Identifying Potential Subjects for NASH Clinical Trials Using the HepQuant®-SHUNT and HepQuant®-STAT Quantitative Liver Function Tests
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Background: NASH trials require screening biopsies to confirm diagnosis and establish histological stage. Many drug trials focus on advanced fibrosis (F3) and compensated cirrhosis (CompF4), because early-stage and decompensated late-stage patients may be unresponsive. HepQuant’s unique liver function tests correlate with histology and could prescreen subjects to avoid those likely to fail biopsy entry criteria. Technology: The HepQuant®-SHUNT test uses simultaneous oral d4-cholate and IV 13C-cholate with mass spec analysis of 5 serum samples to accurately measure function (Disease Severity Index, DSI). The simpler HepQuant®-STAT test utilizes only oral d4-cholate and a single serum sample to estimate function (STAT).

Results: Identifying Potential Subjects with NASH
50 healthy controls (including normal weight, overweight, and obese) and 39 biopsied NASH patients were tested. The optimum cutoff on the DSI ROC was set by Youden Index. The cutoff on the STAT ROC was set at the same Specificity.

HepQuant®-SHUNT Test

\[
\text{DSI ROC Curve}
\]

\[\text{AUC c-statistic = 0.96}\]

\[
\text{DSI > 16.5}
\]

The high Specificity (98%) and the high PPV (97%) at the optimum cutoff (DSI >16.5) indicate that almost all of the potential subjects above the cutoff will likely have biopsy-proven NASH. The high Sensitivity (87%) means few NASH patients will be missed.

HepQuant®-STAT Test

\[
\text{STAT ROC Curve}
\]

\[\text{AUC c-statistic = 0.94}\]

\[
\text{STAT > 0.65 µM}
\]

The cutoff (STAT >0.65 µM) was chosen to maintain the high Specificity (98%) and this keeps a high PPV (96%). Almost all of the potential subjects above the cutoff will likely have biopsy-proven NASH, but reduced Sensitivity (69%) yields fewer patients.

Results: Identifying Potential Subjects with NASH F3/Compensated F4
The NASH cohort included 8 patients with early stage fibrosis (F1/F2), 9 patients with advanced fibrosis (F3), 13 patients with compensated cirrhosis (CompF4), and 9 patients with decompensated cirrhosis (DecompF4). The optimum ranges for DSI (17.2 – 30.0) and STAT (0.81 – 2.5 µM) to select F3/CompF4 NASH patients were set by ROC curve Youden Indices. The distribution of the NASH patients by histological staging and by DSI and STAT ranges are shown in the tables below.

HepQuant®-SHUNT Test

<table>
<thead>
<tr>
<th>Histology</th>
<th>DSI &lt;17.2</th>
<th>17.2&lt; DSI &lt;30.0</th>
<th>DSI &gt;30.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decompensated Cirrhosis (F4)</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Compensated Cirrhosis (F4) Advanced Fibrosis (F3)</td>
<td>3</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Early Fibrosis (F1-2)</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Most of the potential subjects in the DSI range (PPV 88%) will be F3/CompF4. The high Sensitivity (82%) means few NASH F3/CompF4 patients will be missed.

HepQuant®-STAT Test

<table>
<thead>
<tr>
<th>Histology</th>
<th>STAT &lt;0.81</th>
<th>0.81&lt; STAT &lt;2.5</th>
<th>STAT &gt;2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decompensated Cirrhosis (F4)</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Compensated Cirrhosis (F4) Advanced Fibrosis (F3)</td>
<td>9</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Early Fibrosis (F1-2)</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Most of the potential subjects in the STAT range (PPV 87%) will be F3/CompF4. The reduced Sensitivity (59%) will yield fewer of the patients with those stages.

Conclusions:

- Prescreening potential subjects with HepQuant®-SHUNT or HepQuant®-STAT could identify those most likely (high PPV) to meet biopsy enrollment criteria and thus avoid many unnecessary biopsies that are painful and risky for patients and very costly for sponsors.
- HepQuant®-SHUNT could yield a higher number of subjects (higher Sensitivity) likely to meet biopsy enrollment criteria, but HepQuant®-STAT could be utilized as a cost-effective alternative.