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

1.0 Introduction and Objectives

1.1 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 biopsy are designed to diagnose different chronic liver diseases, do not provide direct hepatic functional readout.

2.0 Methods

- Prolonged permanence of limonene in circulation leading to its increased secretion in the enzymes CYP2C9 and CYP2C19 to trans-carveol and perillyl alcohol, respectively
- Beverages and flavored food. It is rapidly absorbed and metabolized in the liver by the CYP2C9/19
- Reduce background contamination
- The CASPER Portable Air Supply™ to reduce background contamination
- The protocol prior to breath collection
- Monitoring of liver metabolism using Breath Biopsy® exogenous volatile organic compound

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 predicting 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).
- Breath limonene predicts intake and drugs metabolized by CYP2C9 and CYP2C19 affects limonene levels in cirrhotic patients’ breath (Fig. 5).

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.

5.0 Perspective

- Ensuring participants have the same exposure to limonene over 24 hours prior to breath sampling may increase the potential of a 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.

6.0 References

- First reference
- Second reference
- Third reference
- Fourth reference
- Fifth reference
- Sixth reference

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy</th>
<th>Cirrhosis</th>
<th>Cirrhosis + HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 (39-89)</td>
<td>56.5 (39-79)</td>
<td>62.5 (54-79)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>90 (57-145)</td>
<td>90 (57-145)</td>
<td>90 (57-145)</td>
</tr>
<tr>
<td>Child-Pugh class</td>
<td>A/B/C/na</td>
<td>8/1/0/1</td>
<td>8/1/0/1</td>
</tr>
<tr>
<td>MELD score</td>
<td>45.7 (39.3-51.5)</td>
<td>45.7 (39.3-51.5)</td>
<td>45.7 (39.3-51.5)</td>
</tr>
<tr>
<td>Child-Pugh score</td>
<td>2/1/1/0</td>
<td>2/1/1/0</td>
<td>2/1/1/0</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>34.9 (28-38)</td>
<td>26.1 (20-32)</td>
<td>26.1 (20-32)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.0 (0.3-1.5)</td>
<td>1.0 (0.3-1.5)</td>
<td>1.0 (0.3-1.5)</td>
</tr>
<tr>
<td>INR</td>
<td>1.2 (1.0-1.5)</td>
<td>1.2 (1.0-1.5)</td>
<td>1.2 (1.0-1.5)</td>
</tr>
<tr>
<td>Liver function index (LFI)</td>
<td>6.0 (4.0-8.0)</td>
<td>6.0 (4.0-8.0)</td>
<td>6.0 (4.0-8.0)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>3.9 (2.8-4.2)</td>
<td>3.9 (2.8-4.2)</td>
<td>3.9 (2.8-4.2)</td>
</tr>
<tr>
<td>Plasma bilirubin (mg/dL)</td>
<td>1.0 (0.3-1.5)</td>
<td>1.0 (0.3-1.5)</td>
<td>1.0 (0.3-1.5)</td>
</tr>
<tr>
<td>Plasma albumin (g/L)</td>
<td>3.9 (2.8-4.2)</td>
<td>3.9 (2.8-4.2)</td>
<td>3.9 (2.8-4.2)</td>
</tr>
<tr>
<td>Plasminogen (mg/L)</td>
<td>150 (100-200)</td>
<td>150 (100-200)</td>
<td>150 (100-200)</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>150 (100-200)</td>
<td>150 (100-200)</td>
<td>150 (100-200)</td>
</tr>
<tr>
<td>Blood limonene (ng/L)</td>
<td>10.2 (5.0-20.0)</td>
<td>10.2 (5.0-20.0)</td>
<td>10.2 (5.0-20.0)</td>
</tr>
</tbody>
</table>

Figure 1: Limonene metabolism and proposed mechanism of accumulation in cirrhosis.

Healthy

Cirrhosis

Biomarker: Limonene

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

Figure 3: ROC plot obtained utilizing log10 transformed limonene levels (Fig. 2).

Figure 4: Least square regression analysis of log10 transformed limonene against (A) Bilirubin, (B) Albumin, (C) INR, (D) Child-Pugh class 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 CYP2C19.

Find out more about Exogenous Organic Compound (EVOC®) Probes and targeted assessment of biological pathways.

owlstonemedical.com/evoC