Diagnosis of *Clostridioides Difficile* Infection Using Breath Analysis

BREATH® BIOPSY

Volatile organic compounds (VOCs) produced as a consequence of *Clostridioides Difficile* infection (CDI) could act as a non-invasive and on-demand means of diagnosis.

BACKGROUND AND OBJECTIVES:

CDI is a leading cause of hospital-acquired diarrhea that poses challenges in timely diagnosis and management. Traditional diagnostic methods for CDI include enzyme immunoassays and PCR (polymerase chain reaction), however these suffer from limitations. Enzyme assays have variable sensitivities, and PCR is unable to differentiate between infection and colonization. Breath analysis could act as rapid, on-demand solution to this unmet clinical need.

The primary objective of this study was to investigate the potential of VOCs in exhaled breath as biomarkers for detecting the presence of CDI. By analyzing breath samples from patients with and without CDI the researchers aimed to identify specific VOCs associated with the infection and develop classification algorithms to accurately differentiate between CDI patients and controls.

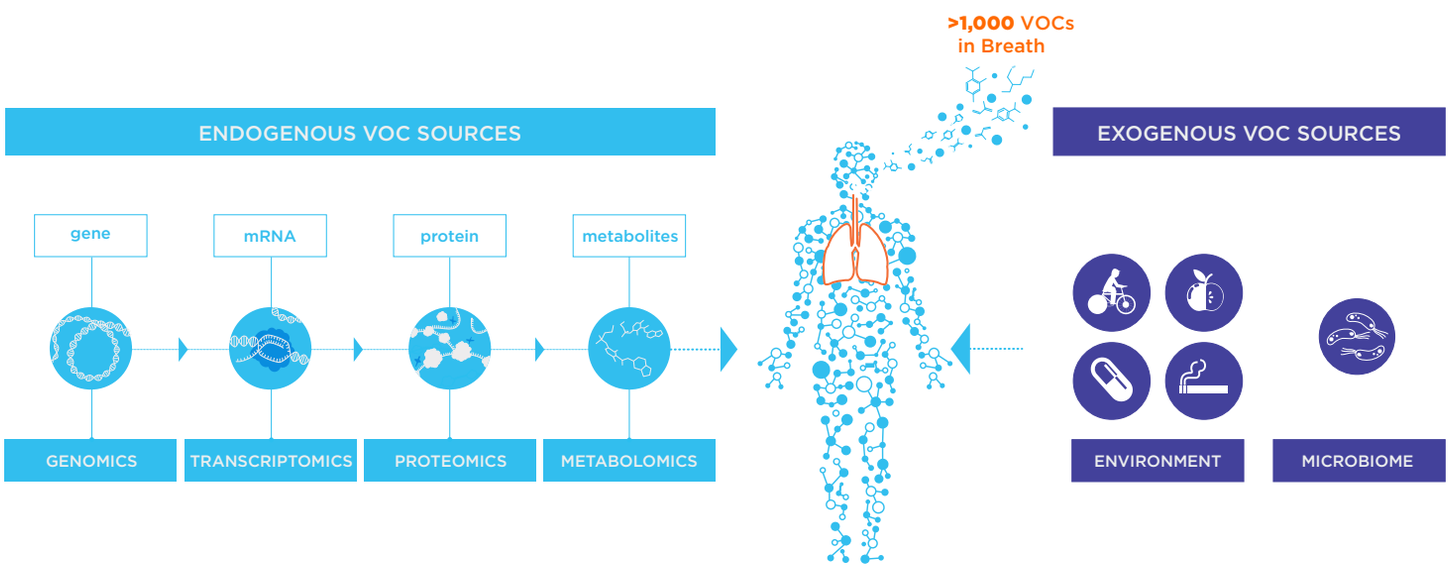

Figure 1: VOCs are low molecular weight metabolites that are excreted in breath as a result of metabolic processes in the body, and can be of endogenous and/or exogenous origin. *Bacterial and viral infections affect the endogenous metabolites and exogenous compounds found in breath.*

METHODS:

Breath samples were collected using the ReCIVA® Breath Sampler and analyzed using TD-GC-MS. Principal component analysis (PCA) was used to visually check for any underlying structure in the data. Paired testing was performed using the Wilcoxon sign-rank test and the Mann-Whitney U-test was used for unpaired group comparisons. For molecular features of interest, a tentative ID was assigned by comparison to the NIST (National Institute of Standards and Technology) library.

Clinical Variable	Controls	Cases	Full Cohort
Number of patients	17	17	34
Male/Female	9/8	8/9	17/17
Age	55.06 ± 13.49	59.71 ± 16.70	57.38 ± 14.91
BMI	29.23 ± 6.91	27.24 ± 6.99	28.24 ± 6.82
Current or Ex-Smoker	6	8	14

Table 1: Participant characteristics.

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RESULTS:

34 samples were analyzed in total, with 17 from patients with CDI and 17 from patients without. In total, 65 VOCs were detected and 11 showed evidence of association with CDI. The most significant of these were 2-Ethyl-1-hexanol, p-Xylene, Isophorone, and Tetrachloroethylene.

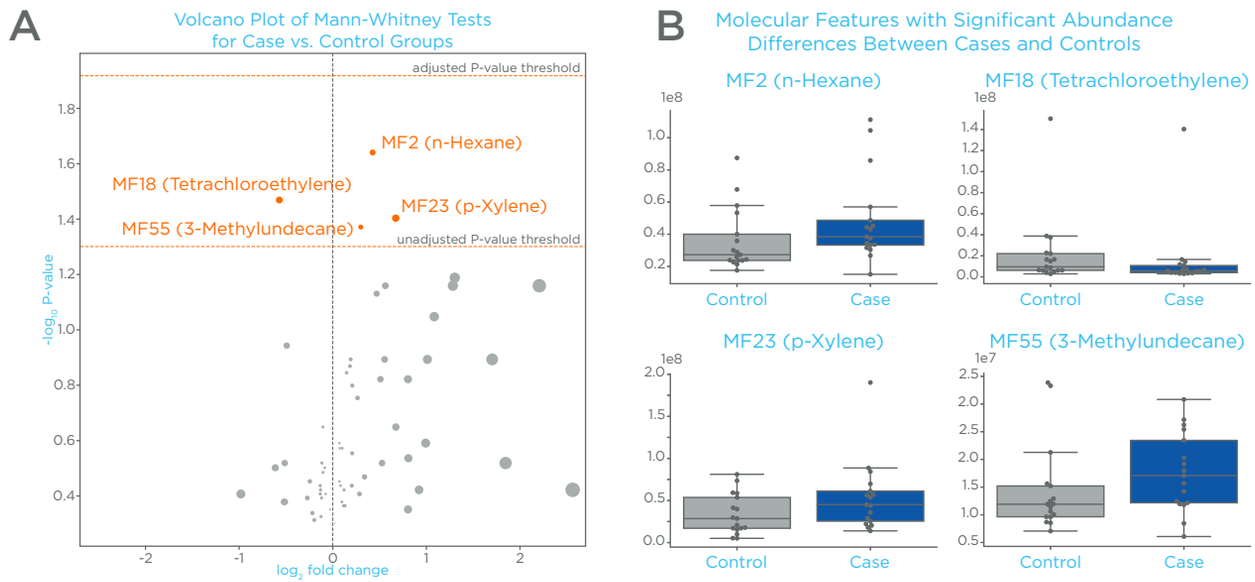

Figure 2: *(A) A volcano plot of all molecular features showing a higher abundance of 3 molecular features, MF23 (p-Xylene), MF2 (n-Hexane), and MF55 (3-Methylundecane), in the Clostridioides difficile infection group. (B) Box plots of the molecular features showing significant differences between groups.*

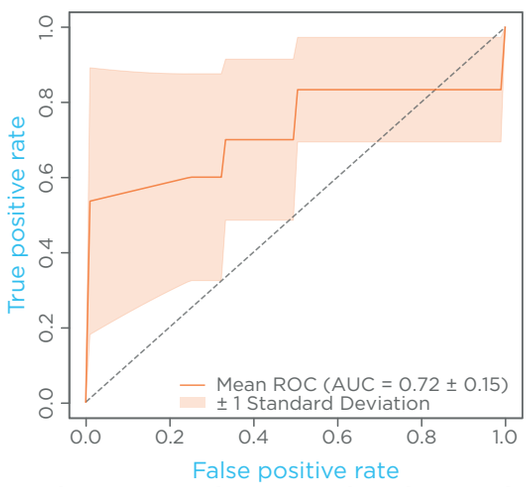

Figure 3: Receiver operating characteristic (ROC) curve for the QDA model. *A model with 9 VOCs had an AUC of 0.72, sensitivity 0.71, and specificity 0.76.*

MF	NIST Tentative ID	NIST % Match	Mann-Whitney *P*-value	Wilcoxon sign-rank *P*-value	Effect Size
MF2	n-Hexane	81.93	0.023	0.480	0.522
MF18	Tetrachloroethylene	97.59	0.034	0.099	-0.219
MF53	2-Phenyl-2-propanol	80.87	0.065	0.308	0.682
MF26	o-Xylene	84.98	0.069	0.136	0.549
MF25	4-Heptanone	93.79	0.069	0.136	0.720
MF41	1-Hexanol, 2-Ethyl	94.98	0.128	0.050	0.132
MF46	7-Octan-2-ol, 2,6-dimethyl-	78.71	0.128	0.272	0.451
MF31	Benzene, 1,2,4-trimethyl-	71.41	0.151	0.480	0.266
MF50	3-Octanol, 3,7-dimethyl-	91.29	0.176	0.136	0.251

Table 2: Summary of the volatile compounds selected by the QDA classifier model. 21 MFs with P-value < 0.2 were considered for model building A non-linear quadratic discriminant analysis model delivered the best accuracy of the methods tested. QDA models with 9-11 VOCs had the greatest accuracy. A model with 9 VOCs features was selected to prevent the likelihood of overfitting.

CDI patients appeared to have higher levels of VOCs related to lipid peroxidation in their breath than controls, demonstrated by elevated levels of n-hexane and 3-methylundecane. Lipid peroxidation has been recorded to occur during colonic damage caused by CDI. Further validation would be required to confirm that the source of these VOS was not environmental in nature.

Some species of Clostridioides have been reported to metabolize tetrachloroethylene. Therefore, the lower levels of tetrachloroethylene in CDI patients may have been related to this metabolic process.

To find out more about incorporating breath analysis into your research workflow please get in touch at: breathbiopsy@owlstone.co.uk

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