Identifying and characterizing VOCs in exhaled breath from SARS-CoV-2 positive individuals

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Aims

- Identify VOCs differentiating asymptomatic COVID-19 patients, symptomatic COVID-19 patients and controls
- Identify dynamic trends in candidate biomarkers over the course of COVID-19 disease progression

1. Background and Objectives

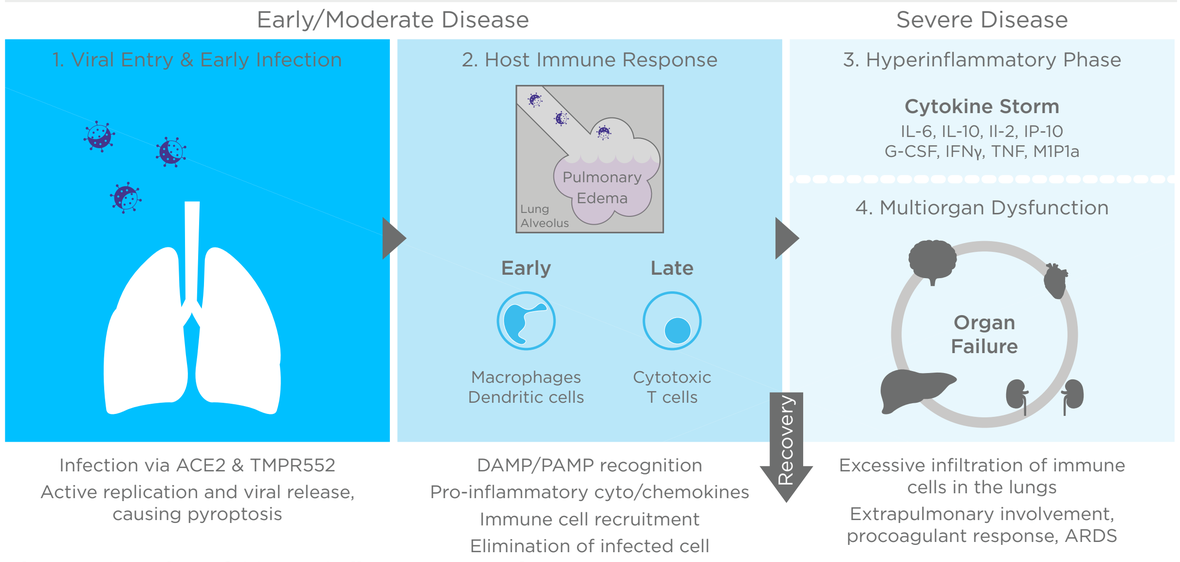

Figure 1. Overview of COVID-19 disease progression.

The coronavirus SARS-CoV-2, which causes the disease COVID-19 belongs to a family of viruses that produce symptoms including fever, cough, fatigue, and nausea [1]. Identifying infected individuals early in the course of the disease is essential for minimizing spread of COVID-19 and remains a critical part of continuing efforts to address the pandemic.

To date, there is no quick and effective way to test large groups of people in order to isolate and treat those suspected of being infectious. Current rapid tests generally take at least 15 minutes to provide results and are based on detection of SARS-CoV-2 antigens or antibodies in nasal swabs or blood. A breath-based test has the potential to be faster, more accurate, and less objectionable/invasive than current rapid tests.

Volatile metabolites are uniquely suited to report on disease status in the lung.

Analyses of breath-borne volatile organic compounds (VOCs) have been conducted for multiple respiratory diseases, including COVID-19, and several studies have established VOC analyses as a promising avenue for the development of COVID-19 diagnostics [2]. A recent meta-analysis of published studies found that VOCs can be used to accurately identify patients infected with SARS-CoV-2 with a cumulative sensitivity of 98.2% (97.5% CI 93.1%–99.6%) and specificity of 74.3% (97.5% CI 66.4%–80.9%) [3], with sensor-based analyses displaying a higher overall sensitivity than gas chromatography-mass spectrometry (GC-MS) based analyses.

This case-control study was initiated to collect, analyze, and compare breath samples from patients with or without COVID-19 to identify specific VOCs on breath that contributed to signals associated with SARS-CoV-2 infection using Owlstone Medical's Breath Biopsy® technology.

2. Methods

This study consists of cross-sectional and longitudinal components with the joint objectives of identifying and characterizing VOCs in exhaled breath from SARS-CoV-2 positive individuals and describing changes in VOCs over the course of the disease.

Samples from this study were derived from an epidemiological case-control study (test-negative design) including suspected and confirmed COVID-19 cases in the Valencia region of Spain. Patients enrolled in the cross-sectional study were between the ages of 18 and 51, had a SARS-CoV-2 RT-PCR test result (positive or negative) or had at least one symptom of COVID-19, and provided breath samples using the ReCIVA® Breath Sampler.

A sub-population of 19 subjects (17 Symptomatic and 2 Control) was enrolled in a longitudinal study arm. Participants in the longitudinal arm returned to the hospital to provide breath samples on both days 7 and 14 after the baseline collect. Swabs for SARS-CoV-2 PCR testing were also collected at these timepoints. Analysis of longitudinal samples includes only those collected from the Symptomatic cohort. Mean values are presented for continuous variables with low and high values in parentheses, and categorical variables are presented as percentages.

Samples were analyzed using the Breath Biopsy OMNI® process in the Breath Biopsy Laboratory.

	COVID(+)		COVID(-)
Design	**Symptomatic**	**Asymptomatic**	**Control**
Cross-sectional	52	45	41
Longitudinal	17	-	2
Variable	**Symptomatic**	**Asymptomatic**	**Control**
Age	29 (18-50)	35 (18-51)	36 (19-48)
BMI	23.7 (17.1-31.6)	25.1 (18.4-37.3)	23.7 (18.4-48.5)
Sex (%Female)	40%	62%	66%
PCR C_T Value	20.6 (9-39.5)	27.2 (16-35.7)	47.4 (47.4-47.4)
% Vaccinated	6%	50%	50%

Table 1. Overview of study design. *Numbers of subjects are shown for each group collected in the study. Subjects enrolled in the longitudinal arm of the trial had collects at baseline (used in the cross sectional analysis), day 7, and day 14.*

References

1. Wiersinga, W.J., et al., Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA*, 2020. 324(8): p. 782-793.
2. Abumeeiz, M., L. Elliott, and P. Olla, Use of Breath Analysis for Diagnosing COVID-19: Opportunities, Challenges, and Considerations for Future Pandemic Responses *Disaster Med Public Health Prep*, 2021: p. 1-4.
3. Subali, A.D., et al., The potential of volatile organic compounds-based breath analysis for COVID-19 screening: a systematic review & meta-analysis. *Diagn Microbiol Infect Dis*, 2021. 102(2): p. 115589.

3. Results

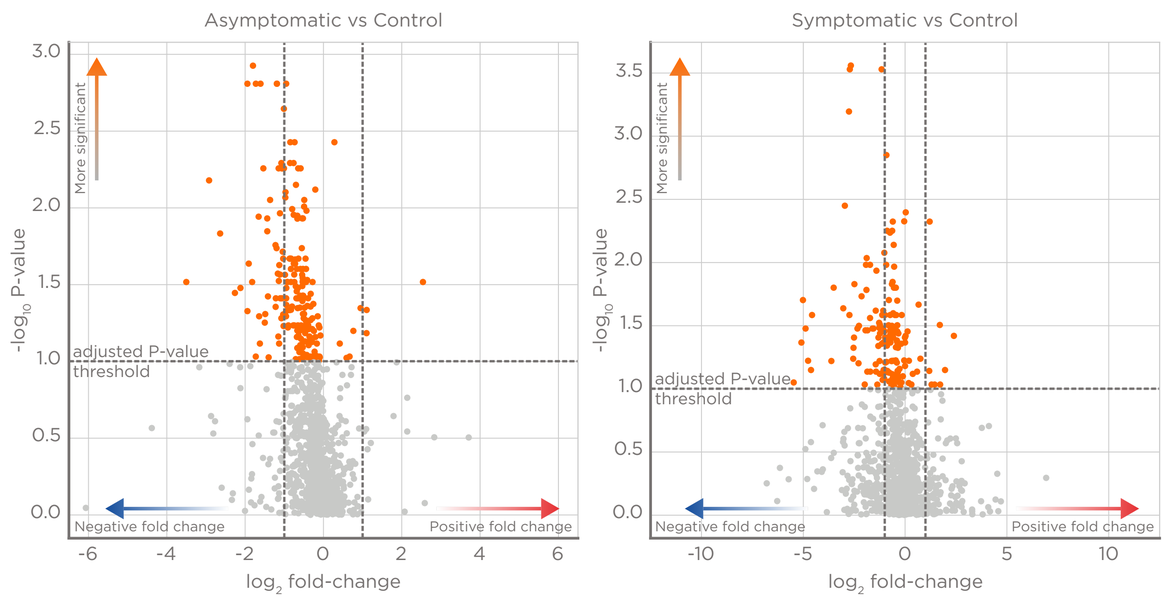

Figure 2: Volcano plots showing high-level trends observed in cross-sectional comparisons. *Comparing Asymptomatic or Symptomatic COVID(+) to Control led to a large number of negative fold-changes, suggesting an overall lower abundance in signal in COVID(+) groups. A trend toward negative changes was also observed when comparing Asymptomatic to Symptomatic COVID(+) subjects which suggests an overall greater abundance of signal in Symptomatic subjects.*

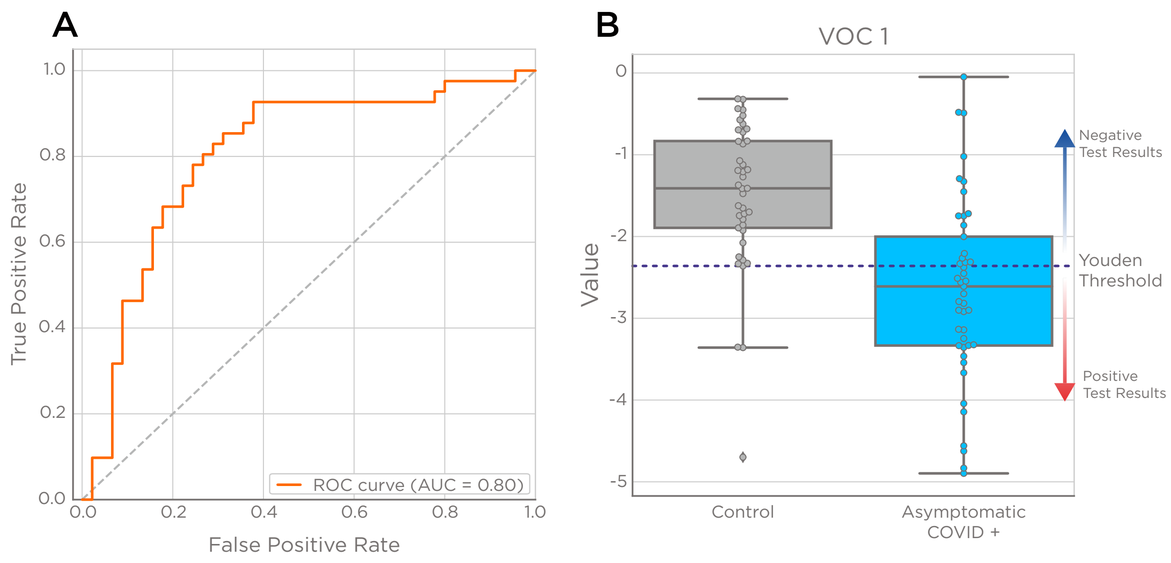

Figure 3: Evaluating the predictive ability of a single VOC. *Plots shown describe (A) ROC-AUC for a single VOC and (B) a box plot with Youden threshold marked. Model-predicted control-case status was generated using the optimal intensity cutoff selected by maximizing the Youden's Index, a summary statistic that combines test sensitivity and specificity and reflects the performance of a ROC. In this case, values above the Youden threshold (B) are classified as control and values below the Youden threshold are classified as asymptomatic COVID(+).*

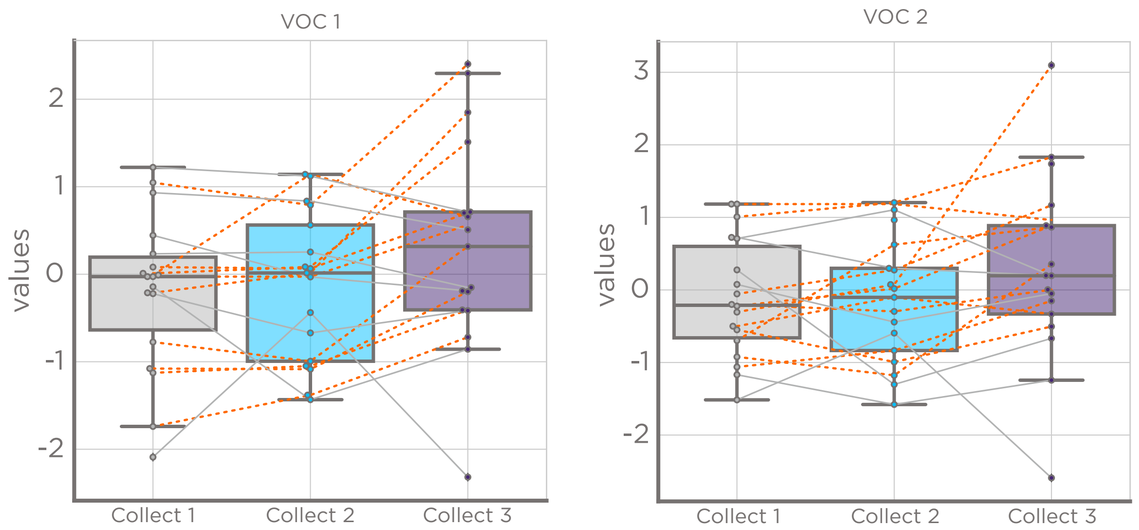

Figure 4: Statistical results from longitudinal comparisons argue for a recovery of downward trends with time. *An evaluation of changes in individual VOCs in recovering symptomatic patients showed overall trends toward an increase in VOCs over time, as could be expected if disease was responsible for decreased signal in COVID(+) groups.*

4. Conclusions

Over 1100 individual molecular features were identified by untargeted analysis of the breath samples and included in the final dataset. VOC levels were generally lower in the COVID(+) subgroups than in control COVID(-) patients, likely related to the biology of infection.

Two candidate biomarkers were identified (ROC-AUC > 0.8). Candidate biomarkers tended to decrease in this dataset which may represent lung impacts associated with respiratory disease (e.g. edema) present in both asymptomatic and symptomatic subjects.

Several important points were discovered in this analysis during candidate evaluation:

- 91 candidates showed significant (Benjamini-Hochberg adjusted P < 0.1) differences in both Symptomatic and Asymptomatic COVID(+) subjects compared to controls.
- Eight of the top 20 candidate biomarkers (sorted by ROC-AUC for the comparison of Asymptomatic vs Control) were placed in the alkane class.
- Alkanes are thought to result from interaction of reactive oxygen species with lipid species, reporting on oxidative stress often associated with inflammation.

Taken together, these results provide several paths forward for continued development of a diagnostic test for SARS-CoV-2 infection.

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