



Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Adverse drug reactions and organ damage: The liver

Anna Licata*

Sezione di Gastroenterologia & Epatologia, Dipartimento di Medicina Interna e Specialistica, DiBiMIS, Università di Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy

ARTICLE INFO

Article history:

Received 18 December 2015

Accepted 21 December 2015

Available online xxx

Keywords:

Drug induced liver injury

Epidemiology

Risk factor

Clinical presentation

Diagnosis

Biomarkers

ABSTRACT

Drug-induced liver injury (DILI) is among the most challenging acute or chronic liver conditions to be handled by physicians. Despite its low incidence in the general population, DILI is a frequent cause of acute liver failure. As such, the possibility of DILI should be considered in all patients who present with acute liver damage, independent of any known pre-existing liver disease. DILI can be classified as intrinsic/dose-dependent (e.g., acetaminophen toxicity) or idiosyncratic/dose-independent, with the latter form being relatively uncommon. Amoxicillin-clavulanate is the antimicrobial that is most frequently associated with idiosyncratic DILI. Large, ongoing, prospective studies in western countries have reported other drugs associated with DILI, including nonsteroidal anti-inflammatory drugs, statins, and herbal and dietary supplements. An important safety issue, DILI is one of the most frequently cited reasons for cessation of drug development during or after preclinical studies and for withdrawal of a drug from the market. This review summarizes the epidemiology, risk factors, commonly implicated drugs, clinical features, and diagnosis of DILI, with the aim of aiding physicians in the management of this debated problem. Old and new biomarkers for DILI and pharmacogenetic studies are also described.

© 2016 European Federation of Internal Medicine. Published by Elsevier B.V. All rights reserved.

1. Introduction

Drug-induced liver injury (DILI) encompasses adverse drug reactions (ADRs) involving the liver after the administration of a xenobiotic, such a drug or herbal and/or dietary supplement (HDS), at the usual dose interval [1]. Theoretically, most drugs are capable of inducing DILI, owing to the liver's pivotal role in drug metabolism.

The two main categories of DILI are intrinsic/dose-dependent and idiosyncratic/dose-independent, with the latter form being relatively uncommon. Liver injuries due to acetaminophen (N-acetyl-p-aminophenol [APAP]) are typically associated with an excessive dose. APAP overdose is a frequent cause of acute liver failure (ALF) requiring liver transplantation [2,3]. Most cases of DILI are due to unpredictable reactions and, traditionally, were believed to be dose-independent. However, drugs with a daily dose of less than 50 mg are very rarely

associated with DILI, as demonstrated independently by two different research groups [4,5]. Thus, many drugs that were previously associated with idiosyncratic DILI are now considered to act in a dose-dependent manner. Nevertheless, the possibility of idiosyncratic DILI should always be considered in a patient with acute or chronic liver damage, independent of a possible underlying liver condition (e.g., non-alcoholic steatohepatitis or non-alcoholic fatty liver disease). As the diagnosis of DILI remains one of exclusion, DILI is one of the most challenging disorders to be managed by physicians and hepatologists [6].

Many drugs have been associated with DILI. Antibiotics and antimicrobials accounted for more than 46% of all DILI cases in the Drug-Induced Liver Injury Network (DILIN) cohort from the United States [7]. Among antibiotics, amoxicillin-clavulanate (AMC) was the most frequently implicated agent, a finding confirmed in prospective DILI studies in Iceland and Spain [8,9]. Non-steroidal anti-inflammatory drugs (NSAIDs), statins, and HDS can also lead to DILI. In recent years, there has been an increase in the number of people who prefer alternative therapies, such as HDS, to commonly used drugs whose safety and efficacy have been demonstrated by controlled clinical studies. For example, the use of HDS has increased to 3.7% in Italy, 20% in the United Kingdom, and 40% in the United States [11,12]. Given the easy availability of HDS via the Internet, people purchase what they deem to be the correct preparation directly, without consulting a physician or pharmacist. Unfortunately, patients, even those previously diagnosed with chronic liver disease, are reluctant to disclose their use of nonconventional therapies [10].

An important safety issue, DILI is one of the most frequently cited reasons for cessation of drug development during or after preclinical

Abbreviations: DILI, Drug-induced liver injury; ADRs, Adverse drug reactions; HDS, Herbal and/or dietary supplement; APAP, N-acetyl-p-aminophenol; ALF, Acute liver failure; NSAIDs, Non-steroidal anti-inflammatory drugs; AMC, Amoxicillin-clavulanate; INH, Isoniazid; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; RUCAM, Roussel Uclaf causality assessment method; DIAIH, Drug-induced autoimmune hepatitis; NAC, N-acetyl cysteine; miRNAs, MicroRNAs; ABC, ATP binding cassette; Th, T helper; IL, Interleukin; MDR-3, Multi drug resistance-3; HLA, Human leukocytes antigen; GWAS, Genome wide association studies; NAT-2, N-acetyltransferase-2; SNPs, Single-nucleotide polymorphisms; SLC01B1, Solute carrier organic anion transporter family member 1B1; OATP1B1, Organic anion transporting polypeptide-1.

* Corresponding author at: Sezione di Gastroenterologia ed Epatologia, DiBiMIS, Università di Palermo, Piazza delle Cliniche 2, 90127, Palermo, Italy. Tel.: +39 091 655 2280; fax: +39 091 6552156.

E-mail address: anna.licata@unipa.it.

<http://dx.doi.org/10.1016/j.ejim.2015.12.017>

0953-6205/© 2016 European Federation of Internal Medicine. Published by Elsevier B.V. All rights reserved.

Please cite this article as: Licata A, Adverse drug reactions and organ damage: The liver, Eur J Intern Med (2016), <http://dx.doi.org/10.1016/j.ejim.2015.12.017>

studies and for withdrawal of a drug from the market [13]. Recent examples of drug withdrawal in the United States and in Europe include troglitazone, bromfenac [14] and others. DILI is the most common cause of ALF in the United States [15] and Europe [16]. To increase awareness of DILI, facilitate clinical management, and improve prognosis, the American College of Gastroenterology developed practice guidelines for the diagnosis and management of idiosyncratic DILI, using an evidence-based approach [6]. Currently, however, there are no guidelines in Europe regarding when to suspect DILI, how to confirm a diagnosis of DILI, or how to manage DILI once it has been recognized. Many researchers in Europe are investigating DILI, and there is a need for a larger European network.

The purpose of this review is to prompt physicians to consider DILI whenever they are managing a patient with an unexplained liver injury. This review should assist physicians in accurately diagnosing DILI and improving outcomes, given that the disorder is sometimes responsible for ALF and occasionally requires liver transplantation. The use of biomarkers and the impact of pharmacogenetic studies are also discussed.

2. Epidemiology and risk factors for idiosyncratic DILI

The clinical diagnosis of DILI is one of exclusion, owing to a lack of reliable and confirmatory tests. Accordingly, it is difficult to establish the exact burden of DILI on the healthcare system. Registries of idiosyncratic DILI cases have been established in different Western [8, 9,17,18] and Asian [19] countries. Registry data have greatly improved knowledge regarding the etiologies, pathogenic mechanisms, and clinical outcomes of DILI [20]. Patients enrolled in DILI registries are usually middle-aged women (mean age: 50 years) who require hospitalization. The clinical presentation is most often consistent with hepatocellular liver damage, followed by a cholestatic or mixed presentation. In population-based studies, the crude annual incidence of DILI has been reported as 19.1 cases per 100,000 inhabitants in Iceland [8], and 13.9 cases per 100,000 inhabitants in France [18].

Some routinely used drugs are more hepatotoxic than others. AMC, which has been associated with cholestatic liver injury, is the most frequently prescribed antimicrobial agent worldwide, with more than 70 million prescriptions each year in the United States alone. In a study of DILI in an Icelandic population, AMC was the most frequently implicated agent, with an estimated DILI risk of 1 case per 2350 AMC users. Using data from the DILIN database, Fontana [20] showed that AMC was responsible for almost 120 cases of DILI each year. In contrast, isoniazid (INH), with fewer than 200,000 prescriptions per year, was responsible for 50 DILI cases per year. Therefore, INH seems to be more hepatotoxic than AMC. Patients treated with INH should have their aminotransferase levels checked after 15 days of therapy and monthly thereafter. An elevation of less than five-fold above the upper limit of normal (ULN) is typical and will often normalize without discontinuation of the drug. By contrast, in DILI registries such drugs as beta-blockers or calcium channel blockers, despite their large use among patients suffering from cardiovascular disease, are poorly responsible of DILI, probably due to their scarce hepatotoxicity. Statins, although rarely showed idiosyncratic liver injury, sometimes can be associated with severe outcome [13,21].

Multifactorial mechanisms appear to underlie DILI [22]. Drug-related risk factors include the drug's chemical features, dose, route, and duration of administration. Host-related risk factors include the patient's age, sex, genetics, previous episodes of DILI, and underlying chronic liver disease. Environmental risk factors include the patient's metabolic features (e.g., obesity), diet type, alcohol, coffee, and tobacco consumption, multidrug therapy, immune state (e.g., immunocompromised), and nutritional status [20,22,23] (Fig. 1). According to the international literature, women develop DILI more often than men, perhaps because women are the main consumers of herbal products. Polypharmacy is a frequent cause of ADRs and longer

hospital stays in the elderly [24]. Aging results in a broad range of physiological changes that decrease the tolerability profile of drugs. In addition, cognitive disorders may worsen compliance to with multidrug therapy [25].

3. Clinical features and diagnosis of DILI: causality assessment systems

Clinically, DILI represents a wide spectrum of diseases with diverse biochemical and histologic features. A clinical picture resembling acute hepatitis is the most common clinical presentation of acute liver disease of all etiologies and is pathognomonic of DILI. Other clinical features of hepatotoxicity may also be present, such as cholestatic hepatitis, nodular regeneration, cirrhosis, non-alcoholic fatty liver disease, veno-occlusive disease, and vanishing bile duct [20]. Liver biopsy may support the clinical diagnosis when typical histopathologic findings are detected [26]. However, histologic evaluation is infrequently performed in favor of Fibroscan[®], and DILI patterns are often defined through biochemical data alone. Due to the lack of pathognomonic features or specific diagnostic criteria, there is no consensus regarding adequate terminology for defining the various clinical patterns of DILI.

Consensus criteria for a diagnosis of DILI [27] include a chronological relationship between drug intake and hepatitis onset, as well as the presence of any one of the following conditions: an alanine aminotransferase (ALT) level of more than five-fold above the ULN; an alkaline phosphatase (ALP) level of more than two-fold above the ULN; or an ALT level of more than three-fold above the ULN, with the simultaneous elevation of bilirubin levels to more than two-fold above the ULN. The liver injury pattern can be assessed by the R value, where $R = (ALT/ULN)/(ALP/ULN)$, with $R \geq 5$ reflecting a hepatocellular pattern, $2 < R < 5$ reflecting a mixed pattern, and $R \leq 2$ reflecting a cholestatic pattern of liver injury.

Although many patients with mild liver damage are diagnosed in the outpatient clinic, those with severe liver injury commonly require hospitalization and, occasionally, intensive care as a bridge to liver transplantation. The hepatocellular pattern of liver injury exposes patients to a risk of ALF. Hy's rule [28] predicts a mean rate of mortality (or liver transplantation, as a surrogate marker) of 10% for jaundiced patients with acute hepatocellular damage. This rule, originally observed by Zimmerman, was recently confirmed in at least two studies [9,21]. These studies identified additional variables, such as older age, female sex, and AST levels, to be independently associated with poor outcome. APAP, halothane, and cocaine toxicity, as well as mushroom poisoning, typically result in acute or subacute liver failure. Toxicity by NSAIDs, such as diclofenac and nimesulide, results in massive necrosis.

Patients with cholestatic liver damage usually present with jaundice and itching. The canalicular pattern is characterized by an increase in conjugated bilirubin, ALP, and γ -glutamyltranspeptidase levels, with minimal to no elevation of aminotransferase levels. Steroids typically produce this pattern of hepatotoxicity. The mixed pattern of liver damage resembles features of hepatocanalicular damage, recalling acute biliary obstruction. Hypersensitivity features sometimes occur and are important clues to aid in the diagnosis. Typical examples of drugs that cause mixed liver damage are AMC, macrolides, neuroleptics, and similar compounds [29].

From a nosographic perspective, there is a propensity to try to distinguish the various patterns of liver damage and to associate specific drugs with each pattern. However, it is not always possible to make such associations. The clinical pattern of hepatotoxicity may vary depending on the interaction between drug factors (e.g., dose, bioavailability, and duration of treatment) and host factors (e.g., age, sex, and drug absorption). For example, AMC frequently causes acute cholestasis or cholestatic hepatitis in men over 65 years old, whereas a mixed pattern is more likely in younger patients [7]. Nimesulide, an NSAID that is commonly used for osteoarticular pain relief, can cause hepatocellular damage in young women, sometimes leading to ALF requiring

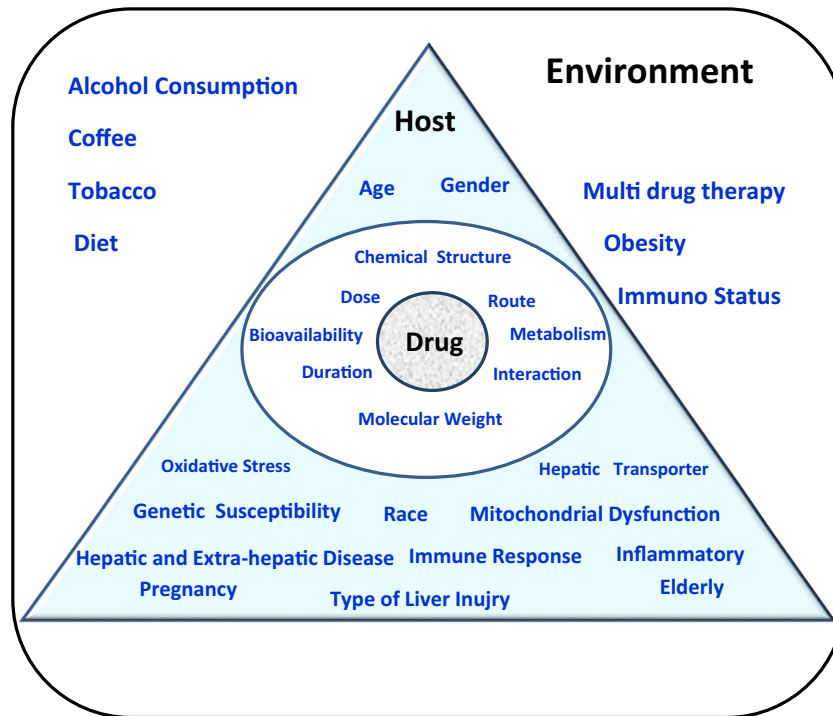


Fig. 1. Pathogenesis of DILI is multifactorial and depends on complex interplay made by the interaction among drug, host and environmental factors. The multi-drug therapy and the immune compromised status are features mainly characterizing the elderly, which consequently become an “at risk condition” for ADRs.

liver transplantation. In men, however, cholestatic liver damage is more frequently observed [30] (Fig. 2).

DILI is largely diagnosed through a detailed clinical history, biochemical tests, hepatobiliary imaging, and liver biopsy [6]. Some diagnostic algorithms are available, such as the Roussel Uclaf causality assessment method (RUCAM) [31,32] and the Maria & Victorino assessment method. RUCAM scores employ the temporal relationship between drug intake and clinical presentation (latency), the clinical course after drug discontinuation (dechallenge), and the response upon rechallenge. The Maria & Victorino assessment score [33] subsequently published adds to the parameters the exclusion of alternative causes of liver injury and the coexistence of extrahepatic and immune-allergic manifestations (e.g., fever, rash, arthralgia, eosinophilia). Hepatotoxicity causality assessment scores show low concordance

with each other, because they assign different weights to each criterion. The clinical utility of RUCAM results from the topics that it requires the clinician to address in cases of suspected hepatotoxicity, which improve the consistency of clinical judgment [34].

4. Drugs frequently involved in DILI

Almost any class of drug can be involved in idiosyncratic DILI. Nonetheless, in the United States, antimicrobials and antidepressant/antipsychotics are the most commonly implicated drug classes, followed by NSAIDs, antiplatelet agents, statins, and HDS [6]. Since 2000, we have followed a prospective cohort of consecutive DILI patients at our tertiary referral center. This cohort primarily consists of women (54%), with a mean age of 54 years. We reported that almost 25% of patients in the

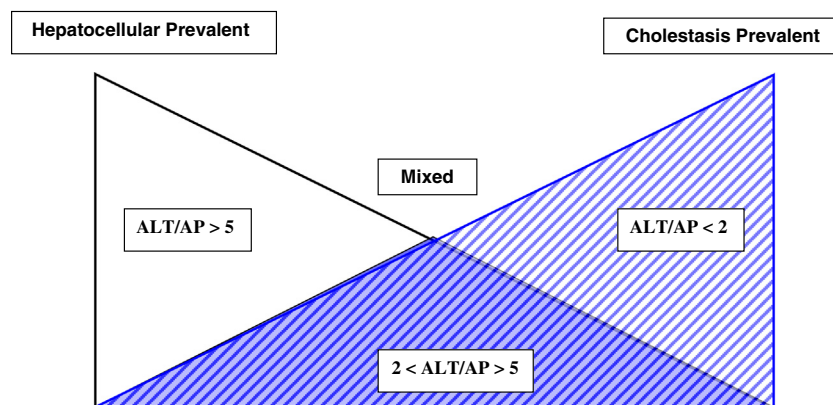


Fig. 2. Pattern of liver damage is not always the same with a given drug. For example, AMC frequently causes acute cholestasis or cholestatic hepatitis in men over 65 years old, whereas a mixed pattern is more likely in younger. Nimesulide causes hepatocellular damage in young women, sometimes leading to ALF requiring liver transplantation. In men cholestatic liver damage is more frequently observed.

Table 1
Clinical features of 157 patients with drug induced liver injury seen at our tertiary referral center: the impact of NSAIDs.

	NSAIDs (n = 61)	Other drugs (n = 96)	p
Age (years)	48.1 ± 18.4	55.1 ± 17.6	0.02
Duration of drug intake (days)	11.2 ± 24.6	60.1 ± 114.2	<0.001
Latency (weeks)	29.2 ± 16.2	57.1 ± 90.6	0.02
Hepatic encephalopathy at diagnosis	5 (8.2%)	1 (1.1%)	0.02
Pattern of DILI			
Hepatocellular	42 (68.8%)	41 (42.7%)	0.006
Cholestatic	11 (18.0%)	32 (33.3%)	
Mixed	8 (13.2%)	23 (24.0%)	
ALT (IU/L)	744.2 ± 755.7	427.6 ± 512.2	0.02
Eosinophils (%)	1.9 ± 1.9	3.1 ± 4.2	0.02
Stiffness (KPa)	9.4 ± 6.6	11.3 ± 8.4	0.6

DILI cohort had preexisting compensated chronic liver disease. A hepatocellular pattern was observed in 53% of cases, followed by cholestatic (27%) and mixed patterns (20%). The most frequently involved drugs were NSAIDs (~40%), followed by antibiotics, immunosuppressants, antiplatelet agents, antidiabetic drugs, and statins. In the 24% of DILI cases, two or more drugs were involved [35] (see Table 1 and Fig. 3). Nimesulide was the most commonly implicated NSAID, responsible for hepatocellular DILI in women and the cholestatic/mixed type in men [30,36]. Among antibiotics, we reported the impact of AMC-induced [37] and fluoroquinolone-induced [38] liver injury.

From our prospectively followed DILI cohort, we recently identified a small group of 12 patients who had drug-induced autoimmune hepatitis (DIAIH). Liver biopsy specimens from these patients featured severe portal inflammation and lymphoplasmacytic infiltrates. Despite their briefer drug exposure period compared to DILI patients, DIAIH patients displayed higher aminotransferase and γ -globulin values [39]. These data support the importance of correctly distinguishing between DIAIH and DILI, and emphasize the key role of liver biopsy in the diagnosis, prognosis, and follow-up of affected patients [26,39].

5. APAP-induced liver injury

APAP-induced hepatotoxicity represents one of the best examples of predictive DILI, causing rapid liver injury in the centrilobular region [14]. Liver damage can be intentional (e.g., attempted suicide) or accidental (e.g., inadvertent drug intake in excess of the intended dose over several days). APAP is widely used as an analgesic and is available in many nonprescription and prescription products, including in combination with opioids [40]. For this reason, APAP overdose is the most common cause of ALF and death due to analgesics [41,42] in Anglo-Saxon countries, with over 500 deaths per year in the United States.

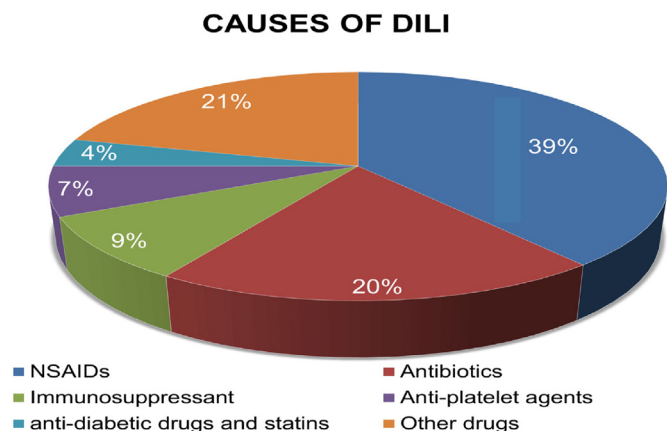


Fig. 3. Drugs commonly involved as causes of drug induced liver injury in a cohort of 157 patients prospectively followed at our tertiary referral center.

The inappropriate use of APAP results in 78,000 visits to the emergency room annually, at an annual cost of more than \$86 million [43,44].

Patients presenting to the emergency room with a report of APAP ingestion are assessed for treatment with N-acetyl cysteine (NAC), which is effective in most cases if given early. Onset of liver damage can only be prevented within about 8 to 10 h of APAP ingestion. Thus, the time to NAC administration is the most relevant factor in preventing injury by APAP. A careful medical history, clarifying the quantity ingested, the blood concentration of APAP, and the extreme values of ALT (up to 3000 UI/L), help clinicians to differentiate between APAP poisoning and other causes of ALF (e.g., viral hepatitis, ischemia) [14]. There are limitations to the use of ALT as a biomarker for assessing patient status after APAP ingestion. Although serum ALT levels are elevated as a rule, they may take more than 72 h to peak. For the best outcome, NAC should be administered as soon as possible after APAP ingestion, while promptly diagnosing the patient.

Recently, Ward et al. [45] identified a set of 11 small, noncoding microRNAs (miRNAs) whose profiles and dynamics can discriminate APAP hepatotoxicity from ischemic acute hepatitis. Wang et al. [46] demonstrated that, in mice overdosed with APAP, blood levels of miRNA 122 and 192 were increased. The same miRNAs were subsequently detected in human plasma as well [47].

Although dose is a predictor of outcome in patients with APAP toxicity, the actual toxic dose remains unclear [48]. Most international guidelines recommend 200 mg/kg or 10 g as the toxic dose. Currently, the gold standard for starting NAC therapy is a single serum APAP concentration above the nomogram between 4 and 24 h from ingestion [49]. A recently published Australian study on patients presenting with APAP overdose at a tertiary referral center showed that the median dose of APAP ingested was 10 g among 1303 patients (1140 women, median age of 27 years). In 22% of patients, the APAP concentration was above the nomogram line. For many years, guidelines for patients presenting with APAP overdose within 8 h of ingestion recommended a “wait and see” approach while testing the serum APAP concentration. However, current guidelines suggest that more than 90% of patients with a reported dose greater than 50 g will require treatment with NAC. Therefore, NAC should be started immediately in these patients [48].

Finally, ALF due to APAP toxicity has a better overall survival rate (70%) compared to ALF due to DILI by other drugs (58%), despite the initial severity of APAP overdose. This phenomenon could be due to the rapid evolution of the clinical course, which also induces rapid hepatocyte regeneration and, thus, resolution of liver damage [6,15].

6. Pathogenic mechanisms of DILI

After biotransformation by the cytochrome P450 system, a drug can become an active metabolite that is less toxic, more hydrophilic, and ready to be eliminated. Subsequently, UDP-glucuronosyltransferase, sulfotransferase, and glutathione-S-transferase hydrolyze the metabolite. Transport of the metabolite out of hepatocytes is mediated by the ATP binding cassette (ABC) transporter superfamily [50]. In general, drugs can either directly affect hepatocytes/cholangiocytes or elicit an innate or adaptive immune reaction [14]. In most cases bioactivation of a drug leads to a reactive metabolite that causes mitochondrial dysfunction, cytoskeletal breakdown, and cell membrane disruption. In other cases, however, bioactivation can influence the transport of proteins (e.g., MDR-3), resulting in an interruption of bile flow and bilirubin excretion, causing cholestasis [51–53].

As an alternative to direct action, hepatocyte stress results in the activation of the innate immune system through natural killer cells of the liver, which kill cells through the Fas/Fas ligand pathway. Kupffer cells contribute to the progression of liver damage by producing proinflammatory mediators, cytokines, and chemokines, mediating cytotoxicity by degrading the extracellular matrix, and promoting cell adhesion and leukocyte infiltration. This mechanism, commonly known as apoptosis, is considered a non- or low-inflammatory process,

due to the rapid removal of apoptotic cells [54]. The adaptive immune system is also involved in the pathogenesis of liver damage. If an active metabolite is able to act as a hapten and covalently bind host proteins, then the immune system will perceive the metabolite as foreign, triggering an inappropriate T-cell response [55]. This necrotic process is the type of liver damage observed after APAP hepatotoxicity. A third form of cell death, necroptosis, incorporates features of both necrosis and apoptosis [56]. Necroptosis is better characterized in the setting of TNF-induced cell death (e.g., patients with acute hepatocellular DILI), although it may also occur in acutely developing conditions, such as ischemia-reperfusion injury.

7. Biomarkers of DILI

Currently, there are no clearly identified biomarkers of DILI that are useful for monitoring patients on drug therapy for early detection of hepatotoxicity or for making an appropriate diagnosis. Traditionally, ALT, ALP, and total bilirubin levels have been used clinically to identify the various types of DILI. However, patterns of liver injury may be categorized by the pathological conditions and underlying events, such as apoptosis, necrosis, and necroptosis, inflammation, oxidative stress, and immune system activation, all of which contribute to liver injury.

Studies have recently proposed candidate biomarkers for liver injury and mitochondrial dysfunction, all of which are related to the potential mechanisms involved in DILI pathogenesis. Not all of these biomarkers are used clinically [20,54] (Table 2). For example, cytokines T helper (Th) 1-type (IL-12, IFN- γ , IL-2, IL-15) and Th2-type (IL-4, IL-5, IL-13) are usually activated in response to acute immune stimuli. If the inflammatory state does not resolve, then chronic inflammation could evolve into an adaptive immune response, with activation of Th17/Th9 cell types, resulting in a poor prognosis [57]. Recently, miRNA 122 and

miRNA 192 have emerged as liver-specific biomarkers in DILI. Levels of these miRNAs parallel ALT levels during hepatotoxicity, increasing early in the course of liver damage. These miRNAs may have prognostic significance in patients with ALF after APAP overdose [46]. Another group of biomarkers includes molecules related to the apoptotic and/or necrotic process [20,58,59]. Finally, an emerging area being studied for biomarkers in patients with DILI is proteomics, with studies examining both serum and urine [60].

8. Pharmacogenetic impact of DILI

Susceptibility to drugs depends on the presence of genetic variations among gene classes, such as those involved in drug disposition/transport, cellular stress, and inflammatory and immune response genes, including the human leukocyte antigen (HLA) system [61,62]. Different drugs may share specific genetic susceptibility variants [63]. Furthermore, as some patients experience multiple DILI episodes caused by different drugs, there may exist a subgroup of individuals that is predisposed to DILI [63]. Because genetic susceptibility is an important feature of severe ADRs [64,65], there is a growing interest in developing genetic tests to identify people at risk for adverse events before prescription.

The success of genome-wide association studies (GWAS) in identifying susceptibility genes for polygenic diseases [66] has led to an interest to applying GWAS to serious ADRs [67]. In general, GWAS are particularly suitable for detecting small effects. However, the small number of analyzed cases limits the ability of GWAS to detect significant effects [68]. To overcome this limitation, large networks have been established in the United States and Europe, facilitating recruitment of patients with DILI [17,69].

8.1 HLA, immune response, and DILI susceptibility

A traditional example of HLA association with idiosyncratic DILI is represented by halothane, a general anesthetic that was an important cause of acute hepatitis in the operation room. Previous studies have described associations between HLA genes and liver damage caused by halothane [70], diclofenac, and/or chlorpromazine [71]. More recently, a study of the HLA genotyping has been carried out on patients with AMC-related DILI. Although this form of DILI usually does not manifest typical immune-related features, an association with the HLADRB1*15:01 allele was reported [72]. Subsequent studies using candidate genes and GWAS methods identified multiple HLA class I and II associations [73]. In our study of 12 patients with DIAIH, we identified a genetic susceptibility to nimesulide and other xenobiotics [39], supporting the hypothesis of an autoimmune pathogenesis in these patients (Table 3). Recently, the concept that HLA alleles are risk factors for flucloxacillin-, ximelagatran-, and lumiracoxib-induced liver injury [74–76] has become more widely accepted. Hepatotoxicity arising from these agents appears to involve the immune system, with DILI resulting from inappropriate T-cell responses, possibly due to skewing of the T-cell repertoire [77–79].

A few studies have investigated the association between cytokine genetic polymorphisms and the risk of DILI. In 2004, Aithal [80] reported that the frequencies of variant alleles for interleukin (IL)-10 and IL-4 were significantly higher in patients with diclofenac-induced liver damage compared to subjects who received diclofenac but did not develop hepatitis. Liang et al. [81] showed that IL-10-592 AA and IL-10-819 TT genotypes significantly increased the incidence of DILI in breast cancer patients. However, another study failed to confirm these results [82]. Nonetheless, it seems that cytokine genetic polymorphisms are biologically likely risk factors that may contribute to the risk of DILI, given that polymorphisms for IL-6, STAT3, and HSPA1L confer a higher risk of developing hepatotoxicity from antitubercular drugs [83].

GWAS of DILI have failed to identify novel single-nucleotide polymorphisms (SNPs) in HLA genes showing strong effects [68].

Table 2

Established and emerging biomarkers associated with acute and chronic liver damage (modified from Fontana [20]).

Marker	Pattern of injury	Disease
<i>Liver injury biomarkers</i>		
ALT, AST, AP bilirubin	Acute and chronic liver injury	All liver disease, hepatitis, viral and autoimmune, NASH, NAFLD
Sorbitol dehydrogenase	Acute liver injury	Acute liver disease
Glutathione S-transferase	Liver and kidney injury (mitochondrial damage)	Chronic liver disease
Glutamate dehydrogenase	Chronic liver injury	Chronic liver disease
<i>Serum cytokines pattern</i>		
Th 1 (IL-2, IFN γ , IL-12, IL-15)	Acute liver injury	Acute liver disease
Th2 (IL-4, IL-5, IL-13)	Acute liver injury	Acute liver disease
Th9/Th17	Chronic liver injury	Chronic liver disease
<i>miRNAs</i>		
miR-122	Acute and chronic liver injury	APAP overdosing
miR-192	Acute and chronic liver injury	APAP overdosing
<i>Cell death biomarkers</i>		
HMGB-1 (High mobility group box1)	Acute liver injury (Necrosis)	APAP overdosing
Cytokeratin (CK) 18 fragments	Acute and chronic liver injury	ALD, NASH, viral hepatitis
M-30	Acute liver injury (Apoptosis)	Acute liver failure
M-65	Acute liver injury (total hepatocytes death)	Acute liver failure
<i>Proteomics biomarkers</i>		
Fructose biphosphonate aldolase B, apolipoprotein E, apolipoprotein A	Acute and chronic liver injury	Acute and chronic liver disease

Table 3
HLA and non-HLA genes associated with drug induced liver injury (DILI) (modified from Daly [79]).

HLA associated genes			
	Pattern of reaction	Drugs	HLA loci
Liver	Hepatocellular/Cholestatic type	Amoxicillin–clavulanate	DRB1*1501-DQB1*0602 A*0201
	Cholestatic type	Flucloxacillin	B*5701
	Hepatocellular type	Ximelagatran	DRB1*0701-DQA1*0201
	Cholestatic type	Ticlopidine	A*3303
	Cholestatic type	Lumiracoxib	DRB1*1501-DQB1*0602
	Hepatocellular/Mixed type	Nimesulide	DRB1*0708-DQB1*0204 DRB1*0713-DQB1*0206
	Hepatocellular type	Ketoprofen	DRB1*0413-DQB1*0306
	Hepatocellular type	Green Tea	DRB1*0103-DQB1*0205
<i>Non-HLA associated genes</i>			
Drug metabolism and transporters (ATP binding cassette)	Liver injury	Isoniazid	Phase II
		Diclofenac	NAT2
	Myopathy/Liver injury	Simvastatin	UGT2B7
		Diclofenac	UGT1A
			Transporters SLCO1B1
			ABCB11 ABCC2
	Cholestatic		1549
			1774
	Liver injury	Diclofenac	IL-4 C590A
			IL-10 C627A
Immune and inflammatory system	Liver injury	Anti tubercular drugs	IL-6
	Liver injury	Amoxicillin–clavulanate	STAT-4
		Flucloxacillin	STAT3
		Anti tubercular drugs	ST6GAL1

However, further analysis of data obtained from patients with DILI induced by flucloxacillin [84] showed that patients with HLA-B*57:01 have a significant association with a SNP close to ST6GAL1, a gene contributing to B-cell activation responses [85]. Moreover, Lucena et al. [73] showed a significant association for a SNP in the STAT4 gene in their examination of DILI cases. STAT4 triggers activation of IL-12 and IL-23 signaling during T-cell responses [86]. This SNP has been previously associated with other autoimmune diseases, representing another likely risk factor for DILI.

8.2 Drug disposition and transporter gene susceptibility

A polymorphism of the N-acetyltransferase-2 (NAT-2) gene represents a well-known example of a genetic predisposition to DILI. NAT-2 is the enzyme responsible for the metabolism of INH. Several studies have reported that patients who are slow acetylators (i.e., homozygous for both NAT-2 alleles) completely lack enzyme activity and, therefore, are at risk for developing liver injury [87]. However, being a slow acetylator is not sufficient [88] to cause the toxicity, because many of the patients analyzed showed only mild increases of aminotransferase levels. These elevations resolved easily after transient drug withdrawal and usually did not recur when INH therapy resumed.

Transporter genes in the ABC superfamily are biologically reasonable candidates for genetic susceptibility to DILI [89]. Some inherited forms of cholestasis result from specific mutations in the ABCB4 (MDR3) and ABCB11 (BSEP) genes [90]. In addition, a great deal of evidence correlates cholestatic liver injury from diverse drugs to the presence of a polymorphism in the ABCB11 gene, which is also associated with intrahepatic cholestasis of pregnancy [91]. The ABCC2 (MRP2) gene has an important role in the biliary excretion of diverse glucuronide conjugates. Daly et al. [92] reported that a polymorphism in ABCC2 (C24T) was common among patients with diclofenac-induced liver injury. This result was consistent with the increased levels of active metabolites of diclofenac, largely toxic, in the absence of the polymorphism C24T, which results in low production of the MRP2 protein and, thus, the accumulation of the glucuronide. However, two polymorphisms of ABCC2 have been identified, one – 1549 in linkage disequilibrium with C-24T, already known risk factor for hepatocellular liver

injury, and a second at – 1774 of ABCC2, which indeed is a risk factor for cholestatic and mixed liver damage [93].

There are reports of associations between the UGT genotype and DILI. UGT2B7*2 is associated with an increased susceptibility to diclofenac-related DILI. The pathogenic mechanism could be due to high levels of the metabolite, diclofenac acylglucuronide [92].

Statins are the most commonly prescribed agents for lowering low-density lipoprotein cholesterol levels, because of their excellent tolerability and safety. However, in some individuals, statins can cause liver and muscle damage, as evidenced by increased levels of aminotransferase and creatine-phosphokinase. These elevations are promptly reversed on drug discontinuation. Although typically asymptomatic, the increased levels can also become severe, leading to rhabdomyolysis [94]. A GWAS involving simvastatin-induced myopathy cases identified an SNP in SLCO1B1. This gene encodes a transporter, organic anion transporting polypeptide-1 (OATP1B1), which is responsible for the transport of statins and other drugs from the bloodstream into the cell [95]. The association between SLCO1B1 and statin-induced myopathy was subsequently confirmed [96]. Because the overall contribution of SLCO1B1 to hepatic transport is likely to vary across statins, the contribution of the SLCO1B1*5 polymorphism may vary among different statins [97].

Learning points

- An important safety issue, DILI is one of the most frequently cited reasons for cessation of drug development during or after preclinical studies and for withdrawal of a drug from the market. It is the most common cause of ALF in western countries.
- Almost all drug classes can be involved in idiosyncratic DILI. Antimicrobials, antidepressant/antipsychotics and NSAIDs are the most common implicated drugs classes followed by, anti-platelets agents, statins and HDS; recently, body building products and slimming aids have shown a relevant increase of consumption.
- Hepatotoxicity by acetaminophen (APAP) represent the best example of predictive DILI, causing rapidly hepatocellular liver injury. Patients claimed APAP ingestion have to be assess for treatment with N-acetyl cysteine (NAC). Timing of NAC administration (8–10 H) is the most relevant factor in preventing injury by APAP.
- There are no clearly identified biomarkers of DILI that are useful for

monitoring patients on drug therapy for early detection of hepatotoxicity or for making an appropriate diagnosis. AST, ALT, AP and bilirubin are able to identify hepatocellular, cholestatic and/or mixed pattern of liver damage.

- Susceptibility to drugs mainly depends on the presence of genetic variation among gene classes, such as drug disposition/transporters genes, inflammatory and immune response genes, including HLA system. There is growing interest in developing genetic tests to identify people at risk for adverse events before prescription.

Concluding remarks

This review was aimed at highlighting knowledge regarding the epidemiology, risk factors, clinical features, and diagnostic criteria of idiosyncratic and dose-related DILI. Diagnosis of DILI remains a debated issue, despite many efforts to identify proper diagnostic criteria and useful biochemical markers to assist physicians and predict outcomes. In this article, I have reported the most commonly implicated medications, the increasing use of HDS, the difficulties of diagnosis with currently available causality assessment methods, and the new biomarkers being developed for DILI. I have reviewed the issue of APAP overdose-related ALF and its management. I have also provided an update of the literature and discussion of the genetic susceptibility risks of patients with DILI. Nevertheless, substantial research is still needed before useful predictors of risk can be established to define patients susceptible to developing DILI.

Conflict of interest

None.

Acknowledgment

I am really thankful to Antonio Craxi for his valuable advice and support in reviewing the manuscript.

References

- [1] Einar S. Björnsson. Drug-induced liver injury: an overview over the most critical compounds. *Arch Toxicol* 2015;89:327–34.
- [2] Lee W. Acetaminophen-related acute liver failure in the United States. *Hepatol Res*; 38 (S1):S3–8.
- [3] Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* 2002; 137:947–95.
- [4] Lucena MI, Andrade RJ, Fernández MC, Pachkoria K, Pelaez G, Durán JA, et al. Determinants of the clinical expression of amoxicillin–clavulanate hepatotoxicity: a prospective series from Spain. *Hepatology* 2006;44(4):850–6.
- [5] Lammert C, Einarsson S, Saha C, Niklasson A, Björnsson E, Chalasani N. Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. *Hepatology* 2008;47(6):2003–9.
- [6] Chalasani N, Hayashi PH, Bonkovsky HL, Navarro VJ, Lee WM, Fontana RJ, et al. Practice Parameters Committee of the American College of Gastroenterology. ACG Clinical Guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. *Am J Gastroenterol* 2014;109(7):950–66.
- [7] Chalasani N, Fontana RJ, Bonkovsky HL, et al. Causes, clinical features, and outcomes from a prospective study of drug induced liver injury in the United States. *Gastroenterology* 2008;135:1924–34.
- [8] Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. *Gastroenterology* 2013;144(7):1419–25 1425.
- [9] Andrade RJ, Lucena MI, Fernández MC, Pelaez G, Pachkoria K, García-Ruiz E et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology* 2005;129(2):512–21.
- [10] Vitalone A, Menniti-Ippolito F, Moro P, Firenzuoli F, Raschetti R, Mazzanti G. Suspected adverse reactions associated with herbal products used for weight loss: a case series reported to the Italian National Institute of Health. *Eur J Clin Pharmacol* 2011;67:215–24.
- [11] Navarro VJ, Barnhart H, Bonkovsky HL, Davern T, Fontana RJ, Grant L, et al. Liver injury from herbals and dietary supplements in the U.S. Drug-Induced Liver Injury Network. *Hepatology* 2014;60(4):1399–408.
- [12] A Licata A, Macaluso FS, Craxi A. Herbal hepatotoxicity: a hidden epidemic. *Intern Emerg Med* 2013;8(1):13–22.
- [13] Navarro VJ, Senior JR. Drug-related hepatotoxicity. *N Engl J Med* 2006;354:731–9.
- [14] Lee WM. Drug-induced hepatotoxicity. *N Engl J Med* 2003;349(5):474–85 31.
- [15] Reuben A, Koch DG, Lee WM. Acute Liver Failure Study Group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology* 2010; 52(6):2065–76.
- [16] Wei G, Bergquist A, Broomé U, Lindgren S, Wallerstedt S, Almer S, et al. Acute liver failure in Sweden: etiology and outcome. *J Intern Med* 2007;262(3):393–401.
- [17] Hoofnagle JH. Drug-Induced Liver Injury Network (DILIN). *Hepatology* 2004;40:773.
- [18] Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guilleminet C, et al. Incidence of drug-induced hepatic injuries: a French population-based study. *Hepatology* 2002;36(2): 451–5.
- [19] Takikawa H, Murata Y, Horiike N, Fukui H, Onji M. Drug-induced liver injury in Japan: an analysis of 1676 cases between 1997 and 2006. *Hepatol Res* 2009;39(5): 427–31.
- [20] Fontana RJ. Pathogenesis of idiosyncratic drug-induced liver injury and clinical perspective. *Gastroenterology Apr.* 2014;146(4):914–28.
- [21] Björnsson E, Olsson R. Outcome and prognostic markers in severe drug-induced liver disease. *Hepatology* 2005;42(2):481–9
- [22] Chen M, Suzuki A, Borlak J, Andrade RJ, Lucena MI. Drug-induced liver injury: interactions between drug properties and host factors. *J Hepatol.* 2015 ; 63(2):503–14
- [23] Ghabril M, Chalasani N, Björnsson E. Drug-induced liver injury: a clinical update. *Curr Opin Gastroenterol* 2010;26(3):222–6.
- [24] Nobili A, Licata G, Salerno F, Pasina L, Tettamanti M, Franchi C, et al. Polypharmacy, length of hospital stay, and in-hospital mortality among elderly patients in internal medicine wards. The Reposi study. *Eur J Clin Pharmacol* 2011;5:507–19.
- [25] Mannucci PM. NobiliA: Reposi investigators. *Intern Emerg Med* 2014;9:723–34.
- [26] Suzuki A, Brunt EM, Kleiner DE, et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. *Hepatology* 2011;54:931–9.
- [27] Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, et al. Case definition and phenotype standardization in drug-induced liver injury. *Clin Pharmacol Ther* 2011;89:806–15.
- [28] Zimmerman HJ. Hepatotoxicity. The adverse effects of drugs and other chemicals on the liver. Philadelphia: Lippincott Williams & Wilkins 2nd ed. ; 1999.
- [29] Andrade R, Robles M, Fernández-Castañer A, López-Ortega S, López-Vega MC, Lucena MI. Assessment of drug-induced hepatotoxicity in clinical practice: a challenge for gastroenterologists. *World J Gastroenterol* 2007;13(3):329–40 21.
- [30] Van Steenberghe W, Peeters P, De Bondt J, Staessen D, Büscher H, Laporta T, et al. Nimesulide-induced acute hepatitis: evidence from six cases. *J Hepatol Jul.* 1998; 29(1):135–41.
- [31] Danan G, Benichou C. Causality assessment of adverse reactions to drugs – I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol* 1993;46:1323–30.
- [32] Benichou C, Danan G, Flahault A. Causality assessment of adverse reactions to drugs – II. An original model for validation of drug causality assessment methods: case reports with positive rechallenge. *J Clin Epidemiol* 1993;46:1331–6.
- [33] Maria VA, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. *Hepatology* 1997;26:664–9.
- [34] Lucena MI, Camargo R, Andrade RJ, Perez-Sanchez CJ, Sanchez De La Cuesta F. Comparison of two clinical scales for causality assessment in hepatotoxicity. *Hepatology* 2001;33:123–30.
- [35] Licata A, Butera G, Maida M, Calvaruso V, Cappello M, Craxi A, et al. Drug-induced liver injury: the impact of non steroidal anti-inflammatory drugs (NSAIDs). *J Hepatol* 2013;58(S6):S227 # 539.
- [36] Licata A, Calvaruso V, Cappello M, Craxi A, Almasio PL. Clinical course and outcomes of drug-induced liver injury: nimesulide as the first implicated medication. *Dig Liver Dis* 2010;42(2):143–8.
- [37] Licata A, Randazzo C, Butera G, Calvaruso V, Cali A, Cappello M, et al. Pattern of drug induced liver injury: the impact of amoxicillin–clavulanate on severity of clinical course. *J Hepatol* 2011;54(S1):S203 # 497.
- [38] Licata A, Randazzo C, Morreale I, Butera G, D'Alessandro N, Craxi A. Fluoroquinolone-induced liver injury: three new cases and a review of the literature. *Eur J Clin Pharmacol May* 2012;68(5):525–32.
- [39] Licata A, Maida M, Cabibi D, Butera G, Macaluso FS, Alessi N, et al. Clinical features and outcomes of patients with drug-induced autoimmune hepatitis: a retrospective cohort study. *Dig Liver Dis Dec.* 2014;46(12):1116–20.
- [40] Doyon S, Klein-Schwartz W. Hepatotoxicity despite early administration of intravenous N-acetylcysteine for acute acetaminophen overdose. *Acad Emerg Med* 2009 Jan;16(1):34–9.
- [41] Craig DG, Lee A, Hayes PC, Simpson KJ. Review article: the current management of acute liver failure. *Aliment Pharmacol Ther* 2010;31(3):345–58.
- [42] Bronstein AC, Spyker DA, Cantilena Jr LR, Green JL, Rumack BH, Giffin SL. Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 26th annual report. *Clin Toxicol (Phila)* 2009;47(10):911–1084.
- [43] Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hyman LS, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology Dec.* 2005;42(6):1364–72.
- [44] Budnitz DS, Lovegrove MC, Crosby AE. Emergency department visits for overdoses of acetaminophen containing products. *Am J Prev Med* 2011;40(6): 585–92.
- [45] Ward J, Kanchaga C, Veksler-Lublinsky I, Lee RC, McGill MR, Jaeschke H, et al. Circulating microRNA profiles in human patients with acetaminophen hepatotoxicity or ischemic hepatitis. *Proc Natl Acad Sci U S A* 2014;111(33):12169–74 19.
- [46] Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, Hu Z, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci U S A Mar.* 14 2009;106(11):4402–7.

- [47] Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DG, Antoine DJ, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. *Hepatology* 2011;54(5):1767–76.
- [48] Duffull SB, Isbister GK. Predicting the requirement for N-acetylcysteine in paracetamol poisoning from reported dose. *Clin Toxicol* 2013;51(8):772–6.
- [49] Daly FF, Fountain JS, Murray L, Graudins A, Buckley NA. Panel of Australian and New Zealand clinical toxicologists. Guidelines for the management of paracetamol poisoning in Australia and New Zealand — explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australasian poisons information centres. *Med J Aust* 2008;188(5):296–301 3.
- [50] Moitra K, Dean M. Evolution of ABC transporters by gene duplication and their role in human disease. *Biol Chem Jan*. 2011;392(1-2):29–37.
- [51] Yun CH, Okerholm RA, Guengerich FP. Oxidation of the antihistaminic drug terfenadine in human liver microsomes. Role of cytochrome P-450 3A(4) in N-dealkylation and C-hydroxylation. *Drug Metab Dispos* 1993;21(3):403–9.
- [52] Beaune P, Dansette PM, Mansuy D, Kiffel L, Finck M, Amar C, et al. Human anti-endoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis are directed against a human liver cytochrome P-450 that hydroxylates the drug. *Proc Natl Acad Sci U S A* 1987;84(2):551–5.
- [53] Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med* 1998;22(339):1217–27.
- [54] Luedde T, Kaplowitz N. Schwabe R. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. *Gastroenterology* 2014;147(4):765–83.
- [55] Festjens N, Vanden Berghe T, Vandenberghe P. Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. *Biochim. Biophys. Acta* 2006;1757(9-10):1371–87.
- [56] Linkermann A, Green DR. Necroptosis. *N Engl J Med Jan*. 2014;370(5):455–65.
- [57] Steuerwald NM, Foureau DM, Norton HJ, Zhou J, Parsons JC, Chalasani N. Profiles of serum cytokines in acute drug-induced liver injury and their prognostic significance. *PLoS One Dec*. 27 2013;8(12):e81974.
- [58] Rutherford AE, Hynan LS, Borges CB, Forcione DG, Blackard JT, Lin W, et al. Serum apoptosis markers in acute liver failure: a pilot study. *Clin Gastroenterol Hepatol Dec*. 2007;5(12):1477–83.
- [59] Chung RT, Stravitz RT, Fontana RJ, Schiodt FV, Mehal WZ, Reddy KR, et al. Pathogenesis of liver injury in acute liver failure. *Gastroenterology Sep*. 2012;143(3):e1–7.
- [60] Bell LN, Vuppalanchi R, Watkins PB, Bonkovsky HL, Serrano J, Fontana RJ, et al. Serum proteomic profiling in patients with drug-induced liver injury. *Aliment Pharmacol Ther Mar*. 2012;35(5):600–12.
- [61] Andrade RJ, Robles M, Ulzurrun E, Lucena MI. Drug-induced liver injury: insights from genetic studies. *Pharmacogenomics* 2009;10:1467–87.
- [62] Daly AK. Drug-induced liver injury: past, present and future. *Pharmacogenomics* 2010;11:607–11.
- [63] Lucena MI, Kaplowitz N, Hallal H, Castiella A, Garcia-Bengochea M, Otazua P, et al. Recurrent drug-induced liver injury (DILI) with different drugs in the Spanish Registry. The dilemma of the relationship to autoimmune hepatitis. *J Hepatol* 2011.
- [64] Wilke R, Lin DW, Roden D, Watkins PB, Flockhart D, Zineh I, et al. Identifying genetic risk factors for serious adverse drug reactions: current progress and challenges. *Nat Rev Drug Discov* 2007;6(11):904–16.
- [65] Becquemont L. Pharmacogenomics of adverse drug reactions: practical applications and perspectives. *Pharmacogenomics* 2009;10:961–9.
- [66] Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature* 2007;447:661–78.
- [67] Daly A. Using genome-wide association studies to identify genes important in serious adverse drug reactions. *Annu Rev Pharmacol Toxicol* 2012;52:21–35.
- [68] Urban TJ, Shen Y, Stolz A, Chalasani N, Fontana RJ, Rochon J, et al. Limited contribution of common genetic variants to risk for liver injury due to a variety of drugs. *Pharmacogenet Genomics* 2012 Nov;22(11):784–95.
- [69] Molokhia M, McKeigue P. EUDRAGENE: European collaboration to establish a case-control DNA collection for studying the genetic basis of adverse drug reactions. *Pharmacogenomics* 2006;7:633–8.
- [70] Otsuka S, Yamamoto M, Kasuya S, Ohtomo H, Yamamoto Y, et al. HLA antigens in patients with unexplained hepatitis followinghalothaneanesthesia. *Acta Anaesthesiol Scand* 1985;29:497–501.
- [71] Berson A, Freneaux E, Larrey D, Lepage V, Douay C, Mallet C, et al. Possible role of HLA in hepatotoxicity — an exploratory study in 71 patients with drug-induced idiosyncratic hepatitis. *J. Hepatol.*1994, 20:336–342.
- [72] O'Donohue J, Oien KA, Donaldson P, Underhill J, Clare M, MacSween RM. MillsPR: co-amoxiclav jaundice: clinical and histological features and HLA classII association. *Gut* 2000;47:717–20.
- [73] Lucena MI, Molokhia M, Shen Y, Urban TJ, Aithal GP, Andrade RJ, et al. Susceptibility to amoxicillin–clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. *Gastroenterology Jul*. 2011;141(1):338–47.
- [74] Monshi M, Faulkner L, Gibson A, Jenkins RE, Farrell J, Earnshaw CJ, et al. HLA B*57:01 restricted activation of drug-specific T-cells provides the immunological basis for flucloxacillin-induced liver injury. *Hepatology* 2013;57(2):727–39.
- [75] Kindmark A, Jawaid A, Harbrun CG, Barratt BJ, Bengtsson OF, Andersson TB, et al. Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. *Pharmacogenomics J* 2008;8:186–95.
- [76] Singer JB, Lewitzky S, Leroy E, Yang F, Zhao X, Klickstein L, et al. A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nat Genet* 2010;42:711–4.
- [77] Illing PT, Vivian JP, Dudek NL, Kostenko L, Chen Z, Bharadwaj M, et al. Immune self-reactivity triggered by drug-modified HLA peptide repertoire. *Nature* 2012;486:554–8.
- [78] Ostrov DA, Grant BJ, Pompeu YA, Sidney J, Hamdahl M, Southwood S, et al. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc Natl Acad Sci U S A* 2012;109:9959–64.
- [79] Daly A. Pharmacogenomics of adverse events. *Gend Med* 2013;5(5):2–12.
- [80] Aithal GP, Ramsay L, Daly AK, Sonchit N, Leathart JB, Alexander G, et al. Hepatic antibodies, circulating antibodies, and cytokine polymorphisms in patients with diclofenac hepatotoxicity. *Hepatology* 2004;39:1430–40.
- [81] Liang X, Zhang J, Zhu Y, Lu Y, Zhou X, Wang Z, et al. Specific genetic polymorphisms of IL10–592 AA and IL10–819 TT genotypes lead to the key role for inducing docetaxel-induced liver injury in breast cancer patients. *Clin Transl Oncol* 2013;15:33.
- [82] Pachkoria K, Lucena MI, Crespo E, Ruiz-Cabello F, Lopez-Ortega S, Fernandez MA, et al. Analysis of IL-10, IL-4 and TNF-alpha polymorphisms in drug-induced liver injury (DILI) and its outcome. *J Hepatol Jul*. 2008;49(1):107–14.
- [83] Wang J, Chen R, Tang S, Lv X, Wu S, Zhang Y, et al. Analysis of IL-6, STAT3 and HSPA1L gene polymorphisms in anti-tuberculosis drug-induced hepatitis in a nested case-control study. *PLoS One* 2015;10(3):e0118862.
- [84] Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet Jul*. 2009;41(7):816–9.
- [85] Nasirikerani M, Segal BH, Ostberg JR, Urbasic A, Lau J, et al. Altered granulopoietic profile and exaggerated acute neutrophilic inflammation in mice with targeted deficiency in the sialyltransferase ST6gGal1. *Blood* 2006;108:3397–405.
- [86] Korman BD, Kastner DL, Gregersen PK, Remmers EF. STAT4: genetics, mechanisms, and implications for autoimmunity. *Curr Allergy Asthma Rep* 2008;8(5):398–403.
- [87] Metushi IG, Cai P, Zhu X, Nakagawa T, Uetrecht JP. A fresh look at the mechanism of isoniazid-induced hepatotoxicity. *Clin Pharmacol Ther* 2011;89:911–4.
- [88] Daly AK, Day CP. Genetic association studies in drug-induced liver injury. *Drug Metab Rev* 2012;44(1):116–26.
- [89] Geier A, Wagner M, Dietrich CG, Trauner M. Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. *Biochim Biophys Acta* 2007;1773:283–308.
- [90] Noe J, Kullak-Ublick GA, Jochum W, Stieger B, Kerb R, Haberl M, et al. Impaired expression and function of the bile salt export pump due to three novel ABCB11 mutations in intrahepaticcholestasis. *J Hepatol* 2005;43:536–43.
- [91] Lang C, Meier Y, Stieger B, Beuers U, Lang T, Kerb R, et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug induced liver injury. *Pharmacogenet Genomics* 2007;17:47–60.
- [92] Daly AK, Aithal GP, Leathart JB, Swainsbury RA, Dang TS, Day CP. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABC2C genotypes. *Gastroenterology* 2007;132:272–81.
- [93] Choi JH, Ahn BM, Yi J, Lee JH, Nam SW, Chon CY, et al. MRP2 haplotypes confer differential susceptibility to toxic liver injury. *Pharmacogenet Genomics* 2007;17:403–15.
- [94] Dalakas MC. Toxic and drug-induced myopathies. *J Neurol Neurosurg Psychiatry* 2009;80:832–8.
- [95] Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. SLC10B1 variants and statin-induced myopathy — a genome wide study. *N Engl J Med* 2008;359:789–99.
- [96] Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, et al. The SLC10B1*5 genetic variant is associated with statin-induced side effects. *J Am Coll Cardiol* 2009;54:1609–16.
- [97] Niemi M. Transporter pharmacogenetics and statin toxicity. *Clin Pharmacol Ther* 2010;87:130–3.