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Biomarkers of drug-induced liver injury: progress and utility in research, medicine, and regulation

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Abstract

Introduction: The difficulty of understanding and diagnosing drug-induced liver injury (DILI) has led to proliferation of serum and genetic biomarkers. Many applications of these biomarkers have been proposed, including investigation of mechanisms, prediction of DILI during early trials or before initiation of therapy in patients, and diagnosis of DILI during therapy.

Areas covered: We review the definition and categories of DILI, describe recent developments in DILI biomarker development, and provide guidance for future directions in DILI biomarker research.

Expert commentary: There are major obstacles to DILI biomarker development and implementation, including the low prevalence of idiosyncratic DILI (IDILI), weak associations of IDILI with genetic variants, and lack of specificity of many biomarkers for the liver. Certain serum biomarkers, like miR-122, may have clinical utility in early-presenting patients with either intrinsic or idiosyncratic DILI in the future, while others likely will not find use. Future research should focus on implementation of biomarkers to predict later injury and outcome in early presenters with intrinsic DILI, and on development of biomarkers of adaptation and repair in the liver that can be used to determine if a liver test abnormality is likely to be clinically significant in IDILI.

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1. Introduction

Drug-induced liver injury (DILI) is a major problem for patients, regulators, and the pharmaceutical industry. In the US, DILI remains the primary etiology of acute liver failure (ALF). It accounts for approximately 60% of all ALF cases [1]. It is also one of the most common reasons for post-marketing drug withdrawal, as well as new black box warnings, in this country [2,3]. Globally, it was the single most common reason for withdrawal of drugs and other medicinal products from the market from 1953 to 2013 [4].

Due to the clinical and regulatory burden of DILI, there has been a surge of interest in DILI biomarkers over the last decade. Many novel biomarkers have been introduced. However, many remain in the development stages, and we are only now beginning to understand their clinical utility. As discussed in detail later, recent studies have reported values for sensitivity, specificity, and predictive values that can be used to compare them. The purpose of this review is to summarize recent developments in DILI biomarker research and discuss future directions based on clinical and regulatory applications. We will begin by defining and describing DILI. We will then discuss recent findings regarding both serum biomarkers and genetic testing. Finally, we will offer our expert opinion on how and when these biomarkers should be used.

2. Overview of drug-induced liver injury

DILI is often broken down into two types: 1) intrinsic and 2) idiosyncratic. This categorization is thought to have been introduced and popularized decades ago by the hepatologist Hyman Zimmerman [5]. Whereas intrinsic DILI is said to be due to intrinsic properties of a drug or its metabolite(s) (e.g. reactive functional groups), idiosyncratic DILI (IDILI) is largely blamed on some aspect of the recipient's response to the drug. According to this model, intrinsic DILI is predictable and dose-dependent, while IDILI is rare (usually said to occur in less than 1 in 10,000), non-dose-dependent, and unpredictable. Some authors have mentioned a third form of DILI, in which generalized drug hypersensitivity that predominantly affects other organs (e.g. skin) also targets the liver in a small proportion (1–20%) of patients who experience adverse reactions [6], while others make no such distinction [7]. However, despite the popularity of this categorization, it has been pointed out that there is no universally accepted definition of either intrinsic DILI or IDILI [5]. Furthermore, it has been recognized for more than a decade that IDILI does display some dose-response relationship, as it tends to be caused by drugs that have low potency and therefore require relatively high daily doses [8–10]. There is also evidence that lipophilic drugs and drugs that undergo extensive metabolism in the liver (>50%) tend to cause it [11,12]. Thus, there are aspects of IDILI that depend on the intrinsic properties of drugs [13]. Overall, the relationships between drug properties, genetics, host factors and DILI development are complex [14]. Nevertheless, the intrinsic vs. idiosyncratic model is useful

to simplify discussion of the topic and will be used throughout this review. DILI can also be broken down into three categories based on the type of liver injury. Some drugs cause predominantly hepatocellular injury, which typically means necrosis of hepatocytes. Others cause a predominantly biliary phenotype, resembling cholestasis. Finally, some drugs cause a mixed hepatocellular and biliary phenotype.

3. Intrinsic DILI

APAP hepatotoxicity is the archetype of intrinsic DILI and the most common cause of DILI in general. It is reproducible and displays a clear dose-response in both rodents and human hepatocytes [15–17]. For ethical reasons, it is impossible to perform dose-response studies for APAP hepatotoxicity in humans. However, available evidence consistently points to a threshold of toxicity around 6–10 g/day for adults [18]. Furthermore, the correlation between peak values for serum APAP-protein adducts and plasma aminotransferases [19] strongly suggests that there is a granular dose-response in humans, similar to mice and cultured human hepatocytes [15–17]. Although the results of a few studies indicate that a minority of patients taking therapeutic doses of APAP develop transient elevations in liver injury markers [20–23], those patients do not experience significant clinical sequelae suggestive of true liver injury and never develop liver failure [20–23]. Even populations thought to be at increased risk of APAP hepatotoxicity due to chronic alcohol consumption show no evidence of liver injury after ingestion of the maximum therapeutic dose [24–27]. Clinically, few other drugs are as menacing as APAP. Numerous estimates of the number of emergency department visits and hospitalizations for APAP overdose per year are available for the US and range from 40,000 to 80,000 and 20,000 to 40,000, respectively [28–31]. Incidence of APAP hospitalization per population is approximately 10–15 per 100,000 [30], while the rate of poison center calls or emergency department visits is likely greater. APAP overdose is also responsible for approximately half of all ALF cases in the US, UK, Australia, and various other regions [1,32]. Although outcomes are somewhat better after ALF caused by APAP than other etiologies, mortality in APAP-induced ALF is still high at 20–40% [33,34]. Despite the significant clinical problem of APAP hepatotoxicity, it needs to be kept in mind that APAP is one of the most consumed drugs worldwide and liver injury and acute liver failure is caused by intentional or unintentional overdosing of the drug but not by therapeutic use.

The mechanism of APAP hepatotoxicity begins with formation of a reactive metabolite that binds to proteins [35]. Mitochondrial proteins are especially important targets [36,37]. The resulting mitochondrial dysfunction and oxidative stress activate a mitogen-activated protein kinase cascade that can exacerbate the injury [38–41]. Eventually, the mitochondrial membrane permeability transition occurs, and the mitochondrial membrane potential is lost [42–44]. Swelling and lysis of mitochondrial membranes causes release of mitochondrial macromolecules, including endonucleases that translocate to the nucleus and then cleave nuclear DNA [45,46]. Finally, the cells die by oncotic necrosis [47]. Importantly, there is evidence that these mechanisms are the same in humans [15,17,48–51].

APAP is by the far the most common cause of intrinsic DILI in humans, but there are others. Halothane is one example. Although halothane hepatotoxicity can be delayed and involve

adaptive immunity like IDILI, severe acute cases are known to occur. Acute halothane hepatotoxicity appears to be due to metabolism to reactive acyl chlorides that bind to proteins and cause mitochondrial damage and oxidative stress [52]. Rare cases of severe acute hepatotoxicity have been reported for numerous other drugs, including cocaine [53], methotrexate [54], amiodarone [55,56], and others.

4. Idiosyncratic DILI

Typical IDILI is severe liver injury that occurs in a small proportion of patients using a particular drug at recommended doses. The drugs that are known to cause it belong to multiple classes, including antibiotics (e.g. amoxicillin-clavulanate), cardiovascular drugs (amiodarone), CNS agents (e.g. phenytoin), NSAIDs (e.g. diclofenac), and others [57–59]. Herbal products and dietary supplements may also cause many cases of IDILI [57–59]. In most cases, the injury is delayed and develops suddenly after months of asymptomatic exposure and quickly resolves upon discontinuation. However, there are rare cases of both very rapid and extremely latent response [7], and a subset of patients experience delayed resolution [7,60,61]. The rarity, latency of injury, variety of causative drugs, and lack of uniformity in presentation make IDILI difficult to study. Nevertheless, some clues are available.

Most toxicologists agree that the latency of injury indicates a role for the adaptive immune system in IDILI. The delay after initial exposure is likely due to the time required for antigen-specific lymphocytes to respond and proliferate [7,62]. The fact that some drugs induce injury much more rapidly upon re-exposure is also consistent with an immune-mediated mechanism [7]. There is also limited evidence for CD4⁺ and CD8⁺ T-cell activation and infiltration in the liver after IDILI [63,64]. Of course, in order to elicit an immune reaction, there must be a unique antigen. The creation of novel antigens in idiosyncratic drug toxicity is thought to be due to interactions of the drug and either various endogenous proteins or specific antigen presenting-complexes. The long-standing hapten hypothesis states that the drugs are converted to reactive metabolites that covalently bind to proteins, forming novel epitopes that are foreign to T-cells and can therefore elicit an immune response. However, the fact that certain drugs like APAP can bind extensively to proteins even at subtoxic doses yet do not cause toxicity through adaptive immunity [15,65,66] argues against that. Two alternative hypotheses have been proposed. First, in some cases direct activation of T-cell receptors by drugs or their metabolites may elicit a response. This idea is consistent with the fact that lymphocytes from some patients with idiosyncratic injury seem to be directly activated by exposure to the offending drug [63]. However, in that case, one might not expect a delay for months. Second, changes in the repertoire of self-peptides that HLA complexes present due to interactions between the complexes and drugs or their metabolites could lead to an immune response [62,67]. A problem with all three hypotheses in IDILI is that most associations with HLA variants that have been discovered are weak and cannot explain much of the risk [62]. The issues with these hypotheses have led some to suggest that non-immune-mediated mechanisms are involved, or at least precipitate the immune response. For example, though controversial, it has been suggested that potentiation of subclinical mitochondrial dysfunction may be responsible for IDILI in some patients [68,69]. A role for innate immunity and damage-

associated molecular patterns has also been suggested, though there is also evidence against that [70]. Overall, the mechanisms of IDILI are not well understood, but most likely involve adaptive immunity.

5. Biomarkers of drug-induced liver injury

Biomarkers of DILI can be divided in two categories: 1) serum biomarkers and 2) genetic biomarkers (Fig. 1). Serum biomarkers of DILI can be useful for several reasons. First, some, often referred to as “mechanistic biomarkers,” provide insight into the fundamental mechanisms underlying the injury [50,51]. Such markers may aid identification of therapeutic targets to treat DILI patients, or lead to a better understanding of how drugs cause hepatotoxicity in humans so future drugs can be designed to avoid it. Other biomarkers appear to be sensitive for detection of acute liver injury in early-presenting patients who do not yet have elevated liver function tests [71,72]. Those markers may allow clinicians to determine which patients require hospital admission and treatment and which do not. Biomarkers that can discriminate between serious liver injury and transient changes in liver function tests would also be useful in early clinical trials to avoid unnecessarily terminating the development of a promising new drug. Finally, some serum biomarkers may be useful for prediction of patient outcome after the onset of liver injury [50,51,73]. A liver transplant is the only life-saving treatment for most non-APAP DILI patients, as well as the only effective option for late-presenting APAP overdose ALF patients. Markers that predict which patients will need a liver transplant early in the course of injury could allow clinicians to make treatment decisions faster and more accurately. It has also been suggested that serum biomarkers could be useful to identify drugs with the potential to cause IDILI during pre-clinical and early clinical trials. However, as we will see, that may not be a wise approach. It should be noted that the vast majority of research into serum biomarkers of DILI have been performed using either animal models of APAP overdose or samples from APAP overdose patients, but there is no reason at present not to believe that those markers would not be useful for IDILI shortly before or after injury develops.

The primary purpose of genetic biomarkers is risk management. For the same reasons that trying to use biomarkers to predict IDILI may not work, genetic testing of a candidate for treatment with a drug known to cause DILI may not be practical. However, once injury develops in a patient taking that drug, genetic testing may help to determine if the injury is likely to be due to the drug or to some other disease or exposure. In this way, genetic tests may serve as useful companion biomarkers in patient treatment.

6. Serum biomarkers

6.1 Mechanistic biomarkers

Glutamate dehydrogenase (GLDH) is an enzyme present in the mitochondrial matrix of most eukaryotic cells. In humans, its expression is highest in liver tissue [74], and it is concentrated in the centrilobular area within the liver [76]. Although it is also expressed in the kidney, expression is much higher in the liver [74,75]. GLDH is important for amino acid metabolism, the urea cycle and the Krebs cycle. It catalyzes the reversible deamination of glutamate to α -ketoglutarate and ammonia. The α -ketoglutarate can then feed into the Krebs

cycle, or it can be used by aminotransferases to make pyruvate and re-synthesize glutamate, while ammonia proceeds to urea synthesis for excretion. The reaction catalyzed by GLDH also produces NADH, which can be used in numerous other reactions including ATP synthesis through the electron transport chain. The enzyme can be measured in plasma using spectrophotometry based on its reverse reaction that consumes NADH [50].

GLDH has been tested as a mechanistic biomarker of mitochondrial damage in acute liver injury [50,51,77], as a biomarker to predict outcome of intrinsic DILI [51] and as a biomarker for the prediction of later liver injury in early-presenting APAP overdose patients [71,72,78]. Rodent studies comparing plasma GLDH activity between mice treated with APAP or another hepatotoxicant that does not affect mitochondria revealed that GLDH release is greater when mitochondrial damage has occurred [50], suggesting that GLDH release indicates at least some degree of mitochondrial dysfunction. Clinically, GLDH has been shown to predict outcome in APAP-induced ALF [51]. Similarly, it was found to predict later development of liver injury in APAP overdose patients who presented with normal ALT values [71,72]. However, in all cases, the sensitivity of GLDH for prognosis was very poor. Moreover, in the only study to attempt to provide positive and negative predictive values (PV), the positive PV for injury in patients with normal ALT at presentation was extremely low and the negative PV was probably not good enough to rule out injury [72], making it useless for that purpose in the real world. This finding is not surprising, as the kinetics of circulating GLDH closely resemble that of ALT [50]. The real utility of GLDH may be in identifying liver injury in the context of muscle disease or damage. Unlike ALT, GLDH is not highly expressed in muscle tissue, which has led to the suggestion that it could be used to differentiate between ALT elevations due to liver and muscle effects [73].

Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) fragments have also been tested as mechanistic biomarkers and as biomarkers to predict patient outcome in DILI. mtDNA can be measured by quantitative PCR, while nDNA fragments can be measured using an anti-histone immunoassay. Like GLDH, both are elevated in circulation after APAP overdose in mice and humans with a time course similar to ALT [50,51,79,80], and mtDNA may be specific for mitochondrial damage [50]. Although few studies have tested their clinical utility, both predict outcome in APAP-induced ALF with similar or marginally better performance than GLDH [51]. Nevertheless, like GLDH, their usefulness for prognosis is probably limited based on the available data.

Long-chain acyl-carnitines are also elevated in circulation after APAP overdose in mice and increase before ALT [81]. They are also elevated in APAP overdose patients with delayed N-acetylcysteine (NAC) treatment [82]. Animal studies demonstrated that NAC treatment attenuated the increase in acyl-carnitine plasma levels after APAP overdose [81] most likely through the support of the mitochondrial bioenergetics by converting NAC to Krebs cycle intermediates [83]. Like GLDH and mtDNA, long-chain acylcarnitines may be specific for mitochondrial damage [81]. Although ALT was also higher in the patients who received delayed treatment [82], it is not currently known if these acyl-carnitines have any prognostic utility.

High mobility group box 1 (HMGB1) is a protein that is normally found in the nucleus and is involved in gene transcription, nucleosome assembly, and DNA replication and repair [84,85]. However, various post-translational modifications can shift HMGB1 to the cytosol and even promote its secretion [85]. For example, acetylation of HMGB1 occurs during inflammation and the acetylated form can then be secreted [86,87]. Thus, acetylated HMGB1 (acHMGB1) can be considered a biomarker of inflammation, while total HMGB1 may be viewed as a biomarker of necrosis with passive release. Total HMGB1 is higher in early-presenting APAP overdose patients who later develop elevated ALT [71], and there is evidence that normal values at presentation may be useful to rule out risk of injury [72]. Unfortunately, the integrity of data regarding acHMGB1 in serum in DILI patients has recently been called into question and will not be discussed here. However, it may still be a promising biomarker, as it has been reported to increase in pre-clinical models of acute liver injury [88].

Like HMGB1, keratin 18 (K18) can be measured in circulation in two forms. K18 is a structural protein, part of the cytoskeleton. During apoptosis, caspases cleave K18 to reveal a novel epitope that is recognized by an antibody called M30 [89]. Both total and caspase-cleaved K18 are elevated in circulation of APAP overdose patients [71], though total is far higher in both APAP overdose and other DILI [73] indicating that the major mode of cell death is oncotic necrosis. Importantly, total K18 is one of the best prognostic biomarkers identified so far for both prediction of later injury in early-presenting APAP overdose patients [72] and for patient death/survival in DILI [73], though the results are still modest. Interestingly, plasma levels of total K18 and the ratio of cleaved-to-total K18 are also predictive of early stage mortality in alcoholic hepatitis [90].

6.2 MicroRNAs

MicroRNAs (miRs) are among the most promising DILI biomarkers so far. The use of circulating miRs as biomarkers of intrinsic DILI has been investigated by several groups. Multiple studies have demonstrated that certain miRs, particularly miR-122 and miR-192, are elevated in serum samples from mice and humans after APAP overdose before ALT [71,72,91–96]. Elevated miRs, particularly miR-122, have also been reported in patients with non-APAP DILI [97]. Interestingly, there is some evidence that circulating miR profiles may be useful to discriminate acute liver injury caused by different etiologies. One study revealed different profiles between APAP overdose patients and patients with hypoxic hepatitis [93]. The mechanism of miR release also differs between DILI and other causes of injury [98]. Importantly, along with total K18, miR-122 is one of the best prognostic markers for prediction of later injury in APAP overdose patients who present with normal ALT [71,72]. Furthermore, another study found a modest association between serum miR values and survival in non-APAP DILI [97]. Surprisingly, however, the miRs were lower in non-survivors than in survivors [97].

Despite the promise of miR-122 and other miRs as circulating biomarkers of DILI, most methods available to measure miRs involve sequencing, PCR or microarrays and questions surround the proper way to normalize the data [99]. Another issue is that miR-122 has considerable intra- and inter-individual variation [73]. This complexity has caused some

researchers to pivot away from studying miRs as biomarkers. However, the recent demonstration that miR-122 can be measured in capillary blood specimens [100] or using an automated immunoassay platform [101] may signal a shift toward development of simpler and more rapid approaches in the future. Overall, miR-122 may be the biomarker best positioned for future clinical use.

6.3 Serum bile acids

Bile acid changes have been reported in serum and plasma after intrinsic DILI in both humans and rodent models [102–107]. Several bile acids are elevated in DILI and correlate with ALT. One study revealed that serum glycodeoxycholic acid values are higher in non-survivors of APAP-induced ALF [103]. Unfortunately, no attempt has yet been made to test the utility of circulating bile acid values in any other clinical context involving DILI.

6.4 Repair and adaptation biomarkers

Liver repair and regeneration are critical for survival of acute liver injury, including both intrinsic and IDILI. This has led to the emerging idea that biomarkers of regeneration may be better for prediction of patient outcome than the markers of injury or inflammation described above. Moreover, the US Food Drug Administration (FDA) and the European Medicines Agency (EMA) have recently expressed support for development of biomarkers to assess adaptation and repair after liver injury during pre-clinical and clinical drug trials in order to differentiate between drugs that cause significant liver injury and those that are likely to cause only transient changes in injury markers. It has already been known for over a decade that serum α -fetoprotein (AFP), a marker of hepatocyte proliferation, predicts survival in APAP overdose patients [108]. More recent data have confirmed that in non-APAP DILI patients [73]. However, a major drawback of AFP is that its values cannot be used to differentiate survivors from non-survivors until 2–3 days after the peak of injury [108,109]. By that time, a patient may no longer be a candidate for a liver transplant due to encephalopathy or other complications. Thus, other biomarkers are needed to more rapidly guide treatment decisions. It has been reported that serum leukocyte cell-derived chemotaxin 2 (LECT2) increases during liver regeneration in humans [110] and that it is lower in non-survivors of non-DILI ALF [111]. Based on that, LECT2 was measured in serum from DILI patients. Unfortunately, it failed to accurately identify patients with DILI due to various different drugs compared to healthy volunteers based on receiver operating characteristic curve analysis, so no further testing was done [73]. Interestingly, there is evidence that osteopontin (OPN) promotes liver regeneration after partial hepatectomy in rodents [112,113]. Counterintuitively, it was recently reported that circulating OPN is higher in non-survivors of DILI than in survivors and may predict death better than all other novel candidate DILI biomarkers tested [73]. Adding to the confusion, an earlier study reported the opposite result in a cohort of ALF patients with mixed etiologies [114]. The reason for the discrepancy between these studies is not clear. Additional studies of the prognostic utility of OPN are advisable. Finally, it was recently demonstrated that the lipid phosphatidic acid (PA) is elevated in liver tissue and plasma from both mice and humans after APAP overdose, and that it is important for liver regeneration signaling [115]. Thus, PA may be a promising new biomarker of liver regeneration. Comparison of PA values between survivors and non-survivors of DILI is needed to test that.

Immune tolerance appears to be critical for avoidance of IDILI [116], so biomarkers of tolerance may be useful to determine if modest changes in clinical liver test values portend severe IDILI. Unfortunately, no such biomarkers are currently available, but this could be an important area of emphasis for future research.

6.5 Other biomarkers

Several other biomarkers have been proposed and/or tested in DILI, including argininosuccinate synthetase [117], paraoxonase 1 (PON1) [73,78], glutathione-S-transferase α (GST α) [78,118,119], liver-type fatty acid binding protein 1 (FABP1) [73,120], cadherin 5 [73,120], macrophage colony stimulating factor receptor (M-CSFR) [73,121], aldolase B [122] and many more. To date, these additional markers have either not been thoroughly tested for use in DILI, or they have, and their performance is no better than the markers described above. In the future, a few of these biomarkers may provide additional mechanistic insight in some cases of DILI. For example, M-CSFR may be a biomarker of inflammation. However, at the moment, the mechanistic significance of these markers in the context of DILI has not been well tested.

7. Molecular diagnostics

The advent of genome-wide association studies (GWAS) has facilitated discovery of numerous genetic associations with IDILI (Table 1). Not surprisingly, a number of HLA variants are among them [62,123]. HLA variants have been suspected to be important in DILI for several decades [124–127], but the difficulty of identifying DILI patients and acquiring enough samples to achieve necessary statistical power prevented discovery of any firm associations until the 1990s. Perhaps one of the earliest widely accepted associations was with amoxicillin-clavulanate hepatotoxicity [128]. Since then, a number of others have been identified [64,129–156]. Currently, however, the only HLA testing that is routinely performed in clinical laboratories to check for risk of DILI before administration of a specific drug is PCR for HLA-B*5701 in candidates for abacavir treatment, which by itself has positive predictive values reported to be in the range of 50 to 80% [129,141]. However, the high predictive values achieved with that test are possible because DILI is not the only immunoallergic reaction and the percentage of patients exposed to abacavir who have one or more adverse reactions is relatively high. It should also be noted that for some drugs, variation in other genes (e.g. phase I and II enzymes) likely also plays a role in DILI [142–144]. One example is Gilbert's syndrome patients who have a gene mutation in glucuronosyl transferase 1A1 (UGT1A1), which results in a 70–80% reduction in its enzyme activity affecting mainly bilirubin conjugation [144]. Although glucuronidation of APAP is critical to limit toxicity, especially since glucuronidation is not saturated even after an overdose [145], UGT1A1 does not metabolize APAP. However, a subset of Gilbert's syndrome patients has additional mutations of UGT1A6 and 1A7, which are responsible for APAP glucuronidation [144]; these patients have a higher risk for increased APAP toxicity after an overdose [146]. Furthermore, it has been known for many years that slow acetylators may be at increased risk of isoniazid hepatotoxicity [147], and tests for *NAT2* variants are available for clinical use. Still, no genetic tests specifically for DILI risk have been widely adopted for

clinical use due to the low prevalence of DILI for most drugs that cause it and because of low prevalence of the associated HLA variants even among patients who do develop IDILI.

8. Expert commentary

Biomarkers show promise for several uses in DILI. However, one of the major obstacles to routine use of DILI biomarkers by both clinicians and regulators is the low prevalence of DILI. When thinking about biomarkers specifically for IDILI, it is important to recall the basic concept of predictive values. Positive predictive value (PPV) is the probability that a patient with a positive test result (above some pre-specified cutoff) has the condition for which they are being tested, while negative predictive value (NPV) is the probability that a patient with a negative test result does not have the condition. These predictive values depend upon the sensitivity and specificity of the biomarker, and on the prevalence of the condition in a given population. As mentioned, an often-cited figure for prevalence of DILI among users of a given DILI-causing drug is 1 in 10,000 (or 0.01%). Using that number, we can look at a plot of predictive values versus prevalence (Fig. 2) and immediately see that there will likely never be a useful biomarker for the identification of IDILI. Even for a nearly perfect marker with 95% sensitivity and 95% specificity, the PPV is less than 20%. For more realistic values of sensitivity and specificity (90%), the PPV drops below 10%. Thus, most positive test results will actually be false positives when testing for IDILI. PPV of 10–20% may be better than flipping a coin (sensitivity and specificity of 50%), but it is clearly not enough to confidently make a diagnosis in the clinic. For regulatory personnel, this means that most drugs that are terminated during pre-clinical or early clinical trials due to a high serum biomarker value would actually be perfectly safe for the liver (which is likely the current situation with use of “Hy’s Law” in regulatory decision making). It may be tempting to point out that the NPV is very high in our example, so a biomarker could be used to rule out IDILI. However, it is already known that IDILI is rare and therefore unlikely. Thus, a negative result would only reaffirm what we already know. It has already been pointed out by others that the problem of prevalence is compounded when considering genetic DILI biomarkers, such as HLA variants [62]. Not only is IDILI itself rare, even within a population that has suffered IDILI due to a given drug the prevalence of those variants is low. Nevertheless, if a genetic marker with very high NPV could be used to avoid overutilization of laboratory testing by reducing regular monitoring of liver function tests in a significant portion of patients receiving a drug known to cause IDILI, that could be beneficial.

Even when considering the specific case of serum biomarkers to predict later injury in early-presenting APAP overdose patients, prevalence is limiting. Prospective studies suggest that only about 10–30% of patients who present to a hospital and are suspected of APAP overdose actually develop liver injury as indicated by elevated ALT [71,72]. Looking at the graph again (Fig. 2), it should be obvious that current biomarkers like miR-122, which had PPV of 70% in one cohort of a recent study [72], are already approaching the highest possible PPV for acute injury in APAP overdose patients.

Lack of specificity for the liver is another problem with use of existing DILI biomarkers. While miR-122 and GLDH are relatively liver-specific, the standard liver function tests (e.g.

ALT, AST, bilirubin) as well as the novel DILI biomarkers K18, HMGB1, OPN, and most others are not. That could lead to problems in clinical care or in early drug trials; the clinician or regulator must determine if the cause of the biomarker elevation(s) is DILI, another adverse reaction, or a condition entirely unrelated to the drug. Differentiating between liver injury and muscle damage is one application that has been proposed for GLDH as a DILI biomarker [73,77]. GLDH has even been used clinically for that purpose, though in our experience that is not common.

9. Five-year view

In our opinion, the plausible clinical, regulatory and research uses of DILI serum biomarkers are 1) prediction, or at least rule-out, of later injury in APAP overdose patients presenting with liver function test values in the normal range; 2) prediction of either adaptation (e.g. immune tolerance in IDILI) or repair/regeneration in patients with DILI; and 3) investigation of DILI mechanisms. Based on the available data, the most promising biomarkers to rule out later liver injury in early-presenting APAP overdose patients are miR-122, total K18 and total HMGB1, while miR-122 may also be somewhat useful to predict it [72]. To our knowledge, a cost-effectiveness analysis has never been carried out for clinical use of DILI biomarkers, but it is possible that in the future screening patients with a rule-out test would be beneficial. For example, discharging patients with low circulating miR-122 who present to the emergency department with suspected APAP overdose but normal liver function test results may avoid prolonged and unnecessary monitoring of many patients. However, a faster and more standardized approach to miR-122 measurement would be needed to make that possible. As of yet, no biomarkers perform particularly well for prediction of adaptation or survival, though recent research into markers of liver regeneration after both intrinsic and IDILI shows promise [73,115]. Also, several mechanistic biomarkers have already been described to explore mitochondrial damage, mode of cell death and inflammation, and more will likely be developed in the future. Finally, while genetic biomarkers specifically for DILI are unlikely to find clinical use, they will continue to help shed light on DILI mechanisms, and genetic associations with adverse reactions in general – as in the case of abacavir – will continue to be important in the clinic.

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10. Key issues

- Many novel biomarkers of DILI have been introduced
- Serum microRNA-122 can predict injury at presentation
- Serum biomarkers of regeneration may predict survival after injury
- Numerous genetic biomarkers of idiosyncratic DILI have been described
- Biomarkers of adaptation / immune tolerance should be developed
- The utility of all biomarkers depends upon disease or outcome prevalence

Figure 1. Serum biomarkers of DILI.

Promising biomarkers of DILI include mechanistic biomarkers (necrosis: HMGB1, K18, miR-122; Inflammation: acHMGB1; mitochondrial damage: GLDH, mtDNA, acylcarnitines; apoptosis: caspase cleaved K18), markers for early prediction of acute injury, and markers of repair and survival/death.

Figure 2: Plot of predictive values vs. prevalence.

Solid lines show positive predictive values and dashed lines show negative predictive values over a range of prevalence at set values of sensitivity and specificity. Each line represents a specific combination of prevalence of equal sensitivity and specificity from 0.8 to 0.95, as shown in the figure legend. For example, the line with the darkest circles shows predictive values vs. prevalence when sensitivity and specificity both equal 0.95 (or 95%). (A) Full range of prevalence. (B) Zoomed in on the range of prevalence relevant to drug-induced liver injury.

Darkened area shows the commonly cited prevalence of idiosyncratic DILI (1 in 10,000 or 0.01%).

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Table 1.

Major or recent genetic associations with IDILI.

Drug	Variant	Reference
HLA genotypes		
Abacavir	B*57:01	[129]
Amoxicillin/clavulanate	A*02:01 DRB1*15:01-DQB1*06:02	[128;132;135]
Flucloxacillin	B*57:01 DRB1*07:01-DQB1*03:03	[131,132]
Flupirtine	DRB1*16:01-DQB1*05:02	[140]
Minocycline	B*35:02	[138]
Terbenafine	A*33:01	[139]
Ticlopidine	A*33:03	[130]
Anti-tubercular drugs	DQB1*02:01	[148]
Lapatinib	DRB1*07:01-DQB1*03:03	[134]
Nevirapine	DRB1*01:02	[149]
Ximelagatran	DRB1*07-DQA1*02	[150]
Drug metabolizing enzymes/transporters		
Bosentan	CYP2C9*2	[151]
Diclofenac	UGT2B7, ABCC2	[152]
Efavirenz	CYP2B6*6	[153]
Isoniazid	CYP2E1*C2, NAT2	[154]
Nevirapine	ABCB1	[155]
Other		
Interferon- β	IRF6	[156]