to operate correctly – hampering both their deployment in clinical scenarios and the transition of VOCs from research laboratories to deployable clinical tests.

New Technology

Recent advances in breath sampling and analysis technology are now addressing the field's long-standing issues and herald the arrival of an era in which breath biopsy will be as ubiquitous as blood and urine tests. Arguably, the crucial step of technological standardisation in breath sampling has now been taken with the development of a breath sampler designed by the Breathe Free Consortium, a community of more than 100 breath researchers (see Figure 2) (4).

Pressure and CO_2 sensors provide real time monitoring of patient breathing, allowing specific breath fractions to be selected. The device enables different fractions such as bronchial or end-tidal to be stored independently on sorbent tubes, which can then be analysed immediately or saved for later use. Sorbent tubes ensure reproducible collection of VOCs, unlike other commonly used collection media such as Tedlar® bags (5). The sampler is also designed to incorporate a clean air supply in order to prevent interference from unwanted local VOC contaminants.

Another key advance in breath analysis is the miniaturisation of technologies which have the potential to be deployed at the point-of-care. 'Electronic Nose' (eNose) systems have been developed, using arrays of non-selective chemical sensors to detect VOCs. However, they often suffer from unwanted chemical interactions between VOCs and the sensors, low intra-device repeatability, limited temporal stability and poor chemical selectivity (6).

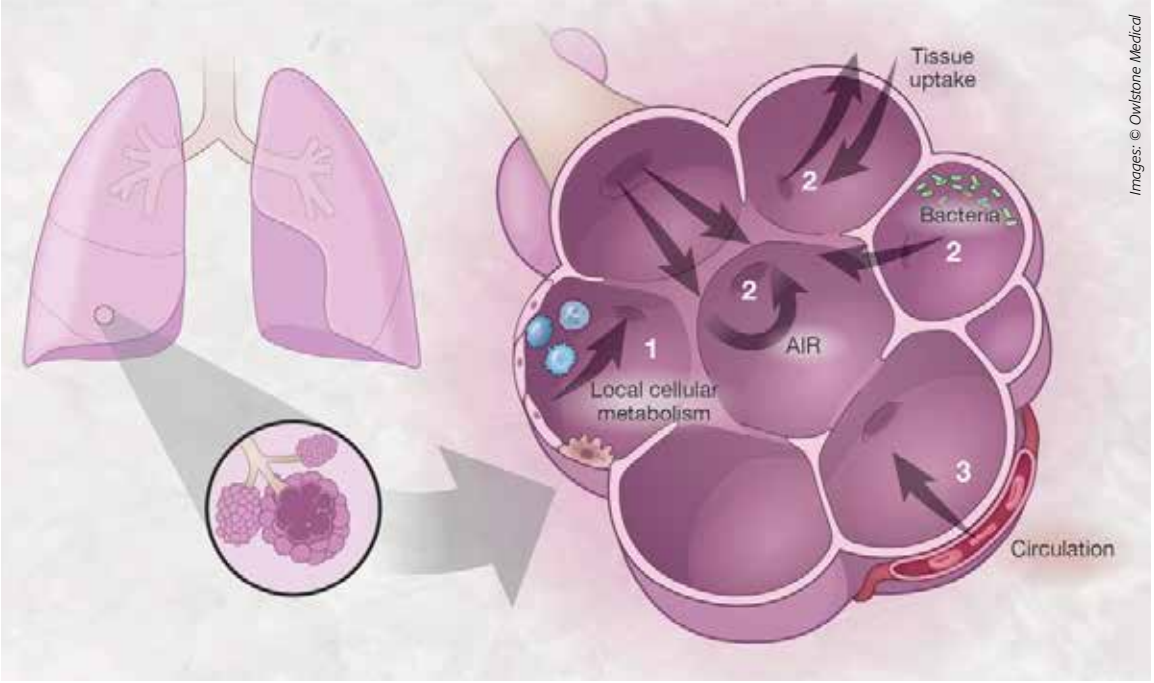

Figure 1: Schematic representation of alveolus detailing origins of VOCs. 1. Local cellular metabolites 2. Exogeneous compounds, eg microbiota 3. Compounds originating from elsewhere in the body (1)

Field asymmetric ion mobility spectrometry (FAIMS) is now available in a chip-based miniaturised form that offers improved stability and sensitivity when compared to eNose systems. FAIMS distinguishes ionised VOCs according to differences in the speed that they move through a buffer gas under the influence of an oscillating electric field (7). The FAIMS chip is also programmable in software, making it suitable for a wide range of medical applications (6). Along with the Breathe Free sampler, FAIMS is being incorporated into large clinical trials relating to lung cancer, colorectal cancer and asthma (8-10).

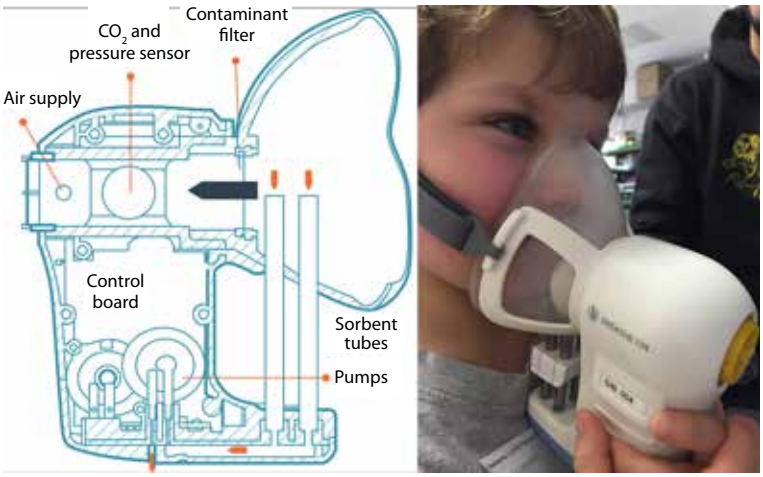

Figure 2: The Breathe Free open source breath sampler, designed by over 100 collaborating breath scientists

Asthma: A Case Study

As many as 334 million people have asthma and up to 14% of the world's children experience its symptoms (11). Asthma management is focused on achieving control of symptoms to minimise the risk of future exacerbations. However, a lot of patients do not respond sufficiently to treatment, so their symptoms are not kept adequately under control. In the UK, 4.4% of patients fail to respond to standard therapies even at high doses, and account for more than 50% of asthma costs to the NHS.

Asthma symptoms are caused by chronic inflammation in the small airways. Many underlying mechanisms are responsible for this inflammation, with different treatments required for different inflammation types. There is no single diagnostic test for asthma at present, in part because of its heterogeneous underlying pathophysiology.

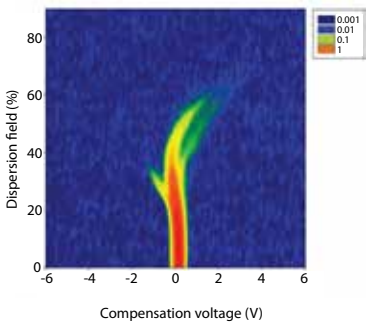

Figure 3: A typical FAIMS spectrum for exhaled breath (7)

The difference in cell activity related to different inflammatory subtypes is reflected by the metabolites produced by these cells, and consequently by the VOCs in exhaled breath. This is why asthma

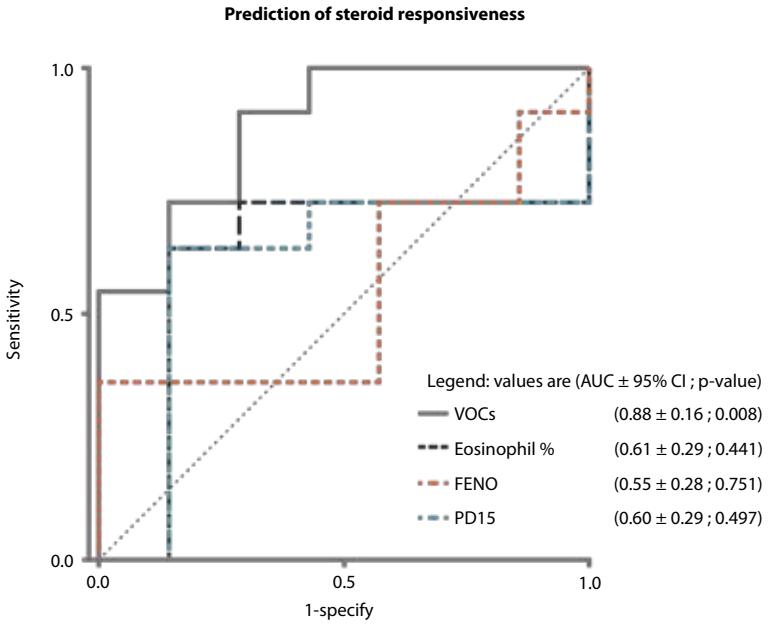

Figure 4: Predicting steroid responsiveness in steroid-naive patients (18)

Source: van der Schee et al, CEA, 2013

has become one of the most studied diseases with regard to breath VOCs, and improvements in technology are accelerating progress in the field.

Improved Diagnostics

One breath-based biomarker, fractional exhaled nitric oxide (F_ENO), is already being used to support asthma diagnosis. It is useful because it reflects a protective biochemical pathway of the lungs, but it does lack specificity as it is a single biomarker affected by many processes other than asthma. A study of VOCs in asthma found that they outperform F_ENO and lung function tests in discriminating asthmatics and healthy controls. Furthermore, combining VOCs with F_ENO gave an even higher diagnostic performance (12).

It has also been shown that VOCs can be used to discriminate with a high degree of accuracy between asthmatic children and those with transient wheezing (13). In both this and two other studies, increases in methylated alkanes were associated with asthma prevalence (14,15). These VOCs are potential markers of oxidative stress that have been linked to airway inflammation.

Data collected so far in one clinical trial indicate that VOC biomarkers collected using the Breathe Free sampler and measured using GC-MS combined with FAIMS can differentiate patients with asthma from patients with a wide range of other pulmonary diseases with a high degree of accuracy (8).

The existing evidence suggests that VOCs are strongly affected by the airway inflammation that characterises obstructive pulmonary diseases like asthma, and that they could be useful tools in non-invasive asthma diagnosis. This method is particularly valuable for diagnosis in children as other known biomarkers – F_ENO and blood immunoglobin E (IgE) levels, for example – require exhalation at a continuous flow or invasive sampling.

Asthma Phenotyping

While the diagnostic power of breath VOCs could provide a valuable new tool for clinicians, the most impactful application of an asthma breath biopsy is likely to be in the phenotyping of the disease by characterisation of underlying inflammation.

There is evidence that VOCs can be used to classify patients by asthma phenotype with high accuracy (eg 15-17). VOCs will therefore allow different phenotypes of asthma defined by different inflammatory responses to be distinguished. This clearly points towards the use of breath biopsy as a companion diagnostic for therapy stratification. There is already evidence showing that VOCs can be used to predict steroid responsiveness in mild to moderate asthma sufferers (18). In this study, VOCs were considerably more accurate at predicting responsiveness to steroid treatment (AUC 0.88 ± 0.16) than either F_ENO or measurements of eosinophil cells from sputum samples (see Figure 4).

Personalised Medicine

A reliable tool to tailor treatment to asthma phenotype would be an important development. The past decade has seen the approval of biological drugs targeted at specific inflammatory subtypes, yet their approval for clinical use has been delayed due to their high costs and the difficulty of identifying patients with the correct asthma phenotype who would benefit.

The lack of stratifying diagnostics means that current guidelines advocate a 'trial and error' approach, resulting in increased healthcare costs, prolonged periods of poor disease control and an increased risk of exacerbations.

Using biomarkers to characterise asthma subtypes has so far not found widespread adoption due to the invasive nature of existing cell-harvesting techniques, such as bronchoalveolar lavage and the limited applicability of F_ENO testing at identifying responders to new targeted biologic treatments aimed at patients who remain uncontrolled under traditional asthma therapies. The blood-based biomarker IgE

has shown positive predictive potential for treatment response to the targeted anti-IgE treatment XOLAIR®/Omalizumab (19). Therapy failure still occurs in a high percentage of adult and paediatric patients however, and a significant fraction of sufferers lose responsiveness to XOLAIR® over time (20). Additionally, a large portion of patients that would benefit are currently not eligible based on the IgE entry criteria. Similar issues have arisen with the use of periostin and blood eosinophils to identify potential responders to novel asthma therapies.

Promising initial results from the U-BIOPRED consortium project show that VOCs measured by a FAIMS device could be used to stratify asthmatic patients into treatment subgroups. For example, VOCs discriminated between anti-IgE-treated XOLAIR®/Omalizumab and non-treated severe asthma patients with 83% accuracy (21,22).

Future Outlook

VOCs in exhaled breath have enormous potential as biomarkers for disease diagnosis, phenotyping and monitoring. Promising results in the field of asthma indicate that they reflect specific immunological processes that underlie chronic inflammation, making them ideal biomarkers for personalised medicine and treatment stratification.

Highly powered studies such as the MRC EMBER, LuCID and STRATA programmes will generate key evidence for using VOC biomarkers in diagnostic tests (23,8,10). The standardisation of breath sampling technology by the Breathe Free Consortium and the development of analytical techniques like FAIMS with the potential for deployment at point-of-care will allow the widespread adoption of breath biopsy in many clinical applications (4,6).

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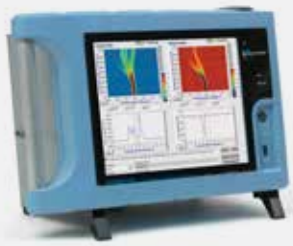
VOC analysis



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