Breath Biopsy® to Discover Volatile Organic Compound (VOC) Biomarkers for Chronic Liver Diseases

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1. Background and Objective

- Cirrhosis is the end-stage condition of necroinflammation and fibrogenesis of the liver induced by chronic hepatic injury[1]. Disease progression is often asymptomatic with 50% of the cases diagnosed at advanced stages when episodes of liver decompensation occur[2]. Diagnosis of cirrhosis through non-invasive quantifications of breath biomarkers represents an attractive means for early detection and monitoring of the disease in point-of-care settings.
- We have recently shown that limonene, an exogenous volatile organic compound (EVOC*) we are exposed to through the diet and environment, is elevated in the breath of patients with cirrhosis, and may serve as a biomarker for the detection of chronic liver diseases (Fig 1)[3].
- The goal of this study is to identify additional EVOC Probes with a diagnostic potential for cirrhosis that coupled with limonene improve discriminatory performance for liver cirrhosis.

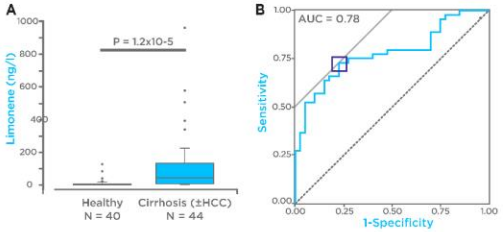

Figure 1: (A) Breath limonene levels are significantly elevated in the breath of patients with cirrhosis compared to controls. (B) Receiver operator characteristic (ROC) plot obtained utilizing breath limonene levels of healthy individuals and patients with cirrhosis. The purple square indicates the Youden index corresponding to an amount of 10.2 ng limonene on breath.

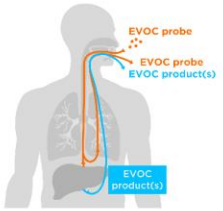

Figure 2: The exogenous volatile organic compound (EVOC) concept. A subset of ingested compounds is metabolized by the liver. However, when liver function is reduced, because of chronic liver diseases, abundance of these compounds in breath changes. These changes can be used for the non-invasive detection of chronic liver diseases.

2. Methods

- Participants were not assigned any specific protocol prior to breath collection.
- Breath Biopsies were collected by using the ReCIVA breath sampler system, developed by Owlstone Medical, and analysed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS)*.

Feature	Healthy	Cirrhosis
Number of patients	42	46 (14 with HCC)
Age median [range] (years)	62 [34-81]	58 [35-79]
Male/Female	21/21	29/17
Aetiology	-	14 NASH^, 19 alcoholic, 13 other
Child-Pugh class A/B/C/na	-	30/12/1/3
MELD median [range]	-	5 [1-16]
UKELD median [range]	-	48 [44.7-60.5]
BCLC 0/A/B/C/na	-	3/7/2/1/1
Total bilirubin median [range] (µmol/L)	-	17 [7-86]
Serum Albumin median [range] (g/L)	-	35 [24-45]
PT INR median [range]	-	1.07 [0.82-1.78]
ALT median [range] (UI/L)	-	27 [14-105]
ALP median [range] (UI/L)	-	100 [40-440]
Creatinine (µmol/L)	-	67.5 [38-147]
Sodium (mM)	-	139[126-144]

Table 1: Participant characteristics. ^ NASH = non-alcoholic steatohepatitis; Na = not available.
** For more info visit https://www.owlstonemedical.com/science-technology/breath-biopsy/*

3. Results

- Nineteen of 277 identified features showed discriminatory potential between cirrhosis and control.
- Combination of limonene with 4 additional molecular features increases the area under the ROC curve (AUC) from 0.78 (limonene only) to 0.88 and 0.94 respectively for the training and test sets.
- The spectrum of compounds altered in breath relates to the severity of liver disease determined as Child-Pugh score and correlates with blood metrics of liver function.

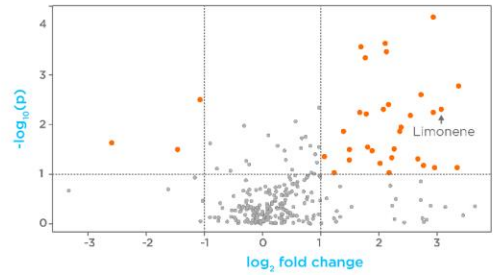

Figure 3: Several breath features show alterations between cirrhosis and control subjects. Volcano plot highlighting molecular features with a fold change >2 and a row p-value > 0.1. Limonene was identified among the upregulated features in the breath of patients with cirrhosis.

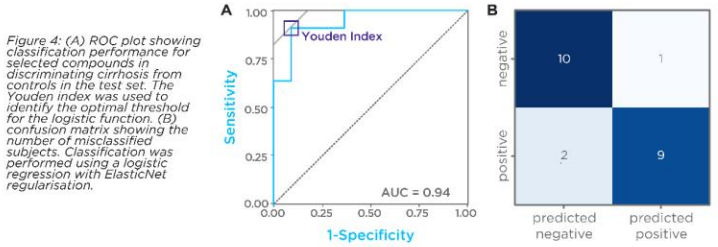

Figure 4: (A) ROC plot showing classification performance for selected compounds in discriminating cirrhosis from controls in the test set. The Youden index was used to identify the optimal threshold for the logistic function. (B) confusion matrix showing the number of misclassified subjects. Classification was performed using a logistic regression with ElasticNet regularisation.

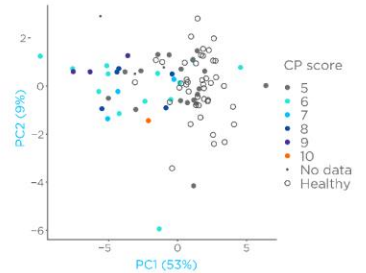

Figure 5: Plot of the first two components of a principal component analysis (PCA) on the 19 compounds that showed discriminatory performance between cirrhosis and control.

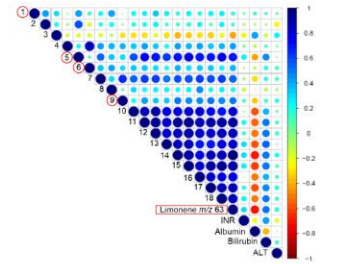

Figure 6: Correlation plot of the 19 features, including limonene, and blood metrics related to liver function/damage. Circle size and colour scale indicate magnitude and sign of the correlations. Features used for classification are highlighted.

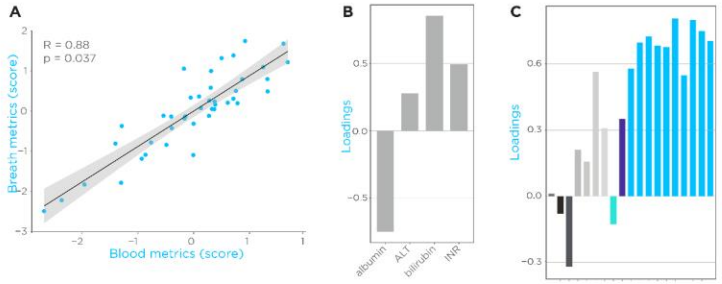

Figure 7. (A) Canonical correlation score plot using the first pair of canonical variates of discriminatory volatile metabolites in breath and blood metrics shown in fig 6. Each point represents the combined information of a single breath and blood sample collected from patients with cirrhosis. (B and C) Canonical loadings for, respectively, the blood and breath set of variables.

4. Conclusions

- A subset of EVOCs coupled with limonene improves diagnostic performance for the classification of patients with cirrhosis compared to the utilization of limonene alone.
- Alteration of breath metrics is a consequence of the severity of liver cirrhosis and correlates with alterations in blood metrics for liver function.
- Future work will expand these observations into larger cohorts that include patients with earlier stages of liver diseases, such as NASH and will elucidate the chemical structure of identified molecular features. Ultimately these potential biomarkers may serve for non-invasive diagnostic and prognostic purposes.

5. References

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