Application of Breath VOCs in Asthma

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Every time you breathe out there are hundreds of chemicals called volatile organic compounds (VOCs) on your breath. These compounds are potential biomarkers for health and disease as their presence and concentration are products of underlying metabolic activity that reflect the state of cells and tissues. Additionally, exogenous sources such as microbiota and environmental exposures contribute to the mixture of exhaled biomarkers. These varied sources open up a wide realm of possibilities for the use of VOCs as the basis for a 'breath biopsy' in disease diagnosis, phenotyping and monitoring.

1 Introduction

Exhaled VOCs are typically analysed by high-end laboratory based tools such as mass-spectrometry coupled to gas-chromatography (GC-MS), with the potential to translate to point of care by means of either miniaturised gas sensors such as a field asymmetric ion mobility spectrometer (FAIMS), or Electronic Nose-type pattern recognition based sensors. A metabolomics approach such as using VOCs creates possibilities that range wide beyond a genomic analysis which provides the starting blueprint without details on actual disease activity. Taken together with the non-invasive nature of breath analysis this makes VOCs promising tools in a very wide range of diseases.

Asthma is one of the most studied diseases in terms ofits effects on VOCs, largely because of the wide range of clinical applications for which VOCs might provide a solution. Firstly, local inflammatory activity in the airways is known to alter the exhaled biomarkers and reflect the type and severity of the chronic inflammation, potentially guiding treatment decisions. Secondly, exogenous compounds produced through infections or shifts in resident microbiota could help identify and monitor exacerbations. Finally, systemic VOCs passing through the lungs from the bloodstream provides insight into systemic effects of the disease and its treatment. The purpose of the current paper is to provide an overview of the clinical applications of VOCs in asthma in relation to currently available alternatives.

2 The clinical need for better biomarkers in asthma

Asthma is characterised by a combination of reversible airway obstruction and characteristic symptoms such as wheeze and dyspnea. The chronic airway inflammation that underlies these symptoms has diverse origins and triggers. Combined with the poor correlation between airway inflammation and symptoms this inherent heterogeneity creates severe challenges for the management of asthma.

3 Disease diagnosis

There currently is no single diagnostic test for asthma reflecting its heterogeneous underlying pathophysiology. Challenges surrounding diagnosis of asthma are especially poignant in the pediatric population. Thirty percent of children under six years of age visit a physician for wheezing symptoms that could indicate the onset of asthma. Treating all such children, however, would be inappropriate and would cause

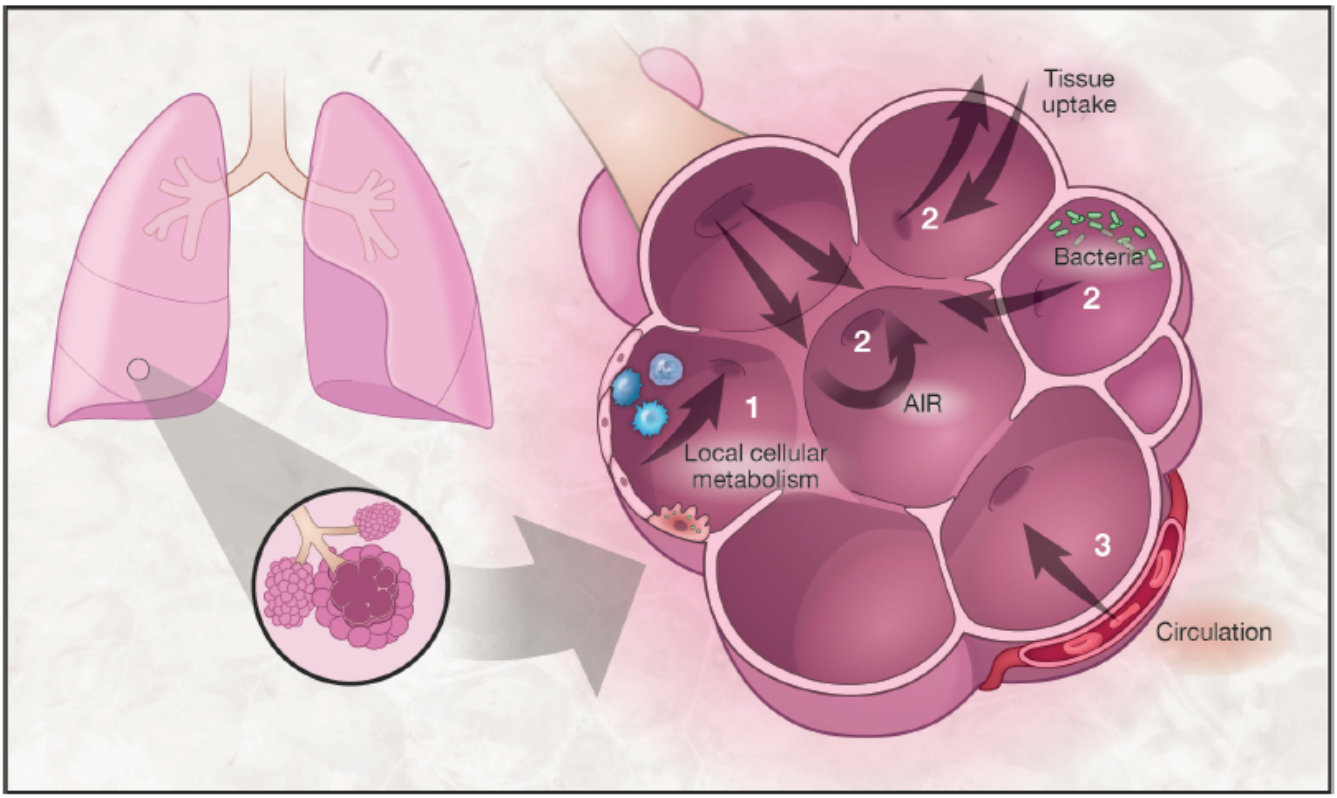


Figure 1: Schematic representation of alveolus detailing origins of VOCs. 1. Local cellular metabolites 2. Exogeneous compounds e.g. microbiota and 3. Compounds originating from the systemic compartment. [1]

unwanted side effects as only one in three of them will continue to wheeze and develop asthma.

Initial research on VOCs in asthma focussed on the diagnostic potential of VOCs to discriminate asthmatic patients from various groups of controls. These studies have shown that VOCs are an accurate diagnostic tool in their own right, and they combine well with other measurements to achieve even higher accuracy. In a study comparing the diagnostic performance of VOCs detected with an electronic nose device, fractional exhaled nitric oxide (F_ENO), and lung function tests between 27 intermittent or persistent mild asthma patients and 24 healthy subjects, VOCs (88%) outperformed either F_ENO (79%) or lung function tests (71%) in classifying between asthmatics and healthy controls.[2] Furthermore, combining VOCs with F_ENO had even higher diagnostic performance (96%).

In a study of 202 children between two and four years, the addition of a VOC test significantly improved the predictive value of the Asthma Predictive Index-assessed clinical information from receiver operating characteristic area under the curve (AUC) values of .61 ± .09 to .89 ± .06. In the same study other biomarkers associated with gene expression improved asthma prediction to a lesser extent (an increase to AUC .75 ± .1) and exhaled breath condensate biomarkers or airway resistance tests did not contribute to improvement in asthma diagnosis.[3] Combining the VOCs, genetic markers, and clinical information gave the highest predictive accuracy (AUC .95 ± .04). Developing accurate, non-invasive biomarker tests is particularly relevant in the case of pre-school children, where other known biomarkers such as F_ENO or blood IgE levels are not appropriate as they either require exhalation at a continuous flow or invasive sampling.

While the above results are promising, clinical utility can only be demonstrated by testing the biomarkers in a population with a clinical suspicion. Initial steps in this direction have been demonstrated in a cross-sectional study looking at 37 asthmatics, 31 chronic obstructive pulmonary disease (COPD) patients, 31 lung cancer patients along with 45 controls. In this study an electronic nose array using four VOC markers combined with spirometry data could classify between asthmatics and controls with 87% accuracy, between asthma and COPD with 81% accuracy, and with similar (78%-88%) cross-validation values between the other patient groups.[4] In a study of 252 children between two and six years of age, a set of 12 VOCs analyzed with a GC-MS discriminated asth-

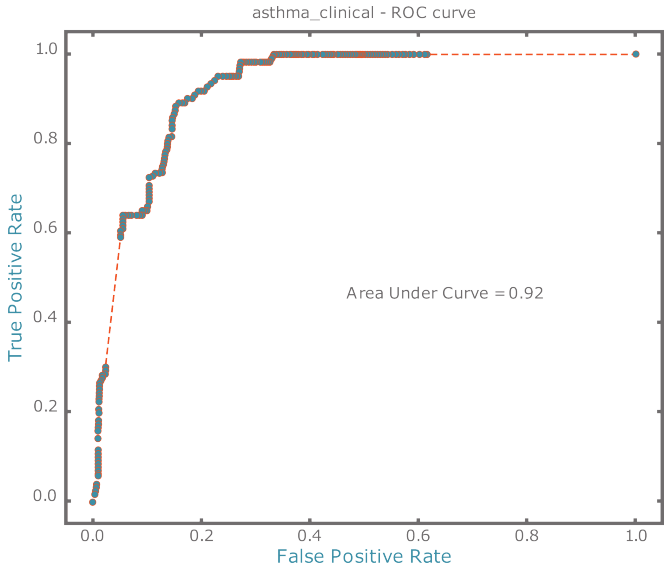


Figure 2: Discriminating patients with asthma in LuCID clinical trial population

matics from transient wheezing children (AUC .78) and 12 VOCs discriminated patients with asthma from healthy controls (AUC .86).[5] In both this and two other studies,[6,7] increases in methylated alkanes were associated with asthma prevalence: these chemicals are potential markers of oxidative stress which have been linked to airway inflammation.

To extend confidence in these results, further studies are required which would obtain breath samples containing VOCs from larger patient cohorts. An example of this is Owlstone Medical's NHS funded Lung Cancer Indicator Detection program (LuCID) which will recruit up to 3,000 patients across 21 sites in the UK and the rest of Europe to validate VOC biomarkers for the early detection of cancer, and for differentiation between benign and malignant tumours. As part of this project, a detailed medical history is obtained: preliminary results indicate that VOCs can discriminate between asthmatic patients and non-asthmatic patients (AUC .92, Figure 2) in a heterogeneous population of patients with a wide variety of medical conditions. Such an approach allows identification of disease specific volatiles greatly facilitating translation to clinical practice.

In summary, current data suggest that VOCs are strongly affected by the airway inflammation that characterizes obstructive pulmonary diseases such as asthma. Interestingly, the value of these biomarkers is partly complementary to other biomarkers. In view of the non-invasive nature and the ease of collecting samples the value of early diagnosis in the pediatric population is very apparent. For adults the optimal clinical positioning of a diagnostic test for asthma has yet to be determined. Such a test may have value at the general practitioner, potentially as an adjunct to spirometry. However, the ultimate value of a breath test is probably not diagnosis but rather characterisation of the underlying inflammation as it allows a personalised medicine approach to asthma therapy.

4 Asthma phenotyping

Over the past decade knowledge concerning the different inflammatory subtypes of asthma[8] has resulted in the development of targeted biological drugs that have fewer side effects and greatly enhanced efficacy, especially in patients with severe asthma.[9,10] Attempts to use biomarkers as guides for this therapy stratification have so far seen only partial success. The lack of stratifying diagnostics means that current international asthma guidelines advocate using lung function metrics and symptoms to guide a trial and error approach with progressive escalation of treatment towards higher dosage with add-on of adjuvant drugs.[11] This results in increased healthcare costs, prolonged periods of poor disease control, and an increased risk of exacerbations.

Initial approaches to using biomarkers to characterize asthma types involved invasive procedures such as bronchoalveolar lavage or cumbersome procedures such as sputum induction through inhalation of nebulised saline. Both techniques allow a direct count ofinflammatory cell types in the airways to help identify the type ofinflammation (for example eosinophilic or neutrophilic). Identification of the cell type allows physicians to make an informed decision concerning the appropriate therapy. Despite this clear advantage these techniques have not found widespread adoption due to patient discomfort as well as resource intensity. Furthermore the procedure for obtaining a reliable sputum sample is considered too invasive to be used regularly in children.

The observation that fractional exhaled nitric oxide (F_ENO) is produced locally in the airways as a protective mechanism against airway constriction and inflammation sparked extensive research into its use as a non-invasive biomarker for asthma. Initial research pointed towards strong potential for this biomarker in eosinophilic or Th2 driven asthma. Unfortunately, being a single biomarker F_ENO is affected by a wide variety of processes in the airway which are only partly related to asthma. This has resulted in only limited utility for F_ENO to guide asthma phenotyping and treatment decisions. Also,

DUE TO **LACK OF STRATIFYING DIAGNOSTICS** CURRENT **INTERNATIONAL ASTHMA GUIDELINES** ADVOCATE A '**TRIAL** AND **ERROR**' APPROACH WITH PROGRESSIVE ESCALATION OF TREATMENT

a reliable F_ENO sample depends on the patient being able to provide a fixed flow exhalation. This can be very challenging in patients during an acute exacerbation or for children under six years of age, and it is debatable whether F_ENO sampling for children under twelve is sufficiently reliable.

The observation that exhaled volatiles allowed differentiation between asthmatic patients and controls sparked research into understanding the underlying dynamics. The first study showing a link between inflammatory activity and selected VOC concentrations was a study of 35 asthma patients in which 15 VOCs analyzed by GC-MS were successfully used to classify patients by:

1. the presence of asthma (86% cross-validation accuracy),
2. whether symptoms were uncontrolled as defined by an Asthma Control Questionnaire score 1 (80% accuracy),
3. whether the patient had a sputum eosinophil cell count of 2% (83% accuracy),
4. and whether the sputum neutrophil cell count was 40% (72% accuracy).[7]

Further in vitro investigations of eosinophil and neutrophil cells cultured from healthy volunteers supported the notion that exhaled VOCs are linked to inflammatory activity. Non-activated eosinophil and neutrophil cell cultures could be discriminated from one another with 100% accuracy using either two or three VOCs (benzylalcohol and 3-methlyfuran for neutrophil cells, with an unidentified VOC elevated for eosinophils).[12] Furthermore, cell cultures stimulated with phorbol 12-myristate 13-acetate could be 96% accurately classified from each other using five VOCs, different biomarkers from the above three.

Discrimination of eosinophilic and neutrophilic inflammation

Reciever operating characteristics of the multivariate models for the asthma phenotypes of interest

	Eosinophilic versus non-eosinophilic	Neutrophilic versus non-neutrophilic	Controlled versus not controlled
Sensitivity	0.75	0.80	0.89
Specificity	0.90	0.75	0.88
Positive predictive value	0.86	0.80	0.89
Negative predictive value	0.82	0.75	0.88
AUROC (95%CI)	0.98 (0.91to 1.00)	0.90 (0.76 to 1.00)	0.97 (0.93 to 1.00)
Cross-validation accuracy	83%	72%	80%

Leave-one-out cross-validation accuracy from discriminant fuction analysis also shown.
AUROC, area under the receiver operating characteristic.

Ibrahim et al, Thorax 2011

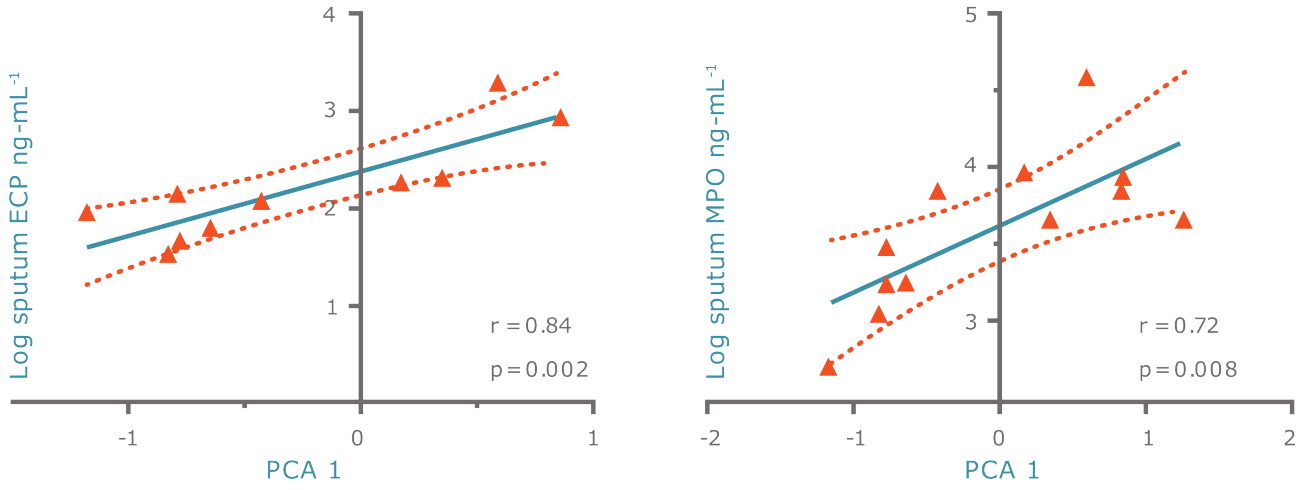
Figure 3: VOC discrimination by asthma phenotype (cell presence and symptom control status) [7]

In line with this, in vivo research assessed the association between VOCs and eosinophil cationic protein (ECP). ECP is produced by eosinophil cells as part of their inflammatory response. The concentration of these inflammatory markers has been shown to correlate more closely to the tissues inflammatory activity. In a study of 129 patients with mild or moderate asthma, both eosinophils and ECP were analysed from sputum. Both eosinophils and ECP showed a weak correlation to asthma severity, while ECP was more closely related to lung function parameters such as FEV1 and peak expiratory flow (PEF) than eosinophil cell counts.[13]

In a study of 28 COPD patients, VOCs in exhaled breath were measured with both GC-MS and also electronic nose devices: a composite of 19 VOCs were found to be highly associated with ECP, while another 4 were highly associated with myeloperoxidase (MPO), an activity marker of neutrophilic cells (Figure 4).[14] Analysis showed high sensitivity and specificity (AUC 1.00 for ECP, 0.96 for MPO) for the 12 mild COPD patients but not for 16 moderate COPD patients: since mild COPD is more commonly typified by airways inflammation while moderate COPD is less associated with inflammation and more associated with airways remodeling, this suggests that these VOCs may be used for measuring activity in airway inflammation.

In another study, potential biomarkers that could discriminate between asthma inflammatory phenotypes were discovered using GC-MS in a cohort of 245 asthma patients. Levels of 3,7-dimethylnonane, 1-propanol, and nonanal were higher in the neutrophilic than eosinophilic subtype (AUC 0.92). The combination of hexane and nonanal showed the best classification performances between the two subtypes (AUROC 0.71, accuracy 0.7, sensitivity 0.45, and specificity 0.85).[31]

VOCs correlate with ECP and MPO as inflammatory cell markers



Fens et al, ERJ 2011

Figure 4: Strong VOC correlation with inflammation activity markers in COPD

Taken together this evidence suggests that both base metabolic activity as well as changes to that activity can be detected by distinct populations of VOCs. By choosing the correct set of VOCs to measure, different phenotypes of asthma defined by different kinds of inflammatory response can be distinguished.

5 Treatment stratification

The clinical need for therapy stratification is very apparent as many patients with asthma currently do not receive optimal therapy. This is particularly true for patients with severe refractory asthma, whose symptoms may be better controlled by being initiated on novel, expensive biologicals. The challenge is to determine which patient populations would benefit from each targeted biological therapy.

5.1 Alternative biomarkers

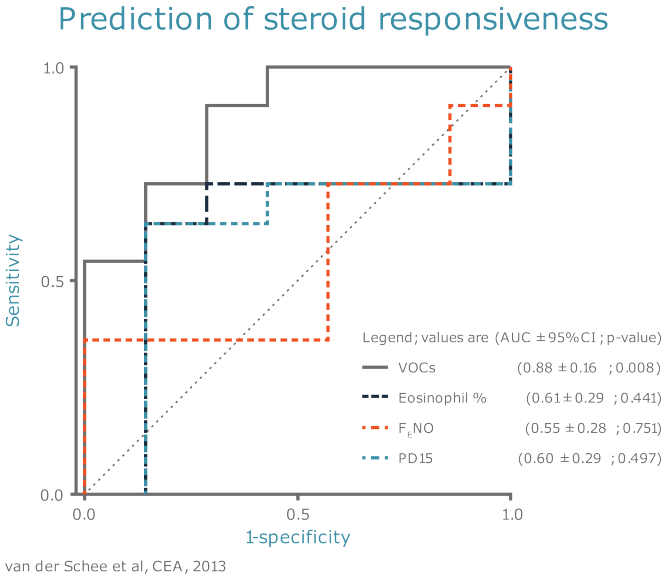
To address this clinical challenge, blood based biomarkers such as blood eosinophils, IgE, and periostin have been investigated. Blood IgE levels have shown good positive predictive potential for treatment response to anti-IgE treatment XOLAIR/Omalizumab.[15] Therapy failure, however, occurs in 28-48% of the adult and 26% of the pediatric population (NICE guidance - Omalizumab for treating severe persistent allergic asthma). Furthermore, a significant fraction (2-19% of adult patients and 20% of pediatric patients) lose responsiveness to Xolair over time. This results in wasteful treatment and delayed disease control in patients.

Similarly issues have arisen with the use of blood eosinophils to identify potential responders to Mepolizumab. This might be a consequence of the fact that circulating eosinophils only mildly correlated with resident lung cell concentrations and as such do not well represent cellular activity there.[16] A potential biomarker for anti-IL-13 treatments could be blood periostin levels, but studies to date have however not yet shown a correlation with treatment response in asthmatics.[17]

As described earlier the use of F_ENO to guide therapeutic decision has been plagued by the multitude of process that can affect its levels. The recently proposed F_ENO suppression test (evaluating a decrease in F_ENO after a steroid injection) to assess steroid therapy adherence may be a notable exception.[18]

5.2 Potential value of VOCs in stratification

The fact that exhaled VOCs allow identification of asthma phenotypes clearly points towards its use as a companion diagnostic for therapy stratification. In one study, 25 mild to moderate asthma patients were taken off inhaled steroid treatment for 28 days, then treated with oral prednisone for 14 days. VOCs measured using an electronic nose device discriminated between asthma and controls with similar accuracy to F_ENO measurements or sputum eosinophil counts, but were considerably more accurate at predicting responsiveness to steroid treatment (AUC .88 ± .16) than either F_ENO (AUC .55 ± .28) or sputum eosinophils (AUC .61 ± .29) (Figure 5).[19] Both the VOCs (AUC .81 ± .17) and the sputum eosinophils (AUC .87 ± .17) were able to distinguish between patients who experienced loss of control while off their steroid treatment, whereas the F_ENO measure-



van der Schee et al, CEA, 2013

Figure 5: Predicting steroid responsiveness in steroid naive patients.

ments did not (AUC .67 ± .22). Furthermore, while patients were taking the inhaled steroid treatment, VOCs had much higher specificity in distinguishing asthmatics from healthy controls (80% to 65%, with 84% sensitivity for both), showing that VOCs were more accurate at detecting the underlying inflammation and thus predicting potential loss of control even when symptoms were controlled. This exemplifies how the utility of a single biomarker such as F_ENO has limitations in certain clinical settings.

Steroid insensitivity is a problem seen in cases of severe asthma. Biomarkers that are sensitive to inhaled corticosteroids (ICS) could ensure more personalized and specific treatment options. In one study 17 patients with severe asthma and elevated FeNO were recruited to investigate how exhaled biomarkers change over two hours and one week following ICS dosing. Levels of size VOCs ($q > 0.1$) fell over the 2 hours after dosing, and after one week two VOCs ($p < 0.05$) fell. These observed changes in exhaled VOCs show potential as markers of ICS use and its effectiveness.[32]

The anti-IgE monoclonal antibody omalizumab can reduce asthma exacerbations, however there is currently no way to predict which patients will benefit from this. One study recruited 191 patients with severe asthma who received omalizumab and gave exhaled breath, blood, sputum, and urine samples before and after 16 weeks of treatment. 173 participants were also assessed 52 weeks after treatment. Five VOCs in exhaled breath (2-ethyl-1-hexanol, toluene, 2-pentene, nonanal, and one unknown VOC) were able to predict a reduction in exacerbations, and a second set of VOCs in exhaled breath (benzothiazole, acetophenone, 2-pentylfuran, methylene chloride, and 2-methylbutane) were able to predict early responses. These VOCs together had the ability to differentiate between mild/moderate asthmatics and atopic severe asthmatics (AUROC 0.931). This data is meaningful as the difference between mild/moderate asthmatics and severe asthmatics is the cut-off point for treatments such as omalizumab.[33]

With respect to novel biological treatments, results from the U-BIOPRED consortium (funded by the Innovative Medicines Initiative) presented at the September 2015 ERS Congress showed that VOCs measured using a Field Asymmetric Ion Mobility Spectrometry (FAIMS) device could be used to stratify asthmatic patients into relevant treatment sub-groups. For example, VOCs discriminated between anti-IgE-treated XOLAIR/Omalizumab and non-treated severe asthma patients with an 83% accuracy.[20,21]

This promising initial result suggests that VOCs can be used as companion or complementary diagnostics through its potential to allow detailed phenotyping. Such applications are directly valuable to allow a personalised medicine approach to asthma: matching the right treatment to the right patient first time around. Furthermore, there is a clear case for the use of VOCs as companion biomarkers during the development of novel targeted biologicals. Pre-selection of patients with specific inflammatory phenotypes reduces the number of patients that need to be studied and increase chances ofidentifying clinically relevant therapeutic effects.

6 Exacerbation prediction and monitoring

Reducing the number of exacerbations is the most urgent unmet need in asthma treatment, greatly impacting the overall disease burden in terms of preventable deaths, patient quality oflife, and treatment expense. Unfortunately symptoms show only poor correlation with disease activity and typically show a lag period of several days. Biomarkers that correlate with inflammatory activity are prime candidates to be used for disease monitoring and therapy titration. The rationale behind this is that matching treatment to inflammatory activity enables the patients to take the minimally required dose to control symptoms and prevent exacerbations whilst also minimising treatment side-effects.

Early attempts to use biomarkers for therapy titration include a randomized control trial involving 74 moderate to severe asthma patients, in which ei-

ther British Thoracic Society (BTS) guidelines or sputum eosinophil counts were used to manage therapy: increasing or decreasing dosage ofinhaled or oral corticosteroids. [22] While there was no difference in average daily dose between the two groups, the sputum-control group had fewer severe exacerbations (35 compared to 109) and fewer hospital admissions (one compared to six). This approach has only been adopted in a select number of highly specialised clinics due to resource intensity and the requirement for highly trained staff. Attempts to replicate a similar approach using non-invasive FENO testing have been inconclusive.[23–26]

Current research using VOCs has focused on early detection of exacerbations. In a study of 40 children over one year, breath samples were taken prospectively; six VOCs (ρ-xylene, 3-methylpentane, 2-ethyl-4-methyl-1-pentanol, 1-phenyl-1-butene, and 4,6,9-nonadecatriene, and one other) were able to classify with 100% sensitivity and 93% specificity between baseline and exacerbation samples for a given patient. [27] Between patients, however, the best classification comparing those who had exacerbations and those who did not had 79% sensitivity and 100% specificity using a different seven VOCs (2-ethyl-1,3-butadiene, cyclohexane, 2-octen-1-ol, 1,2-methyl-4H-1,3-benzoxathiine, benzene). [28] An earlier examination of exhaled pentanea product oflipid oxidationin asthma patients detected similar levels between stable asthmatics and healthy controls, but elevated levels during exacerbations. [29]

This data suggests that patient-specific changes in VOCs may be a more powerful way to detect exacerbations early. While not examined in this study this may be a consequence of the difference in the underlying inflammatory subtypes.

A study with 178 pre-school children demonstrated clear differences between children with and without wheezing complaints. Interestingly, those children with an elevated risk to develop asthma had distinct exhaled VOCs irrespective of the presence or absence of symptoms. The latter underpin that chronic airway inflammation can be reflected by exhaled biomarkers in the absence of symptoms. [30]

A study with 96 asthmatic children aimed to identify a set of VOCs that can predict exacerbation. A relationship was found between a set of VOCs and the onset of exacerbation 14 days after breath sampling (AUROC 0.9, sensitivity 0.88, and specificity 0.75). The VOCs included 2 aldehydes, 1 hydrocarbon, 1 aromatic compound, 1 ketone, and 1 other unidentified VOC. This data highlights that VOCs in exhaled breath show potential for predicting asthma exacerbations in children within 14 days after breath sampling. [34]

There are current studies underway within the Medical Research Council (MRC) East Midlands Breathomics Pathology Node (EMBER) and at Imperial College London that are further investigating changes in VOCs between baseline measurements and during exacerbations. Being able to identify patients who are at risk for exacerbations would enable early intervention, greatly improving patient outcomes. Especially in combination with the potential to phenotype asthma patients this would open up the potential for targeted treatments focused on preventing or easing an imminent exacerbation.

7 Summary and roadmap

Exhaled volatile organic compounds reflect the underlying metabolic state of an individual. This means these metabolites are potential biomarkers for disease diagnosis, phenotyping and monitoring. The use of VOCs for these purposes in the field of asthma has seen considerable research interest with promising results for each of these applications. Result indicate that VOCs reflect the type of immunological process that underlies the chronic inflammation in asthma. The exhaled biomarkers appear to change as a consequence of the presence of disease but also during disease exacerbations. This variety of potential application is a reflection of the large number of exhaled biomarkers (more than 3000) that can be detected on breath.

Challenges faced by the field mainly revolve around standardisation of breath collection techniques, sufficiently powering studies, and the need to study the biomarkers ofinterest in the clinically relevant populations. Significant steps into standardisation of breath sampling have recently been undertaken (www.breathe-free.org). The coming years will see maturation of breath sampling in asthma and will identify where the technology fits best in the pathway. Sufficiently powered studies such as the MRC EMBER and STRATA (www.owlstonemedical.com/strata) programs will generate key evidence required to bring VOC breath analysis into widespread adoption.

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BREATH® BIOPSY

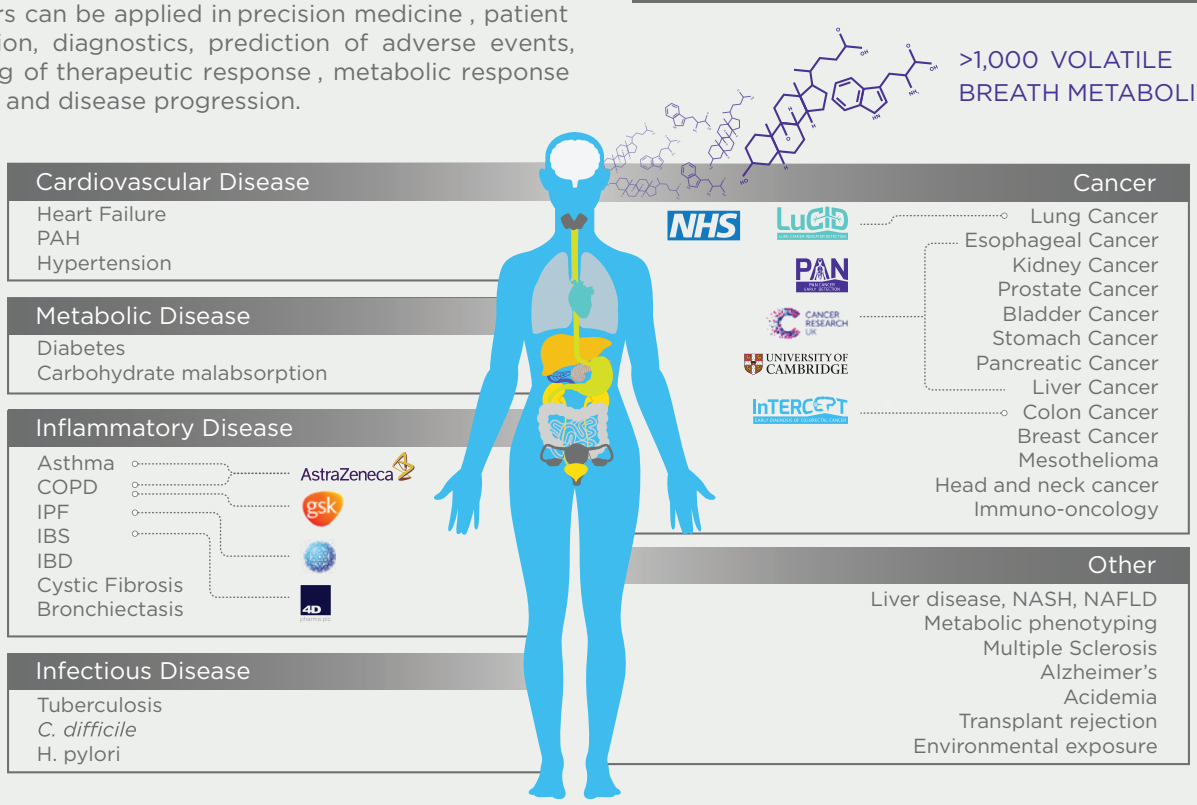
Discovery Services

Breath Biopsy Clinical Trials - A Wide Range of Application Areas

Breath VOCs have been reported for a wide range of applications. The diagram below shows the Breath Biopsy clinical trials completed or in progress, and additional areas where Breath Biopsy is applicable. Breath biomarkers can be applied in precision medicine , patient stratification, diagnostics, prediction of adverse events, monitoring of therapeutic response , metabolic response to stimuli, and disease progression.

Integrate Breath Biopsy Into Your Clinical Trial

Our Breath Biopsy Services integrate seamlessly into clinical trials, enabling you to discover breath-based biomarkers for early disease detection and precision medicine applications.



Volatile Chemicals in Breath

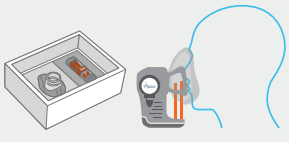
- Endogenous metabolites, some of which originate from the airways, but most of which are derived from the blood.
- Exogenous compounds related to drug metabolites, diet, air pollutants and metabolites from the microbiome.

Why Breath?

- A real-time dynamic source of valuable information about the health of an individual, as well as lifestyle and environmental factors.
- Completely non-invasive sample collection; samples shipped under ambient conditions.

Our Breath Biopsy Services combine breath collection with analysis in our established Breath Biopsy Laboratory

The Breath Biopsy Laboratory analyzes breath samples shipped from clinical sites around the world, including our own clinical trials of up to 4,000 patients.



Install Breath Biopsy Collection Station with ReCIVA Breath Sampler at clinical trial sites; Breath Biopsy Kits provided to sites for sample collection

Send Breath Biopsy samples to Owlstone Medical for comprehensive VOC analysis

Owlstone Medical performs statistical analysis such as machine learning and delivers a report including biological interpretation

Learn more at: owlstonemedical.com/services

Breath Biopsy Services: Three Steps to Robust Breath Biomarker Discovery

Breath Collection

We install the Breath Biopsy Collection Station including ReCIVA Breath Sampler at clinical sites and provide everything required for reliable, reproducible breath collection in clinic, including training and support of trial staff, and scheduled maintenance throughout the study lifetime.

- Pre-concentrates VOCs onto a Breath Biopsy Cartridge for high sensitivity.
- High patient safety and comfort.
- CE Marked.
- In use in the world's largest breath-based clinical trials, at over 100 clinical sites around the world.
- CASPER Portable Air Supply provided to minimize contamination of breath samples by external VOCs.
- We provide site initiation (installation and training) and site support.

Sample Analysis

We provide a regular supply of 2.0 Breath Biopsy Discovery VOC Kits , manufactured and quality checked to the exacting standards required for the analysis of VOC biomarkers in breath. After sampling, the Breath Biopsy Cartridges are returned to Owlstone Medical for analysis.

Each Breath Biopsy Discovery VOC Kit includes:

- One conditioned Breath Biopsy Cartridge.
- One disposable Breath Biopsy Mask.
- Comprehensive VOC analysis performed in Owlstone Medical's Breath Biopsy Laboratory using our thermal desorption gas chromatography mass spectrometry (TD-GC-MS) Breath Biopsy platform.

Data Analysis

Our data science team uses statistical analysis including machine learning algorithms to analyze the VOC profile, combined with subject medical history and clinical labels, in order to distinguish between patient populations. Choose from the following three types of analysis:

- Cross sectional (identification of differences between Group A vs. Group B).
- Longitudinal analysis of changes in the VOC profile of a subject over time (studies including more than 2 samples per subject).
- Pre-/Post- (analysis of differences between samples collected from the same subject e.g. pre- vs. post-intervention).

We provide a comprehensive report detailing the results of our analysis including biological interpretation of the results. Our data analysis includes the following:

- Univariate feature exploration including volcano plots and box plots.
- Machine Learning such as Random Forest and Partial Least Squares Discriminant Analysis.
- Consideration of potential covariates including clinical and sampling variables.

Download the Example Report:

owlstonemedical.com/discovery-report

DUE TO LACK OF STRATIFYING DIAGNOSTICS CURRENT INTERNATIONAL ASTHMA GUIDELINES ADVOCATE A 'TRIAL AND ERROR' APPROACH WITH PROGRESSIVE ESCALATION OF TREATMENT

THERE IS AN URGENT NEED FOR

PRECISION MEDICINE TOOLS

TO MATCH THE RIGHT PATIENT WITH THE RIGHT TREATMENT AT THE RIGHT TIME

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