The notion of “biomarker” is not new. FDA defines a biomarker as the measurable endpoint that can be used as an indicator of a particular disease or some other physiological state of an organism. There are numerous publications in the literature addressing the importance of biomarkers in clinical trials, and especially in drug development. In a clinical laboratory, testing for biomarkers can vary from a simple test such as blood glucose to a more sophisticated test like genetic analysis. In this article, the term “biomarker” will refer to the toxicity biomarkers, which have the potential to detect organ toxicity.

It is widely admitted that, the conventional biomarkers everyone is familiar with, have their limitations. Basic and clinical research in the past years draws the attention to potential new biomarkers, which are more sensitive and specific than the conventional ones. Modern technology allows central laboratories to offer testing for some of these novel biomarkers. At ACM Global Central Laboratory, we would like to integrate this type of testing in “biomarkers of toxicity panel”, or “safety panel”, adapted in collaboration with the sponsor according to the specificity of the study and updated periodically.

Drug developers need to be aware of the latest options for liver and kidney laboratory safety testing, and understand the importance of testing a combination of novel, early predictive toxicity biomarkers, along with the conventional ones.

**WHAT ARE THE CHARACTERISTICS OF AN IDEAL BIOMARKER?**
The current literature describes the ideal biomarker as: accessible, non-invasive, sensitive, specific, inexpensive, translational (able to cross the bridge between basic and clinical research), predictive of the extent of injury and accurate.

**SAFETY BIOMARKERS AND DRUG-INDUCED LIVER TOXICITY (DILI)**
A careful selection of the laboratory biomarkers which best identify organ toxicity, should happen as early as Phase I or Phase II of the clinical trials. Safety laboratory tests include basic metabolic along with liver, kidneys, bone marrow, and other target organs biomarkers. Most drug candidates are discontinued because of induced organ toxicity and half of them owe this to liver toxicity. Initially, DILI involves direct drug or drug metabolites injury. The injury is then propagated via cell stress, mitochondrial inhibition and/or specific reactions, leading to apoptosis or necrosis. In order to monitor liver functions, several tests can be currently performed. Among the conventional safety liver functions tests ordered in clinical trials are:

- Serum alanine transaminase (ALT) and aspartate transaminase (AST) to monitor hepatocellular injury
- Bilirubin, alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) to monitor cholestasis,
- Prothrombin time and albumin for liver synthetic capacity.

Reports in the literature picture DILI as predominately hepatocellular, signaled by an increase in transaminases and bilirubin. However, this increase happens days after the onset of injury and makes these markers more confirmatory than predictive, because they signal toxicity after irreversible pathological damage has occurred. The increase in transaminases can also be transient only and has been shown as an
unreliable predictor of potential for progression to severe DILI. In some cases, a self-limited liver injury resolves even if the treatment with the drug continues in a process called “adaptation”. The phenomenon was first described with isoniazid, but confirmed with drugs like trioglitazone, tacrine, heparins, or statins.

The conventional approach to DILI is evolving with the “multiple determinant hypothesis”, which postulates that the idiosyncratic (unpredictable) drug injury is related to the sum of factors like drug exposure, host metabolism, immunity and genetic polymorphism. In order to better understand the implications of all these factors and to discover novel biomarkers, different areas of study like pharmacogenetics, toxicogenomics, metabolomics, transcriptomics, and proteomics have been utilized.

Despite of all the efforts, only a few, novel potential biomarkers of liver injury have been developed. Some of them are more predictive than ALT (like miR-122), others more specific (like alanine transaminase ALT1 and serum protein F), others more sensitive (like glutathione S transferase α-GST). In Table 1 there are some of the novel liver injury biomarkers that show promises for future testing implementation.

Table 1. Novel biomarkers of acute liver injury (7,15)

<table>
<thead>
<tr>
<th>NOVEL BIOMARKERS FOR ACUTE LIVER INJURY</th>
<th>SOURCE</th>
<th>INJURY LOCATION</th>
<th>BENEFITS</th>
<th>ASSAYS AVAILABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT1</td>
<td>Serum</td>
<td>Liver restricted</td>
<td>Possible new standard</td>
<td>Immuno-enzymatic</td>
</tr>
<tr>
<td>α-GST</td>
<td>Serum</td>
<td>Liver (centrolobular distribution)</td>
<td>- Highly specific for liver damage - Rapid removal from circulation - Differentiates liver injury from muscle injury</td>
<td>ELISA</td>
</tr>
</tbody>
</table>

SAFETY BIOMARKERS: DRUG-INDUCED ACUTE KIDNEY INJURY (DIAKI)

The lack of sensitivity and specificity of conventional biomarkers has an impact also on detecting DIAKI. Relative large pathological damage can occur before the conventional biomarkers of kidney injury like serum creatinine (sCr) and blood urea nitrogen (BUN) to be detected. Drug-induced acute renal toxicity affects primarily the proximal tubule. A secondary proximal tubule injury may occur in case drug toxicity affects initially the glomerulus or to the distal parts of the nephron. Current urinary biomarkers for nephrotoxicity respond primarily to damage of glomerulus or proximal tube. However, their limited sensitivity and specificity prompted the search for novel biomarkers.

Table 2 lists some of the promising candidates show better sensitivity and specificity for glomerulus and/or proximal tubule injuries as well as for the distal or collecting tube. For example, neutrophil-associated gelatinase lipocalin (NGAL) is elevated in proximal and distal tubule ischemia or injury, matrix metallopeptidases (MMP-9) in renal collecting duct injury. A higher degree of sensitivity is achieved by novel biomarkers like kidney injury molecule (Kim-1), Cystatin C or urinary GST, which can be detected within hours from the onset of the injury.
The “ideal” biomarkers have yet to be discovered, therefore we cannot totally stop testing for conventional biomarkers and switch to the new ones abruptly. We also cannot ignore the fact that the conventional biomarkers do not perform ideally. What needs to be done is parallel testing, starting as early as Phase I and II of the clinical trials, in which the conventional biomarkers would be compared with some of the novel ones best suited for the purpose of each study. Reports in the literature show that sensitivity and specificity for diagnosing DIAKI increased when a combination of novel biomarkers was used, instead of adding only a single one to the conventional testing. The idea is to combine the biomarkers in optimal safety panels, according to the needs of each study, in order to maintain low costs.

### Enabling Safer Drugs

While limited by the number of subjects, the results from a well-planned Phase I or Phase II biomarker testing could depict host genetic factors, elucidated by in more depth testing or by genetic analysis of the affected patients. It could also mean designing a better Phase III trial with increasing chances of achieving post marketing success. One of the drawbacks is that most of the novel biomarkers assays are ELISA and only few are automated, thus increasing the costs. While initially this can be viewed as an extra expense, in the end, correctly interpreted data can prove very useful to drug developing companies, patients, clinical laboratories and why not, to science, with the goal of conquering better and safer drugs.

### Table 2. Novel biomarkers of acute kidney injury (5, 6, 9, 11, 14, 16, 17, 18)

<table>
<thead>
<tr>
<th>NOVEL BIOMARKERS FOR ACUTE LIVER INJURY</th>
<th>SOURCE</th>
<th>INJURY LOCATION</th>
<th>BENEFITS</th>
<th>ASSAYS AVAILABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL</td>
<td>Urine</td>
<td>Proximal and distal tubule injury</td>
<td>- Early detection (hours) - Easily detected</td>
<td>ELISA and automated assays</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>Urine, blood</td>
<td>Glomerulus and proximal tubule</td>
<td>- Early detection - Very good estimator of glomerular filtration rate - Outperformed BUN and sCr in detecting glomerular injuries</td>
<td>ELISA and FDA approved automated assays</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Urine</td>
<td>Proximal tubule</td>
<td>- Early detection - Upregulated in proximal tubule injury</td>
<td>ELISA and dipstick assay</td>
</tr>
<tr>
<td>α/γGST</td>
<td>Urine</td>
<td>Proximal/distal tubule</td>
<td>- Early detection and specific indicator of renal injury</td>
<td>ELISA</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Urine</td>
<td>Renal collecting duct</td>
<td>- MMP inhibitors suppress ischemic AKI</td>
<td>ELISA</td>
</tr>
<tr>
<td>Clusterin</td>
<td>Urine</td>
<td>Proximal tubule epithelium, Glomerulus</td>
<td>- Outperformed BUN and sCr in detecting drug-induced proximal tubule injury</td>
<td>ELISA</td>
</tr>
<tr>
<td>IL-18</td>
<td>Urine</td>
<td>Glomerulus</td>
<td>Early detection</td>
<td>ELISA</td>
</tr>
</tbody>
</table>
ROLE OF CENTRAL LAB

A central laboratory’s responsibility is to ensure the quality of testing, which is very important with all tests, but vital for novel safety biomarkers in order for the data to be credible, accurately interpreted and for the correct decision to be made. Central laboratories also need to make sure that the assays testing for the novel biomarkers are validated and globally standardized, in order to minimize errors. They should develop flexible safety biomarker panels, in which both conventional and novel biomarkers testing will be offered to sponsors. The idea is to divide and combine the biomarkers in optimal safety panels according to the needs of each study, in an effort to maintain low costs and support better and safer drugs.

ABOUT THE AUTHOR

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