Breath Biopsy® Reveals Promising VOC Biomarkers for Early Detection and Monitoring of Acute Pulmonary Exacerbations in people with Cystic Fibrosis

Hsuan Chou[1], Amy Craster[1], Ibrahim Karaman[1], Lucy Godbeer[1], Billy Boyle[1], Max Allsworth[1], Lucy Gale[2,3], Andres Floto[2,3]

[1]Owlstone Medical, Cambridge, UK. [2]Department of Medicine, University of Cambridge, Cambridge, UK. [3]Cambridge Centre for Lung Infection, Royal Papworth Hospital NHS Foundation Trust

Aim:

- The purpose of this study is to utilize the Breath Biopsy® technology to identify novel VOC biomarkers for early detection of acute pulmonary exacerbation in cystic fibrosis to enable diagnosis and treatment monitoring.

1. Background and Objectives

- Cystic fibrosis (CF) is a life-limiting genetic condition in which the transport of cell surface fluids is impaired in individuals. This makes it difficult for mucus hydration and clearance at the cell surface, resulting with an environment susceptible for bacterial infection. Acute pulmonary exacerbation (APE) is common in CF and is the single most important cause of morbidity, strongly predicting for 5-yr survival.
- Early detection of APE is crucial for providing appropriate clinical intervention and minimizing lung function decline. The challenge remains in both diagnosis of APE and treatment response monitoring due to insensitive measurements, such as inflammatory marker C-reactive protein, as well as subjective self-reported symptoms from patients.
- Volatile organic compounds (VOCs) are metabolites produced by various physiological processes, and VOCs in exhaled breath has demonstrated great potential as a non-invasive tool for disease diagnosis and monitoring. The metabolic changes captured in breath could provide early signals for APE, as opposed to lung function measurements, which changes occur in later developmental stages of an exacerbation.
- **This study utilized the Breath Biopsy® technology to identify novel VOC biomarkers for early detection of APEs in CF to enable diagnosis and treatment monitoring. A longitudinal profiling of exhaled breath VOCs was performed shortly after the onset of an acute pulmonary exacerbation (APE) through to the resolution back to a stable baseline.**

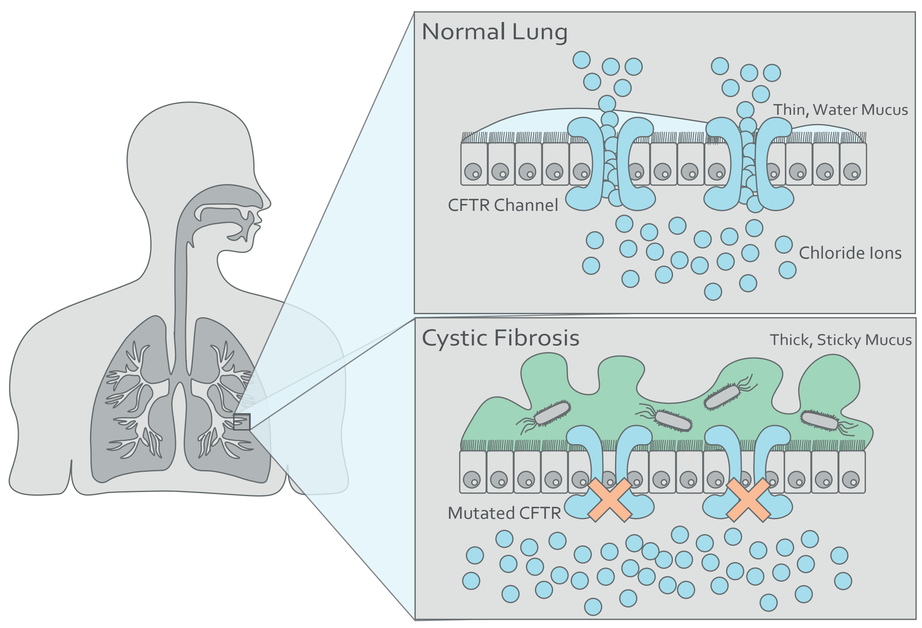


Figure 1: Adapted illustration of a normal lung and a cystic fibrosis lung[1].

2. Methods

Five adult subjects (median age =36) participated in this study, each providing four breath samples over time (Table 1). 80% of the subjects were female.

The study utilized ReCIVA® Breath Sampler (developed by Owlstone Medical, Figure 2) for collection. The samples were analyzed using the Breath Biopsy® OMNI® Platform via TD-GC-MS.

VOCs presented in less than nine of the 20 samples were removed from the analysis. Semi-present VOCs were imputed with 80% of the minimum value of that specific VOC and 1007 compounds were moved forward for identification and statistical analysis.

Compound ID was assigned using three different tiers: validated tier 1 IDs (with reference standards run either in the same sequence or equivalent sequence), putative tier 2 IDs (assigned through comparison to Owlstone Medical's HRAM library), and tentative tier 3 IDs (assigned through comparison to the NIST library).

The Friedman test was selected for longitudinal analysis of individual VOC changes across all timepoints in all subjects. The Wilcoxon signed-rank test was selected to compare individual VOC changes in subjects between recruitment and at the end of treatment, and between recruitment and the follow-up appointment.

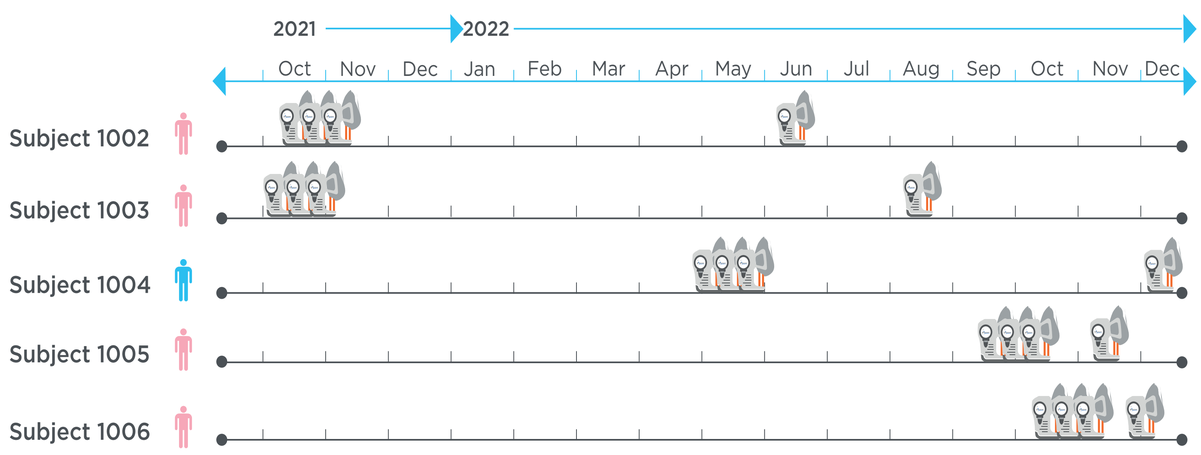


Table 1: Overview of the study design. The first three breath samples were collected at seven-day intervals while subjects were taking antibiotics and being monitored. The last breath sample was collected as a follow up to treatment completion.

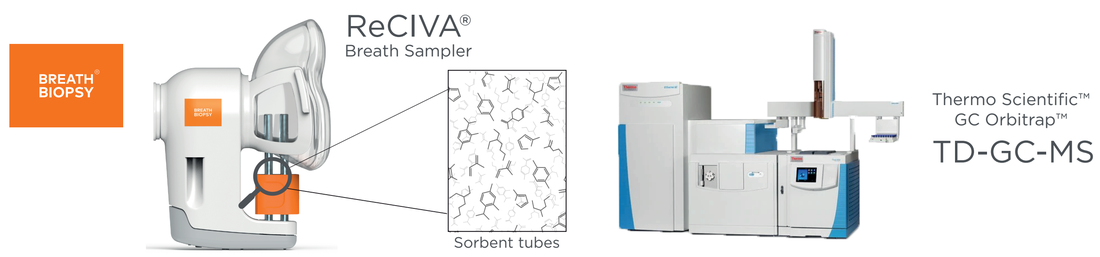


Figure 2: The breath analysis equipment used, including the ReCIVA® Breath Sampler (left) and the Thermo Scientific™ GC Orbitrap™ TD-GC-MS (right).

3. Results

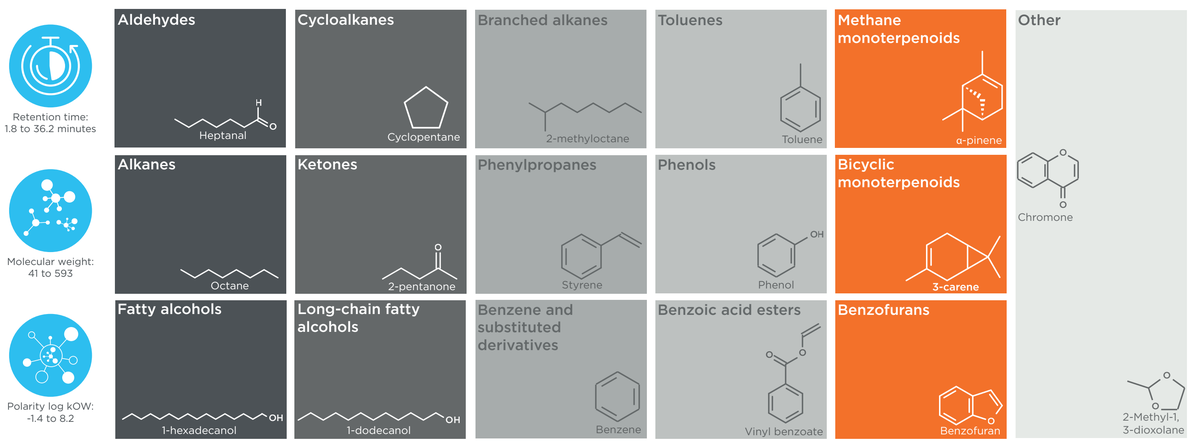


Figure 3. Summary of the key breath VOCs observed in the study, with chromatographic properties on the left, and the multiple chemical classes on the right.

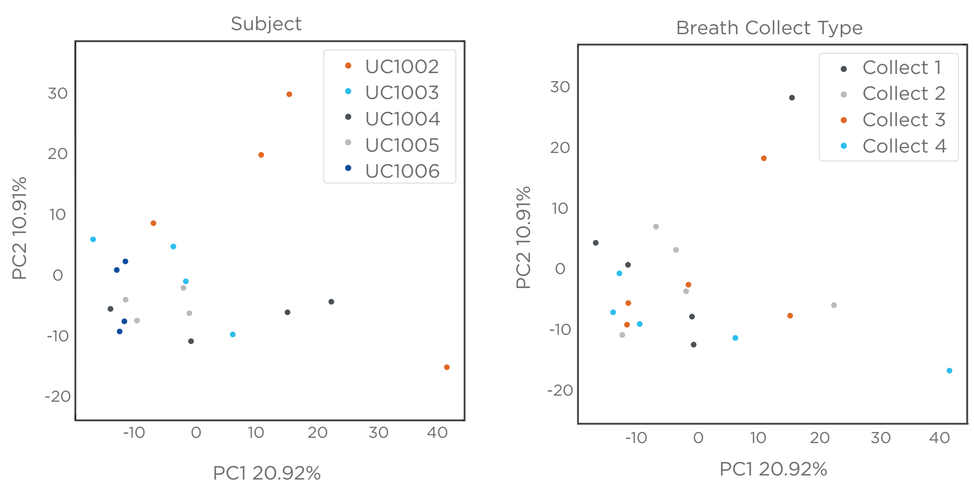


Figure 4. Principal component analysis (PCA) of breath samples colored by subject (left) and collection type (right). The results suggest a modest metabolic differences in breath VOCs across different subjects and different collect types as subgroups do not cluster together. Regardless, individual VOCs found to be significantly different in further analysis showed interesting results with disease treatment relevance.

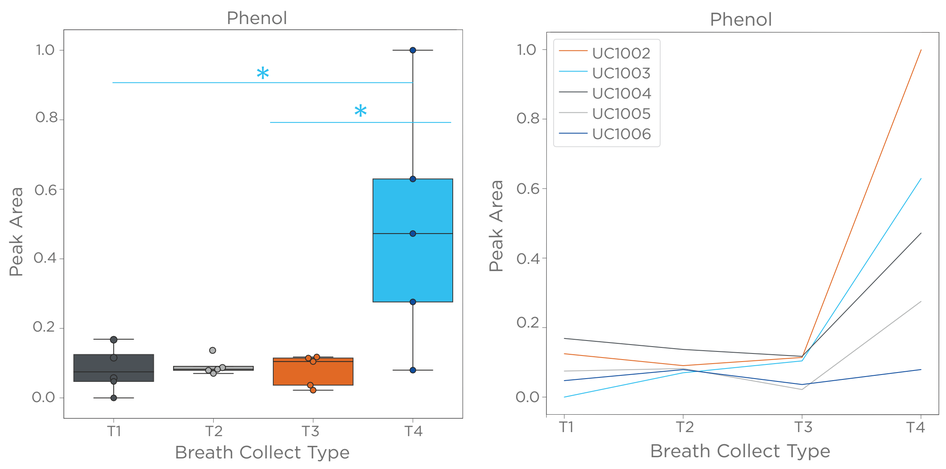
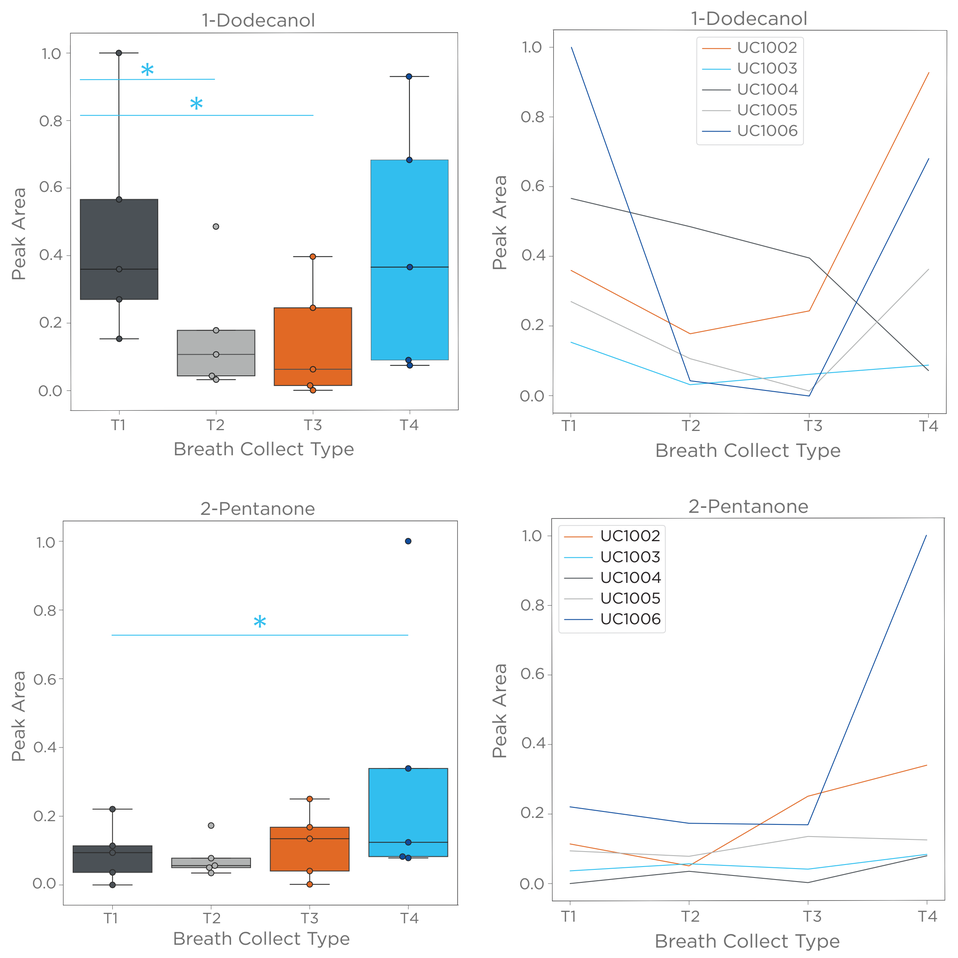


Figure 6. Phenol is a known VOC product of microbial metabolism. The observed changes may reflect the effects of antibiotic treatment on microbial composition in the lung or gut during initial exacerbation.



2-Pentanone Overall Correlation with FEV1	T1	T2	T3	T4
-0.582	-0.6	0	-0.7	-0.8

Figure 5. Of the 39 compounds exhibiting a large effect size (Kendall's W value≥ 0.5) in the longitudinal analysis, 1-dodecanol and 2-pentanone are potential biomarkers for monitoring treatment response and P. aeruginosa infection[2], respectively. Timepoints with significant VOC changes in cross-sectional analysis are indicated with an asterisk. The trends within some individuals and the moderate negative correlation between 2-pentanone and lung function (FEV1) may suggest re-occurring events at follow-up.

4. Conclusions

In this study, longitudinal analysis with post hoc pairwise comparison suggests breath VOCs have the potential to discriminate CF exacerbation from baseline, as well as antibiotic treatment response. Despite some limitations, VOCs identified may suggest the involvement or influence of inflammation, bacterial infection and impact of antibiotics on the (gut) microbiome. Validation of findings in an independent cohort with a larger sample size and the inclusion of blank samples is needed. VOC headspace analysis from sputum in parallel of breath VOCs will also help validate the relation of VOCs and infection as well as antibiotic treatment monitoring.

5. References

1. Chirico, V., et al., Acute pulmonary exacerbation and lung function decline in patients with cystic fibrosis: high-mobility group box 1 (HMGB1) between inflammation and infection. Clin Microbiol Infect, 2015. 21(4): p. 368 e1-9.
2. Kos, R., et al., Targeted exhaled breath analysis for detection of Pseudomonas aeruginosa in cystic fibrosis patients. J Cystic Fibrosis, 2022. 21 (1): p. e28-e34