Liver Toxicity Biomarkers in Patients with Muscle Damage

Drug-induced liver injury (DILI) remains the single greatest cause for termination of development of drug candidates and withdrawal of approved drugs from the market. Alkaline aminotransferase (ALT) and aspartate aminotransferase (AST) serum activity measurements are used as gold standard biomarkers for the identification of liver injury in clinical practice, and ALT is commonly used to assess risk of liver injury during drug development. However, both biomarkers are present in muscle as well as liver.

- As ALT and AST are present in myofibers, serum ALT and AST activities increase in subjects with muscle injury
- This severely limits the utility of ALT/AST as markers of liver damage in subjects with underlying muscle impairments such as those with muscular dystrophies or myositis
- Increased levels of ALT/AST due to underlying muscle damage may potentially mask a hepatotoxic signal, creating a diagnostic challenge for clinicians
- Persistent transaminasemia is frequently misdiagnosed as liver injury in patients with inherited muscle disorders such as DMD and idopathic inflammatory myopathies.

GLDH correlates with liver damage

**Table 1: Liver safety biomarkers ALT and AST are high in boys with DMD even when there is no liver injury. GLDH levels increase with liver injury (APAP toxicity), but not in patients with only muscle disease.**

<table>
<thead>
<tr>
<th>GLDH (u/L)</th>
<th>ALT (u/L)</th>
<th>AST (u/L)</th>
<th>CK (u/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults (18–64)</td>
<td>3 ± 2</td>
<td>20 ± 6</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Healthy boys (18–64)</td>
<td>3 ± 0</td>
<td>21 ± 10</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>DMD boys (41)</td>
<td>5 ± 3</td>
<td>376 ± 212</td>
<td>235 ± 145</td>
</tr>
<tr>
<td>APAP tox adults (8)</td>
<td>963 ± 1,000</td>
<td>3,788 ± 1,730</td>
<td>3,614 ± 2,824</td>
</tr>
</tbody>
</table>

**Figure 1: Correlation of DILI biomarkers with muscle damage: ALT levels increase with creatine kinase (A); GLDH levels do not (B).**

**Figure 2: GLDH as a DILI biomarker in patients with underlying muscle damage. GLDH levels are increased with liver injury, then increases in a patient with rhabdomyolysis.**

**Table 2: Comparative diagnostic performance of GLDH and ALT.**

<table>
<thead>
<tr>
<th>GLDH &gt; 2.5x</th>
<th>GLDH &gt; 5x</th>
<th>GLDH ≤ 2.5x</th>
<th>GLDH ≤ 5x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity:</td>
<td>99%</td>
<td>99.6%</td>
<td>99.4%</td>
</tr>
<tr>
<td>Specificity:</td>
<td>99.3%</td>
<td>99.8%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>

For these analyses, subjects selected had both AST and ALT levels. In the 2.5x ULN threshold for GLDH (25 U/L), there is 98% agreement with the 3x U/L threshold for ALT (123 U/L). Sensitivity and specificity of GLDH at this threshold are 99% and 99.5%, respectively. Similarly, there is 97% agreement for the 5x ULN cutoffs.

**Assay Characteristics**

The Random GLDH assay was validated according to Clinical Laboratory Improvement Amendments (CLIA) guidelines for Laboratory Developed Tests (LDT). It has been tested for: accuracy, analytical sensitivity, long term stability, freeze/thaw stability, analytical specificity to include interfering substances, reportable range, and reference interval.

**Next Steps:** We will complete analyses for qualification, submit the final qualification package, and work towards in vitro diagnostic status for a GLDH assay.

**Use of GLDH as a DILI biomarker**

When GLDH is utilized in clinical studies:

1. GLDH activity should be utilized as a complement to standard methods for assessing drug induced liver injury (DILI).
2. The mechanism by which GLDH and ALT appear in serum following hepatocellular injury is similar; their enzymatic activity is highly correlated in humans and animals with a diversity of liver injuries and diseases.

GLDH activity levels 2.5x and 5x above upper limit of normal (ULN) are estimated to correspond to 3x and 5x above ULN for ALT, though this still requires further confirmation. These fold changes of GLDH could be utilized, along with the standard hepatic injury monitoring panel, for the assessment of DILI, in the same manner as 3x and 5x ULN ALT.

**GLDH correlation**

PSTC and D-RSC are seeking qualification of GLDH as a biomarker of liver toxicity independent of muscle damage.

**Context of use:** Elevated serum enzymatic activity of GLDH is a measure of hepatocellular injury, and can be used in healthy subjects and patients as an adjunct to ALT in all stages of drug development trials. When ALT increases are observed from suspected extrastomachic sources such as muscle, GLDH can lend weight of evidence to confirm or rule hepatocellular injury.

**Figure 3: Correlation of GLDH and ALT. Serum GLDH cutoffs are 25 U/L and 48 U/L, which can also be expressed as 2.5x and 5x ULN GLDH. Confidence intervals (95%) for these cut-offs are (3.0, 3.4) and (3.5, 3.6), respectively.**

**Figure 4: Decision scheme for GLDH use in drug development**

**Timeline for qualification**

- GLDH from EMA
- GLDH from FDA
- GLDH from EMA
- GLDH from FDA

**Glutamate Dehydrogenase (GLDH)**

- Converts glutamate to ketoglutarate in mitochondria
- Tissue distribution: Liver, kidney, pancreas and intestinal mucosa
- Utility of GLDH as a liver specific biomarker of liver injury shown in preclinical species

**GLDH thresholds levels**

**Figure 2: GLDH as a DILI biomarker in patients with underlying muscle damage. GLDH levels are increased with liver injury, then increases in a patient with rhabdomyolysis.**

**Table 3: Studies completed in support of GLDH as a DILI biomarker. More confirmation is ongoing.**

**References:**

1. GLDH threshold levels
2. GLDH correlates with liver damage
3. GLDH activity levels 2.5x and 5x above upper limit of normal (ULN) are estimated to correspond to 3x and 5x above ULN for ALT, though this still requires further confirmation. These fold changes of GLDH could be utilized, along with the standard hepatic injury monitoring panel, for the assessment of DILI, in the same manner as 3x and 5x ULN ALT.