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COMMENTARY

Novel biomarkers for drug-induced liver injury

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ABSTRACT

Introduction: Liver toxicity due to medicines (drug-induced liver injury) is a challenge for clinicians and drug developers. There are well-established biomarkers of drug-induced liver injury, which are widely used and validated by decades of clinical experience. These include alanine aminotransferase and bilirubin. Limitations of the current biomarkers are well described, and this has resulted in global efforts to identify and develop new candidates. This process has been aided by regulatory pathways being established for biomarker qualification. This article aims to provide a broad overview of the mechanisms of liver toxicity and discuss emerging novel biomarkers. There is a focus on the recent advances in the identification and validation of novel biomarkers, their potential applications in drug development and clinical practice, and the challenges and opportunities in translating these biomarkers into routine clinical use.

Current gold-standard biomarkers: Alanine and aspartate aminotransferase activities perform well in diagnosing established drug-induced liver injury but may lack specificity and are not prognostic.

The burden of proof for novel biomarkers: The amount of evidence required for a new biomarker will depend on its context-of-use, specifically on the impact on patient outcome of a false negative or false positive result.

Leading potential biomarkers: Cytokeratin-18, glutamate dehydrogenase, microRNA-122, high-mobility group box 1 proteins, osteopontin, and macrophage colony-stimulating factor receptor 1 are examples of lead candidates.

Potential applications of novel biomarkers: The early detection of drug-induced liver injury, interpretation of an alanine aminotransferase activity increase, and decisions about dose escalation in clinical trials may all be informed by new biomarkers.

Conclusions: There have been numerous exploratory studies describing differences in biomarkers and their potential value in risk-stratifying populations or identifying specific patients who may be failed by current assessment protocols. Additionally, the use of exploratory biomarkers to guide clinical trial decision-making is becoming routine. The challenge is now clinically validating leading candidate biomarkers in the assessment of patients presenting with conditions such as paracetamol overdose, which place them at risk of acute liver injury. This will require robust clinical trials. If the use of these biomarkers is to be widely adopted, they will need to unequivocally demonstrate benefit in overall cost, morbidity or mortality.

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Introduction

Liver toxicity is a significant concern in drug development and clinical practice due to its potential to cause liver failure. Traditional methods for detecting liver toxicity, such as alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity and liver biopsies, have limitations in terms of early sensitivity, specificity, and invasiveness. It is inevitable that the role of the liver in metabolism and detoxification makes it vulnerable to damage. As such, the clinical and economic impact of drug-induced liver injury (DILI) will persist without better identification of DILI in drug development and clinical presentations. Consequently, there is a growing need for the development of novel biomarkers that can accurately detect liver toxicity and provide mechanistic insights into the underlying pathophysiology.

Aminotransferases are primarily an indicator of hepatocellular injury resulting in intracellular contents leaking into serum, but this interpretation can be complicated by the multitude of other potential causes (primarily acute or chronic liver disease, or rhabdomyolysis), the rarity of DILI presentations (approximately 1 in 1,000 subjects studied for new drug applications for even the most potent hepatotoxins) and potential for some drugs to cause significant liver impairment even without increases in aminotransferase activities [1]. The rarity of DILI requires that clinical trials are required to actively look for any and all signs of potential

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DILI risk, using criteria such as Hy's Law (patient is at high risk of a fatal drug-induced liver injury if given a medication that causes hepatocellular injury with jaundice). However, while aminotransferases are in common use for the detection of hepatotoxicity, many medications with low potential for causing severe DILI can also cause mild elevations.

The United States Food and Drug Administration (FDA) defines a biomarker as a 'defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions' [2]. Novel biomarkers may be intended to be diagnostic or prognostic clinically, used in predicting DILI risk for novel drugs or monitoring clinical trial populations for safety or efficacy. Both the FDA and the European Medicines Agency (EMA) have frameworks for the regulatory qualification of biomarkers. For both, this process begins with a context-of-use statement, which defines both the novel methodology and the purpose of use [3].

The subsequent qualification of novel biomarkers faces multiple challenges. Biomarkers validated in animal models may not translate to humans; potential mechanistic biomarkers identified on biopsy or single-cell studies may not be appreciably different in serum; and the pursuit of increasingly mechanism-specific biomarkers may limit their breadth of application.

To date, no human liver safety biomarkers have been fully qualified by regulators, though protein biomarkers, including cytokeratin-18 (K18) and glutamate dehydrogenase, have received significant regulatory support and are the subject of a large qualification programme by the Innovative Medicines Initiative funded TransBioLine consortium. This process is intensive, requiring letters of intent, collaborative discussions with regulators, and a wealth of validation data establishing reference ranges and evidencing performance across a range of clinical scenarios [4].

This article aims to provide a broad overview of the mechanisms of liver toxicity and discuss emerging novel biomarkers. There is a focus on the recent advances in the identification and validation of novel biomarkers, their potential applications in drug development and clinical practice, and the challenges and opportunities in translating these biomarkers into routine clinical use.

Current gold-standard biomarkers

While tests of liver function (e.g., total bilirubin concentration, prothrombin time) offer insight into the impact of acute liver injury, current gold-standard biomarkers used to detect acute liver injury (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities) demonstrate heterogeneity of response in both the timing and magnitude of their rise, and although they are included in most 'liver function tests' do not reflect liver function [5]. It may be surprising that biomarkers developed in the 1950s have continued in routine clinical use, but understandable; clinical decision-making requires established consensus standards, and when used correctly, aminotransferase activities are extremely sensitive for the detection of DILI. The use of ALT in the assessment of DILI was endorsed by the FDA as recently as 2009 [1]. The current FDA-recommended approach to identifying the risk of severe DILI is described in Table 1.

The principal issue with the use of aminotransferase activities and bilirubin concentration as biomarkers is that, while they are useful in predicting the risk of hepatotoxicity at a population level if rises occur during drug trials, they are not prognostic for clinical severity at an individual level [6]. Additionally, the detected value does not reflect the degree of liver damage [7]. There are multiple mechanisms by which ALT/AST activity rises may occur, and specifically in DILI, it has been demonstrated that there are alternative biomarker candidates which rise more quickly, which could logically result in earlier detection of DILI [5,8]. As an additional benefit, many of these candidates hold the promise of improved specificity for DILI [7].

The burden of proof for novel biomarkers

Regardless of their issues, established biomarkers have decades of use in clinical and regulatory practice, and there is no doubt that their use is well established. The developmental and regulatory pathway to qualify biomarkers for use in non-experimental settings is a long one, and though the majority of drug development programmes now involve the use of biomarkers, relatively few make the leap to become routinely available diagnostic tests [9].

Table 1. Current United States Food and Drug Administration recommendations on identifying the risk of severe drug-induced liver injury [1].

Major indicator of risk	Explanation	Limitation
Increased incidence of aminotransferase elevation >3x upper limit of normal activity compared to control group	Aminotransferase activity rises are often an indication of drug-induced liver injury. Nearly always occur versus control group in drugs that have ultimately been shown to cause severe drug-induced liver injury.	Insufficient data to predict how much greater incidence should be. May not predict idiosyncratic drug-induced liver injury.
Increased incidence of aminotransferase elevation to >5x upper limit of normal activity in modest numbers compared to control group	Most drugs that induce severe drug-induced liver injury demonstrate this finding.	May occur in drugs which do not cause severe drug-induced liver injury.
Cases of serum bilirubin concentration >2x upper limit of normal with hepatocellular injury and an increased incidence of ALT activity >3x upper limit of normal activity compared to control group.	The liver has an excess of bilirubin excreting capacity. Injury sufficient to raise bilirubin indicates extensive liver injury. This rule has a relatively high sensitivity for identifying drug-induced liver injury risk.	Drug-induced liver injury with an incidence of 1:10,000 would still require 3,000 exposed subjects to have a 95% probability of detecting risk.

ALT: alanine aminotransferase.

The burden of proof for novel biomarkers is variable and is related to the level of risk that the planned context-of-use creates [10]. For example, if a negative test result is used to decide to discharge a patient with a potentially fatal presenting complaint, then a substantial body of evidence of safety would be required. Conversely, if a positive result was used to expedite specialist involvement, while negative results resulted in current standard care, false negative results would result in no change to current practice, and the burden of proof is likely to be lower.

The pathway for biomarker qualification is clearly described by the FDA [11]. The process begins with a Letter of Intent, which starts a collaborative process to create a Qualification Plan. Once agreed, the required data are obtained to be submitted as part of the Full Qualification Package, which will lead to a formal Qualification Recommendation by the FDA. The regulatory burden means that interested parties tend to form consortia to approach the process, for example, the current Innovative Medicines Initiative funded Translational Safety Biomarker Pipeline (TransBioLine) consortium.

There is an additional challenge in the validation of biomarkers in human populations: paracetamol (acetaminophen) toxicity is a regular occurrence with well-explored pathophysiology (and, due to the number of presentations, forms a reliable test-bed for exploring novel biomarker detection). However, biomarkers validated in this population may not translate well to patients presenting with DILI from less understood causes, and the frequency of rarer DILI causes may make targets difficult to identify and validation studies challenging to complete [12]. There will be an element of pragmatic decision-making in the application of novel biomarker tests, which will need to be supported by a sound theoretical basis and the best evidence reasonably achievable.

Selecting biomarkers based on mechanism: host or dose?

When selecting a biomarker for a population, it is important to recognize that there are multiple mechanisms that may lead to acute liver injury. The biomarker(s) selected should reflect the mechanism(s) of interest.

Intrinsic DILI is a dose-dependent response to drug exposure. At population levels, it is a predictable response, with paracetamol as the leading cause in both the United Kingdom and the US. There are several implicated mechanisms, which may be achieved *via* several pathways and do not necessarily occur in isolation:

1. Inhibition of mitochondrial function;
2. Reactive oxygen species accumulation; and
3. Bile acid accumulation (likely related to inhibition of the bile salt export pump protein) [13].

Paracetamol overdose, for example, can deplete glutathione due to excessive concentrations of N-acetyl-p-benzoquinone imine (NAPQI), causing accumulation of reactive oxygen species and consequent mitochondrial injury by both direct impact of reactive oxygen species and NAPQI binding to mitochondrial proteins [14]. This results in mitochondrial permeability transition pores opening, leading to decreased adenosine triphosphate synthesis, intermembrane protein release, deoxyribonucleic acid fragmentation, and cell necrosis [15].

While at first glance, these three mechanisms may appear to generate the same outcome (hepatocyte death), it is unlikely that the biochemical fingerprint of each occurring is identical. Nor is it likely that there are identical biomarker signatures in the prodromal phase – the different processes make it highly likely that examining the correct biomarkers could generate valuable mechanistic insights. It should also be noted that the risk of these mechanisms leading to DILI may be due to pharmacokinetic variation (whether by functional polymorphism in genes coding for drug transport and metabolism, age, nutritional status, biological sex, etc.) and are likely to become increasingly predictable [16,17].

In contrast to intrinsic mechanisms, idiosyncratic DILI is a rare response to drug exposure without a clear link to dose and is the leading determined cause of DILI after paracetamol toxicity [18]. Idiosyncratic reactions are associated with variants of human leukocyte antigen (HLA) alleles, and there is significant interest as they are a leading cause of post-market drug withdrawals [19]. However, patients with specific alleles are not guaranteed to have DILI on specific drug exposure, nor are implicated alleles necessarily rare [20]. This makes use of HLA testing prior to exposure of limited value, and the rarity of idiosyncratic DILI events means that any biomarker testing is likely to be post hoc for patients suspected of having suffered DILI or focussed on early damage-associated molecular pattern detection in drug trials in order to prevent repeat dose administration, in which extensive post-dosing testing is less likely to be considered overly burdensome.

Leading potential biomarkers

The potential value of novel biomarkers was recognized in 2016 by both FDA and EMA and, though one early candidate molecule (the hyperacetylated form of high mobility group protein B1) has unfortunately been determined to have been affected by academic misconduct tempering support, many other biomarkers are now beginning to deliver on their perceived potential [21–23].

The ideal biomarker for the detection of DILI would be liver injury-specific, rise early in acute liver injury, be rapidly measurable, and have regulatory qualification. Table 2 describes some of the leading novel biomarkers for DILI. Some are liver-specific, while others are not. Lack of specificity may not ultimately prevent the use of these biomarkers in DILI but will mean that any translation into routine clinical use will require additional measures to either clearly describe the populations in which they should be applied or assist with clinical correlation in those the test identifies [24]. Placing non-specific results in context and excluding other causes of their rise will continue to be required. It should

Table 2. Candidate novel biomarkers for the detection of drug-induced liver injury.

Biomarker	Liver specific?	Marker of	Suggested advantage versus ALT activity
Caspase-cleaved cytokeratin-18 (ccCK-18)	No	Thought to enter circulation when cells undergo apoptosis (rather than necrosis) [26].	May have mechanistic value when identifying hepatocyte injury.
Cytokeratin-18 (K18)	No	Epithelial cell filament released in cell necrosis. Highest concentrations found in liver.	Identified as a potential single biomarker to identify acute liver injury [25]. May have prognostic value [26].
Glutamate dehydrogenase	Relatively	Mitochondrial protein, concentrations higher in liver than other tissues [27].	Shorter half-life than ALT. May also provide mechanistic insight into mitochondrial toxicity as a mechanism of drug-induced liver injury.
High mobility group box-1 protein (HMGB1)	No	Identifies patients with paracetamol toxicity who require additional treatment [8].	May more accurately predict the development of hepatic synthetic dysfunction.
Macrophage colony-stimulating factor receptor 1	No	Originates from infiltrating macrophages in drug-induced liver injury [28].	May more accurately represent level of inflammation. May have prognostic value in paracetamol toxicity [29].
MicroRNA-122	Yes	Most common microRNA in the liver, serum concentrations rise in liver injury [30]. May also be released in response to stress in the absence of hepatocyte death [26].	Highly liver specific.
Osteopontin	No	Originates from infiltrating macrophages and lymphocytes.	May therefore more accurately represent level of inflammation. Concentrations may prognosticate outcome in drug-induced liver injury, and predict death/transplant [26].

also be noted that biomarkers validated for prognostication do not necessarily translate to clinical detection and vice-versa.

While Table 2 discusses biomarkers in isolation, the value of biomarkers used in panels has also been demonstrated. A combination of novel biomarkers can be superior to the use of novel biomarkers in isolation and has also been shown to outperform the use of ALT in the prediction of acute liver injury due to paracetamol toxicity [8,25]. These results have consequently informed the design of clinical trials [31].

It is perhaps surprising that while our understanding of the mechanisms of hepatotoxicity has evolved significantly, the majority of promising biomarker candidates are molecules that are released from their intracellular locations secondary to apoptosis or necrosis. As we seek earlier detection of DILI and prediction of which patients are likely to experience liver failure, there are potential benefits in examining paracrine signal molecules in the immune system. Being better able to read the behaviour of these cells, which are both the effector of apoptosis and early responders to necrosis, could allow the identification of emerging hepatocyte damage before significant hepatocyte death occurs. The value of interpreting the behaviour of the immune system has been recognized in some form for years – for example, the degree of reduction in monocyte count in paracetamol toxicity (reflecting monocyte infiltration to the liver) is associated with mortality [32].

As multiplex cytokine assays become less expensive and more widely available as a research tool, it may be that biomarker recognition begins to move beyond (or expands on) damage-associated molecular pattern recognition and instead focuses on serum concentrations of paracrine effector molecules describing the scale of sterile inflammation (e.g., interleukin-6), anti-inflammatory activity (e.g., interleukin −10), or the restoration of innate immunity after acute liver injury (e.g., macrophage colony-stimulating factor). Recent studies have demonstrated significant differences in inflammasome behaviour between survivors and non-survivors after paracetamol overdose [33]. However, the role of the inflammasome in paracetamol overdose remains contested. Animal models have not translated well to humans, and cytokines which are identified in biopsy samples may not be detectable in serum. The understandable absence of simultaneous serum samples and human biopsies, as well as variation in serum assay sensitivities, further complicates the picture.

To a degree, the distinction between classes of some of the current leading biomarkers and cytokines may be artificial. For example, it is easy to class microRNAs as damage-associated molecular patterns which simply describe damage, but there is considerable evidence supporting the direct role of microRNA-122 in the activation of anti-inflammatory processes [34]. Rather than being a simple marker of damage, the immune response is directly linked to microRNA-122 release. This may go some way to explaining why it appears to be predictive at an earlier stage than ALT activity, though it should be recognized that if assay sensitivity for ALT can be improved, the difference may become insignificant.

New isolation methods may also allow clearer identification of damage-associated molecular patterns before they are released in necrosis. It has been suggested that the analysis of exosomes may provide insight into emerging DILI before obvious cell injury develops [35].

Biomarkers predicting idiosyncratic DILI present a significant challenge and are unlikely to be used outside trial settings. While the evidence base for pharmacogenomic-guided prescribing is growing, the economic case is predicated on improving clinical outcomes by optimizing drug response phenotypes rather than identifying extremely rare complications [36].

Table 3. Examples of potential applications of novel biomarkers in drug-induced liver injury. Categories are based on the Council for International Organizations of Medical Sciences drug-induced liver injury guidelines [3].

Context of use	Example
Diagnostic biomarker: *Used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease.*	MicroRNA-122 or cytokeratin 18 rapid test for patients presenting following paracetamol overdose. If significantly elevated, immediate antidote therapy might be indicated rather than waiting for laboratory values.
Efficacy/pharmacodynamics biomarker: *Used to show that a biological response has occurred in an individual who has been exposed to a medicinal product or an environmental agent.*	Glutamate dehydrogenase could be used in patients with paracetamol hepatotoxicity to demonstrate response to acetylcysteine more rapidly than ALT activity, due to the shorter half-life of glutamate dehydrogenase.
Monitoring/safety biomarker: *Typically measured at baseline and serially during drug therapy to assess the status of a disease or medical condition.*	MicroRNA-122 or cytokeratin18 rapid test used in resource poor settings to monitor patients receiving tuberculosis medication for early stage drug-induced liver injury.
Predictive/susceptibility biomarker: *Used to identify individuals who are more likely than similar individuals without the biomarker to experience a favourable or unfavourable effect from exposure.*	Patients who present with paracetamol overdose and are found to have CYP3A5 polymorphism rs776746 may be at increased risk for acute liver failure [37].
Prognostic biomarker: *A biomarker used to identify the likelihood of a future clinical event or disease recurrence or progression in patients.*	High mobility group box 1 protein concentrations predict death/transplant in drug-induced liver injury patients [26].

ALT: alanine aminotransferase.

Potential applications of novel biomarkers

Possible biomarker uses may be considered proprietary, and many biomarker consortia have carefully constructed data-sharing agreements to ensure that commercially sensitive information is not released. Table 3 illustrates the potential applications of novel biomarkers in each of the potential contexts of use.

Conclusions

While significant progress has been made in identifying potential biomarkers, the reality is that the journey from identification to clinical use is a long one. While we are increasingly well-placed to select candidate biomarkers which reflect specific mechanisms of interest, their use should not be permitted without appropriate validation, through comparison to the current gold-standard within the specific population of interest. The reality is that there should be no new 'general purpose' novel biomarker – using one would entirely miss the point. Novel biomarkers will achieve increased sensitivity and specificity by being increasingly mechanism and population-specific, and in adopting them, clinicians and scientists should not expect them to outperform ALT across all patient populations.

The same journey can occurred with cardiac biomarkers, as AST was superseded by creatine kinase, then creatine kinase-myocardial band (CK-MB), and consequently troponin. The biomarker of choice is increasingly specific [30]. There is, however, perhaps a note of warning from cardiac biomarkers for the development of DILI-biomarkers; once a specific biomarker is identified, the use of high-sensitivity assays makes cut-off values, and the selection of patients in whom it is used, increasingly important.

There have been numerous exploratory studies describing differences in biomarkers and their potential value in risk-stratifying populations or identifying specific patients who may be failed by current assessment protocols. Additionally, the use of exploratory biomarkers to guide clinical trial decision-making is becoming routine. The challenge is now clinically validating leading candidate biomarkers in the assessment of patients presenting with conditions such as paracetamol overdose, which place them at risk of acute liver injury. This will require robust clinical trials, and as discussed above, these are likely to start by 'ruling in' patients to enhanced care rather than 'ruling out' patients from treatment. If the use of these biomarkers is to be widely adopted, they will need to unequivocally demonstrate benefit in overall cost, morbidity or mortality in this context.

Disclosure statement

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