Monitoring of Liver Metabolism using Breath Biopsy® Exogenous Volatile Organic Compound (EVOC®) Probes - Towards Improved Detection

[1]Giuseppe Ferrandino*, [1,2]Isabel Orf*, [1]Rob Smith*, [1]Marzia Calcagno, [1]Anita Kaur Thind, [3]Irene Debiram-Beecham, [1]Megan Williams, [1]Olga Gandelman, [1]Alexandra de Saedeleer, [3]Graham Kibble, [3]Anne Marie Lydon, [4,5]Christopher A. Mayhew, [1]Max Allsworth, [1]Billy Boyle, [1]Marc P. van der Schee, [6,7]Michael Allison, [7,8,9]Matthew Hoare, [10]Rebecca C. Fitzgerald, [7]Victoria K. Snowdon

*Equal contribution; [1] Owlstone Medical, 183 Cambridge Science Park, Milton Road, Cambridge CB4 0GJ, UK; [2] Current affiliation: Human Metabolome Technologies, J.H. Oortweg 21, 2333 CH Leiden, The Netherlands; [3] Department of Oncology, University of Cambridge, Hutchison/MRC Research Centre, Box 197 Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0XZ, UK; [4] Institute for Breath Research, Leopold-Franzens-Universität Innsbruck, Rathausplatz 4, A 6850, Dornbirn, Austria; [5] Molecular Physics Group, School of Physics and Astronomy, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK; [6] Department of Medicine, Cambridge Biomedical Research Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [7] Addenbrooke's Hepatology and Liver transplantation Unit, Addenbrooke's Hospital, Cambridge, UK; [8] CRUK Cambridge Institute, Cambridge, UK; [9] Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; [10] MRC Cancer Unit, Hutchison/MRC Research Centre, University of Cambridge, Cambridge, UK.

1.0 Introduction and Objectives

- The so-called liver function test routinely used in clinical practice relies mainly on blood biomarkers that measure liver damage rather than liver function
- Imaging techniques and histopathology of liver biopsies, used to diagnose different chronic liver diseases, do not provide direct hepatic functional readout
- The lack of non-invasive tests to monitor improvement of liver function adversely affects drug discovery for the treatment of chronic liver conditions
- Limonene is a Volatile Organic Compound (VOC) that is elevated in the breath of patients with liver cirrhosis1 (Fig 1) and has the potential to be used as biomarker for a breath-based, non-invasive, liver function test

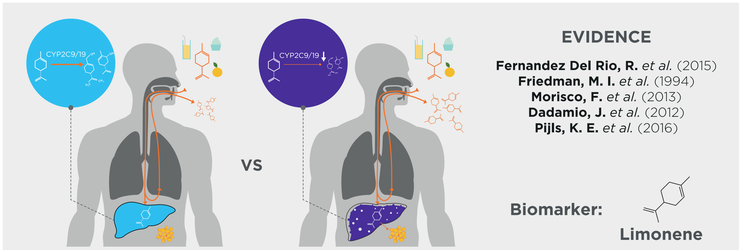

Figure 1: ***Limonene metabolism and proposed mechanism of accumulation in cirrhosis.*** *Limonene is an abundant constituent of our diet. It is ingested with fruit, fruit juices, beverages and flavored food. It is rapidly absorbed and metabolized in the liver by the enzymes CYP2C9 and CYP2C19 to trans-carveol and perillyl alcohol, respectively[2]. In the presence of liver cirrhosis, CYP2C9 and CYP2C19 activity is downregulated, leading to accumulation of limonene in adipose tissue. Gradual release from adipose tissue results in prolonged permanence of limonene in circulation leading to its increased secretion in the breath. This mechanism has been proposed by independent studies[1-5] that found increased breath limonene in patients affected by cirrhosis compared to non-cirrhotic individuals.*

2.0 Methods

- Participants were not assigned any specific protocol prior to breath collection
- Breath samples were collected with the ReCIVA Breath Sampler® coupled with the CASPER Portable Air Supply™ to reduce background contamination
- Limonene was quantified by thermal desorption–gas chromatography–mass spectrometry (TD-GC-MS)
- A Mann-Whitney U-test was used to test for significant changes between groups
- Breath samples were obtained from 40 controls, 32 cirrhosis patients, and 12 hepatocellular carcinoma (HCC) cirrhotic patients (see patient details in Table 1)

Feature	Healthy	Cirrhosis	Cirrhosis + HCC
Number of patients	40	32	12
Age median [range] (years)	62 [34-81]	56.5 [35-78]	69.5 [55-79]
Male/Female	21/19	20/12	7/5
Child-Pugh class A/B/C/na	-	21/8/0/3	9/2/1/0
MELD median [range]	-	5 [1-16]	6 [1-11]
UKELD median [range]	-	48 [44.7-58.9]	48.2 [45.2-50.5]
BCLC 0/A/B/C/na	-	-	3/6/1/1/1
Total bilirubin median [range] (µmol/L)	-	17 [7-58.9]	17 [9/80]
Serum Albumin median [range] (g/L)	-	37 [24-45]	35 [26-40]
PT INR median [range] (%)	-	1.11 [0.82-1.78]	1.03 [0.88-1.33]
ALT median [range] (UI/L)	-	27 [14-105]	30 [14-70]
ALP median [range] (UI/L)	-	95 [40-440]	109 [42-334]
Creatinine (µmol/L)	-	67 [38-147]	68 [48-92]
Sodium (mM)	-	139.5 [126-144]	139 [128-142]

Table 1: ***Participant characteristics***

3.0 Results

- Median exhaled limonene levels in patients with cirrhosis and patients with cirrhosis complicated by HCC were significantly higher than that of controls. However, the presence of HCC in study subjects with cirrhosis did not impact exhaled limonene levels (Fig. 2)
- An exploratory ROC-analysis resulted in an area under the curve (AUC) of 0.78 for prediction of cirrhosis with a threshold of 10.2 ng/L giving optimal accuracy (Youden index) with a sensitivity of 0.73 and specificity of 0.77 (Fig. 3)
- Levels of cirrhosis severity correlate with breath limonene, as well as serum bilirubin, albumin, and PT-INR, but not with alanine aminotransferase (ALT) (Fig. 4)
- Frequency of citrus products intake and drugs metabolized by CYP2C9 and CYP2C19 affects limonene levels in cirrhotic patients' breath (Fig. 5)

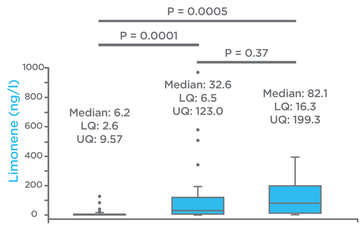

Figure 2: ***Breath limonene concentration in healthy volunteers, patients with cirrhosis and patients who progressed to HCC.***

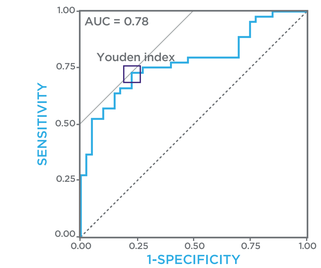

Figure 3: ***ROC plot obtained utilizing breath limonene levels of healthy individuals and patients with cirrhosis regardless of the presence of HCC. The purple square indicates the Youden index.***

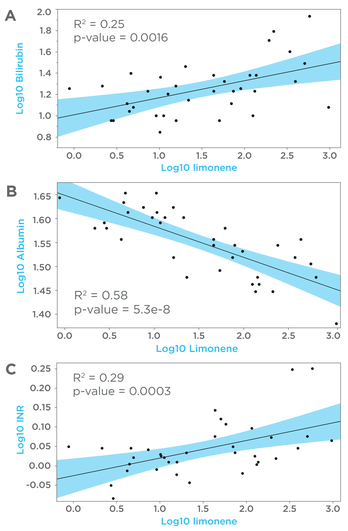

Figure 4: ***Least square regression analysis of Log10 transformed limonene against (A) Bilirubin, (B) Albumin, (C) INR, (D) ALP and (E) Levels of breath limonene in patients stratified by Child-Pugh class.***

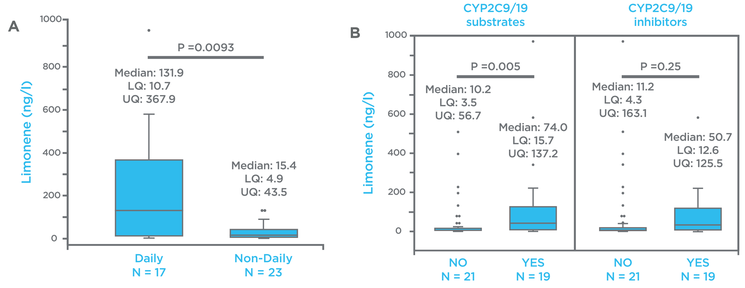

Figure 5: ***Breath limonene concentration of patients with cirrhosis related to frequency of citrus products consumption (A) and intake of medications (B) that are known substrates and/or inhibitors of CYP2C9 and/or CYP2C19.***

4.0 Conclusions

- Exhaled limonene discriminates groupwise patients with cirrhosis from healthy volunteers
- The wide spread of limonene levels observed in patients with cirrhosis is dependent on residual liver function and uneven exposure
- Breath limonene concentration reflects cirrhosis severity, detoxifying capacity, and protein synthesis, as demonstrated by the correlations with CP score, bilirubin, albumin and INR serum levels

Find out how Exogenous Organic Compound (EVOC®) Probes enable targeted assessment of biological pathways.

owlstonemedical.com/evoc

5.0 Perspective

- Ensuring participants have the same exposure to limonene over 24 hours prior to breath sampling may increase the performance of a potential test for the diagnosis of cirrhosis
- Administering limonene in an active test design may increase sensitivity and allow detection of earlier stages of chronic liver diseases such as non-alcoholic steatohepatitis (NASH)
- A limonene-based breath test could provide a cost-effective, non-invasive means for the monitoring of improved liver function to test effectiveness of new drugs for chronic liver diseases

Want to find out more?

Join the Breath Biomarkers for Liver Disease session at the Breath Biopsy Conference 2020

owlstonemedical.com/breath-biopsy-conference

6.0 References

1 Fernandez Del Rio, R. et al. Volatile Biomarkers in Breath Associated With Liver Cirrhosis - Comparisons of Pre- and Post-liver Transplant Breath Samples. EBioMedicine 2, 1243-1250, doi:10.1016/j.ebiom.2015.07.027 (2015).

2 Dadamio, J. et al. Breath biomarkers of liver cirrhosis. J Chromatogr B Analyt Technol Biomed Life Sci 905, 17-22, doi:10.1016/j.jchromb.2012.07.025 (2012).

3 Friedman, M. I. et al. Limonene in expired lung air of patients with liver disease. Dig Dis Sci 39, 1672-1676, doi:10.1007/bf02087774 (1994).

4 Morisco, F. et al. Rapid "breath-print" of liver cirrhosis by proton transfer reaction time-of-flight mass spectrometry. A pilot study. PLoS One 8, e59658, doi:10.1371/journal.pone.0059658 (2013).

5 Pijls, K. E. et al. A profile of volatile organic compounds in exhaled air as a potential non-invasive biomarker for liver cirrhosis. Sci Rep 6, 19903, doi:10.1038/srep19903 (2016).

6 Miyazawa, M., Shindo, M. & Shimada, T. Metabolism of (+)- and (-)-limonenes to respective carveols and perillyl alcohols by CYP2C9 and CYP2C19 in human liver microsomes. Drug Metab Dispos 30, 602-607, doi:10.1124/dmd.30.5.602 (2002).