Breath Diagnostics

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.

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 CO₂ 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).
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).

**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.

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...
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 immunoglobulin 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
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clinical applications (4,6).

adoption of breath biopsy in many of-care will allow the widespread potential for deployment at point-of-care by analytical techniques like FAIMS with the Consortium and the development of technology by the Breathe Free evidence for using VOC biomarkers programmes will generate key MRC EMBER, LuCID and STRATA Highly powered studies such as the 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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Breath Biopsy® Products

Bring VOC Biomarker research to your own lab with our complete range of products for breath collection and analysis.

**ReCIVA™ Breath Sampler**
ReCIVA provides an out-of-the-box means to collect breath samples reliably and reproducibly.
- Collect different breath fractions (enriched bronchial or end-tidal)
- Pre-concentrate a large volume of breath onto sorbent tubes, enabling even VOCs present at low levels to be detected
- Developed in conjunction with a multi-disciplinary group of 100 breath analysis experts

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**CASPER Clean Air Supply**
CASPER provides a consistent, portable supply of clean air, ideal for use with the ReCIVA™ Breath Sampler.
- Ensure breath samples are not contaminated by external VOCs
- Remove the need for a clean air supply line
- Portable – to wherever the patient is in clinic

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**Lonestar VOC Analyzer**
The Lonestar VOC Analyzer is an easy to use instrument for the detection of VOC biomarkers in clinical samples such as breath and other biospecimens.
- Rapid, sensitive, and selective VOC analyzer
- Based on proven FAIMS technology
- Biomarker discovery and targeted analysis

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“*We have found FAIMS technology able to non-invasively detect a broad range of diseases with high sensitivity and specificity.*”

*Prof. J. Covington, Warwick University*
Breath Biopsy® Biomarker Services

Discover novel non-invasive biomarkers for cancer, inflammatory and infectious disease with our comprehensive Breath Biopsy® Biomarker Services.

Overview

Owlstone Medical’s Biomarker Services enable academics, clinicians and pharmaceutical companies to identify novel breath biomarkers for diagnostics as well as precision medicine applications including therapeutic response, patient stratification and outcome prediction.

We offer a complete set of services for analysis of volatile organic compound (VOC) biomarkers in breath and other biospecimens. Our services include everything from consultation on optimal study design to biomarker discovery and external blinded validation of the test in the context of the clinical use case.

Breath collection

We ensure that breath samples are collected reliably and reproducibly by installing the ReCIVA™ Breath Sampler at clinical sites, and providing training on optimal breath collection. Breath samples are pre-concentrated on stable sorbent tubes and shipped to Owlstone Medical’s service laboratory for analysis.

Biomarker discovery

Owlstone Medical offers the largest laboratory for breath analysis. VOC Biomarkers are analyzed using our proven FAIMS technology. Our analysis experts develop robust classification algorithms from rich spectral FAIMS data. Our exceptional analytical platforms, rigorous quality standards and experienced staff deliver reliable, reproducible and rapid results.

In silico verification and validation

Classification algorithms are verified in silico using our proprietary database containing thousands of patient VOC profiles, to provide early evidence of likely clinical performance. Finally, an external blinded test validation is performed in patient groups representative of the intended use population.