BREATH® BIOPSY
VOC Atlas

A breathomics database to identify, contextualize, and validate biomarkers of respiratory conditions

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1. Introduction and Aims

Volatile organic compound (VOC) biomarkers in exhaled breath offer a promising route to safe, non-invasive diagnostics in multiple disease settings. Many breath-based studies have been carried out in a range of conditions, including respiratory diseases, but there are few validated breath-based biomarkers currently in clinical use[1]. The complex and unknown composition of the breath matrix makes identification and validation of on-breath VOC biomarkers a significant challenge. On-breath compounds are thought to be associated with normal variation in the population, whereas off-breath compounds in the VOC Atlas® are compounds that are not regularly observed in healthy individuals.

The Atlas study was designed to provide a comprehensive catalogue of VOCs identified on exhaled human breath, the Breath Biopsy VOC Atlas, to support development of breath VOC biomarkers and diagnostics. Here we present the study cohort, breath sampling procedure, and analysis leading to the Breath Biopsy VOC Atlas. The Atlas will support future breath studies, particularly in respiratory disease, by providing insight and scientific context to identified compounds to enable the confident selection of candidate biomarkers for a variety of diseases.

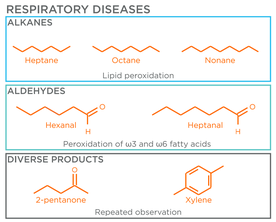

Figure 1: Example volatile compounds associated with respiratory inflammation. These compounds are present in the VOC Atlas.

2. Methods

94 adult volunteers were enrolled in January/February 2022 in Cambridge, UK. All volunteers provided written informed consent and breath samples were collected according to an IRB-approved protocol at Owlstone Medical's Breath Biopsy Laboratory.

Volunteers provided breath samples using Owlstone Medical's Breath Biopsy OMNI sample collection and analysis platform. A paired equipment blank was generated by sampling room air immediately prior to each breath sample collection. The ReCIVA® Breath Sampler is shown in Figure 2. The final analysis cohort consists of 90 volunteer breath samples and paired equipment blanks (Table 1).

Samples were analyzed using thermal desorption gas chromatography mass spectrometry (TD-GC-MS) (Thermo Scientific, Waltham, MA, US).

Molecular features were determined to be originating from breath or "on-breath" using three criteria. 1) Sample signal > 3 standard deviations higher than background 2) Paired t-test p-value <0.05 and fold difference >2 between breath and paired blank 3) receiver operating characteristic area under the curve (ROC-AUC) >0.8. VOCs confirmed to be on breath were identified using purified chemical standards.

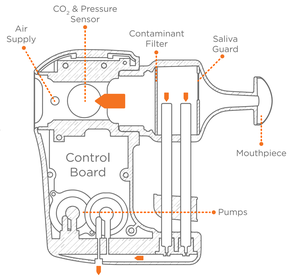

Figure 2: ReCIVA® Breath Sampler.

Study Cohort

Age Group (Yr)	Male (n)	Female (n)
18-30	10	14
31-50	19	18
51-70	12	15
71+	4	2

Table 1: Study cohort displayed by age and sex.

References

1. Haworth, J. J., et al. (2022). "Breathing new life into clinical testing and diagnostics: perspectives on volatile biomarkers from breath." Critical Reviews in Clinical Laboratory Sciences 59(5): 353-372. DOI: 10.1080/10408363.2022.2038075)
2. Crone, E., et al., Investigating allergen-induced acute lung inflammation in asthmatic individuals using Breath Biopsy - Poster ; Nov 10, 2020; Online.
3. Smolinska, A., et al., Volatile metabolites in breath strongly correlate with gut microbiome in CD patients. Anal Chim Acta, 2018. 1025: p. 1-11. DOI: 10.1016/j.aca.2018.03.046

3. Results

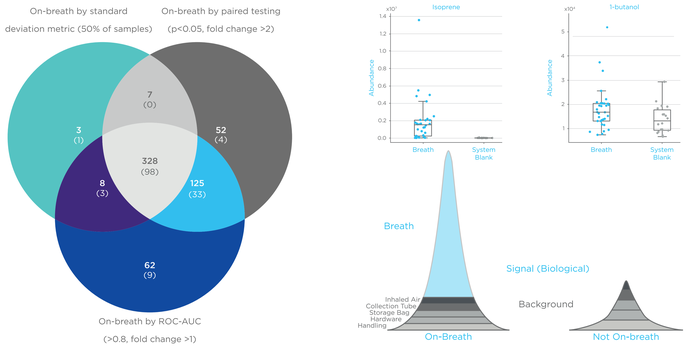

Figure 3: On-breath features (n) by each criterion. 1471 total features were identified in breath samples, of these 328 are on breath by all 3 criteria (left). An on-breath compound must be distinguishable from background noise: *(Individual) area under peak ≥ mean$_{background}$ + 3 x standard deviation$_{background}$* (right).

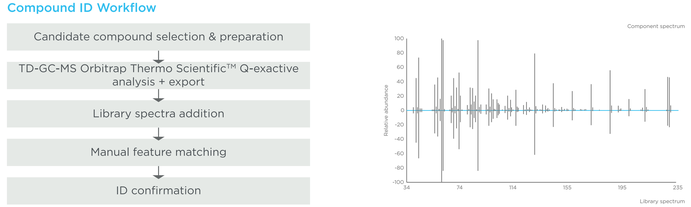

Figure 4: VOC Identification process workflow (left). Example comparison of standard and on-breath feature spectra for VOC identification (right).

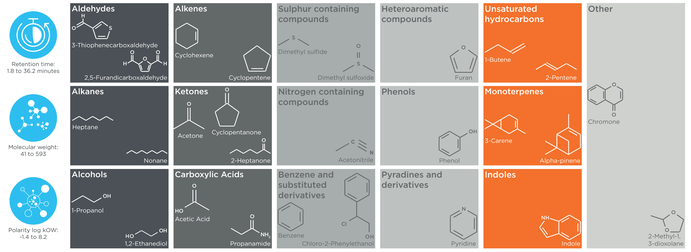

Figure 5: Summary of Breath Biopsy Atlas contents. Currently, >180 on-breath VOCs have been identified using comparisons to purified chemical standards. On-breath VOCs reflect multiple chemical classes (right) and chromatographic properties (left). Categories will expand as additional VOCs are added to the Atlas.

What Pathways Do Breath VOCs Originate From?

Lipid peroxidation is a major origin of characteristic VOCs in the breath of those with respiratory conditions. Chronic inflammation generates oxidative stress, resulting in the generation of reactive oxygen species that can react with unsaturated fatty acids in the cell (lipid peroxidation) (Figure 6), and the subsequent release of characteristic volatile compounds such as alkanes, aldehydes, hydrocarbons, and carbonyls. These are several groups of compounds in the VOC Atlas that are hypothesized to originate from lipid peroxidation

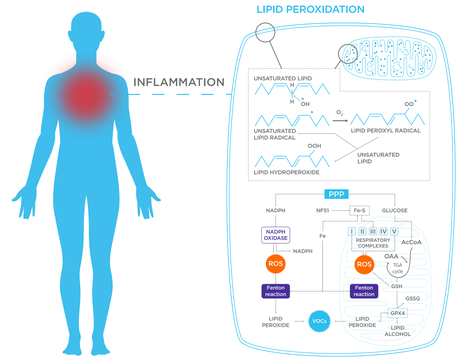

Figure 6: VOCs in the Breath Biopsy Atlas are potentially associated with lipid peroxidation. Additional experimentation will elucidate mechanistic associations between on-breath VOCs.

In a previous collaboration with McMaster University, we investigated VOCs in exhaled breath that are associated with lung inflammation induced by the allergen challenge in asthmatic individuals. Several VOCs related to inflammation show significant correlation with blood and/or sputum markers. These may provide valuable insights into the underlying biological processes and interactions between the immune system and metabolic pathways[2]. This study found that 1-dodecanol and 2-pentanone are potential biomarkers for monitoring treatment response and *P. aeruginosa* infection in cystic fibrosis patients. These compounds are also found in the Atlas.

Several of the VOCs in Atlas (such as acetone) belong to the fatty acid beta-oxidation pathway. Lungs are organs with active fatty acid metabolism, and during acute lung injury (ALI), inflammation and oxidative stress can lead to a series of metabolic reprogramming such as impaired fatty acid oxidation. This can therefore alter the composition of VOCs in the breath and be an indicator of disease.

VOCs can also be produced by the gut microbiome. Dietary fibers, which cannot be processed by humans, are fermented by microbes in the intestines, resulting in the production of VOCs such as short chain fatty acids (SCFAs) such as acetate, butyrate, and propionic acid, all of which are in the VOC Atlas. These VOCs have roles in several signaling contexts including the central nervous system, immunity, and inflammation.

4. Conclusions

The Breath Biopsy VOC Atlas is an ever-growing list of VOCs that exist on-breath, identified using purified chemical standards, and differentiated from background contamination using system blanks generated at the time of breath sample collection. This tool will facilitate future VOC biomarker discovery efforts in respiratory diseases by providing a comprehensive suite of on-breath VOCs and reference ranges in a diverse healthy population that can be used as a comparison in multiple disease contexts and to characterize relationships between physiology and breath VOCs.