

Drug-induced liver injury: the dawn of biomarkers?

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Abstract

Drug-induced liver injury (DILI) is a potentially fatal adverse event with significant medical and economic impact. Many drugs, especially anti-infective, neurologic or pain-modifying substances, act as hepatotoxins. With cardiovascular toxicity, liver toxicity is one of the two leading causes for drug withdrawal from the market. The liver can be affected directly, in a predictable and dose-dependent manner, or idiosyncratically, independent of the dose and therefore unpredictable.

Currently DILI is a diagnosis of exclusion that physicians have to bear in mind in patients with an unexplained increase of liver enzymes. The type of injury is categorized into hepatocellular, cholestatic, or mixed by the respective enzyme pattern of injury. Symptoms of affected patients can mimic any other liver disease.

Therefore, new diagnostic and prognostic biomarkers for early liver injury are currently being evaluated in multi-centre clinical trials that are conducted by international consortia and other initiatives. Pharmacogenetic testing, next-generation sequencing, proteomics, metabolomics and mechanistic markers can help to preselect susceptible patient populations and tailor drug therapy to individual patients. Proposed DILI indicators that are under investigation include microRNAs, cytokeratin-18 (CK18), high mobility group box protein 1 (HMGB-1), and several other biomarkers. These developments can change clinical practice, and improve patients' safety and management. However, they have not been translated into clinical practice or approved for routine use yet. Management of DILI usually consists of initial withdrawal of the suspected drug and—if applicable—administration of specific antidotes, such as N-acetylcysteine. However, the overall management of DILI could change in the near future with the advent of novel diagnostic and prognostic DILI markers.

Introduction

DILI is a potentially fatal adverse event. It is the most frequent cause of acute liver failure when acetaminophen overdose is included and is therefore medically relevant, exhibiting a high morbidity and mortality [1]. It is also of economic importance and represents the second most frequent cause for drug withdrawal from the pharmaceutical market.

Many agents, for example, anti-infectives, neurologic, cholesterol-lowering or pain-modifying substances, can act as hepatotoxins [2] (Table 1). They can either damage the liver directly in a dose-dependent and therefore predictable fashion, or idiosyncratically by immunologic

mechanisms or metabolic activation independent of the dose. The latter is unpredictable, but unfortunately the more common event, if serious liver injury caused by acetaminophen/paracetamol (APAP) overdose is not accounted for. Symptoms of affected patients are non-specific and include nausea, discomfort in the right upper abdominal quadrant, dark urine, or fatigue.

To substantiate the relationship between an administered drug and liver injury, other causes of acute hepatocellular injury need to be excluded. These comprise viral hepatitis (hepatitis A virus [HAV], hepatitis B virus [HBV], hepatitis C virus [HCV], hepatitis E virus [HEV], Epstein-Barr virus [EBV], cytomegalovirus [CMV]),

Table 1. Liver injury patterns and examples of hepatotoxic drugs

Type of liver injury	Hepatocellular	Mixed	Cholestatic
	Injury predominantly to hepatocytes	Hepatocellular and cholestatic injury	Injury to bile ducts or affecting bile flow
R-Value [$R = \frac{ALT/U_{LN_ALT}}{ALP/U_{LN_ALP}}$]	≥5	>2 and <5	≤2
Examples Drug Classes			
Antiinfectives	Ciprofloxacin, isoniazid, rifampicin, tetracyclines, ketoconazole	Amoxicillin/clavulanic acid, clindamycin, erythromycin, nitrofurantoin, sulfonamides, cotrimoxazole	Amoxicillin/clavulanic acid, erythromycin
Pain medication	Paracetamol (Acetaminophen), Aspirin, NSAIDs		
Hormones		Anabolic steroids	Anabolic steroids, estrogen, oral contraceptives
Cardiovascular and metabolic drugs	Lisinopril, losartan, statins, allopurinol	Enalapril, verapamil	Clopidogrel, irbesartan
Neurologic/Psychiatric drugs	Valproic acid	Carbamazepine, phenytoin	Chlorpromazine, tricyclic antidepressants

ALT, alanine aminotransferase; ALP, alkaline phosphatase; R, ratio; ULN, upper limit normal.

ischemic liver injury, autoimmune hepatitis, Budd-Chiari syndrome, Wilson's disease and, in cases of cholestatic liver injury, other causes of extrahepatic or intrahepatic cholestasis, such as choledocholithiasis, malignancy, primary biliary cirrhosis, or sclerosing cholangitis. After exclusion of these differential diagnoses, physicians have to consider DILI in patients with an unexplained increase in liver enzymes [3,4]. Currently DILI is categorized into hepatocellular (R-value $[\text{ALT}/\text{ULN} \div \text{ALP}/\text{ULN}]$ [alanine aminotransferase/upper limit of normal \div alkaline phosphatase/upper limit of normal] ≥ 5), cholestatic (R-value ≤ 2), or mixed-type injury (R-value 2-5) by the respective enzyme pattern in blood (Table 1). The temporal relation to the administration of a potential hepatotoxin and the exclusion of other differential causes are the cornerstones for establishing a diagnosis of DILI. For causality assessment, different tools such as the RUCAM or Maria and Victorino score are available [5–7].

However, new diagnostic biomarkers for liver injury are currently under investigation in various mechanistic and pre-clinical models and also in large clinical trials.

Diagnosing DILI: future biomarkers

Several consortia are dedicated to evaluating new biomarkers in the diagnosis and management of DILI. Although the objectives of the various endeavours differ, the translation of preclinical mechanism-based findings into clinical liver safety assessment offers a unique opportunity to move the field forward.

The US Drug-Induced Liver Injury Network (DILIN) recruits patients with idiosyncratic DILI in both

retrospective and prospective study registries. The international Drug-Induced Liver Injury Consortium (iDILIC) is a multinational scientific group coordinated by the University of Newcastle and University of Nottingham [8]. Genetic associations with DILI are investigated in different centres in Europe and the US. Predicting DILI by genetic risk factors and identifying potential hepatotoxins before they lead to severe liver injury are the primary goals of investigation. For this purpose, DNA isolated from patients with DILI is analysed, for example, in genome-wide association studies (GWAS) [9]. Genetic associations have been shown for flucloxacillin-induced DILI and the human leukocyte antigen (HLA)-B*5701 allele [10,11]. With this predisposition, an 80-fold increase in the risk of developing DILI was attributed, resulting in an absolute risk of 1:500–1:1000. Pharmacogenetic testing of HLA-B*5701 is mandatory in patients scheduled to receive abacavir to prevent hypersensitivity [12,13]. Donaldson and co-workers [14] identified HLA class II genotypes in association with amoxicillin/clavulanate associated DILI. For lumiracoxib, a cyclooxygenase-2 (COX-2) selective inhibitor, HLA-DR and HLA-DQ genotypes were found to be predictive for DILI [15,16]. This drug was withdrawn from the market because of hepatotoxicity. Lapatinib, a tyrosine kinase inhibitor, has been associated with DILI in carriers of the HLA allele DQA*02:01 [17,18]. An association between DILI caused by ximelagatran, a direct thrombin inhibitor, and HLA-DRB1*0701 and HLA-DQA1*02 was reported [19]. In the case of ALT elevations caused by ximelagatran, colony-stimulating factor 1 receptor (CSF1R), the receptor for the respective cytokine, was increased significantly in plasma [20], leading to a new quest for early identification tools.

The SAFE-T Consortium (Safer and Faster Evidence-based Translation) is focused on the identification of new biomarkers for DILI [21]. Funded by the Innovative Medicines Initiative (IMI) the consortium aims to identify new tools to predict, detect and monitor drug-induced organ toxicity in a more specific, sensitive and predictive manner. Academic centres, pharmaceutical companies and small- to medium-sized enterprises collaborate to promote a personalized medicine approach in the prediction and management of DILI. Toxicities to other organs of interest are analysed in drug-induced kidney injury (DIKI) and drug-induced vascular injury (DIVI) work packages. Another IMI project, called MIP-DILI (Mechanism-Based Integrated Systems for the Prediction of Drug-Induced Liver Injury), focuses on the development of preclinical test systems that integrate multiple preclinical data types to improve prediction of DILI in man.

The Predictive Safety Testing Consortium (PSTC) was created by the Critical Path Institute and aims to qualify new biomarkers for the detection and monitoring of drug-induced toxicity in preclinical and clinical studies [22,23]. In this respect, PSTC is the preclinical counterpart of the SAFE-T consortium, since both aim to qualify biomarkers that allow the separation of patients with a self-limiting course of DILI from those that progress to severe DILI and ultimately liver failure. This prognostic assessment is currently performed by use of Hy's law that predicts a 10% risk of fatality in cases in which a 3-fold elevation of ALT above the upper limit of normal (ULN) is accompanied by a 2-fold elevation of bilirubin [24,25]. The elevation of bilirubin in these cases is the sequel of progressive hepatocellular death. For the rapid identification of potential cases of Hy's law, the U.S. Food and Drug Administration (FDA) introduced the eDISH program (evaluation of Drug-Induced Serious Hepatotoxicity) in 2004, which has since been implemented to assess DILI in clinical trials [26].

New biomarkers should improve patient safety and also reduce drug attrition due to toxicity. Further investigated and proposed markers for DILI include liver injury markers such as microRNAs (miRNA), mechanistic biomarkers such as HMGB-1 and Cytokeratin-18 fragments, and metabolites from urine and serum [27]. Circulating serum miRNAs such as miR-122 and miR-192 are liver-specific [28,29] and are proposed as potentially sensitive and specific markers of liver injury [27]. With a short half-life they are released into plasma in acute and chronic liver injury [30]. HMGB-1 is a molecular pattern protein marking necrosis: in the acetylated form it indicates immune activation [31]. CK18 and miRNA-122 were evaluated as superior

biomarkers compared to ALT in terms of sensitivity and specificity in DILI [32]. CK18 together with HMGB-1 point towards the mechanism of hepatocellular death in APAP overdose, notably the degree of necrosis vs. apoptosis [31,33]. The performance of combinations of biomarkers is likely to surpass that of a single biomarker alone, depending on the context of its use. Thus, the balance between apoptosis and necrosis may be indicative of the subsequent course the patient will follow, given that the liver is unique in its ability to activate defence mechanisms against toxic injury.

Idiosyncratic DILI is usually detected in a late stage of drug development—often after drug approval in phase IV studies or pharmacovigilance surveillance. An understanding of the pathophysiologic processes in this type of hepatotoxicity is of special interest to both pharmaceutical companies and regulatory authorities. In cases of APAP hepatotoxicity, levels of mitochondrial and nuclear DNA could differentiate patients who developed acute liver failure, but subsequently recovered, from those who died [34]. These mechanistic markers could provide further insight into the pathophysiological mechanisms of DILI, allowing early detection and outcome prediction. *In vitro* generated hepatocytes derived from skin biopsies of patients with an idiosyncratic reaction could be used to study the unique features of the affected individuals' hepatocytes, as well as the genotype-phenotype correlation in DILI [35]. These new techniques and models hold great promise to improve drug safety—with back-translation from bedside to bench.

Management and outlook

Until now, the management of DILI has usually consisted of initial withdrawal of the suspected drug. Targeted therapy with antidotes, such as N-acetylcysteine in paracetamol (acetaminophen) overdose, is possible in defined cases. Re-challenge with the causative agent can lead to an augmented adverse effect, especially in immunologically mediated DILI, and is therefore not recommended. Preventive or prophylactic measures against DILI include patients' education and alerting health care professionals towards liver enzyme monitoring under therapy with a potentially hepatotoxic drug. Pharmacogenetic tests, including next-generation sequencing, proteomic or metabolomic approaches for preselecting susceptible patient populations, and tailoring drug therapy to individual patients, have not yet been approved for routine clinical practice. To what extent these new markers will change clinical practice for the prevention of DILI remains to be seen. Ongoing efforts aim to overcome the gap between bench and bedside towards the early detection of DILI and the identification

of patients at risk. Important results from major consortia such as DILIN, SAFE-T, MIP-DILI, PSTC and iDILIC can be expected in 2015.










Abbreviations

ALT, alanine aminotransferase; APAP, acetaminophen/paracetamol; CK18, cytokeratin-18; COX-2, cyclooxygenase-2; DILI, drug-induced liver injury; DILIN, Drug-Induced Liver Injury Network; GWAS, genome-wide association studies; HLA, human leukocyte antigen; iDILIC, International Drug-Induced Liver Injury Consortium; IMI, Innovative Medicines Initiative; MIP-DILI, Mechanism-Based Integrated Systems for the Prediction of Drug-Induced Liver Injury; miRNA, microRNA; PSTC, Predictive Safety Testing Consortium; SAFE-T, Safer and Faster Evidence-based Translation; ULN, upper limit of normal.

Disclosures

The authors declare that they have no disclosures.

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