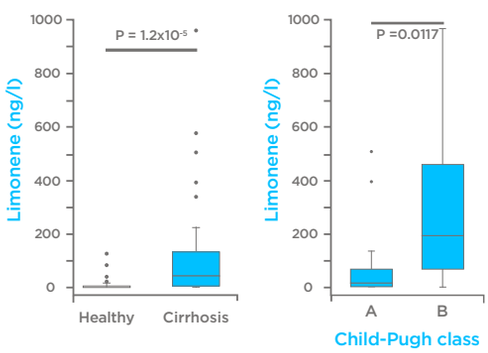
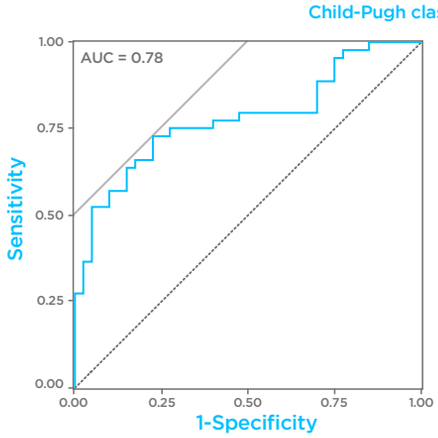
Breath Biopsy® to Identify Exhaled VOC Biomarkers for Liver Cirrhosis Detection

DOI: 10.14218/JCTH.2022.00309

BREATH® BIOPSY

The progression of chronic liver diseases to cirrhosis is often asymptomatic and is regularly diagnosed at advanced stages when therapeutic interventions are ineffective...





In a previous study, the compound limonene was found to be elevated in the breath of patients with cirrhosis and showed diagnostic potential. This compound is metabolized in the liver by the enzymes CYP2C9 and CYP2C19, and in cirrhotic patients, reduced activity of these enzymes impairs hepatic clearance and results in an extended half-life of limonene in the bloodstream, which raises its abundance in the breath.

The complexity of hepatic metabolic pathways does not allow a comprehensive evaluation of liver function from a single biomarker. Therefore, a combination of multiple VOCs generated by alterations of different metabolic pathways is needed to provide a more exhaustive picture of the liver's condition.

In this study Breath Biopsy OMNI® was used to discover differentially abundant VOCs in patients with cirrhosis in order to identify potential disease-related biomarkers that could be used to diagnose patients with progressive liver disease.

The investigation is a cross-sectional case-control study conducted as part of the PAN-study funded by Owlstone Medical and Cancer Research UK. A total of 46 subjects with cirrhosis and 42 controls were recruited. Patients had an established histological or radiological diagnosis of cirrhosis. Control subjects had no known liver disease and were excluded if they were under medical investigation or had a history of non-skin malignancy in the last two years.

Breath samples were collected using the ReCIVA® Breath Sampler and analyzed using Breath Biopsy OMNI®. Compounds were tentatively identified using the National Institute of Standards and Technology (NIST) Library and in-house High Resolution Accurate Mass Library. The identity of compounds was validated by standards injection.

Figure 1: Fernandez del Rio et al. (2020) showed that a successful liver transplant can reduce the abundance of limonene on breath. This study was a targeted analysis of exhaled limonene, demonstrating limonene correlates with blood biomarkers of liver function (bilirubin, albumin, INR) but not of liver damage (ALT), and also increases in cirrhotics, correlated with Child-Pugh classifications.

DOI: 10.14309/ctg.0000000000000239

...existing diagnostic tests, such as liver biopsy are inadequate for population screening. Surrogate imaging techniques and serological markers rely on anatomical alterations rather than hepatic function and are predominantly only effective at advanced disease stages...

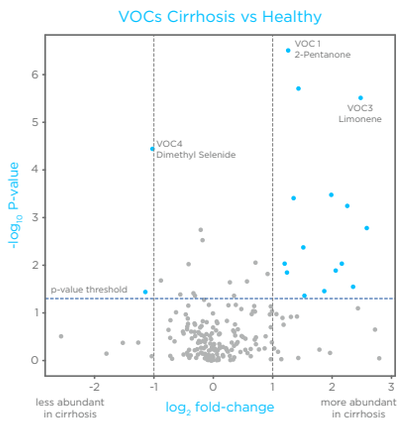


Figure 2: Volcano plot of exhaled VOCs. Limonene and 2-pentanone were elevated in the breath of patients with cirrhosis, while dimethyl selenide was reduced.

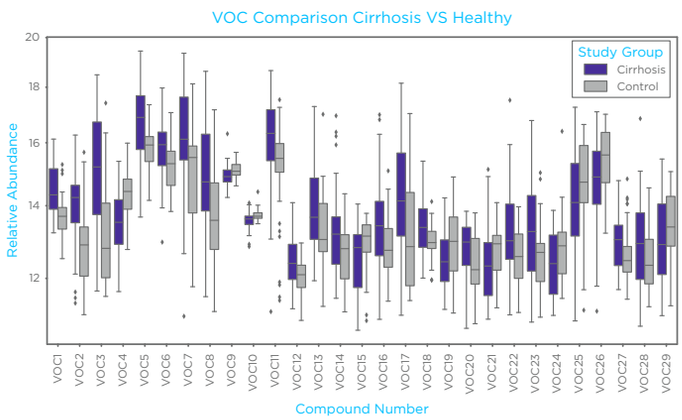


Figure 3: Box plots of discriminatory VOCs between cirrhosis and controls. A total of 29 on-breath VOCs were found significantly different between control and cirrhosis groups.

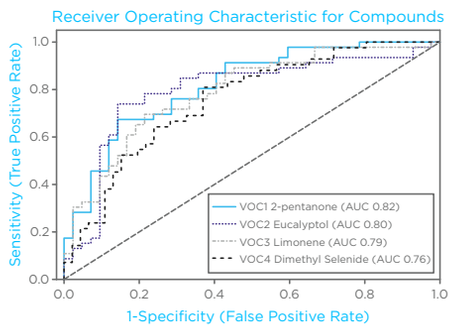


Figure 4: Receiver operating characteristic plots of the four top single VOCs comparing cirrhosis vs controls. The top 4 ROC plots for on-breath VOCs were calculated to explore their discriminatory performance. 2-pentanone, limonene, and dimethyl selenide were found among them.

An exploratory, cirrhosis-associated VOC profile, resulted in 15 upregulated and 2 downregulated VOCs in the breath of cirrhosis patients compared with controls. Limonene (m/z 91.05, RT ~11 min), and 2-pentanone (m/z 71.04, RT ~5 min) were found upregulated, and dimethyl selenide (m/z 109.96, RT ~3 min) was found downregulated in the breath of patients with cirrhosis (Fig. 2).

A total of 29 on-breath VOCs were differentially abundant between controls and cirrhosis groups and were first used to generate individual receiver operating characteristic (ROC) plots to estimate individual diagnostic performance predicting the presence of cirrhosis (Fig. 3). The top four compounds with area under the ROC curve (ROC-AUC) of 0.82, 0.80, 0.79, and 0.76 are shown in Figure 4.

...impaired hepatic function shifts the spectrum of compounds detoxified by the liver, and a subset of these can be measured in breath as volatile organic compounds (VOCs) and are potential biomarkers...

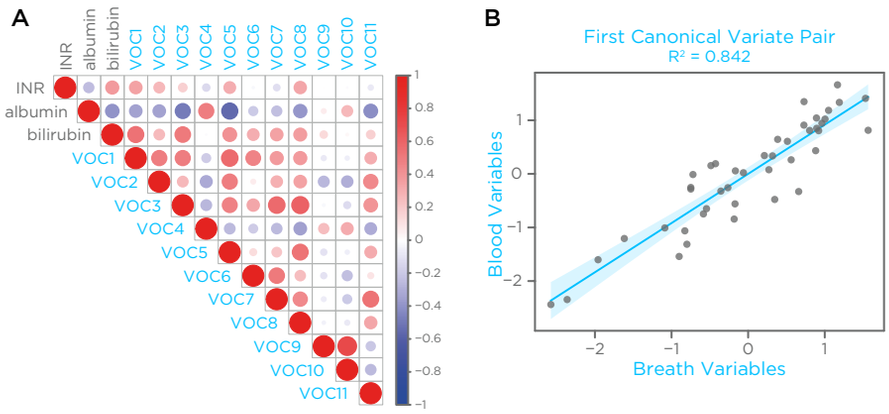


Figure 5: Correlation of breath VOCs with blood metrics of liver function in cirrhosis subjects. (A) Correlation plot of identified VOCs and serum bilirubin, albumin, and INR. Blue indicates a negative and red a positive correlation. Circle size and color intensity of the colour represents the magnitude of the correlation. (B) The CCA score plot using the first canonical variates of selected sets of VOCs and blood metrics of liver function. Each projected data point represents the combined information of breath VOCs and blood metrics of one cirrhotic patient.

11 VOCs were reported with an adjusted $p < 0.1$. Correlations between these selected breath compounds and blood metrics of liver function within the cirrhosis group, namely bilirubin, albumin, and prothrombin time, expressed as international normalized ratio (INR), were first investigated by generating a Pearson correlation matrix visualized in Figure 5A.

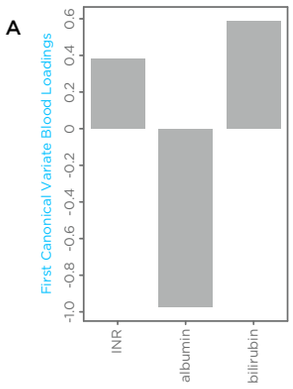
Limonene (VOC3) had a positive correlation with bilirubin and INR, and a negative correlation with albumin. Other molecular features with a retention time of ~11 minutes had a similar pattern. Consistently, dimethyl selenide (VOC4) and VOC10, that were downregulated in the breath of patients with cirrhosis (Fig. 3), had a positive correlation with albumin.

Collective correlations between breath and blood metrics of subjects with cirrhosis were further investigated by using the canonical correlation analysis (CCA) The resulting CCA score plot of the first canonical variates showed that the set of variables measured in blood significantly correlated with the set of variables measured in breath ($R^2 = 0.842$ Fig. 5B).

...therefore analysis of VOCs in exhaled breath has the potential to develop functional, non-invasive tests for early detection of chronic liver disease.

The relationship between individual exhaled VOCs and blood metrics of liver function was investigated by computing the canonical loadings. These parameters represent the correlation between each variable and its resulting canonical variate, reflecting the contribution of the variable to the overall correlation. Among the set of blood metrics, albumin had a correlation that was the reverse of bilirubin and INR, as expected. Albumin contributed the most to the overall correlation, while INR had the least contribution (Fig. 6A). Eight upregulated VOCs, including limonene (VOC3) and 2-pentanone (VOC1) showed positive loading, while dimethyl selenide (VOC4) and VOC10, which were found downregulated had a negative loading, and VOC9 had a loading near zero (Fig. 6B).

Given that bilirubin, albumin, and INR are used to calculate the Child-Pugh (CP) score, as a readout of hepatic function impairment associated with cirrhosis, we investigated the relation of the identified VOCs and CP score. The subset of 11 VOCs were used to perform PCA and projected data points for each patient were colored by CP score (Fig. 7).



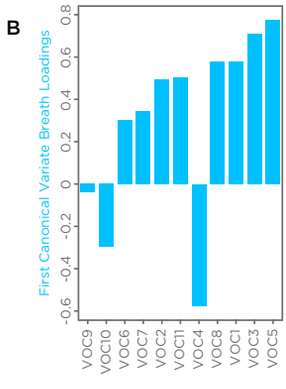


Figure 6: Canonical loadings of set of variables. (A) Loadings for blood variables, albumin has opposite correlation than bilirubin and INR. (B) Loadings for breath VOCs, limonene and 2-pentanone had a positive contribution. Dimethyl selenide (DMSE), which was downregulated in the breath of patients with cirrhosis, had a negative. correlation.

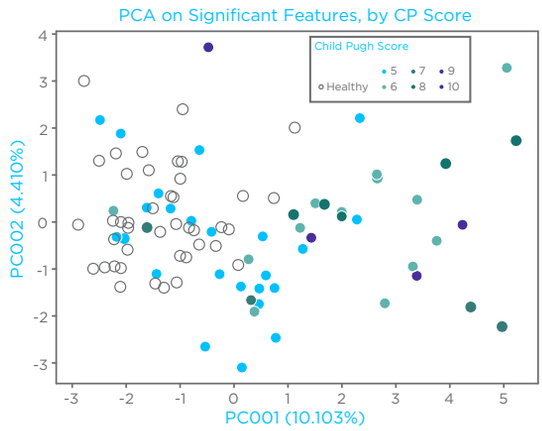


Figure 7: VOC alterations in relation to cirrhosis severity. Projected data points of the first two components of a PCA calculated using breath variables with a BH adjusted $p < 0.1$. PC1 explains 10.1% and PC2 4.4% of variance and shows the separation of cirrhotic patients based on their CP score. About 50% of the patients with a CP = 5 cluster with controls.

In summary, this study identified a set of exhaled VOCs with alterations that seem to be driven primarily by functional impairment of the liver. The results underpin earlier observations that downregulation of different hepatic metabolic pathways occurring in cirrhosis, and early stages of liver disease, may be the underlying mechanism. Interestingly, most of the VOCs were of exogenous origin. Further investigation will establish if adjustment of the exposure to these VOCs allows detection of more subtle metabolic alterations that occur in earlier stages of liver diseases such as NASH.

Contact us to find out more about collaborating with Owlstone Medical and to discuss incorporating Breath Biopsy in your biomarker research.

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