Clinical Practice Guidelines

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EASL Clinical Practice Guidelines: Drug-induced liver injury☆

European Association for the Study of the Liver*

Summary

Idiosyncratic (unpredictable) drug-induced liver injury is one of the most challenging liver disorders faced by hepatologists, because of the myriad of drugs used in clinical practice, available herbs and dietary supplements with hepatotoxic potential, the ability of the condition to present with a variety of clinical and pathological phenotypes and the current absence of specific biomarkers. This makes the diagnosis of drug-induced liver injury an uncertain process, requiring a high degree of awareness of the condition and the careful exclusion of alternative aetiologies of liver disease. Idiosyncratic hepatotoxicity can be severe, leading to a particularly serious variety of acute liver failure for which no effective therapy has yet been developed. These Clinical Practice Guidelines summarize the available evidence on risk factors, diagnosis, management and risk minimization strategies for drug-induced liver jury.

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Introduction

The focus of these guidelines is idiosyncratic drug-induced liver injury (DILI). However, it is important to recognise that DILI is traditionally classified as intrinsic (or direct) *vs.* idiosyncratic. Intrinsic DILI is typically dose-related and occurs in a large proportion of individuals exposed to the drug (predictable) and onset is within a short time span (hours to days). Idiosyncratic DILI is usually not dose-related, although a dose threshold of 50–100 mg/day is usually required, occurs in only a small proportion of exposed individuals (unpredictable) and exhibits a variable latency to onset of days to weeks. Drugs known to produce intrinsic and idiosyncratic DILI are presented in Table 1. The pathogeneses of these 2 types of DILI share some common features as well as major differences. In both types the chemical characteristics of the drug are important, particularly lipophilicity and drug biotransformation. This exposes the liver to reactive metabolites which can covalently bind to proteins, induce oxidative stress, activate signal transduction pathways (*e.g.* mitogen-activated protein (MAP) kinases) and result in organelle stress (*e.g.* mitochondrial or endoplasmic reticulum (ER) stress), interfere with bile acid transport and either lead to lethal consequences (necrosis or apoptosis) or induce adaptive responses which dampen these processes (*e.g.* antioxidant defence, mitochondrial or ER unfolded protein responses, mitochondrial biogenesis) so that injury does not occur or is very mild.[1–3] However, the stress can provoke innate immune responses which provide a co-stimulation for an adaptive immune response in some individuals with a genetic predisposition to adaptive immunity. Despite the fact that idiosyncratic DILI occurs in a very small proportion of exposed patients, screening for stress in cell systems and isolated mitochondria is predictive of the risk associated with a large proportion of the drugs known to cause idiosyncratic DILI.[3,4] The key feature of idiosyncratic DILI with most drugs is the critical role of the adaptive immune system. Many drugs which cause immune-mediated idiosyncratic DILI exhibit no systemic allergic features such as rash and eosinophilia. Key in the development of an adaptive immune response is the role of restricted human leukocyte antigen (HLA) associations. Nevertheless, in most instances upstream factors include the chemical properties of the drug and the formation of reactive metabolites which serve as haptens. Furthermore, even among those patients with HLA specific associations, only a minority develop DILI. A potential explanation for this is that the development of immune tolerance may suppress or modulate the severity of DILI so that only those with an insufficient adaptive response, such as immune tolerance, progress to liver injury.[5,6]

Some comment about acetaminophen hepatotoxicity is important as it is the most common cause of acute liver failure (ALF) in the US and parts of Europe. It is a prototype of intrinsic DILI. It accounts for more than 50% of cases of ALF. Half the cases are due to single overdoses but half are unintentional cases, usually resulting from individuals taking acetaminophen over several days at daily doses in the range of 4–10 g/day, although a number of cases have been reported at doses ranging from 2–4 g/day.[7,8] Factors such as concomitant drugs, fasting, systemic illnesses, and chronic alcoholic abuse modulate the threshold toxic dose by influencing CYP2E1 (the main enzyme which converts acetaminophen to a reactive metabolite) or glutathione status (main detoxification factor). If glutathione is severely depleted, particularly in mitochondria, the toxic metabolite covalently binds to mitochondrial proteins and induces increased reactive oxygen species (ROS) production. The latter activates the MAPK pathway leading to sustained activation of c-Jun N-terminal kinase (JNK). JNK then interplays with mitochondria to amplify mitochondrial ROS production leading to permeabilization of the mitochondria and release of

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Table 1. Drugs associated with intrinsic *vs.* idiosyncratic DILI.*

Intrinsic	Idiosyncratic	
Acetaminophen	Allopurinol	Lapatinib
Amiodarone§	Amiodarone§	Methyldopa
Anabolic steroids	Amoxicillin-clavulanate	Minocycline
Antimetabolites	Bosentan	Nitrofurantoin
Cholestyramine**	Dantrolene	Pazopanib
Cyclosporine	Diclofenac	Phenytoin
Valproic acid	Disulfiram	Pyrazinamide
HAART drugs	Felbamate	Propylthiouracil
Heparins**	Fenofibrate	Statins§
Nicotinic acid	Flucloxacillin	Sulfonamides
Statins§	Flutamide	Terbinafine
Tacrine**	Halothane	Ticlopidine
	Isoniazid	Tolvaptan
	Ketoconazole	Tolcapone
	Leflunomide	Trovafloxacin
	Lisinopril	

ALT, alanine aminotransferase; DILI, drug-induced liver injury; HAART, highly active antiretroviral therapy.
*Known examples; withdrawn or unapproved drugs not listed
**Mild ALT elevations without jaundice
§Both intrinsic and idiosyncratic.

mitochondrial proteins which damage nuclear DNA and, along with ATP depletion, lead to oncolytic necrosis (Fig. 1).[9–11]

Idiosyncratic DILI is a serious matter with consequences on various levels, including individual patient health, pharmaceutical regulatory decisions and drug development schemes. From the clinical side, DILI can result in illness, hospitalization and even life-threatening liver failure, death or need for liver transplantation. Besides, diagnosis of DILI is one of the most challenging liver disorders faced by hepatologists because of its relatively low incidence, the variety in its clinical phenotype, as well as the absence of specific biomarkers. The hepatotoxic potential of many drugs used in clinical practice can further jeopardize the correct assessment of DILI cases. New immunotherapeutic agents including biologics, and in particular immune checkpoint inhibitors for advanced cancer, are associated with immune-mediated adverse reactions including hepatic damage. These treatments are leading to emerging forms of DILI that pose new challenges for physicians. The aim of the present Clinical Practice Guidelines (CPGs) is to provide guidance to hepatologists, internists and other clinical specialists in the understanding, diagnosis and management of DILI, in order to increase awareness of this condition and improve the rate of early detection and care for affected patients. For this area of knowledge and in view of the limited data from large controlled studies and trials we have used the levels of evidence recommended by the Oxford Centre for Evidence-based Medicine, which are suitable for critical assessment of aetiology, prevalence, diagnostic, prognostic and natural history studies,[12] in line with recent recommendations for EASL CPGs.[13] A much-simplified interpretation of the level of evidence has been shown in Table S1. The grade of recommendation is dependent on the evidence (Table S2), consistency of studies, risk-benefit ratio, patient preferences, ethical obligations and feasibility and reflected in the wording, as advised by Cornberg *et al.*[13] Some further recommendations are based on expert consensus from the panel members, who are experts in the DILI field. To further strengthen its validity both the EASL Governing Board as well as external experts have reviewed the recommendations. All recommendations of this CPG were agreed upon unanimously (100% consensus).

Epidemiology

Demography and drugs

Determining the true incidence of DILI is difficult. Despite increasing awareness of hepatotoxicity and the availability of less toxic alternatives, the absolute frequency of hepatic drug reactions does not appear to decrease, in keeping with the increasing number of prescriptions and range of pharmacological agents available.[14–16] A large proportion of drug-induced hepatotoxicity occurs in an unpredictable manner, wherein a drug has been used as recommended, which defines an idiosyncratic event. As a consequence, the prevalence and incidence of the majority of adverse effects of drugs, such as DILI, are still only partially known.

Clinical trials produce reliable information about the development of abnormal liver biochemistries and DILI if the incidence is high. However, such trials usually include a limited number of patients and are therefore underpowered to detect rare adverse effects such as idiosyncratic hepatotoxicity. Consequently, the majority of data are provided by retrospective studies of databases from pharmacovigilance centres and/or pharmaceutical companies, aimed to determine the most frequently associated drugs and their clinical characteristics. Due to the retrospective nature of these studies, it is clear that many events are overlooked or ignored and what is detected is only the "tip of the iceberg". Studies on the aetiology of ALF have demonstrated that drugs are the main causes of ALF in the US,[17,18] Europe[19,20] and Japan.[21] In the US and Europe, idiosyncratic drug reactions due to conventional medications are the most common causes of DILI, while traditional complementary and dietary supplements are the main causative agents of DILI in Asia.[22]

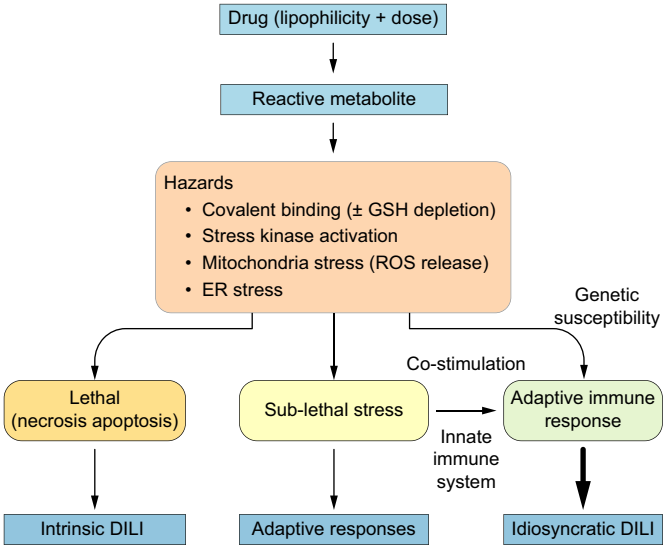


Fig. 1. Mechanistic relationship between intrinsic and idiosyncratic DILI. A common prerequisite for intrinsic toxicity and idiosyncratic DILI is the metabolism of lipophilic drugs in the liver, generating reactive metabolites which lead to initial consequences, such as covalent binding, oxidative stress, stress kinase signalling and organelle stress responses (mitochondria and ER) which either overwhelm defences and lead directly to necrosis or apoptosis or elicit an adaptive immune response to drug-adducts (haptens) in genetically susceptible individuals. DILI, drug-induced liver injury; ER, endoplasmic reticulum; GSH, glutathione; ROS, reactive oxygen species.

The burden of herbal and complementary medicines hepatotoxicity

The awareness of potential hepatotoxicity associated with alternative medicines such as herbal preparations and dietary supplements (HDS) is increasing.[23] The last decades have shown that herbal medicines may cause a large spectrum of liver injury, affecting all cells present in the liver and biliary tree, and ranging from mild asymptomatic liver enzyme elevation to acute hepatitis, chronic hepatitis, cirrhosis, liver failure, acute and chronic cholangitis, macro- and microvesicular steatosis, and vascular lesions.[24–26]

Epidemiological studies of DILI related to HDS products are still limited. In 2005, the Spanish DILI registry showed that herbal medicines ranked 9th in terms of DILI frequency, at the same level as isoniazid.[14] The US Drug-Induced Liver Injury Network (DILIN) has estimated that HDS products account for 16% of DILI cases overall, with an increase in proportion from 7% in 2004–2005 to 20% in 2013–2014,[26] which is similar to 16% prevalence of HDS associated hepatotoxicity found in a prospective study from Iceland.[15]

Hepatotoxicity of herbal remedies is particularly difficult to demonstrate.[27,28] In addition to the usual difficulties in determining a relationship between an adverse event and drug intake largely caused by the absence of clinical specificity, factors such as frequent auto-medication and assumed safety of HDS, causing the patient not to declare HDS use to the physician, can make the causality assessment more difficult. In addition, there are specific risks that contribute to the hepatotoxicity of herbal remedies: misidentification of the plant, selection of a wrong part of the medicinal plant, inadequate storage modifying the native product, adulteration during the processing and mislabelling of the final product.[29] Another difficulty is that the real composition of the herbal preparation may remain unclear, particularly in multicompound products. A safe herbal product may also be contaminated by toxic compounds leading to hepatotoxicity. This may result from adulteration with heavy metals, pesticides, herbicides, microorganisms and even classical pharmaceutical products.[29]

To date, more than 100 medicinal preparations have been reported to be toxic to the liver.[23,27–31] The degree of evidence of toxicity is variable as for classical pharmaceutical agents. Herbal medicines with the highest level of evidence of hepatotoxicity are plants containing pyrrolizidine alkaloids, germander (*Teucrium chamaedris*), *Atractylis gummifera*, plants containing pennyroyal oil (*Mentha pulegium*, *Hedeoma pulegioides*), great celandine (*Chelidonium majus*), kava-kava (*Piper methysticum*), Black cohosh (*Actaea racemosa*), and several Asian medicinal preparations (Table 2). Other compounds with a fair level of evidence for hepatotoxicity are chaparral leaf (*Larrea tridentata*), senna (*Cassia angustifolia*), hydroalcoholic extracts of green tea and Herbalife®.

Pyrrolizidine alkaloids provide a remarkable illustration of the difficulties encountered with herbal medicine-based hepatotoxicity and the particular need to develop biomarkers to identify the problem. These alkaloids are found in more than 6,000 plants worldwide.[29,32] The main species implicated are: *Heliotroprium*, *Senecio*, *Crotalaria*, and *Symphytum* (comfrey) species and more recently, *Gynura segetum.*[33] Pyrrolizidine alkaloids are a concern in Chinese herbal medicines, with at least 21 cases of DILI related to "Tusanqi", a traditional preparation containing *Gynera segetum.*[34] The main liver injury induced by pyrrolizidine alkaloids is veno-occlusive disease, so called sinusoidal obstruction syndrome (SOS). Pyrrolizidine alkaloids account for more than 8,000 cases of SOS worldwide and make up 1 of the major causes of this syndrome.[32,34,35] Another example in which the mechanism of hepatotoxicity has been clearly elucidated is germander (*Teucrium chamaedris*).[29] Here it is possible to make the diagnosis with a biological marker, as the presence of serum anti-hydrolase antibodies may be detected in patients with DILI caused by germander.

Several recent reports have underlined the hepatotoxicity of dietary supplements including a cocktail of products, usnic acid with other product (yohimbine, caffeine, dihydrothyrone, norephedrine) in various preparations: Lipokinetic®, UPC-1®, Lipoliz®, particularly associated with acute hepatocellular hepatitis. Other products reported to cause DILI include OxyELITE® containing several ingredients (dimethylamylamine, aegeline) for weight loss and muscle building, Hydroxycut® (containing green tea, ephedra, caffeine, carnitine, chromium) and linoleic acid.[30] Furthermore, the illicit use of anabolic androgenic steroids is markedly increasing for body-building, improved fitness and exercise performance purposes.[36,37] These compounds may lead to a large variety of liver lesions ranging from acute hepatitis to adenoma and hepatocellular carcinoma.

Recommendation

- Physicians may consider herbal and dietary supplements as potential causative agents associated with liver injury. **Grade C**.

Evidence: Level 4 (case series)

Retrospective studies

Important pharmacoepidemiologic data on DILI have been obtained from the General Practice Research database (GPRD) in the UK. Early case-control or cohort studies using GPRD found antibiotics such as flucloxacillin, erythromycin, amoxicillin, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole to be the most commonly implicated agents.[38,39] A later study from the same source found the strongest association with hepatotoxicity for chlorpromazine, amoxicillin-clavulanic acid, flucloxacillin, macrolides, sulphasalazine, azathioprine, diclofenac and antiepileptics, with the highest incidence rates for chlorpromazine, azathioprine and sulphasalazine (approximately 1 per 1,000 users).[40] Using a Swiss pharmacoepidemiological inpatient database the DILI prevalence at admission to hospital was estimated to 0.7% and the overall DILI incidence during hospitalization to 1.4%. More importantly, liver injury was not mentioned in the diagnosis or in the physicians discharge letter in 52–68% of cases.[41] The estimated incidence of DILI in retrospective studies has been shown to be much lower than in prospective studies. Studies of the UK GPRD and a Swedish hepatology clinic outpatient database have revealed a DILI incidence rate of 2.3–2.4 cases per 100,000 inhabitants and year.[40,42] This is lower than the incidence rate of DILI in prospective national studies, demonstrating an under-reporting of DILI.[15,43] Furthermore, a retrospective study from the US in patients with new-onset jaundice over a 5-year period, found that idiosyncratic DILI was rare and only observed in 0.7% of patients.[44] However, in a prospective study from Iceland among patients with notably raised alanine aminotransferase (ALT) (>500 U/L), DILI was the

Table 2. Herbal and dietary supplements involved in hepatotoxicity.

Herbal and dietary supplements	Type of liver injury
Herbal preparations	
Pyrrolizidine alkaloids, *e.g.* Crotalaria,senecio, heliotrpium, Symphytum officinale (comfrey)	Acute and chronic SOS
Teucrium chamaedrys (germander)	AHH, ACH, ALF, chronic hepatitis, cirrhosis, cholangitis
Teucrium polium	AHH, ACH, A LF
Atractylis gummifera L.	AHH, ACH, ALF
Callilepis laureola L.	AHH, ALF
Mentha pulegium	AHH, ACH, ALF
Hedeoma pulegioides	AHH, ACH, ALF
Chelidonium majus (greater celandine)	AHH, ACH, chronic hepatitis, cholangitis
Piper methysticum (kava-kava)	AHH, ACH, ALF, chronic hepatitis
Camellia sinensis (green tea extracts)	AHH, ACH, ALF
Actaea racemosa (black cohosh)	AHH, ACH
Cimicifuga racemosa	AHH, ACH
Morinda citrifolia (Noni juice)	AHH, ACH, ALF
Serenoa	ACH
Azadirachta indica	Microvesicular steatosis
Catha edulis (khat)	AHH, ACH, ALF
Borago officinalis (borage)	AHH, ACH
Cassia angustifolia (senna)	AHH, ACH
Larrea tridentata (chaparral)	AHH, ACH, cholangitis, chronic hepatitis/cirrhosis
Asian herbal medicine (Chinese, Japanese, ayurvedic medicines)	
Lycopodium serratum (Jin Bu Huan)	AHH, ACH, ALF
Ephedra (Ma Huang)	AHH with autoimmunity
Sho-Saiko-To (Xiao-Chai-Hu-Tang; complex preparation)	AHH/chronic hepatitis
Dai-Saiko-To (complex preparation)	AHH with autoimmunity
Chaso and Onshido	AHH, ACH, ALF
Boh-Gol-Zhee/Bu Ku Zi	ACH
Polygonum multiflorum (Shou-Wu-Pian)	AHH, ACH
Ganoderma lucidum (Linghzi)	AHH
Brena officinalis (Chi R Yun)	AHH
Dysosma pleiantha (Boh-Gol-Zhee)	AHH
Dietary supplements	
Usnic acid with other ingredients:	
LipoKinetix®	AHH, ALF
UCP-1®	AHH, ALF
Oxy ELITE®	AHH, ALF
Hydroxycut®	AHH, ACH, ALF, AHH with autoimmunity
Linoleic acid	AHH
Plethoryl® (vitamin A, thyroid hormones)	AHH, ACH, chronic hepatitis, cirrhosis
Illicit anabolic androgenic steroids	AHH, ACH, liver adenoma, HCC, SOS

ACH, acute cholestatic hepatitis; AHH, acute hepatocellular hepatitis; ALF, acute liver failure; HCC, hepatocellular carcinoma; SOS, sinusoidal obstruction syndrome.

presumed cause in 7% of patients.[45] A retrospective study from Sweden on 784 patients over a long period (1970–2004) analysed the prognosis in patients with DILI and concomitant jaundice.[46] This study along with findings from the prospective Spanish DILI registry[14] were the first studies to validate and confirm the so called Hy's law (see Section *Detecting DILI in clinical trials* for a detailed description), whereby the mortality/-transplantation rate was approximately 10% in patients with drug-induced jaundice.

Prospective studies

Few prospective DILI studies have been undertaken to date, with 3 studies from France, Iceland and the US being the only population-based studies.[15,43,47] Data corresponding to large prospective studies from the Spanish DILI registry and the US DILIN have also been published but are not population-based.[14,16,48]

Population-based studies

A prospective DILI study on the general population of a French district was undertaken over a period of 3 years. All suspected DILI cases were collected in a defined population in a prospective fashion. The incidence of DILI was found to be 13.9 cases per 100,000 inhabitants, which was at least 16 times more frequent than the reactions obtained through spontaneous reporting in France over the same time period.[43] A prospective study on DILI was also undertaken in Iceland over a 2-year study period.[15] The crude incidence rate of DILI was somewhat higher than reported from France, with 19 new cases per 100,000 inhabitants annually. The Icelandic study was able to evaluate the quantitative risk of DILI associated with different causative drugs. Although amoxicillin-clavulanate was the most commonly implicated agent, the risk of DILI was found to be only 1 in approximately 2,300 users, whereas the highest risk of hepatotoxicity was associated with azathioprine and infliximab, in 1 out of 133 and 148 users, respectively.[15] A study from the state of Delaware in the US, found lower incidence of DILI, showing 2.7 cases per 100,000 inhabitants.[47] The cut-off value for ALT in patients with suspected DILI, however, was higher (>5 × the upper limit of normal [ULN] on 2 separate occasions) than in the previous prospective studies (>2 × ULN[43] and >3 × ULN[15]), which might partly explain the lower incidence. The authors speculated that as surveillance was limited to

subspecialists, the actual incidence of DILI would likely be higher.[47] A prospective nationwide study of DILI in Korea was undertaken in 17 referral hospitals.[49] The extrapolated incidence of hospitalization because of DILI at university hospitals in Korea reported in this study was 12 per 100,000 persons. Herbal medications in different forms were the predominant cause of DILI in Korea as in many other parts of Asia.[22]

DILI registries

A cooperative network was created in Spain in 1994 with the aim of identifying DILI patients within the catchment area of the participating hospitals.[14] The Spanish DILI registry started with the intention of creating a collaborative network of specialists in liver disease, internal medicine and clinical pharmacology in Andalusia, but was later expanded to hospitals all over Spain. In the original publication from the Spanish DILI registry, 461 cases fulfilled the causality assessment criteria out of 570 submitted cases.[14] Antibiotics were the dominating drug class and hepatocellular pattern was the most common type of liver damage that was inversely related to age and conferred the worst outcome.[14] The most commonly implicated drug in this study, amoxicillin-clavulanate, was later confirmed to be the most common agent in other prospective studies.[15,16,48] Since the original report from the Spanish DILI registry, several important publications have appeared on different clinical, pharmacological and genetic aspects of DILI and the registry is still enrolling patients. The US DILIN that was initiated in 2004, funded by the National Institutes of Health in the US, is an ongoing observational study of both children (>2 years of age) and adults with suspected DILI.[16,48] The studies undertaken by DILIN have made very important contributions to the field of DILI. Recently, the Latin American DILI Network (LATINDILIN) was initiated. The primary aim of this DILI registry was to prospectively identify *bona fide* DILI cases and to collect biological samples for genetic biomarker studies.[50] This is an ongoing prospective study and is likely to lead to important contributions to the field of DILI in the future. In addition to DILI registries, single-centre cohort studies from India and Turkey have also been reported, with antibiotics/antituberculosis (anti-TBC) drugs being the most prominent causative agents of DILI.[51,52]

Outcomes

The vast majority of patients who experience DILI will fully recover clinically and biochemically. However, idiosyncratic liver injury was implicated in 13–15% of cases with ALF in the US and Sweden.[17,19] Compared with other causes of ALF, patients with idiosyncratic liver injury have worse transplant-free survival.[17,19,53] Many studies have shown that approximately 10% of patients with drug-induced jaundice will either die from ALF or require a liver transplantation.[14,16,46,48,54–56] Thus, although patients present with DILI and concomitant jaundice, approximately 90% are likely to survive. In general, hepatocellular type of DILI is more likely to be associated with a poor outcomes and with a higher liver-related mortality.[14,16,46,48] However, cholestatic liver injury can also be associated with significant mortality,[14,46,48] whereas mixed liver injury seems to have the lowest mortality rate. The higher risk associated with hepatocellular type of liver injury is in accordance with Hy's law (see Section *Detecting DILI in clinical trials* for a detailed description). A recent study from the Spanish DILI registry presented an attempt to improve and optimize the definition of Hy's law and to develop a model for predicting ALF in patients with DILI. Their results suggested that the use of a new R value (nR) using either ALT or aspartate aminotransferase (AST), which ever is highest, improved ALF prediction.[56] Higher positive predictive value for fatality with nR Hy's law was recently confirmed in a study of American DILI cases.[57] Some patients who survive DILI will have a slow liver injury recovery, clinically and biochemically and this is more common in patients who present with cholestatic liver injury.[54,55,58,59] The rate of chronicity in patients who have recovered from DILI during long-term follow-up has been found to vary, partly due to the use of different chronicity criteria in the studies.[54,55,58–61] Chronic DILI is covered in the section on *Prognosis and natural history*.

Risk factors

Host-dependent risk factors

Age

The incidence of serious adverse drug reactions (ADRs) has been reported to rise with increasing age.[62] A large proportion of ADRs in older people are dose-related, and possibly a result of ageing being associated with impaired drug clearance. Older age has also been proposed as a general risk factor for DILI. In fact, the Council for International Organizations of Medical Sciences/Roussel-Uclaf causality assessment method (CIOMS/RUCAM) causality assessment scale gives an extra point to cases involving patients above 55 years of age.[63] Data available from large prospective DILI registries, however, do not support that older age is a general risk factor. In the Spanish DILI registry, 46% of DILI patients were ≥60 years old at the time of the episode and the US DILIN reported 16.6% of their patients with DILI to be 65 years or older.[16,64] However, data from a population-based study in Iceland demonstrates a clear increase in DILI incidence with increased age, whereby 15–29-year olds had an incidence rate of 9 per 100,000 that increased to 41 per 100,000 for patients >70 years old. The effect of age on DILI incidence was also paralleled by an increase in medication use, suggesting that age *per se* might not increase the risk of DILI but rather the fact that the elderly are generally taking more medications.[65]

Nevertheless, age appears to affect the risk of DILI induced by specific causative agents. Several reports are available in which advanced age is demonstrated as a risk factor for isoniazid hepatotoxicity, alone or in combination with other anti-TBC drugs.[66] A retrospective database evaluation of 3,377 adults on isoniazid therapy in the US found almost twice as many cases of hepatotoxicity in patients aged 35–49 years and almost 5 times as many cases in patients ≥50 years old than in patients aged 25–34 years.[67] It has been speculated that altered pharmacokinetics and/or cumulative mitochondrial functional impairment could be involved in the more frequent occurrence of isoniazid-related liver injury in elderly patients.[67,68] In contrast, young age seems to be a risk factor for DILI induced by valproic acid, with children less than 10 years old having a higher risk of developing DILI and children less than 2 having the highest risk of a fatal outcome, possibly due to differences in drug metabolism and reduced plasma protein binding.[69,70]

In addition to susceptibility, age also seems to have an effect on DILI phenotype with younger patients more commonly developing hepatocellular injury, while older patients are more prone to a cholestatic pattern of injury.[64,71] Interestingly, this observation contradicts old age as a risk factor for isoniazid

hepatotoxicity as it predominantly produces the hepatocellular type of injury. This highlights the intricate interplay between DILI risk factors, in which the effect of a single risk factor may vary depending on the presence or absence of additional modulating factors. Furthermore, older age has been associated with increased risk of DILI with persistent/chronic liver biochemical abnormalities, potentially due to a decline in tissue repair functions occurring with age.[58,72]

Statement

- Age may be considered a contributing factor determining the susceptibility to DILI, secondary to particular drugs, and contributing to the phenotype of DILI.

Evidence: Extrapolation from level 2 studies (prospective cohort studies) and level 4 studies (case series)

Sex

Women are reported to have a higher risk of ADRs in general.[73] Differences in male and female incidence rates have also been observed for various hepatic conditions. While women are more prone to develop primary biliary cholangitis and autoimmune hepatitis (AIH), men predominate among patients with primary sclerosing cholangitis and hepatocellular carcinoma.[74] The effect of sex as a risk factor for DILI is however more ambiguous. Epidemiological data from large DILI cohorts in Spain, the US and Iceland demonstrate a relatively equal sex distribution; 49%, 59% and 56% of patients with DILI were female, respectively.[15,16,64] While sex does not appear to be a general risk factor for DILI, increased female susceptibility has been noted for specific causative agents, such as minocycline and nitrofurantoin.[75] This may be related to the fact that these drugs often produce DILI with autoimmune features, and women are more susceptible to idiopathic AIH. In addition, evidence from several studies supports that female patients with DILI may have a higher risk of progressing to ALF.[18,56]

Statements

- Female sex may be considered a risk factor for DILI associated with specific drugs.

Evidence: Level 4 (case series)

- Female sex may be associated with a greater risk of drug-induced ALF.

Evidence: Extrapolation from level 2b studies (retrospective cohort studies)

Race

The influence of ethnicity on an individual's response to drugs has been primarily attributed to variations in single nucleotide polymorphisms (SNPs) among people from different ethnic groups. The influence of heritable epigenetic factors in the regulation of gene expression and hence pathogenesis, and the potential influence of dietary factors directly affecting the comorbidity (such as insulin resistance, lipid metabolism) or indirectly affecting it through the gut microbial environment have not been investigated in relationship to DILI.

A randomized, single-blind, placebo-controlled, 5-treatment, parallel-group, diet-controlled, longitudinal study of 145 healthy adults showed that an initiation of recurrent daily intake of 4 g of acetaminophen is associated with ALT elevations, while concomitant treatment with opioids is not.[76] An exploratory analysis in this study suggested that Hispanic origin is associated with increased susceptibility to this phenomenon of self-resolving aminotransferase elevation (referred to as adaptation).

A recent cohort study reported significantly different causative medications underlying DILI in different ethnic groups. Trimethoprim/sulfamethoxazole, methyldopa and phenytoin were more often the cause of DILI among African-Americans, while amoxicillin-clavulanate was a causative agent in a significantly higher proportion of Caucasians.[77] Although these variations may be related to genetic factors, equally, factors such as indications for these medications as well as variations in prescription patterns may explain the difference between different groups. Conversely, the frequency of severe cutaneous reactions was significantly higher in African-Americans, so were the rates of hospitalization, liver transplantation or liver-related deaths compared to Caucasians after controlling for selected covariates.

A meta-analysis of results from candidate gene studies investigating drug-metabolising enzyme (DME) polymorphisms on the risk of anti-TBC DILI showed a substantial variation in association between SNPs in N-acetyltransferase 2 (*NAT2*) and DILI. A total of 24 studies involving 1,116 cases of DILI (defined variably) and 2,655 controls, found that slow NAT2 genotype was associated with increased risk of DILI among people of East Asian and Middle Eastern origins, but no associations were found in Caucasians.[78] In contrast, a recent genome wide association study (GWAS) involving cases of anti-TBC DILI and drug-exposed controls from India found no genome wide significant signals questioning the role of the *NAT2* genotype in determining DILI susceptibility.[79] However, considering that the latter study involved a high proportion of patients manifesting with jaundice and ALF leading to death or transplantation, it is possible that the *NAT2* genotype may predominantly influence initial steps in the pathogenesis. Hence, it may still be associated with DILI in cohorts enriched by cases with elevated liver enzymes alone.

A GWAS involving 201 White European and US cases of amoxicillin-clavulanate induced DILI and 532 population controls matched for genetic background, showed the strongest association with HLA class II haplotype, HLA-DRB1*15:01-DQB1*06:02 and another novel and independent association with the class I allele, HLA-A*02:01.[80] However, when considered as an individual risk factor, the effect of A*02:01 was seen only in cases of north-western European, and not Spanish origin.

In addition, minor allele frequency of risk alleles in a particular ethnic group may account for some of the variations among different groups' susceptibility to DILI secondary to a particular drug. The HLA-DRB1*15:02 allele is prevalent in only 0.7% of Caucasian populations while its prevalence is 13–18% among South-Asians. A recent report has identified HLA-DRB1*15:02-DQB1*06:01 as a potential risk factor for amoxicillin-clavulanate related fulminant hepatic failure requiring liver transplantation in individuals of South-Asian origin.[81] Interestingly, adverse cutaneous reactions to anticonvulsant drugs, such as carbamazepine, phenytoin and lamotrigine, have been consistently associated with the HLA-B*15:02 haplotype, especially among Asian patients.[82,83]

A recent international collaborative GWAS involving 862 individuals with DILI and 10,588 population-matched controls, associated overall DILI caseswith A*33:01, a HLA class I allele, with an odds ratio (OR) of 2.7; 95% confidence interval (CI), 1.9–3.8.[84] The association was significant in each of the population clusters, with Italians showing a higher OR than northern Europeans and the Spanish displaying the lowest OR of all. Further drug-specific analyses indicated that the association with A*33:01 was driven by large effects from DILI related to certain drugs including ticlopidine (OR 163). In contrast, A*33:03 is a risk factor for ticlopidine DILI among Japanese (OR 13).[85]

Statement

- Ethnicity should be considered a risk factor for DILI.

Evidence: Extrapolation from level 1 (inception cohort) studies.

Alcohol, pregnancy

Similar to age, alcohol consumption is included as a risk factor in the CIOMS/RUCAM causality assessment scale and gives an extra point to patients with a known history of alcohol consumption, although no specific level of consumption has been defined.[63] Alcohol is a recognised CYP2E1 inducer and as such of crucial importance in the formation of N-acetyl-p-benzoquinone imine (NAPQI), the reactive metabolite responsible for acetaminophen hepatotoxicity. However, data to support alcohol as a risk factor for idiosyncratic DILI are only available for selected drugs, such as isoniazid, methotrexate and halothane.[86] Curiously, any alcohol use in the preceding 12 months was a negative predictor of severe DILI (OR 0.33; 95% CI 0.15–0.76) in the DILIN cohort.[48] Nevertheless, the recovery of idiosyncratic DILI induced by any causative agent in patients with an underlying alcohol-induced liver condition may be hampered by the latter condition. A more recent study of the effect of alcohol on DILI by the DILIN group found that heavy alcohol consumption (men: >3 drinks/day, women: >2 drinks/day) was not associated with worse outcomes in DILI patients compared to no alcohol consumption. Anabolic steroids were found to be the most common cause of DILI among the heavy drinkers. However, this could be a behavioural association rather than a pathophysiological link as stated by the authors. Furthermore, this study found no evidence for alcohol consumption being a risk factor for DILI attributed to isoniazid.[87]

Limited evidence is available to support that pregnant women are more susceptible to DILI, despite the inclusion of pregnancy as a risk factor for cholestatic/mixed type of DILI in the CIOMS/RUCAM causality assessment scale.[63] Furthermore, it is important to distinguish DILI during pregnancy from intrahepatic cholestasis of pregnancy, which can have a similar clinical picture. Information on drugs associated with DILI in pregnant women is mainly restricted to antihypertensive agents (such as methyldopa and hydralazine), antihyperthyroidism agents (propylthiouracil) and antimicrobials (in particular tetracycline and antiretroviral agents). The link between pregnancy and DILI due to methyldopa and hydralazine likely stems from the fact that these drugs are used to treat gestational hypertension. A small number of resultant DILI cases have been reported, however the majority of DILI case reports concerning these antihypertensive agents involve non-pregnant patients, in particular for methyldopa.[88–90]

The hepatotoxic potential of propylthiouracil has been recognised in the form of a black box warning issued by the US Food and Drug Administration (FDA) in 2010 and soon thereafter by the European Medicines Agency (EMA).[91] While paediatric patients appear to be at higher risk of propylthiouracil hepatotoxicity, little evidence supports that pregnancy would be a risk factor for this type of DILI.[92] Nevertheless, propylthiouracil DILI resulting in liver transplantation during pregnancy has been reported.[93] Similar to methyldopa and hydralazine, propylthiouracil is most likely associated with DILI during pregnancy because it is advocated as the treatment of choice for pregnant women with hyperthyroidism during the first trimester.

Tetracycline is currently the only known drug for which pregnancy appears to increase the risk of DILI development. Tetracycline is known to cause "microvesicular steatosis of the liver" also referred to as "acute fatty liver of pregnancy", in particular after taking large doses intravenously;[94,95] this has led to removal of intravenous preparations from clinical practice. Hence, tetracycline-associated fatty liver of pregnancy appears to be more dose-dependent than the more typical examples of idiosyncratic DILI. Hepatotoxicity due to tetracycline, however, is not limited to pregnant women, but has likewise been reported for men.[96] Tetracycline depresses cell anabolism by interfering with protein synthesis, inhibiting acetate metabolism and impairing oxidative phosphorylation. It is believed that the increased demands for protein anabolism in the liver during pregnancy, make pregnant women more susceptible to tetracycline-induced hepatotoxicity. In terms of anti-infectives, several studies have been reported in the area of antiretroviral hepatotoxicity in pregnant women.[97,98] However, the role of pregnancy as an independent risk factor for this form of DILI is debatable.

Statement

- Regular alcohol intake may be a contributing factor for DILI associated with specific drugs such as isoniazid, methotrexate and halothane.

Evidence: Level 4 (case series)

Underlying diseases

Comorbidity. Observations that antimicrobials despite their relatively short exposure are among the most common cause of DILI has led to the hypothesis that ongoing systemic inflammatory response may provide a co-stimulatory 'danger signal' that promotes adaptive immune responses involved in the development of DILI. Similarly, an apparent excess risk of DILI with increasing age may also reflect higher comorbidity (as well as increased exposure to drugs) which may influence susceptibility to hepatotoxicity. However, there is limited evidence to support or refute the role of comorbidities in determining susceptibility to acute DILI. This is due to the fact that DILI is a rare event and hence is not identified in randomized controlled trials (RCT) designed to assess the efficacy of the drug, while longitudinal cohort studies involving large populations of people exposed to a particular drug with and without developing DILI are lacking.

However, the effect of comorbidities has been evaluated in relation to drug-associated fatty liver disease (DAFLD). Evidence from well-designed studies indicates that drugs in this context work synergistically with other risk factors, contributing to pathogenesis and progression of liver disease. In a multicentre trial involving more than 5,000 women, tamoxifen therapy was associated with 2-fold risk of developing fatty liver over a 5-year period with an incidence of 0.4% per year in the treated group compared with 0.2% in the placebo group.[99] This association was restricted to overweight and obese women and the increased risk manifested within the first 2 years of treatment. Other factors associated with the development of fatty liver included hypercholesterolemia and arterial hypertension. In a breast cancer registry,[100] 24 out of 1,105 (2.2%) had non-alcoholic steatohepatitis (NASH; defined using a combination of imaging, liver enzyme elevation and biopsy); the odds of developing NASH increased 8.2-fold when patients were treated with tamoxifen and liver enzymes normalised in the majority after tamoxifen was stopped. In addition, the odds of NASH increased by 13% for every 1 kg/m^2 increase in body mass index and decreased by 5% for every 1-year increase in age.

Methotrexate-associated fatty liver disease and its severity has also been associated with alcohol excess, type 2 diabetes and obesity.[101–103] A recent study demonstrated that obesity and type 2 diabetes were associated with patients being listed for transplantation for end-stage methotrexate-related liver disease.[104]

Chronic liver disease. An assumption that chronic liver disease may be associated with reduced metabolism and clearance of medications is not supported by strong evidence. Studies in alcoholic liver disease and non-alcoholic fatty liver disease (NAFLD) have found inconsistent results with induction, down-regulation or no alteration in the activities of different DMEs.[105,106] Some of these variations may be explained by the varying degree of liver injury of individuals studied and others due to the methodologies used, yet, no generalisations can be made with regards to the impact of liver function on drug disposition in chronic liver disease.

Clinical trials involving treatment of human immunodeficiency virus (HIV) infection report a high rate of hepatic adverse reactions ranging from 2% to 18%[107] with lower incidence of DILI in larger studies. The vast majority of these events (84%) only led to either a temporary or no interruption of therapy.[108] The contribution of each particular drug to the development of hepatotoxicity in a 'highly active antiretroviral therapy' (HAART) regimen is difficult to determine; a number of mechanisms including mitochondrial toxicity, inflammatory response to viral infection and adaptive immune response have all been hypothesised. In 16 adult acquired immunodeficiency syndrome (AIDS) Clinical Trial Group studies involving 8,851 patients, hepatitis C (HCV) coinfection and baseline elevations of ALT were associated with an increased risk of DILI (defined as >5 × ULN for ALT or >2.5 × ULN of total bilirubin [TBL]).[109] Another review that grouped studies involving antiretroviral therapy and those including non-nucleoside reverse transcriptase inhibitors demonstrated that pre-existent liver disease including chronic hepatitis B virus (HBV) or HCV infection as well as alcoholic liver disease and elevated liver enzymes prior to initiation of therapy were risk factors for DILI (defined as elevation of 2–3 times above the baseline of ALT or AST).[107] Immune reconstitution could be one of the mechanisms that mediates liver injury under this set of circumstances. It has been hypothesised that the immune deficit caused by HIV infection is responsible for the attenuation of the inflammatory reaction in the liver and antiretroviral therapy by inhibiting HIV replication leads to immune reconstitution which could unmask liver toxicity.

Anti-TBC therapy where patients are regularly monitored permits investigation of risk factors for DILI. A systematic review of 15 studies demonstrated that when ALT elevation >5 × ULN was applied as a threshold, chronic hepatitis B was associated with DILI (OR 3.4) in an analysis restricted to prospective studies.[110] A recent retrospective study involving 379 (including 128 patients with chronic viral hepatitis) receiving anti-TBC therapy found that HCV on its own or in combination with HBV was associated with increased incidence of DILI.[111] HIV infection has also been shown to increase the risk of anti-TBC DILI by 4-fold and coinfection with HCV increased this risk by 14-fold.[112]

In a large cohort of DILI, 10% had pre-existing liver disease, mainly chronic hepatitis C or raised liver enzymes; azithromycin was the implicated agent in a higher proportion of patients with pre-existing liver disease (6.7%) compared to those without liver disease (1.5%).[16] Mortality was significantly higher in those with chronic liver disease (16%) compared to those without (5.2%). In a cohort of 107 patients with chronic liver disease including 58 with cirrhosis receiving anti-TBC therapy, 17% experienced DILI including 24% with chronic hepatitis and 15% with compensated cirrhosis.[113]

Statements

- Components of metabolic syndrome should be considered risk factors for the occurrence and the degree of DAFLD in patients treated with tamoxifen and methotrexate.

Evidence: Level 1b and 2b studies (RCT and individual cohort studies)

- Chronic hepatitis B and C can be considered risk factors for DILI from anti-HIV and anti-TBC therapy.

Evidence: Level 2a studies (systematic review of cohort studies)

Drug-dependent risk factors

Dose and hepatic drug metabolism

Drug dose plays a crucial role in intrinsic DILI, which occurs in patients having taken a drug overdose, for example acetaminophen hepatotoxicity. The fact that idiosyncratic DILI occurs after drug treatments at recommended daily doses initially led to the belief that idiosyncratic DILI is a dose-independent reaction. In 1999, Uetrecht highlighted the fact that drugs given at a daily dose of 10 mg or less are rarely, if ever, associated with a high incidence of idiosyncratic DILI.[114] The idea that drug dose plays a role in idiosyncratic DILI was first demonstrated in a study of 598 Swedish DILI cases reported to the Swedish Adverse Drug Reaction Advisory Committee, which found that 77% of the cases involved a causative agent given at a dose ≥50 mg/day.[115] A preponderance of causative agents with a recommended daily

dose of ≥50 mg has since been confirmed in the Spanish DILI registry and in a nationwide Icelandic DILI study, in which these causative agents constituted 77% and 88% of the 2 cohorts, respectively.[15,64] That said, a large proportion of today's pharmaceuticals require a dosage of >50 mg/day to have a desired effect. It is therefore difficult to say with certainty if idiosyncratic DILI is associated with higher dosage or if the higher identification rate of DILI cases due to pharmaceuticals with a recommended dosage of >50 mg/day, for example antibiotics, is the result of these medications being more frequently used in modern pharmacotherapy.

Nevertheless, it is now assumed that dose in fact does play a role in idiosyncratic DILI, with some form of threshold dose that needs to be exceeded for the reaction to occur. Such a threshold dose may, however, vary among individuals.[116] This is exemplified in DILI cases where a patient tolerates a drug at an initial lower concentration but develops DILI when a dose increase (still within the recommended daily dose range) is required for better pharmacological effect.[117] In addition, DILI induced by causative agents with a daily dose of ≥50 mg has been found to have significantly shorter latency period than DILI induced by drugs taken at lower doses.[118]

In addition to dose, hepatic drug metabolism is believed to affect a drug's hepatotoxicity potential. The majority of drugs require some form of biotransformation to be eliminated and often also to produce active pharmaceutical ingredients. This process commonly entails formation of reactive metabolites that can lead to covalently bound haptens and/or cellular stress in a susceptible cellular environment that may elicit or co-stimulate the development of an adaptive immune response resulting in DILI. Associations between drug metabolic profiles and hepatotoxic potential have been reported. An analysis of 207 widely prescribed oral medications in the US found that drugs with significant hepatic metabolism (>50%) had a higher reported frequency of ALT elevations and liver failure. Furthermore, drugs with significant hepatic metabolism and a daily dose of >50 mg were found to confer a significantly greater risk of hepatotoxicity.[119]

Lipophilicity

Lipophilicity (often measured as the log of octanol-water partition coefficient, LogP) is known to influence various drug-related aspects such as potency, pharmacokinetics and toxicity.[120,121] Drugs with higher lipophilicity appear to have increased off-target binding as well as an increased likelihood of causing toxic events in general.[122] Lipophilicity combined with daily dose, referred to as the "rule-of-two", has been suggested to reflect a drug's hepatotoxic potential, with high lipophilicity (LogP >3) and daily dose (>100 mg) being associated with increased risk of DILI, based on an analysis of 164 approved medications in the US.[123] It has been speculated that higher lipophilicity could facilitate drug uptake into hepatocytes and subsequent hepatic metabolism that may result in increased amounts of reactive metabolites and thereby a potentially higher risk of DILI.[124] Lipophilic drugs generally require hepatic metabolism to be eliminated and LogP may therefore simply be a surrogate for extensive biotransformation and hepatic exposure to reactive metabolites.[125] A more recent analysis of LogP, daily dose and degree of hepatic metabolism across 5 publically available drug datasets found both lipophilicity and hepatic metabolism to be individual DILI risk factors, with increased risk when considered combined with dose.[126] The potential applicability of the rule-of-two as a predictive tool for hepatotoxicity in supporting drug research and development has been demonstrated on direct-acting antiviral medications for chronic HCV infection.[127] However, an independent study analysing 975 oral drugs was not able to confirm the prognostic ability of drug lipophilicity combined with daily dose.[128]

Concomitant drugs, potential interactions

In patients who are polymedicated prior to their DILI episode, it is often possible to determine the most likely causative agent in such cases based on the known hepatotoxic potential of each drug and temporal compatibilities between drug intake and symptom initiation. However, one should keep in mind that concomitant medications are not always innocent "bystanders" but can also affect DILI susceptibility through drug-drug interactions. Concomitant drugs are capable of modulating the metabolism of other drugs through induction, inhibition or substrate competition, in particular of CYP reactions. This could alter the proportion of a drug metabolised by otherwise minor pathways and/or produce increased cellular stress, resulting in increased hepatotoxic potential of a drug that on its own may not have resulted in clinically important DILI. Rifampicin is a strong CYP inducer and has been demonstrated to increase the incidence of hepatotoxicity when given together with isoniazid as an anti-TBC treatment.[129] Concomitant use of CYP 450-enzyme inducing anticonvulsant drugs, such as carbamazepine or phenytoin, has also been reported to increase the risk of valproic acid hepatotoxicity. The reason behind this is assumed to be the increased production of 4-ene valproic acid and (E)-2,4-diene valproic acid, caused by the concomitant anticonvulsant drugs.[130] Retrospective database analyses of liver event reporting frequencies of acetaminophen, isoniazid, valproic acid and amoxicillin-clavulanate in the presence of co-reported medications also support the potential influence that concomitant medications can have on the risk of hepatotoxicity and clinical outcomes.[131,132] The presence of dyslipidaemia and subsequent statin use has similarly been found to affect DILI outcome by providing a protective effect against progression to ALF in an analysis of 771 Spanish patients with DILI.[56] However, it can at times be difficult to determine if the true DILI modulator is in fact the concomitant medication or the underlying condition requiring the concomitant medication(s).

Special chemical moieties

Reactive metabolites and oxidative stress. Reactive metabolites are known risk factors for the onset of DILI.[133] During drug development, formation of reactive metabolites is assessed by covalent protein binding in *in vitro* human liver models. Reactive intermediates show large differences in their reactivity, which reflects how fast and selectively they bind to proteins or other molecules. Possible consequences of covalent binding are (i) alteration of function or location of the target protein, (ii) formation of neo-antigens, or (iii) no adverse effect or clinical impact, for instance if only few proteins are modified. Because reactive metabolites can modify the functionality and structure of cellular proteins, they are classified as an important risk factor for DILI by the health authorities. In addition to their direct toxic effect, reactive metabolites are considered a first step in the onset of idiosyncratic DILI since the covalently bound proteins form immunogenic haptens which can trigger a downstream immune response.[134]

The non-steroidal anti-inflammatory drug (NSAID) diclofenac causes severe hepatotoxicity, in rare instances, due to formation of reactive quinone imines by CYP2C8, CYP3A4 and activation to acyl glucuronides by UDP-glucuronyl transferase (UGT) 2B7.[135] Both oxidative stress and mitochondrial toxicity can ensue. Glucuronidation of the carboxylic acid moiety to acyl glucuronides is also seen with ibuprofen and naproxen, both of which are considered relatively safe from a hepatic perspective. However, a recent publication has highlighted that ibuprofen may have a higher hepatotoxic potential than previously anticipated.[136] Protein adducts of ibuprofen have been detected in human plasma and appear to derive from the acyl glucuronide.[135] Lumiracoxib, which was withdrawn due to fatal cases of hepatotoxicity, structurally resembles diclofenac and also forms reactive quinone imines.[137] Troglitazone, which forms a reactive quinone metabolite,[138,139] was also withdrawn due to fatal cases of hepatotoxicity. The antipsychotic clozapine forms an iminium ion through CYP-mediated metabolism and acute liver injury is estimated to occur in about 1 in 2,000 treated patients according to the LiverTox database.[140] Other hepatotoxic drugs that form reactive metabolites include acetaminophen, tolcapone, nefazodone, zafirlukast, tamoxifen, flutamide, amiodaquine, sulfamethoxazole, isoniazid, terbinafine, felbamate, halothane and carbamazepine.

Direct toxins to hepatocytes induce oxidative organelle stress such as ER and mitochondrial stress, leading to apoptosis or necrosis. The hepatotoxic metabolite of acetaminophen, NAPQI, oxidizes protein thiol groups and generates ROS. Both NAPQI and ROS damage mitochondrial DNA and activate the JNK signalling pathway, further amplifying mitochondrial ROS production, which lead to the opening of the mitochondrial membrane permeability transition pore (MPT). MPT opening results in the collapse of the mitochondrial membrane potential which is required for ATP synthesis, and in the release of intermembrane proteins which trigger necrotic cell death.[2] Although the opening of the MPT leads to the release of cytochrome c, which activates apoptosis, acetaminophen-induced damage is considered to reflect necrosis and not apoptosis, as there is no caspase activation after acetaminophen overdose and caspase inhibitors are ineffective in protecting against acetaminophen liver toxicity.[2,141] This is likely due to ATP depletion and oxidative stress inactivating caspases.

Mitochondrial hazards. Mitochondrial toxicity is exemplified by fialuridine, a nucleoside analogue that caused microvesicular fatty liver and ALF.[142] Fialuridine leads to a depletion of mitochondrial DNA and patients treated for chronic hepatitis B developed weight loss, jaundice, pancreatitis and lactic acidosis.[142] Microvesicular steatosis is also seen with amiodarone, valproate, tetracycline and various antiviral nucleoside analogues and is characterised by reduced numbers of mitochondria. Patients show hypoglycaemia, hyperammonemia and lactic acidosis but only mildly elevated levels of ALT.[143] The majority of nucleoside reverse transcriptase inhibitors used to treat HIV infection inhibit mitochondrial DNA polymerase γ and consequently have a boxed warning regarding potential mitochondrial toxicity. Valproic acid inhibits the mitochondrial β-oxidation of fatty acids and the mitochondrial respiratory chain, thereby reducing oxidative phosphorylation and depleting intracellular ATP levels. This also leads to the generation of excessive ROS that can cause further cellular injury. Superoxide dismutase 2 (SOD2) is the major scavenger of mitochondrial superoxide. A study in 185 patients from the Spanish DILI registry and population controls identified polymorphisms in the *SOD2* as well as the glutathione peroxidase 1 (*GPX1*) genes in patients who developed cholestatic or mixed type DILI in response to drugs believed to generate a reactive quinine-like or epoxide metabolite.[144] Sod2 (+/-) mice have proven useful to elucidate mechanisms of mitochondrial toxicity such as troglitazone-induced liver injury.[145]

Reye's syndrome describes an acute encephalopathy combined with liver injury that occurs in children treated with acetyl salicylic acid (aspirin), usually in the context of a viral infection such as influenza or varicella. Aspirin can uncouple mitochondria and inhibit mitochondrial fatty acid oxidation, resulting in mainly microvesicular steatosis. Laboratory findings include hyperammonaemia, hypoprothrombinaemia and hypoglycaemia. Since the restriction of use of aspirin in children, the incidence of Reye's syndrome has declined sharply.[146]

Troglitazone, nefazodone and benzbromarone, that were withdrawn from the market because of hepatotoxicity and are known mitochondrial toxicants, were also found to inhibit the bile salt export pump, BSEP (see below). Aleo *et al.* studied 72 compounds contained in the FDA's Liver Toxicity Knowledge Base (LTKB) for their effects on mitochondrial respiration and inhibition of human BSEP transport activity.[147] The LTKB contains a benchmark dataset of drugs whose potential to cause DILI is categorised into most-DILI-concern drugs (boxed warning or withdrawn from the market due to hepatotoxicity), less-DILI-concern drugs (DILI risk mentioned in the label) and no-DILI-concern drugs (no DILI indication in the label).[148] This DILI classification has been refined by incorporating the causality assessment from clinical studies together with drug labelling information to improve its accuracy.[149] Drugs with dual potency as mitochondrial and BSEP inhibitors were highly associated with more severe human DILI and appeared more sensitive to drug exposure (C_{max}).[147]

Hepatobiliary transport inhibition. Consistent with the role of bile acid transporter impairment in various liver diseases, inhibition of BSEP by drugs or their metabolites is considered an important mechanism of drug-induced cholestasis and has been reported for cyclosporine A, rifampicin, bosentan, troglitazone and various other compounds.[150] The standard assay to measure BSEP inhibition employs isolated membrane vesicles from Sf9 insect cells that overexpress BSEP.[151] By this approach, several industry groups have systematically assessed the DILI risk of drugs by correlating the inhibitory potential towards BSEP with exposure levels.[152,153] A well characterised BSEP inhibitor is the endothelin receptor antagonist bosentan, approved for pulmonary hypertension but with a boxed warning for hepatotoxicity.[154] Cyclosporine A is a potent BSEP inhibitor[151–153,155] and is associated with drug-induced cholestasis in clinical routine. The major metabolite of the antidiabetic drug troglitazone, troglitazone sulfate, has a high potential to competitively inhibit BSEP and accumulate in hepatocytes.[156]

The Critical Path Institute's Predictive Safety Testing Consortium (C-Path PSTC) hosted a webinar in 2016 focused on BSEP inhibition and perturbation of bile acid homeostasis as mechanisms of DILI and a broad industry-wide consensus was reached on the importance of testing lead compounds in

BSEP inhibition assays so as to identify potential DILI liabilities at an early stage.[157] The EMA recommends interaction testing of drugs with BSEP during development.[158] The FDA guideline recommends testing of BSEP, multidrug resistance proteins (MRPs) and the multidrug and toxin extrusion (MATE) transporters where appropriate.[159] In cases of elevated liver enzymes (ALT or alkaline phosphatase [ALP]) during clinical trials, testing for inhibition of BSEP by the compound is critical for understanding the mechanism of DILI[160] and may help to design the safety plan for clinical trials. BSEP inhibition *per se* is not a show stopper since additional factors such as the mode of uptake into hepatocytes, the metabolism of the drug or the relation of the unbound C_{max} to the inhibitory affinity to BSEP (as expressed by the Ki value or with limited information by the IC_{50} value) are important parameters to be considered. If BSEP interaction has been found during development, determination of serum bile salt levels should certainly complement the clinical parameters needed for the identification of DILI.[160] In case drug metabolites are of concern, a vesicular BSEP assay should be complemented with a system that has the capacity to metabolise drugs, such as sandwich-cultured human hepatocytes.[161,162]

As drug metabolites are substrates of MRP2,[163] this canalicular export system also constitutes a risk factor for drug-induced cholestatic liver disease. Variants of this transporter have been associated with DILI.[164,165] When BSEP function is impaired, basolateral efflux systems (MRP3 and MRP4) are potential salvage systems to lower the burden of bile salts and drug metabolites for hepatocytes. Hence, these 2 transporters are additional potential susceptibility factors for drug-induced cholestasis.[166]

Statement

- A daily dose of >100 mg whatever the drug, predominant hepatic metabolism by cytochrome P450 enzymes, the formation of reactive metabolites, and dual inhibition of mitochondria and BSEP function are properties of drugs that can confer a risk for DILI. Predictive algorithms and selected preclinical testing are recommended to identify these liabilities in drug development.

Evidence: Extrapolation from 2c studies (outcomes research and mechanistic studies)

Diagnosis and causality assessment

Clinical-pathological manifestations

Clinical presentation

Pharmacological therapy has been associated with wide variety of alterations in the structure and functions of the liver and biliary system (Table 3). The majority of these alterations present acutely, identified by elevations of liver enzymes with or without non-specific symptoms, with the development of jaundice or occasionally ALF with coagulopathy and encephalopathy in the presence of jaundice. However, some forms of DILI presentation do not have distinct clinical, imaging or histopathological features, for example acute fatty liver, acute veno-occlusive syndromes, secondary sclerosing cholangitis or drug-induced AIH. Medications have also been associated with chronic liver diseases with more distinct imaging and histopathological features such as fatty liver disease, fibrosis, granulomatous hepatitis and nodular regenerative hyperplasia. Each of these forms is identified using the same characteristic features as those that are used to define the primary condition that is unrelated to drug aetiology. Drugs are recognised risk factors for liver tumours.

Furthermore, drug reactions with eosinophilia and systemic symptoms (DRESS syndrome) are well described. This drug-induced hypersensitivity syndrome involves multiple organs,[167] including the liver in 60–100% of cases, associated with life-threatening complications including mortality in 10% of cases. Withdrawal of the offending medication is critical and systemic steroids are commonly used, although there are no controlled clinical trials to assess the efficacy of this treatment.

Patterns of DILI

Acute liver injury is often detected and confirmed by liver biochemical blood tests. These generally include ALT, ALP, bilirubin, and albumin. Case definitions for DILI include one of the following thresholds: i) ≥5 × ULN elevation in ALT, ii) ≥2 × ULN elevation in ALP (particularly with accompanying elevations in concentrations of gamma-glutamyltransferase (GGT) in the absence of known bone pathology driving the rise in ALP level) or iii) ≥3 × ULN elevation in ALT and simultaneous elevation of TBL concentration exceeding 2 × ULN. In patients with abnormal liver tests prior to starting treatment with the implicated drug, ULN is replaced by the mean baseline values obtained prior to DILI onset and increases should be proportionate to this modified baseline. Liver injury is designated 'hepatocellular' when there is a 5-fold or higher rise in ALT alone or when the ratio of serum activity (activity is expressed as a multiple of ULN) of ALT to ALP is 5 or more. Liver injury is designated 'cholestatic' when there is a 2-fold or higher rise in ALP alone or when the ratio of serum activity of ALT to ALP is 2 or less. When the ratio of the serum activity of ALT to ALP is between 2 and 5, liver injury is termed 'mixed'.[160] As the liver enzyme elevations evolve over a period of time, the pattern of DILI is determined by the first set of laboratory tests available in relation to the clinical event.

Although the correlation between the biochemical categorisation and the pathological pattern of injury is somewhat limited, when liver biopsies were performed, cases with hepatocellular pattern of DILI were associated with higher degree of inflammation, necrosis, and apoptosis on histology.[168] Portal inflammation in these cases had plasma cells and eosinophils more often. In severe cases of hepatocellular DILI zone confluent necrosis usually involved zone 3; in contrast, patients with cholestatic pattern of DILI tended to have canalicular and hepatocellular cholestasis in zone 3. By and large, broad associations persisted even when analysis was limited to cases where the pattern of injury was determined based on the laboratory results within 1 week of presentation. Distribution of histological changes in mixed injury was more similar to that of cholestatic than hepatocellular injury.

Considering the phenotypic characterisation, increasing age has been associated with cholestatic pattern of liver injury and individual patterns of DILI follow different natural history.[14] A recent GWAS demonstrated a significant association between A*33:01, HLA class I allele and cholestatic and mixed DILI, but not for hepatocellular DILI indicating that host genetic factors influence the pattern of DILI.[84] All these factors taken together

Table 3. Definitions, phenotypes and drugs associated with hepatic adverse reactions.

Phenotypes of DILI	Case definition[160]	Medications associated with the phenotype
Idiosyncratic DILI	An adverse hepatic reaction that is unexpected on the basis of the pharmacological action of the drug administered. Three patterns of DILI determined using earliest identified elevation of liver enzymes levels. Initially ALT activity (patients ALT/upper limit of normal (ULN) of ALT) and ALP activity (patients ALP/ULN of ALP) is calculated. Then ALT/ALP ratio (R) is determined. Hepatocellular pattern: If ALT alone is elevated ≥5-fold above ULN or R ≥5. Cholestatic pattern: ALP alone is elevated ≥2-fold above ULN or R ≤2. Mixed pattern: R >2 to <5. Chronic DILI: DILI with acute presentation where there is evidence of persistent liver injury at >1 year after its onset.	Antimicrobials: Amoxicillin-clavulanate, erythromycin, flucloxacillin, interferon alpha/peginterferon, isoniazid, ketoconazole, minocycline, nevirapine, nitrofurantoin, pyrazinamide, rifampicin, co-trimoxazole, and sulfonamides. Central nervous system: Carbamazepine, chlorpromazine, dantrolene, halothane, phenytoin and valproate. Cardiovascular: Amiodarone, hydralazine, methyldopa, quinidine, statins (atorvastatin and simvastatin). Immunomodulatory: Azathioprine/6-mercaptopurine, infliximab, interferon beta, methotrexate and thioguanine. Antineoplastic: Busulfan, floxuridine and flutamide. Rheumatologic: Allopurinol, auronofin/Gold products, diclofenac, ibuprofen, nimesulide and sulindac. Endocrine: Anabolic androgenic steroids, estrogens/progestins and propylthiouracil. Others: Disulfiram and ticlopidine.
Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS syndrome)	Drug-induced hypersensitivity involving multiple organs with systemic manifestations.	Anticonvulsants (carbamazepine, phenytoin and phenobarbitone), minocycline, allopurinol, abacavir and nevirapine.
Drug-induced autoimmune hepatitis	Patient presenting with acute DILI with serological and/or histological markers of idiopathic autoimmune hepatitis.	Diclofenac, halothane, indomethacin, infliximab, methyldopa, minocycline, nitrofurantoin and statins.
Secondary sclerosing cholangitis	Patients presenting with acute DILI with histological and/or magnetic resonance cholangiopancreatography evidences similar to those of primary sclerosing cholangitis.	Amiodarone, atorvastatin, amoxicillin-clavulanate, gabapentin, infliximab, 6-mercaptopurine, sevoflurane and venlafaxine.
Granulomatous hepatitis	Presence on liver biopsy of granulomas (focal accumulation of modified macrophages) that are attributed to exposure to one or more medication.	Allopurinol, carbamazepine, methyldopa, phenytoin, quinidine and sulphonamides.
Acute fatty liver	Clinical syndrome of rapid development of liver and other organ failure associated with extensive microvesicular steatosis.	Amiodarone, didanosine, stavudine, valproate and zalcitabine.
Drug-associated fatty liver disease	Non-alcoholic fatty liver disease attributable to exposure specific medications.	Methotrexate, 5-fluorouracil, irinotecan, tamoxifen, corticosteroids, lomitapide and mipomerson.
Nodular regenerative hyperplasia	Diffuse nodularity within the liver with characteristic arrangements of hepatocytes at the centre and periphery of nodule.	Azathioprine, busulphan, bleomycin, cyclophosphamide, chlorambucil, cysteine arabinoside, carmustine, doxorubicin, 6-thioguanine and oxaliplatin.
Ductopenic (vanishing bile duct) syndrome	Chronic cholestasis associated with bile duct loss.	Azathioprine, androgens, amoxicillin-clavulanate, carbamazepine, chlorpromazine, erythromycin, estradiol, flucloxacillin, phenytoin, terbinafine and co-trimoxazole.
Liver tumours	Characteristics of hepatocellular adenoma or carcinoma based on established histological, computed tomography or magnetic resonance imaging features.	Anabolic androgenic steroids and oral contraceptives.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; DILI, drug-induced liver injury; ULN, upper limit of normal.

support the clinical classification of DILI on the basis of biochemical tests.

Recommendation

- DILI should be classified as hepatocellular, cholestatic or mixed according to the pattern of elevation of liver enzymes based on the first set of laboratory tests available in relation to the clinical event. **Grade B**.

Evidence: Extrapolation from level 2 studies (prospective cohort studies)

Specific phenotypes

Drug-induced autoimmune hepatitis. Many drugs have been associated with the syndrome drug-induced AIH that shares many features of idiopathic AIH. In cohorts of cases with the diagnosis of AIH, 2–9% were considered to be induced by drugs[169,170] and conversely, drug-induced AIH accounts for 9% of all DILI.[171] Most of these drugs have appeared in case reports or small case series and include nitrofurantoin, minocycline, diclofenac, statins and anti-TNFα agents (Table 4).[172]

Simplified Scoring System of the International Autoimmune Hepatitis Group that includes weighted scores for individual serological, genetic and liver histological features has become an accepted tool for the diagnosis of idiopathic AIH.[173] However, in a recent large cohort study, only 65% of those meeting 1999 International AIH Group criteria also met simplified score based criteria.[169] Likewise, when the differential diagnoses include drug-induced AIH, in addition to causality assessment, to assess the strength of association between drug exposure and the clinical manifestation, evaluation with genetic markers and liver biopsy are justified. Thorough characterisation of this particular subgroup of patients is important; histology might highlight features that favour one diagnosis over the other and genotyping would strengthen the diagnosis, assisting clinical decision making. Carriage of HLA alleles DRB1*03:01/*04:01 would

Table 4. Summary of tests utilised for diagnosis of DILI and distinction from AIH and prevalence of variant alleles[81].

Test: antibodies	% positive in AIH cases	% positive in 'normal' population
ANA 1:60	68–75%	15% (<40 ♀)–24% (>40 ♀)
ASMA	52–59%	Up to 43%
IgG >1,600 mg/dl	86%	5%
Anti-LKM	4–20%	1%
Test: HLA type	**% positive in DILI cases**	**% positive in 'normal' population**
*DRB1*15:01*	57–67% (Amoxicillin-clavulanate)	15–20%
*B*57:01*	84–87% (Flucloxacillin)	6%
*A*31:01*	17% (Carbamazepine)	2%
*DRB1*16:01-DQB1*05:02*	25% (Flupirtine)	1%
*A*33:01*	80% (Ticlopidine), 50% (Methyldopa), 50% (Enalapril), 43% (Fenofibrate), 43% (Terbinafine), 40% (Sertraline), 20% (Erythromycin)	1%
*B*35:02*	16% (Minocycline)	0.6%

AIH, autoimmune hepatitis; ANA, anti-nuclear antibody; anti-LKM, anti-liver-kidney-microsomal antibody; ASMA, anti-smooth muscle antibody; DILI, drug-induced liver injury; HLA, human leukocyte antigen; IgG, immunoglobulin G.

favour the diagnosis of idiopathic AIH, while presence of DILI risk alleles would support the diagnosis of drug-induced AIH. Interestingly, one of the DILI risk alleles, HLA DRB1*15:01, occurs less frequently in association with idiopathic AIH than healthy controls, hence genetic testing aids decision making in this scenario (Table 4). The role of liver biopsy and genetic tests in the diagnosis and management of DILI have been discussed in detail under separate sections.

In cases where even liver histological features cannot establish drug aetiology with certainty, it is reasonable to institute corticosteroid therapy in patients who do not show recovery despite drug cessation, with an intention to avoid progression of liver injury. However, once remission has been achieved, withdrawal of immunosuppression and close monitoring would resolve the diagnosis in the majority as drug-induced AIH do not relapse over a follow-up of 3–4 years,[170,174] while patients with idiopathic AIH relapse in 63% of cases in 1 year and 75% in 5 years.[175] Timing of withdrawal of immunosuppression and how one confirms the status of remission before attempting cessation of treatment varies between clinicians and clinical scenarios. It is best that such decisions are individualized by clinicians in discussion with the patient.

Recommendations

- Suspected drug-induced AIH should be evaluated in detail including causality assessment, serology, genetic tests and liver biopsy whenever possible. **Grade B.**

Evidence: Extrapolation from level 2 studies (validating cohort studies)

- In patients with suspected drug-induced AIH who are being treated with corticosteroids, withdrawal of therapy once the liver injury has resolved should be accompanied by close monitoring. **Grade B.**

Evidence: Level 2a studies (retrospective cohort studies with homogeneity)

Liver injury associated with immunotherapy for cancer. Immunotherapy for cancer refers to a new and leading strategy for the treatment of a variety of neoplastic diseases that have improved response rates, response durability, and overall survival rates. Immunotherapeutic agents that act as immune checkpoint blockades increase T cell responses and restore potent antitumour immune responses that are suppressed in cancer, with the goal of inducing tumour rejection. Immune checkpoints are surface molecules present on both immune cells and tumour cells and include among others cytotoxic T-lymphocyte antigen 4 (CTLA-4, target for ipilimumab), programmed cell death 1 (PD-1, target for pembrolizumab and nivolumab) and programmed cell death ligand 1 (PD-L1, target for atezolizumab, avelumab and durvalumab), which are all involved in intrinsic downregulation of immunity. Monoclonal antibodies targeting immune checkpoints are approved for the treatment of metastatic melanoma (ipilimumab, nivolumab and pembrolizumab), non-small cell lung cancer (nivolumab, pembrolizumab and atezolizumab) and urothelial carcinoma (nivolumab, pembrolizumab, atezolizumab, avelumab and durvalumab) among other solid tumours.[176] Nivolumab has recently been approved by the FDA for treatment of patients with advanced hepatocellular carcinoma, in whom sorafenib fails, on the basis of a phase I/II clinical trial.[177] Numerous phase III trials involving either nivolumab, pembrolizumab or tremelimumab are currently ongoing.[178] Although immune checkpoint inhibitors (ICIs) have shifted the paradigm from treating tumour cells directly to enhancing the host's immune system with a significant improvement in patient survival, the break in tumour tolerance is associated with inflammatory side effects and an increase in immune-related adverse events (irAEs), including hepatotoxicity.[179–181] Treatment emergent hepatotoxicity, although less common than other irAEs associated with the use of ICIs, has been detected in clinical trials powered for efficacy,[182] with ipilimumab leading to early treatment-related discontinuation in up to 11% of patients in clinical trials, while the combination of ipilimumab and nivolumab led to early discontinuations in up to 30% of patients.[183,184] A recent meta-analysis of published data found that CTLA-4 inhibitors were related to a higher rate of all-grade and high-grade hepatotoxicity compared with PD-1 inhibitors. In general, anti-PD-1 and PD-L1 therapy appears to have less severe toxicity than ipilimumab.[185] Other risk factors contributing to heightened risk of liver injury might be: a) dose, as a higher dose of ipilimumab (10 mg/kg) was associated with grade 3 and 4 hepatotoxicity in 3% of patients *vs.* 0% for 0.3 mg/kg and 3 mg/kg groups during maintenance treatment in melanoma patients with stable disease;[186]b) a pre-existing autoimmune diathesis that may be

unmasked by the ICIs, as has been suggested by the presence of serum auto-antibodies in some cases of hepatotoxicity; c) a pre-existing liver disease that is associated with increased expression of PD-L1 and PD-L2 or silent hepatic metastases, which might promote the expression of liver self-antigens, pro-necrotic cytokines, and/or activities of pro-inflammatory pathways which synergize with ICI activated T cells.[182] Nevertheless, a recent literature review of clinical trials did not find that elevated levels of aminotransferases in patients with hepatocellular carcinoma prior to nivolumab therapy increased the risk of severe liver toxicity.[178]

Hepatotoxicity ranges in presentation from asymptomatic increases in aminotransferases to acute hepatitis and even fulminant liver failure, with a time to onset of 6 to 14 weeks after treatment initiation (a median of 52 days after a median of 3 doses of immunotherapy) but may occur after longer periods of treatment and occasionally after discontinuation of the agent.[185,187] A published series including 5 cases of severe hepatitis related to ipilimumab with histological information, described a non-specific signature of portal and periportal inflammation and hepatocellular necrosis with infiltrating lymphocytes, plasma cells and eosinophils similar to what is observed with acute viral and AIH.[188,189] The histological pattern of liver injury related to immunotherapy has been further explored in a single-centre large-scale study including a per protocol liver biopsy for patients with hepatotoxicity grade ≥3.[190] This study has defined distinct patterns of liver damage for anti-CTLA-4 and anti-PD-1/PD-L1 agents. Hepatotoxicity caused by anti-CTLA-4 drugs showed a specific pattern of granulomatous hepatitis associated with severe lobular necrotic and inflammatory activity, fibrin deposits and central vein endothelitis. The histological pattern from patients receiving anti-PD-1/PD-L1 agents alone was more heterogeneous and characterised by active hepatitis with spotty or confluent necrosis and mild to moderate periportal activity, which were not associated with granulomatous inflammation.[190] Interestingly, in contrast to idiopathic AIH, ICI-related hepatitis is typically "seronegative", not presenting high titres of anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA) or other AIH-associated auto-antibodies and – upon ICI discontinuation – responds to a course of immunosuppressive therapy with no recurrence.[182,189,190] A large single-centre retrospective analysis of patients with melanoma treated with ipilimumab, pembrolizumab, and/or ipilimumab/nivolumab showed that 17 out of 218 developed hepatotoxicity. The majority of these patients were males (12/17) with a median age of 57 years and presented mainly with hepatocellular damage and experienced concurrent irAEs in 47% of the cases (gastrointestinal, endocrine, dermatological and lung disorders). Autoimmune serology was mostly negative, but the clinical picture improved with immunosuppressive therapy (steroids or cyclosporine in 1 steroid refractory patient). The median time to resolution after immunosuppression initiation was 31 days (range 6–56 days) with a median of 42 days on steroids (range 7–78 days). Fourteen patients out of 17 (82%) discontinued therapy and 2 deaths (12%) were reported.[187]

Strategies for effectively managing specific ICI-associated hepatotoxicity remain to be defined, but risk management measures include pretreatment and routine liver test monitoring during therapy and after treatment discontinuation. It is noteworthy that oncology clinical trials have graded hepatic adverse effect severity using the Common Terminology Criteria for Adverse Events (CTCAE) established by the Cancer Therapy Evaluation Program of the National Cancer Institute, which is based on peak abnormalities of serum liver biochemical indicators, including ALT, AST, ALP, GGT and bilirubin, measured as categorical levels of multiples of ULN.[191] Thus, very high degrees of aminotransferase elevation without concomitant bilirubin elevations, representing many of the cases reported in clinical trials, are considered as grade 4 hepatotoxicity. Hence, this system is less accurate than Hy's law in reflecting instances of potentially serious hepatotoxicity. Liver injury caused by ICIs usually responds to a short trial of immunosuppressive therapy with no recurrence upon discontinuation of the causative agent. However, not all patients developing liver injury would need corticosteroid therapy; a recent study based the decision to start corticosteroids on biological (bilirubin >2.5 mg/dl and/or international normalized ratio [INR] >1.5) or histological indicators of severity. Sixteen patients were assessed according to these pre-established guidelines and 6 (38%) did not receive corticosteroids and spontaneously improved.[190] The need to start steroid therapy in this type of population has also been questioned by other groups.[192] The recommendations on the management approach to suspected ICI-induced liver injury, which rely on clinical experience and the management of AIH, are summarized in Table 5. These recommendations are similar to protocol procedures used in registrational trials and have been incorporated into CPGs for several organisations including the American Society of Clinical Oncology and the European Society of Medical Oncology.

Statement

- Immune checkpoint inhibitors can induce immune-related hepatotoxicity in a substantial proportion of patients, with CTLA-4 inhibitors (ipilimumab) being more hepatotoxic than PD-L1 agents (nivolumab), and combination treatments carrying a greater risk.

Evidence: Level 1a studies (systematic reviews with homogeneity)

Recommendation

- It is suggested that decisions regarding corticosteroid treatment of immune-mediated hepatitis associated with ICIs are made by a multidisciplinary team involving hepatologists if DILI is sufficiently severe based on clinical and histological assessment. **Grade C**.

Evidence: Extrapolation from level 2 studies (individual cohort studies)

Secondary sclerosing cholangitis. Previously sclerosing cholangitis had been described following transarterial infusion of chemotherapeutic agents, as a result of ischaemic injury to the biliary tract rather than toxicity from chemotherapeutic agents themselves. However, recently secondary sclerosing cholangitis with diffuse inflammatory stricturing of the biliary tree on magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP) has been described in a small proportion of patients presenting with acute cholestatic DILI.[193] In this series, all 10 patients were women with a cholestatic or mixed pattern of DILI, 70%

presented with jaundice and time to resolution was longer in these patients compared to other patients with DILI. Drugs implicated were amoxicillin-clavulanate, sevoflurane, amiodarone, infliximab, 6-mercaptopurine, gabapentin, venlafaxin and atorvastatin. Differential diagnosis should include ischaemic injury, especially in critically ill patients[194] and those post-transplantation; HIV-related cholangitis/cholangiopathy (also termed acquired AIDS-related sclerosing cholangitis) should also be considered when appropriate.

Recommendation

- Diagnosis of drug-induced secondary sclerosing cholangitis can be considered in patients with a cholestatic pattern of DILI with slow resolution of liver injury and characteristic changes in the biliary system demonstrated on MRCP or ERCP. **Grade C**.

Evidence: Extrapolation from level 2 studies (retrospective cohort study)

Granulomatous hepatitis. Granulomata are circumscribed accumulation of macrophages some of which may fuse to form multinucleated giant cells, with a surrounding rim consisting of lymphocytes that have developed with stimulation of mononuclear cells from a variety of cytokines. The incidence of hepatic granulomas is reported in 2–15% of liver biopsies; of those with granulomatous hepatitis, 2.5% are considered drug-related.[195] The granulomas, which are usually non-necrotising can occur either in the portal or lobular distribution.[196] A number of infectious (TBC, *Schistosoma* and fungus), inflammatory (sarcoidosis) and immunological (primary biliary cholangitis) conditions are associated with hepatic granulomata and therefore, the diagnosis of drug-related granulomatous hepatitis depends upon a temporal relationship between exposure to the drug and the clinical manifestation, ruling out an alternative explanation for histological changes and previous reports in the literature. Allopurinol, carbamazepine, phenytoin, quinidine, methyldopa and sulphonamides are some of the medications which have been associated with this form of hepatotoxicity.

Recommendation

- Diagnosis of drug-related granulomatous hepatitis is suggested to involve expert evaluation of liver histology as well as exclusion of specific infections, inflammatory and immunological conditions that are well recognised causes of hepatic granulomata. **Grade D**.

Evidence: Level 5 (expert opinion)

Acute fatty liver. This is a rare form of acute hepatotoxicity referred to as 'Reye's syndrome' when seen in children treated with salicylate, although its occurrence has been reduced markedly by restricting the use of aspirin in those under the age of 16 years and the use of parenteral preparations of tetracycline. Microvesicular steatosis and absence of glycogen in the hepatocytes are characteristic histological features as the liver uses glycolysis to compensate for the lack of ATP produced by mitochondria. ALF related to microvesicular steatosis manifests with hypoglycaemia, lactic acidosis, hyperammonaemia and cerebral oedema. Dramatic rapid development of organ failure precedes the clinical syndrome with an acute rise in liver enzymes and jaundice that follow; hence, an index of suspicion is crucial in identifying the drug aetiology when approaching a patient with 'anicteric hepatic encephalopathy'. This mechanism rarely causes DILI on its own; in a recent review of liver biopsies from 249 cases of DILI only 1 case of microvesicular steatosis was identified in a case of fatal injury secondary to erythromycin.[168]

Sodium valproate is 1 of the drugs currently used that has been linked to the development of acute fatty liver; idiosyncratic hepatotoxicity occurs 1 in 37,000 people taking the drug and the risk increases to 1 in 500 in children on combination of multiple drugs. A case-control study demonstrated an association between variation in the polymerase γ gene, *POLG*, which codes for the mitochondrial DNA polymerase c, and valproate-induced hepatotoxicity.[197] Nucleoside analogue reverse transcriptase inhibitors are liable to cause hepatotoxicity by interfering with mitochondrial function.[198] The incidence of severe hyperlactatemia with hepatic steatosis has been reported to be 0.85–3.9 per 1,000 person-years, with 33% mortality in severe cases.[199] Stavudine, zalcitabine and didanosine have a higher affinity for mitochondrial DNA polymerase-γ, leading to the depletion of mtDNA and hence, have a higher rate of hepatotoxicity than abacavir, zidovudine, lamivudine and tenofovir. Microvesicular steatosis and hepatocellular necrosis (resembling 'Reye's syndrome') has also been reported in association with amiodarone.[200]

Recommendation

- Acute drug-induced fatty liver can be recognised based on its distinct clinicopathological characteristics in people exposed to drugs that are known to interfere with mitochondrial function. **Grade C**.

Evidence: Level 2 studies (retrospective cohort studies)

Drug-associated fatty liver disease. NAFLD is an entity associated with accumulation of fat in >5% of hepatocytes with or without inflammation and fibrosis in those who do not consume alcohol over the amount considered moderate (21 units in men and 14 units in women per week). Although initially described as a histological entity, in clinical practice, excess fat is detected through any of the imaging modalities and accepted as evidence of hepatic steatosis. When the condition is associated with characteristic features of metabolic syndrome or no risk factors are obvious it is considered primary NAFLD, while drugs are behind a proportion of 'secondary' NAFLD cases. A high prevalence of obesity and NAFLD in the general population means that the strength of association between individual drugs and fatty liver is variably dependent upon the particular drug. Risk factors associated with DAFLD are described in a separate section above.

Amiodarone: Hepatic storage of amiodarone may cause phospholipidosis with a characteristic histopathological appearance of intracellular lamellar inclusion bodies.[201] Amiodarone and its metabolite are concentrated in the hepatic mitochondria, inhibit electron transport and uncouple oxidative phosphorylation.[202] A amiodarone-related hepatic adverse

Table 5. Recommendations on management of immune-mediated liver injury induced by immune checkpoint inhibitors (modified from[179,181,187,190]).

	Recommendations	Knowledge gaps
Before start of therapy	Assess baseline liver parameters and lipid profile. Check for potential confounding factors such as pre-existing liver diseases and presence of liver metastases, viral infections (HIV, HBV, HCV, HEV). Rule out underlying autoimmune hepatitis and underlying autoimmune conditions.	Limited experience from clinical trials in patients with AIH. Use on a case by case basis. Risk of associated medication on irDILI severity is not established.
During therapy	Monitor liver biochemical parameters every 2 weeks during the first 8 to 12 weeks and then every 4 weeks. If abnormal liver parameters, follow recommendations.	
Grade 1: ALT ≤3 × ULN AST ≤3 × ULN TBL ≤1.5 × ULN ALP ≤2.5 × ULN	**Assessment**: Define type of liver injury according to biochemical parameters R = (ALT level/ALT ULN)/(ALP level/ALP ULN) R ≥5: Hep, R = 2–5: Mix R ≤2: Chol Rule out NASH or other liver diseases (include imaging test). Investigate history of alcohol consumption. Record status of the tumoural disease. Review concomitant medications including herbal supplements. Assess for other irAEs. **Management**: If irAEs are excluded (unlikely or unrelated) continue therapy with close follow-up. Start symptomatic treatment.	The episode could be considered an adaptive response. Time to achieve liver test resolution while on the drug needs to be defined.
Grade 2: ALT 3–5 × ULN AST 3–5 × ULN TBL 1.5–3.0 × ULN ALP 2.5–5.0 × ULN	**Assessment**: Similar to grade 1 **Management** Skip dose and monitor liver parameters, INR and albumin twice weekly. Start symptomatic treatment. If abnormal liver parameters persist longer than 2 weeks, start immunosuppression and discontinue the drug. Upon improvement immunotherapy could be resumed after corticosteroid tapering.	The ALT threshold for a possible signal of irDILI in patients with or without abnormal liver parameters at baseline needs to be defined.
Grade 3 or 4 **Grade 3:** ALT 5–20 × ULN AST 5–20 × ULN TBL 3–10 × ULN ALP 5–20 × ULN **Grade 4:** ALT >20 × ULN AST >20 × ULN TBL >10 × ULN ALP >20 × ULN	**Assessment**: Similar to grade 1 DILI assessment in patients with underlying liver disease or liver metastases is challenging. Liver biopsy to exclude metastatic progression and to assess the pattern of damage and severity. **Management**: Discontinue immunotherapy and monitor liver parameters and INR daily. Hospital admission if biochemical evidence of impending liver failure (bilirubin ≥2.5 mg/dl and/or INR ≥1.5). Stop further immunotherapy until hepatotoxicity is resolved. Consider permanent discontinuation of immunotherapy. Start corticosteroids (methylprednisolone or equivalent) at a dose of 1–2 mg/kg/day depending on severity. If there is no response to corticosteroids within 2–3 days, mycophenolate mofetil should be added at 1,000 mg twice daily. Supportive care Withdraw hepatotoxic drugs. If steroid refractory hepatotoxicity, consider additional immunosuppression: mycophenolate mofetil, cyclosporine, tacrolimus, anti-thymocyte globulin (first line alternative choice for intolerance to steroids). Infliximab is not recommended.	High dose, longer duration of treatment and host characteristics as risk factors for hepatotoxicity are unclear. The effect of immunosuppressive treatment on immune checkpoint inhibitor efficacy and patient survival is unknown. Lack of criteria to identify refractory patients to corticosteroids therapy. Criteria to distinguish irDILI from DILI due to an associated hepatotoxic drug.

DILI, drug-induced liver injury; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; AIH, autoimmune hepatitis; Hep, hepatocellular; Mix, mixed; Chol, cholestatic; irDILI, immune-related DILI; irAE, immune-related adverse event; ULN, upper limit of normal; INR, international normalized ratio.

reaction appears to be related to the total dose; hepatotoxicity can manifest either acutely or chronically. Raised liver enzymes appear within hours and days following initiation of intravenous administration and the enzymes normalise on cessation of the drug. In the context of cardiogenic shock amiodarone-induced liver injury can be difficult to distinguish from ischaemic injury. On oral administration, liver disease manifests after 3 months in the majority and takes weeks to months to reverse on withdrawal. Symptomatic hepatic dysfunction may occur in 1–3% of patients using amiodarone.[203] All of the histological hallmarks of NASH, including ballooning, Mallory-Denk bodies, fibrosis and cirrhosis have been described.[204]

Methotrexate: Reports that long-term methotrexate therapy is associated with fatty infiltration and fibrosis, with a potential to progress to cirrhosis, have resulted in a plethora of publications describing cohorts of patients receiving methotrexate for psoriasis, rheumatoid arthritis and inflammatory disease. The proportion of patients estimated to have any degree of liver fibrosis varies from 6% to 72%; those with advanced fibrosis range from 0% to 33% and cirrhosis from 0% to 26%.[205] Such a wide range of reported pathology is due

largely to heterogeneity of cohorts, study designs, methods of evaluating histological changes and the case mix. A recent study highlighted the rarity of decompensated cirrhosis associated with methotrexate therapy; of over 150,000 adults who had been listed for or received liver transplantation during a period of 24 years, only 117 had methotrexate-associated cirrhosis.[104]

Methotrexate polyglutamate within the cell interferes with pyrimidine and purine synthesis, through which it exerts its therapeutic effect. In addition, methotrexate indirectly affects methylenetetrahydrofolate reductase and hence the generation of methionine from homocysteine. Excess homocysteine induces ER stress, which, when unresolved, leads to fatty infiltration of the liver. Homocysteine, in addition, can also activate pro-inflammatory cytokines and activate hepatic stellate cells, leading to liver fibrosis.[206] Methylene tetrahydrofolate reductase gene polymorphisms (C677T in particular) have been associated with hepatotoxicity due to methotrexate. A meta-analysis demonstrated an OR of 4.19 (95% CI 1.6–10.7) for the TT *vs.* CC genotype.[207] Assessment of the risk-benefit ratio of long-term methotrexate therapy depends upon the efficacy of the drug in an individual weighed against the rate of progression of hepatic fibrosis. Reports that long-term methotrexate therapy is associated with a potential to develop fibrosis, which can progress to cirrhosis, have resulted in numerous guidelines recommending intense monitoring regimens including liver biopsies at regular intervals. The primary objective of monitoring is to detect hepatic fibrosis that is of clinical significance, yet reversible on withdrawal of the drug. Recently, a number of algorithms, serum biomarkers and imaging techniques have been introduced into clinical practice to non-invasively evaluate the severity of chronic liver diseases.[208] Some of these methods are being evaluated as tools to monitor patients on methotrexate treatment.[103,209–211] Large-scale well-designed studies to validate these tools in clinical practice are underway.

Tamoxifen: Treatment with tamoxifen, an oestrogen-receptor antagonist, has been associated with fat accumulation within the liver. The association between tamoxifen and DAFLD is demonstrated in a large clinical trial; incidence of fatty liver disease was 2-fold higher (hazard ratio = 2.0; 95% CI 1.1–3.5) in those exposed to the drug.[99] None in this large trial developed cirrhosis over a median follow-up of 8.7 years although another registry of tamoxifen treated patients reported the presence of NASH in 2.2% of patients (defined by a combination of liver enzyme elevation, imaging features and biopsy in some case), as well as 2 patients with biopsy-proven cirrhosis.[100]

Tamoxifen-induced fatty liver disease occurred only in overweight or obese women with metabolic syndrome[83] which indicates that host risk factors influence the susceptibility to DAFLD. Interestingly, a recent report associated *PNPLA3* and/or *TM6SF2* variant alleles with hepatic steatosis and elevated ALT levels in those exposed to glucagon receptor antagonists.[212]

Chemotherapy-associated steatohepatitis: Reactive oxygen species generated by chemotherapy intended to induce tumour cell apoptosis can also lead to the development of steatohepatitis especially in those with pre-existent hepatic steatosis; obesity is associated with an increased risk. Drugs commonly associated with steatohepatitis include 5-fluorouracil and irinotecan. Chemotherapy-associated steatohepatitis increases the risk of infections, liver failure and overall mortality following major liver resections (for hepatic metastasis).

Recommendation

- Particular drugs, such as amiodarone, methotrexate, tamoxifen and the chemotherapeutic agents 5-fluorouracil and irinotecan, should be considered as risk factors for fatty liver disease and decisions to continue or withdraw the medication rely upon the benefits of the treatment against the risk of progressive liver disease. **Grade B**.

Evidence: Extrapolation from level 1 studies (RCTs and inception cohort studies)

Nodular regenerative hyperplasia and sinusoidal obstruction syndrome. Some drugs can injure endothelial cells of sinusoids and portal venules with consequent occlusion or dropout of smaller radicles. Widespread vascular changes lead to diffuse nodularity within the hepatic parenchyma. The hepatocytes within the nodule are arranged in plates that are more than 1 cell in thickness while hepatocytes are compressed and atrophied into thin, parallel plates between nodules.[213] Characteristically, the nodules are not separated by fibrosis although there could be perisinusoidal fibrosis and incomplete fibrous septae. Magnetic resonance imaging may demonstrate a characteristic pattern with a sensitivity and specificity of 75–80%[214] although there is no consensus on the use of imaging in the diagnosis. In patients on azathioprine therapy, the cumulative rate of development of nodular regenerative hyperplasia has been estimated to be 0.5% over 5 years and 1.5% over 10 years,[215] although nodular regenerative hyperplasia has also been described in the post-liver transplantation setting in the absence of azathioprine therapy.[216] Early recognition and withdrawal of the medication has been shown to lead to histological resolution over a 5-year period.[217] Otherwise, management is focused on surveillance and prevention of manifestations of portal hypertension.

Nodular regenerative hyperplasia as indicated by liver histology has been reported in 8% (8/97) of a HIV-positive cohort receiving HAART.[218] In another case series, it was shown that 11 HIV patients with non-cirrhotic portal hypertension had all been exposed to didanosine for prolonged periods.[219] Other drugs associated with this form of liver disease are 6-thioguanine, busulphan, bleomycin, cyclophosphamide, chlorambucil, cytosine arabinoside, carmustine, and doxorubicin. In recent literature, oxaliplatin is the most common drug associated with this pathology.[220] In a large group of patients treated with oxaliplatin, nodular regenerative hyperplasia was found on histology in 25% and features consistent with SOS in over 50% of patients.[221] SOS has also been related to pyrrolizidine alkaloids as discussed in the Epidemiology chapter on the burden of herbal supplements.

Recommendation

- Drugs may be considered as risk factors for nodular regenerative hyperplasia and when possible it is suggested that the specific drug that has been associated is withdrawn. **Grade D**.

Evidence: Extrapolation from level 4 studies (inconclusive case series)

Liver tumours. The annual incidence of hepatic adenoma is 3–4 per 100,000 among regular users of oral contraceptives,[222] compared to its estimated incidence of 3 per million per year in the population. The hormonal dose and duration of medication have been associated with the risk of adenoma development and is highest in women over 30 years of age after using oral contraceptives for more than 24 months. The risk of hepatic adenoma has been described with contraceptive combination pills and may be lower with newer progesterone only pills.

Causal association between oral contraceptives and hepatic tumours has been accepted as there have been several reports of regression or resolution of adenomas after cessation of the drugs; regression may be less likely when the exposure to oral contraceptives is prolonged. Hormone receptors have also been found in a substantial proportion of hepatic adenomas.[223] However, there have also been reports of progression to hepatocellular carcinoma 3 to 5 years after stopping oral contraceptives. Therefore, surgical resection should be considered based on the site, size, and number of hepatic tumours as well as certainty regarding their nature on imaging.

The morphology of hepatic adenomas with their extensive proliferation of blood-filled sinusoids, supplied by high-pressure arterial flow, makes 20–40% of them bleed spontaneously causing right upper quadrant pain; intraperitoneal bleeds and ruptures leading to deaths have been reported. Progression into hepatocellular carcinoma occurs in about 10% of adenomas.[224]

Ultrasonographic features of hepatic adenomas are non-specific and triple phase computed tomography scanning or magnetic resonance imaging can distinguish them from haemangiomas, fibronodular hyperplasia and hepatocellular carcinomas in the vast majority of patients.

The association of liver tumours with androgens was first described in patients with Fanconi's anaemia on anabolic androgenic steroids. But, hepatic adenomas, hepatocellular carcinomas and others (cholangiocarcinoma and angiosarcoma) occur in those who take androgens for Fanconi's anaemia, other forms of aplastic anaemia as well as for other reasons (such as body builders, hereditary angioedema and immune thrombocytopenia). In a large series including 133 cases, hepatocellular carcinomas were associated with xymetholone and methyltestosterone, while adenomas were associated with danazol.[225] Both oral and parenteral therapies were associated with the development of tumours; these appear after a median period of 4 to 6 years of exposure to the medications. Male predominance among cases may be related to exposure of males to this medication. The causal association between anabolic androgenic steroids and hepatic tumours has been inferred from observations of regression of hepatic lesions upon discontinuation of the medications. However, the occurrence of tumours many years after discontinuation of therapy has been reported. Focal nodular hyperplasia (FNH) is a common differential diagnosis for liver tumours although its association with oral contraceptives has not been established. In a 9-year study in 216 women with FNH, neither the size nor the number of FNH lesions were influenced by oral contraceptive use; size changes during follow-up are rare and were not related to oral contraceptive use.[226]

Statements

- Oral contraceptives may be considered risk factors for the development of hepatic adenoma.

Evidence: Consistent level 2 studies (retrospective cohort studies)

- Androgens and androgenic steroids, particularly in the context of treating bone marrow failure, may be considered risk factors for the development of liver tumours.

Evidence: Level 5 evidence (inconclusive case series)

Recommendation

- Withdrawal of medications is suggested where possible with continued monitoring until regression of adenoma or definitive treatment. **Grade D**.

Evidence: Inconsistent level 4 (case series) evidence.

Laboratory tests

The absence of specific diagnostic biomarkers makes the diagnostic appraisal of suspected DILI cases strongly dependent of the judicious interpretation of serum liver biochemistry and other routine laboratory and imaging tests to carefully rule out alternative explanations of liver disease. Although DILI is a common cause of unexplained persistent abnormal liver tests,[227,228] patients most often present with a viral hepatitis-like syndrome; symptoms not providing any particular clue for suspicion unless associated skin features[229] point to the drug aetiology.

Aminotransferases

Serum aminotransferases (ALT/AST), ALP and TBL levels remain the mainstay for detecting and classifying liver damage in suspected DILI[230] (Table 6). There is now wide consensus that minor increases in ALT or AST that could result from adaptive and reversible liver responses to the drug (*i.e.* statins), or pre-existing liver disease (*i.e.* fatty liver) should not be classified as DILI. In this regard an international expert group[160] recommended specific serum aminotransferase cut-off points to establish a diagnosis of DILI (see Section *Patterns of DILI* for further details). An isolated elevation in TBL does not qualify as DILI[160] as it is usually related to its unconjugated fraction and secondary to Gilbert's syndrome or, if related to its conjugated form, is due to inhibition of bilirubin reuptake. Ideally, the serum aminotransferase values used for suspected DILI assessment should be from blood samples taken when DILI is first recognised. However, abnormal liver biochemistry should be interpreted with caution as it may not represent the true onset time of liver cell injury, which may already be advanced, subsiding or past when first found.[231] Hence, serial aminotransferase measurements are necessary to clarify this important issue.

Elevated serum aminotransferases do not reflect the extent to which the liver is damaged in insidious or atypical varieties of hepatotoxicity, including indolent fibrosis (methotrexate), vascular liver disorders, cirrhosis and microvesicular

Table 6. Standard liver biochemistry to assess suspected DILI (modified from[376]).

Test	Possible clinical implication of abnormality	Specificity for liver disease
Alanine aminotransferase	Hepatocellular damage	Reasonably specific when >3× ULN (low concentrations in tissues other than liver, *e.g.,* skeletal muscle)
Aspartate aminotransferase	Hepatocellular damage	Not specific (skeletal muscle, heart, pancreas, blood)
Total bilirubin	Cholestasis, impaired uptake, conjugation or excretion, biliary obstruction, haemolysis	Not specific. Two forms: indirect (unconjugated) and direct (conjugated)
Alkaline phosphatase	Cholestasis, infiltrative disease, biliary obstruction	Not specific (bone, salivary glands, intestinal, biliary)
Gamma-glutamyltransferase	Cholestasis, biliary obstruction	Not specific (kidney, liver, pancreas, GI tract, lung)
Glutamate dehydrogenase	Hepatocellular (mitochondrial) damage	Specific, helpful to differentiate muscular from hepatic injury
Albumin	Impaired hepatocellular function	Malnutrition, nephrotic syndrome, cirrhosis (any cause)
International normalized ratio	Impaired hepatocellular function	Vitamin K deficiency; anticoagulants
Creatine kinase	Muscular injury	Crucial to differentiate muscular from hepatic injury

GI, gastrointestinal; ULN, upper limit of normal.

steatosis secondary to mitochondrial toxicity. In such instances, the threshold values may not be reached and the diagnosis must be approached on an individual basis, according to histological/imaging findings in the setting of specific drugs/toxicants.

It should be kept in mind that a rise in serum ALT can also be driven by other organ damages, particularly muscle injury, which can be drug-induced (*i.e.* rhabdomyolysis). A disproportionate increase of AST with regard to ALT should prompt testing for creatine phosphokinase (CPK) that can assist in distinguishing between liver and muscle derived ALT elevations. In addition, rises in ALT accompanied by greatly elevated lactate dehydrogenase (LDH) are normally found during acetaminophen hepatotoxicity as well as ischaemic injuries and lymphoma. Nevertheless, a rise in serum ALT is highly sensitive for hepatocyte injury and if accompanied by a concomitant rise in TBL, which has a great specificity for the liver (and measures liver function), becomes a reliable biomarker of liver injury in DILI.[232] Elevated ALP values usually indicate cholestatic damage and testing for GGT if raised provides evidence that the ALP elevation is of hepatic origin. However, GGT elevated in isolation is insufficient to qualify as DILI as it does not indicate liver damage.[160] The reliability of AST and GGT in replacing ALT and ALP, respectively, was assessed in a study of 588 patients included in the Spanish DILI registry. Whereas AST values can reliably substitute ALT in calculating the pattern of injury when the latter is unavailable at DILI recognition, GGT is less reliable as an ALP substitute.[233]

Initial DILI assessment should also include serum albumin and coagulation parameters. Elevated INR values, which suggest impending liver failure, should prompt referral to a liver transplant unit. Liver biochemistry should be routinely tested in patients with DILI until complete normalisation. Steady decline of aminotransferases supports the diagnosis, whereas slow or incomplete resolution of biochemical abnormalities suggests competing aetiologies. In addition, persistently elevated aminotransferases may indicate a chronic outcome. A long-term follow-up in a cohort study involving 298 patients with DILI revealed that persistently elevated TBL (>2.8 × ULN) and ALP (>1.1 × ULN) in the second month from DILI onset significantly predicted chronic DILI.[58]

Recommendations

- ALT, ALP and TBL are the standard analytes to define liver damage and liver dysfunction in DILI. AST values can be used to reliably substitute ALT in calculating the pattern of injury when the latter is unavailable at DILI recognition, whereas GGT is less reliable as an ALP substitute. **Grade C**.

Evidence: Extrapolation from level 2b studies (exploratory cohort studies with good reference standards)

- Persistently elevated TBL and ALP in the second month from DILI onset should be used as a marker for chronic DILI. **Grade B**.

Evidence: Level 1b studies (individual inception cohort studies).

Laboratory workup for excluding alternative causes

The diagnosis of DILI largely relies on the exclusion of alternative causes of liver damage. The pattern of injury can aid in the initial diagnostic approach to rule out the most common causes of hepatitis and cholestasis (Fig. 2). In addition, age and comorbidities, the individual's unhealthy habits and the local burden of infectious diseases potentially affecting the liver can also help in guiding the diagnostic workup (Table 7). Acute hepatitis C is a challenging diagnosis that can be misdiagnosed as DILI because patients can initially be anti-HCV negative. In fact, HCV-RNA tested positive in the first analysis of the DILIN cohort in 1.3% of adjudicated DILI cases.[48] In Western countries hepatitis E (HEV) is an emerging cause of viral hepatitis in association with ingestion of uncooked meat and can subsequently masquerade as DILI.[234,235] Anti-HEV IgM seroprevalence in adjudicated DILI cases has ranged from 3% in the DILIN database[235] to 7% in the Spanish DILI registry.[236] Spanish anti-HEV IgM positive adjudicated DILI cases had less compatible temporal sequences, were exposed to drugs with low hepatotoxicity potential and/or had very high aminotransferase levels.[236] However, anti-HEV IgM as a diagnostic test for active HEV infection is currently questioned.[237] Despite this limitation,

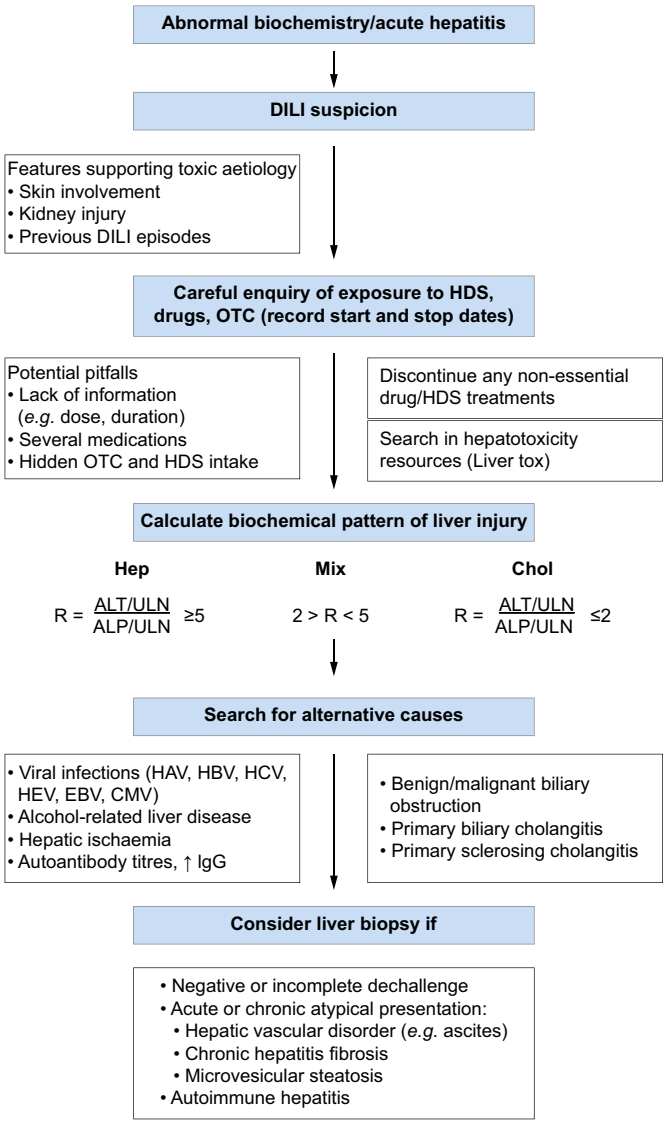


Fig. 2. Stepwise approach to DILI diagnosis. ALP, alkaline phosphatase; ALT, alanine aminotransferase; Chol, cholestatic injury pattern; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDS, herbal and dietary supplements; Hep, hepatocellular injury pattern; HEV, hepatitis E virus; IgG, immunoglobulin G; Mix, mixed injury pattern; OTC, over-the-counter drugs; ULN, upper limit of normal.

HEV infection should be ruled out in patients being assessed for DILI, at least in cases not compatible with the drug signature of the suspected causative agent and in those with high aminotransferase levels in the range of viral hepatitis. HBV DNA should also be tested in patients who are carriers of HBV surface antigen to rule out chronic HBV reactivation as the cause of liver injury. Testing for other viruses less frequently responsible for viral hepatitis such as cytomegalovirus, Epstein-Barr virus or herpes virus would be justified if associated extrahepatic manifestations such as rash, lymphadenopathy and atypical lymphocytes are present.

Screening for auto-antibodies and serum IgG in the hepatocellular pattern is mandatory. However, it is important to keep in mind that a phenotype of AIH with its typical laboratory and pathological features can be seen in association with several drugs including nitrofurantoin, minocycline, anti-TNFα and statins among others,[238–240] making the differentiation from idiopathic AIH a challenge. Similarly, potential DILI adjudication in cholestatic anicteric cases requires appropriate exclusion of primary biliary cholangitis by anti-mitochondrial antibody testing.[241] Alcoholic hepatitis should be excluded on the grounds of prior history of alcohol abuse, a predominance of AST elevation with ALT values not usually reaching values greater than 300 IU/L and other biochemical features of chronic alcoholism such as high values of GGT and erythrocyte mean corpuscular volume.

In younger patients (<40 years) Wilson's disease should be ruled out by screening ceruloplasmin levels. However, ceruloplasmin – an acute phase reactant – may be normal or only slightly decreased in Wilson's disease presenting as acute hepatitis; in these cases other tests such as 24 urine cooper, ophthalmologic examination for Kayser-Fleischer rings and genetic testing of the *ABCB7* gene are required.[242] Ischaemic hepatitis is an obvious competing aetiology in frail older individuals or those with severe, pre-existing, cardiac comorbidity.[243] While these patients should be scrutinized for prior hypotension or syncope, this could only be documented in 53% of the cases in a recent systematic review.[244] Besides, towering values in serum aminotransferases with a predominance of AST over ALT elevation followed by a faster decrease compared with other aetiologies is strongly suggestive of liver ischaemia.[245]

Recommendation

- Tests for HCV-RNA and ant-HEV IgM (or HEV-RNA) are suggested in patients with suspected DILI to exclude acute hepatitis C and/or E, particularly in those cases not compatible with the drug signature of the suspected causative agent and/or with high aminotransferase levels. **Grade C**.

Evidence: Extrapolation from level 2 studies (retrospective cohort study)

Imaging

As DILI is a diagnosis of exclusion, some form of liver imaging is usually undertaken in the diagnostic workup of a patient with suspected DILI. Liver imaging in DILI is typically normal. All patients with suspected DILI should at least undergo an abdominal ultrasound to exclude focal changes in the liver and biliary obstruction. The choice of additional abdominal imaging depends heavily on the clinical context such as symptomatology of the patients and the pattern of liver injury. If the patient presents with "hepatitis-like" syndrome with fatigue, nausea and abdominal discomfort and hepatocellular pattern of liver injury, imaging modalities other than liver ultrasound are usually not necessary. If abdominal pain is a prominent feature and/or the type of liver injury is cholestatic, other imaging tests might be required despite normal abdominal ultrasound. Thus, computerized tomography and magnetic resonance cholangiography are sometimes required to exclude gallstone disease and other competing aetiologies. However, morphological changes have been reported in the hepatic parenchyma and the biliary tree in patients with DILI.[170,193,246–256] Sclerosing cholangitis-like changes on imaging have been described with chemotherapeutic agents such as 5-fluorodeoxyuridine after hepatic intra-arterial infusions for treatment of hepatic metas-

Table 7. Exclusion of underlying diseases in DILI diagnosis.

Disease	Assessment
Hepatitis A, B, C, E	IgM anti-HAV; HBsAg, IgM anti-HBc, HBV DNA; anti-HCV, HCV RNA, IgM & IgG anti-HEV, HEV RNA
CMV, HSV, EBV infection	IgM & IgG anti-CMV, IgM & IgG anti-HSV; IgM & IgG anti-EBV
Autoimmune hepatitis	ANA & ASMA titres, total IgM, IgG, IgE, IgA
Alcoholic hepatitis	Ethanol history, GGT, MCV
Non-alcoholic steatohepatitis	Ultrasound or MRI
Hypoxic/ischaemic hepatopathy	Medical history: acute or chronic CHF, hypotension, hypoxia, hepatic venous occlusion. Ultrasound or MRI
Biliary tract disease	Ultrasound or MRI, ERCP as appropriate.
Wilson disease	Ceruloplasmin
Hemochromatosis	Ferritin, transferrin saturation
Alpha-1-antitrypsin deficiency	Alpha-1-antitrypsin

ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; CD, carbohydrate deficient; CHF, congestive heart failure; CMV, cytomegalovirus; DILI, drug-induced liver injury; EBV, Epstein-Barr virus; ERCP, endoscopic retrograde cholangiopancreatography; GGT, gamma-glutamyltransferase; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis virus; HSV, herpes simplex virus; Ig, immunoglobulin; MCV, mean corpuscular volume; MRI, magnetic resonance imaging.

tases.[249,254–256] Cholangiopathy is well documented after abuse of ketamine, both small duct changes and cholangiographic abnormalities.[246,247,256] Secondary sclerosing cholangitis associated with other drugs such as methimazole and docetaxel-induced secondary sclerosing cholangitis have also been reported.[248,250] A recent study of cholangiographies performed on unselected patients with DILI suggested that up to 10% of DILI cases may have secondary sclerosing cholangitis-like changes on MRCP.[193] Thus, the presence of biliary tract strictures in a patient with suspected DILI does not exclude the possibility of DILI and is not necessarily primary sclerosing cholangitis but can be secondary sclerosing cholangitis caused by a drug-induced cholangiopathy.

Recommendation

- An abdominal ultrasound should be undertaken in all patients suspected of DILI. The use of additional imaging studies relies on the clinical context. **Grade B**.

Evidence: Level 2a studies (retrospective cohort studies with homogeneity)

Liver biopsy

Liver biopsy is an integral part of the specific investigations performed by clinicians to establish the diagnosis of parenchymal liver disease; it has a limited role when the condition presents with typical manifestations and the non-invasive tests are considered diagnostic. When DILI is suspected, liver injury may resolve promptly on cessation of the causal medication; the course after drug withdrawal itself is informative and is a part of causality assessment in DILI. In chronic parenchymal liver diseases, liver biopsy has been used for decades to assess the degree of liver pathology; with the recent adoption of non-invasive markers of liver fibrosis into clinical practice, prognostication and monitoring of chronic liver diseases has become less reliant on serial liver biopsies. DILI, however, shares clinical features with other parenchymal liver diseases and lacks definitive tests and hence, liver histology can provide complementary information and assist in the process of securing an accurate diagnosis.[196]

When faced with acute presentation of liver injury, the vast majority of cases of acute viral hepatitis can be identified using specific serological markers and biliary obstruction is excluded using a variety of imaging modalities. However, diagnosis of AIH is made using a combination of serum and genetic markers as well as liver histology. Therefore, liver biopsy can be justified when it is performed to distinguish DILI from AIH; considering the high prevalence among asymptomatic individuals of ANA (15–24%), ASMA (up to 43%), anti-liver-kidney-microsomal antibody (anti-LKM, 1%) and raised immunoglobulin G levels (5%),[257–259] clinicians often encounter such diagnostic conundrums.

In 9% of cases DILI is indistinguishable from AIH even following detailed investigations[171] and 9% of AIH cases are thought to have been triggered by drugs;[170] both of these groups are classified as drug-induced AIH. Even if patients with drug-induced AIH were to be started on immunosuppressive therapy in an acute setting due to diagnostic uncertainty, treatment can be withdrawn safely once the liver injury resolves,[174] while patients with idiopathic AIH relapse on complete withdrawal of immunosuppressive agents.[175]

In a small comparative study involving 35 cases of DILI and 28 cases of AIH, hepatocellular cholestasis and portal neutrophils were indicative of DILI, while the presence of fibrosis was suggestive of AIH.[260] In another study where portal inflammatory infiltrates were characterised using dual immunohistochemistry staining of liver biopsies from 32 acute DILI cases and 25 cases of acute liver injury due to other aetiology (including 9 cases of AIH), portal infiltrates in DILI were formed predominantly by cytotoxic (CD8+) T cells, while in AIH there were predominantly mature B cells (CD20+).[261]

A systematic review of liver biopsies from 249 patients with DILI from a prospective observational cohort showed more severe inflammation and cell death in association with hepatocellular pattern compared to higher frequency of bile plugs and ductal paucity in those with cholestasis.[168] In addition, hepatic failure and death (n = 46) were associated with higher degrees of necrosis, fibrosis stage, microvesicular steatosis, and ductular reaction, whereas eosinophils and granulomas were more often found in those with a milder degree of DILI. Similar observations have been made previously; eosinophilia in liver biopsies has been associated with a higher rate of recovery from DILI, while, the presence of necrosis was associated with a lower rate of survival.[262] Similarly, evidence of bile duct loss in patients with acute DILI (generally presenting with cholestatic pattern) indicates the development of vanishing bile duct syndrome with progressive cholestasis leading to liver failure requiring transplantation or death.[263] Histological features that are indicative of prognosis in DILI are presented in Table 8.

Table 8. Histological features indicative of prognosis.

Features associated with mild or moderate liver injury	Features associated with severe liver injury, liver transplantation or death
Presence of granulomas	Neutrophil infiltration
Eosinophilic infiltration	Higher degree of necrosis
	Higher degree of fibrosis
	Cholangiolar cholestasis
	Ductular reaction
	Portal venopathy
	Microvesicular steatosis

Recommendations

- Liver biopsy may be considered during the investigation of selected patients suspected of suffering from DILI, as liver histology can provide information supporting the diagnosis of DILI or an alternative. **Grade D**.

Evidence: Level 5 (expert opinion)

- Liver biopsy may be performed in patients suspected of having DILI when serology raises the possibility of AIH. **Grade C**.

Evidence: Level 4 studies (case-control studies with non-independent reference standard)

- Liver biopsy may be considered in patients when suspected DILI progresses or fails to resolve on withdrawal of the causal agent, as the liver histology may provide prognostic information assisting clinical management. **Grade C**.

Evidence: Level 4 (case series)

Causality assessment methods and scales

Systematic evaluation is important to be able to attribute a manifestation of liver injury to a drug therapy with confidence. Physician awareness of the association of a particular drug with a pattern of clinical manifestation, the exclusion of alternative aetiologies that could lead to a similar pattern of liver injury and an objective weighing of the circumstantial evidence are involved in the process of evaluation. This process which provides a structure and objectivity has been termed 'causality assessment' and it has become the standard method for the evaluation of suspected DILI. A number of DILI specific causality assessment methods have been developed over the past decades, however, those based on the decision tree model or Bayesian model, although based on sound principles,[264] have not been formally validated.

Council for International Organizations of medical Sciences (CIOMS) scale

This causality assessment method, also called RUCAM (after the host pharmaceutical Roussel-Uclaf) includes weighted scoring of an event according to 7 distinct domains related to the temporal relationship between exposure to a particular drug and the liver injury (both its onset and course), exclusion of alternative non-drug-related aetiologies, exposure to other medications that could explain DILI, risk factors for the adverse hepatic reaction, evidence in the literature regarding DILI from the drug in question and response to re-exposure to the medication. The total score derived (ranging from −9 to +10) from the domain specific assessment classifies the event as highly probable (>8), probable (6–8), possible (3–5), unlikely (1–2) or excluded (≤0) according to its likelihood to be DILI.[63] The CIOMS method was initially validated using a cohort of DILI confirmed on positive rechallenge as well as non-DILI events. The scale cannot be used in 3–24% of cases due to inadequate information when evaluating cases retrospectively (International DILI consortium, unpublished data); its consistent application can be improved and ambiguities reduced by clearly defining individual parameters and agreeing criteria prior to its use. The overall value added by the risk factor domain to the scale is uncertain. Although, not widely used in clinical practice, the majority of studies use the CIOMS scale for DILI case definitions and inclusion criteria. Despite its limitations, the CIOMS scale provides a degree of objectivity and systematic assessment of the probability of the event in question being DILI.[265] American College of Gastroenterology Guidance recommends the CIOMS scale as a guide to the evaluation of patients with suspected DILI.[266] An international working group is currently revising the tool to address the limitations of the CIOMS scale in its current form and to improve its reliability.

Clinical diagnostic scale (CDS)

This is relatively simple scoring system.[267] Two of the domains in the CIOMS scale, risk factors and concomitant medications, are not considered in the CDS; in contrast, a specific domain attributes scores to the presence of extrahepatic manifestations (thought to be reflecting underlying immune mechanisms). Causality is graded according to the final score as definite drug-induced hepatotoxicity (score >17), probable (score 14–17), possible (score 10–13), unlikely (score 6–9) and excluded (score <6). The original validation of CDS used real and fictitious cases and the opinion of a panel of experts as the gold standard.

Although the 2 scales broadly correlate with regards to the classification of events according to their likelihood of being DILI,[268] discrepancies greater than 1 category level were seen in 31% of patients in a systematic comparison involving 215 cases of suspected DILI (185 classified as DILI and 30 non-DILI aetiology) by 3 experts.[269] The CIOMS scale performed better in cases that were deemed highly probable or probable DILI and its concordance with expert review was superior to that of the CDS.

Structured expert opinion process

The DILIN developed a process where expert hepatologists evaluated prospectively collected clinical and laboratory data from cases of suspected DILI, then, categorised the probability of liver manifestations being DILI.[270] Following the assessment, the likelihood of an event being DILI was described using both a percentage figure and a descriptive legal terminology as definite (>95% likelihood), highly likely (75–95%), probable (50–74%), possible

(25–49%), or unlikely (<25%). When compared with CIOMS, structured expert opinion produced higher rates of inter-individual agreement and likelihood score, although authors admitted that substantial inter-observer variability persisted in both methods. However, this causality assessment method has not been externally validated. Considering the fact that CIOMS favours DILI from established drugs and its ability to assess those secondary to new molecular entities is unknown, expert opinion remains the mainstay for causality assessment of emerging adverse liver reactions that have not been fully characterised.[271]

Recommendation

- CIOMS can be used to assess causality, guiding a systematic and objective evaluation of patients suspected to have DILI. **Grade C**.

Evidence: Extrapolation from level 2b studies (exploratory cohort studies with good reference standards)

Rechallenge and recurrent DILI

Once DILI subsides the individual can be exposed again to the same drug usually in an inadvertent way. This is called rechallenge and if followed by a recrudescence of the hepatic damage is a strong argument to incriminate the agent. In fact, a "positive" rechallenge is currently the strongest proof of causality in the adjudication process of suspected DILI cases.[272] Drug and host characteristics associated with high rates of positive rechallenge include a daily dose >50 mg, an increased incidence of ALT elevations in clinical trials, a frequent clinical presentation with immunoallergic features, association with HLA alleles, production of reactive metabolites, mitochondrial hazard and more modestly BSEP inhibition *in vitro*.[273]

The definition of positive rechallenge relies on the threshold reached by aminotransferases upon drug resumption. By common convention it is currently defined as ALT >3 × ULN.[273] However, it is well established that the bulk of instances of aminotransferase elevations upon drug exposure are transient and even reversible despite drug continuation owing to the remarkable adaptive capacity of hepatocytes and the immune system to chemical insults. Thus, many rechallenge episodes probably go unnoticed. Nonetheless, the response of the damaged liver to the culprit drug re-exposure is poorly documented because of a bias towards reporting instances of positive rechallenge as data on "negative" rechallenge are usually not gathered.[231]

Importantly, rechallenge of a patient who showed initial liver injury caused by a drug has traditionally been regarded as a dangerous practice with potentially serious consequences, as it sometimes leads to rapid, worse liver injury or even fulminant liver failure.[274] Deliberate re-exposure to a non-essential drug is hardly justified in DILI, and unintentional rechallenge is more commonly described. Anti-TBC drugs have been largely considered as examples of essential non-replaceable medications and repeatedly tried for rechallenge. In 2 independent prospective controlled clinical trials involving a total of 220 patients with prior DILI related to anti-TBC therapy, who were rechallenged with various anti-TBC drug schemes including isoniazid or rifampin, with or without pyrazinamide or ethambutol, the rate of positive rechallenge was 0–24%[275,276] (with no recurrence of liver injury when pyrazinamide was excluded).[275] Neither the initial DILI influenced the risk of hepatitis recurrence nor were differences in rechallenge rates observed when the drugs were reintroduced simultaneously or sequentially.[276]

Likewise, deliberate rechallenge is increasingly tried in prospective oncology trials with new antitumoural drugs, which are efficacious in inhibiting targeted cancer pathways yet carry a significant risk of hepatotoxicity. For instance, an integrated analysis of phase II and III studies of pazopanib showed that 103 patients out of 2,080 who developed liver injury with no hypersensitivity features and exhibited positive dechallenge were rechallenged with the drug because of presumed clinical benefit; 62 (60%) displayed adaptation (negative rechallenge) and 39 (38%) showed recurrence of the liver damage. No patients developed severe liver injury with positive rechallenge.[277]

In clinical practice the data are scarce. In a retrospective analysis, a comprehensive search of GlaxoSmtihKline (GSK) adverse events (1958–2007) identified 88 positive rechallenge cases that met predefined biochemical criteria. Most drug rechallenges were inadvertent and include unsupervised self-medication and supervised re-administration (for undisclosed reasons) in differing hospital units.[278] The leading drug classes reported in the positive rechallenge cases were: antibiotics (24%), HIV antiviral medications (15%), azathioprine (16%) and H2 antagonists (10%). Amoxicillin-clavulanate was the drug most commonly reported to be responsible for rechallenge events.[278]

In the prospective Spanish DILI registry, 33 out of 520 DILI cases were inadvertently re-exposed to the culprit drug after the initial DILI and 31 (6% of the total number of enrolled cases) fulfilled criteria for positive drug rechallenge (doubling ALT and ALP for hepatocellular and cholestatic/mixed type of injury, respectively). Anti-infectious agents were the most commonly identified class (26%), followed by nervous system and cardiovascular drugs (16% each). Amoxicillin-clavulanic acid was the single most frequently involved drug. In this series, patients showing a positive drug rechallenge developed liver injury on average in less than half the time of the initial episode, were predominantly hepatocellular (71%), frequently exhibited jaundice (64%), and hypersensitivity features (39%). Overall 13% of rechallenge cases either died or underwent liver transplantation.[279]

The term recurrent DILI is restricted to sequential episodes of liver injury caused by different drugs in a given individual. In the Spanish DILI registry, the incidence and characteristics of recurrent DILI were examined; 9 patients out of 742 (1.21%) had evidence of 2 DILI episodes caused by different drugs. In 4 cases the hepatotoxicity events were associated with structurally related drugs and in an additional 2 cases the agents shared the therapeutic target. All but 1 patient exhibited hepatocellular damage and the type of damage was consistent in both DILI episodes. Interestingly, 4 cases presented as AIH in the second DILI episode.[280]

Statement

- Liver injury caused by unintentional rechallenge in clinical practice can confer a higher risk of mortality/liver transplantation than the initial DILI episode.

Evidence: Level 2b studies (extrapolating cohort studies with good reference standards)

Recommendations

- Deliberate rechallenge with the causative drug in clinical practice is not advocated, unless the clinical scenario demands such an exposure, as it can cause more severe hepatotoxicity. **Grade C**.

Evidence: Level 4 (case series)

- Controlled rechallenge after an episode of liver injury is, however, considered justified in relation to oncology and anti-TBC therapy, as they generally do not result in severe recurrence of hepatotoxicity. **Grade B**.

Evidence: Level 1b studies (validating cohort studies with good reference standards)

Genetic testing

Over the past decade, candidate gene studies, initially, and GWAS more recently, have identified several genetic factors associated with DILI.[281,282] While candidate gene studies have focused on SNPs in genes involved in pathways of drug metabolism and excretion, GWAS have identified key HLA alleles that influence the susceptibility to DILI secondary to a number of drugs with wide ranging chemical structures. There are over 15 currently used drugs where HLA genotype or haplotype increases the susceptibility to DILI and some of these associations are strong with high relative risk ratios.

The rarity of an occurrence of DILI in relation to a given drug means that many of these HLA alleles have a negative predictive value of >95%.[283] Consequently, genetic tests can be used to exclude the diagnosis of DILI or to exclude a specific drug as an aetiological agent when more than one potential medication could have caused DILI. Published case reports demonstrate such examples of effective use of genetic tests in clinical practice.[284,285]

While exclusion of alternative causes is an important component of causality assessment in a suspected DILI, HLA genotyping in combination could strengthen the diagnosis of DILI. There are substantial overlaps between DILI and AIH; in routine clinical practice, a combination of clinical features, serological, histological parameters as well as genetic tests are considered in reaching the diagnosis of AIH as none of the individual features is pathognomonic of AIH.[286] An individual's HLA type in particular has been a component of the original International AIH Group score,[287] although, simplified criteria[288] are used more often in routine clinical practice. In a recent nationwide cohort involving 1,267 patients with AIH, only 65% of those meeting original International AIH Group criteria also met simplified International AIH score.[169] Therefore, when a patient suspected of having DILI also tests positive for 1 or more of the liver specific auto-antibodies (ANA, ASMA, anti-LKM) or has raised immunoglobulins, carriage of specific HLA alleles DRB1*03:01 or *04:01 (the former is found in 27–32% of cases of AIH and 13–15% in controls)[289] supports the diagnosis of AIH. Alternatively, detection of a specific HLA allele that has been linked with hepatotoxicity to a particular drug, which the patient has been exposed to, should equally support the diagnosis of DILI. Table 4 illustrates that the potential yield from histogenetic tests in patients suspected of having DILI due to specific drugs (expressed as carriage of risk alleles in patients with DILI compared with that of a reference population) is comparable to the yield from serological tests utilised in achieving the diagnosis of AIH.[80,81,84,290–292] For example, testing for HLA-DRB1*15:01 when amoxicillin-clavulanate DILI is suspected, HLA-B*57:01 in suspected flucloxacillin DILI and HLA-B*35:02 in a possible minocycline DILI case would have similar performance characteristics to ANA, immunoglobulin G estimation and anti-LKM antibody, respectively, in a case of suspected AIH. Considering the importance of clinical decision making, such as permanent withdrawal of an effective medication in a patient and/or initiation of long-term immunosuppressive regimen, incorporating genetic tests into the diagnostic armamentarium is justified and would increase the accuracy and confidence in the diagnosis.

HLA genotyping is widely available and performed routinely prior to transplantation; even high-resolution typing is performed in relation to bone marrow transplantation. Its use as a diagnostic test in the evaluation of suspected DILI in principle would be similar to incorporating the information regarding presence or absence of HLA alleles DRB1*03:01 and *04:01 within the International AIH score. Rapid turnover of a genetic test to facilitate prompt diagnosis is feasible; in circumstances of HLA-B*57:01 genotyping prior to abacavir prescription for the treatment of HIV infection, genotyping can be performed within 1 day of receipt of sample;[293] protocols are commercially available. Use of this method on a nationwide basis in Canada costs approximately 60 Canadian dollars per sample.[294]

Recommendations

- HLA genotyping should be utilised in selected clinical scenarios where genetic tests assist the diagnosis and management of patients. **Grade B**.

Evidence: Extrapolation from level 1 studies (validating high quality case-control studies).

- HLA genotyping may be used to support the diagnosis of DILI due to specific drugs or distinguish DILI from AIH. Further validation of genetic testing is required before routine implementation can be recommended. **Grade D**.

Evidence: Level 5 (expert opinion based on first principles)

New biomarkers

There have been recent efforts mainly by public-private partnerships such as the IMI Safer and Faster Evidence-based Translation (SAFE-T) Consortium together with the Critical Path Institute's Predictive Safety Testing Consortium (C-Path PSTC) and the US DILIN group to develop and qualify new liver safety biomarkers that outperform current standard markers in terms of sensitivity, specificity and predictivity.[295] From the new markers investigated by IMI SAFE-T and PSTC, a subset (see Section *Detecting DILI in clinical trials* for more details) has recently received regulatory support from both the EMA and FDA for more systematic use in an exploratory development setting,[296,297] which will ultimately enable full qualification of the most promising markers. Once qualified in well-controlled trials, regulatory guidance will then also have to account for the new markers and incorporate them into existing guidelines.

Several new biomarkers have been studied in the context of acetaminophen-induced DILI.[298] Liver injury in acetaminophen-induced DILI results in oncotic necrosis. MicroRNA-122 (miR-122) is a hepatocyte-specific miRNA that is elevated in the plasma of patients within hours of an acetaminophen overdose and has been shown to predict the subsequent onset of liver injury at an early time point before ALT is elevated.[299] In mice, mir-122 and mir-192 are enriched in liver tissue and exhibit dose- and exposure-dependent changes in plasma that parallel serum aminotransferase levels and the histopathology of liver degeneration.[300] In human acetaminophen-induced liver injury, miR-122, miR-192-5p and other miRNAs are elevated, but further studies are needed to assess whether drug-induced pathognomonic "signatures" of circulating miRNAs could serve as diagnostic "liquid biopsies".[301]

Specifically in the context of acetaminophen-induced ALF, the mitochondrial matrix enzyme glutamate dehydrogenase (GLDH), mitochondrial DNA and nuclear DNA (nDNA) fragments are mechanistic biomarkers of mitochondrial damage that predict outcome, *i.e.* survival *vs.* non-survival.[302] GLDH has been evaluated in detail by C-Path PSTC with the aim of full regulatory qualification as a biomarker and is proposed to confirm or rule out hepatocellular injury in cases when ALT increases are observed from suspected extrahepatic sources such as muscle. High mobility group box 1 (HMGB1) is a chromatin binding protein released by necrotic cells that targets Toll-like receptors and the receptor for advanced glycation end products (RAGE), thus acting as a damage-associated molecular pattern molecule.[303] Another marker of immune activation is macrophage colony-stimulating factor receptor 1 (MCSFR1). In DILIN patients studied within the IMI SAFE-T consortium, MCSFR1, cytokeratin K18 and osteopontin were identified as biomarkers that predict an unfavourable prognosis in acute DILI, *i.e.* liver transplantation or death from liver failure.[241,295] Further confirmation of the prognostic value of these biomarkers will be required to obtain the level of evidence required for full qualification as liver safety biomarkers.

Glutathione S-transferases (GSTs) are phase II detoxifying enzymes, which metabolise reactive metabolites. Data from the Spanish DILI registry suggest that GST gene polymorphisms confer susceptibility to hepatotoxicity induced by multiple drugs.[304] GSTα comprises 5–10% of total soluble hepatic protein and up to 90% of all glutathione S-transferase in the liver and is expressed in the cytoplasm and nucleus of hepatocytes throughout the centrilobular region.[305] In rats exposed to various hepatotoxicants, GSTα has been shown to have enhanced specificity and sensitivity compared to ALT alone.[305] Humans with acetaminophen overdose show elevated GSTα levels earlier than ALT, and GSTα as a biomarker may offer a better assessment of rapid changes in liver damage due to the shorter half-life of plasma GSTα compared to ALT or AST.[306]

Statement

- Novel biomarkers should be validated to allow early detection and assessment of prognosis of idiosyncratic DILI.

Evidence: Extrapolation from level 2c (mechanistic studies and outcome research) studies.

Prognosis and natural history

Grading severity

Assessment of the degree of severity relies on the presence of symptoms when clinically apparent (jaundice, encephalopathy, bleeding caused by coagulopathy, ascites). Less specific symptoms such as fatigue, weakness, anorexia, nausea, vomiting, fever, chills, abdominal pain, pruritus, skin rash, *etc.* should also be considered, as such symptoms have been shown to affect the risk of a poorer clinical outcome.[307,308] Analytical tests including total and conjugated bilirubin, blood clotting tests (INR, Factor V, prothrombin time), and hypoalbuminemia should likewise be considered in the assessment of severity, in which INR is particularly important.[160,309,310] The level of elevation of liver enzymes alone is not sufficient to reflect the severity of liver injury. Elevated aminotransferases in conjunction with jaundice, however, are well-known to reflect a higher risk of a severe outcome. This was pointed out by Hyman Zimmerman several decades ago and became the basis for Hy's law (see Section *Detecting DILI in clinical trials* for a more detailed definition of Hy's law). Various large DILI cohorts in the US and Europe have confirmed Hy's law and demonstrate that patients with hepatocellular damage and jaundice have a mortality/liver transplant rate exceeding 10%.[14,16,20,48]

Two recent DILI severity classifications have been proposed (Table 9). The US DILIN severity index comprises 5 grades (mild, moderate, moderate-severe, severe and fatal) and takes into consideration the need for hospitalization.[309] Meanwhile, the International DILI Expert Working Group's severity index only considers 4 grades (mild, moderate, severe and fatal/transplantation). This classification does not consider hospitalization due to important variability in indications for hospitalization between different hospitals/medical organisations. However, the expert panel behind this index scale recognises the socioeconomic consequences that can be associated with (prolonged) hospitalization.[160]

Chronic DILI

The vast majority of patients who experience DILI will recover clinically with normalisation of liver test abnormalities. However, chronic liver disease and in rare instances the development of liver cirrhosis have been observed during follow-up of the liver injury. Some drugs inducing cholestatic type of injury have been associated with the development of vanishing bile duct syndrome with long-term persistent cholestatic injury and occasionally impaired liver function with jaundice. A retrospective assessment of 33 cases, with clinical features and histology suggesting DILI, revealed persisting abnormalities in liver biochemistries and indicated chronicity on imaging in 13 (39%) of these patients.[61] However, these patients were identified through a histological database, with a potential selection bias as patients with slow improvement in liver tests are more likely to have a liver biopsy performed. Hepatic decompensation and/or liver-related morbidity and mortality were not reported. Furthermore, most patients with protracted course (86%) had cholestatic/mixed type of liver injury with all but 1 patient presenting a normalised liver profile at the last follow-up and thereafter remaining free of liver morbidity.[61] Among patients recruited prospectively in the Spanish DILI registry development of chronic liver injury was observed in 28 of 493 (5.7%), with cholestatic/mixed type of injury patients being more prone to developing chronic injury. The definition of chronic liver

Table 9. DILI severity classifications.

Category	Severity	Description
US Drug-Induced Liver Injury Network[310]		
1	Mild	Elevated ALT and/or ALP but TBL <2.5 mg/dl and INR <1.5
2	Moderate	Elevated ALT and/or ALP and TBL ≥2.5 mg/dl or INR ≥1.5
3	Moderate-severe	Elevated ALT, ALP, TBL and/or INR and hospitalization or ongoing hospitalization prolonged due to DILI
4	Severe	Elevated ALT and/or ALP and TBL ≥2.5 mg/dl and at least 1 of the following criteria: - Hepatic failure (INR >1.5, ascites or encephalopathy) - Other organ failure due to DILI
5	Fatal	Death or liver transplantation due to DILI
International DILI Expert Working Group[160]		
1	Mild	ALT ≥5 or ALP ≥2 and TBL <2 × ULN
2	Moderate	ALT ≥5 or ALP ≥2 and TBL ≥2 × ULN, or symptomatic hepatitis
3	Severe	ALT ≥5 or ALP ≥2 and TBL ≥2 × ULN, or symptomatic hepatitis and 1 of the following criteria: - INR ≥1.5 - Ascites and/or encephalopathy, disease duration <26 weeks, and absence of underlying cirrhosis - Other organ failure due to DILI
4	Fatal/transplantation	Death or liver transplantation due to DILI

ALP, alkaline phosphatase; ALT, alanine aminotransferase; INR, international normalized ratio; TBL, total bilirubin, ULN, upper limit of normal.

injury in this study was persistent abnormalities in liver tests more than 3 or 6 months after stopping drug therapy in patients with hepatocellular and cholestatic/mixed type of injury, respectively.[54] Similarly, in a retrospective single-centre study, 6% of patients diagnosed with DILI had persistently abnormal liver biochemistries after a median follow-up of 48 months.[60] A follow-up study of patients with DILI who presented with acute DILI and concomitant jaundice (mean follow-up of 10 years) revealed that development of clinically important liver disease after severe DILI was rare. In this study a total of 23 out of 685 (3.4%) patients with DILI, who survived the acute DILI episode, were hospitalized for liver disease and 5 had liver-related mortality during the follow-up period.[59] Five out of 8 with cirrhosis had cryptogenic cirrhosis, in which DILI might have played a role. A significantly longer duration of drug therapy prior to the detection of DILI was observed in those who developed liver-related morbidity and mortality,[59] in line with the report from Aithal and Day.[61]

In a study of 598 prospectively identified DILI cases by the US DILIN group, 18.9% of patients had signs of persistent liver damage, with chronic DILI defined as elevations in liver tests, histological or radiological evidence of ongoing liver injury at 6 months after enrolment. African-American race, higher ALP values, prior heart disease or malignancy requiring treatment were independent risk factors for chronic DILI.[55] In line with previous studies, duration of treatment of the implicated drug was significantly longer in those with signs of chronicity, and chronicity was more likely to occur in patients with cholestatic type liver injury. Similarly, patients with persistent liver injury at 12 months were more commonly older patients and those with cholestatic liver injury.[55] The various definitions of DILI chronicity led to the Spanish DILI registry analysing the time to liver profile normalisation in 298 patients with DILI, in order to determine the best cut-off point for chronicity. Complete resolution was achieved in 92% of patients ≤1 year from DILI recognition, suggesting that 1 year is the best cut-off point to define chronic DILI or prolonged recovery. Hence, 25 (8%) patients with DILI were considered as having chronic DILI in this study cohort. Histological examination in these patients demonstrated 7 with cirrhosis and 2 with ductal lesion. Older age, presence of dyslipidaemia and a more severe DILI episode were found to be independent risk factors in this study. In contrast to previous studies, the type of liver injury was not found to be a risk factor for DILI chronicity.[58]

In a minority of patients with drug-induced cholestasis, progressive ductopenia (loss of biliary (interlobular) ducts) can occur. Severe bile duct loss in more than 50% of portal areas, vanishing bile duct syndrome (VBDS), is a rare and serious complication of DILI, and requires a liver biopsy for identification. Out of 363 patients with DILI enrolled in the US DILIN study over a 10-year period who had undergone liver biopsies, 26 (7%) had bile duct loss: 14 with moderate to severe (<50% of portal areas with bile ducts) and 12 with mild bile duct loss.[263] The prevalence of DILI developing into bile duct loss in general is probably lower than that determined by the DILIN study as the DILIN cases are identified by tertiary referral centres. Prognosis of patients with bile duct loss is generally unfavourable. In the DILIN cohort, liver-related mortality was observed in 19% of patients with DILI and bile duct loss compared to 6.2% of patients without bile duct loss, while the need for liver transplantation was observed in 8% *vs.* 4%, respectively.[263] Various drugs have been associated with bile duct loss, including VBDS, with the majority of evidence coming from different case reports.[311–315]

Liver cirrhosis has been reported in association with a number of different drugs. The most widely recognised is probably methotrexate. Information on the development of cirrhosis after an acute DILI episode consists mainly of isolated case reports[316–321] or a small number of patients in large DILI cohort studies.[55,58,59] Most of these cases have inactive cirrhosis and it is difficult to completely exclude the participation of underlying diseases (*e.g.* NASH). Non-cholestatic cases are extremely unlikely to progress to cirrhosis and usually have a normal liver profile within 2–3 years. However, decompensated liver cirrhosis following DILI has been reported.[59,318]

Statement

- Development of chronic liver disease in a very small proportion of individuals should be considered a potential consequence of idiosyncratic DILI.

Evidence: Consistent level 2 (cohort studies and outcome research) studies.

Table 10. Practical approaches towards managing suspicion of DILI.

Medical history	Search for recent therapies even if they have finished (*i.e.* antibiotics). Do not forget to ask about herbs and dietary supplements.
Case characterisation	Classify liver injury based on R ([ALT/ULN]/[ALP/ULN]) using the first blood test available after DILI detection.
Case investigation	If hepatocellular pattern: test for RNA-HCV and IgM anti-HEV in addition to HAV, HBV and autoimmune serology. If cholestatic/mixed damage with jaundice: perform cholangiography in addition to ultrasound.
Case adjudication	Use the CIOMS scale as a guide for complete data requirement, but do not exclusively rely on it for causality assessment.
Liver biopsy	It is not required for diagnosis. Not necessary if the suspected drug is a known hepatotoxic compound and the outcome is favourable.
Follow-up	Careful scrutiny of hepatocellular cases with jaundice in females and all other cases with altered INR for impending liver failure. In the long-term pay attention to abnormal ALP and bilirubin after 30 days for the risk of chronicity.
Therapy	Stop all non-essential drugs. Steroids can be tried if AIH is an option and in cases with marked hypersensitivity features.

Treatment

General measures

The most important initial step in terms of management of suspected DILI is to discontinue the implicated agent. In the large majority of DILI, spontaneous recovery occurs, without the need for any treatment or specific measure. In fact, spontaneous recovery after discontinuation of the offending drug is an important criterion in the causality assessment.[160,310] There is usually a complete or near complete resolution of DILI within a matter of days to weeks. However, improvement may not begin immediately and ongoing or even worsening injury can occur despite withdrawal of the causative agent. At the same time severity of the liver injury should be assessed. Patients with concomitant jaundice should be kept under active surveillance with frequent testing of liver biochemistries. Patients with signs or biochemical indication of ALF such as encephalopathy and/or coagulopathy should generally be hospitalized. Some therapeutic approaches have been proposed and are used in specific circumstances with variable levels of proof of efficacy. Practical approaches towards managing suspected DILI cases are presented in Table 10.

Specific therapies

There are examples of particular therapies targeted for specific forms of DILI.

Cholestyramine

Acute liver injury due to leflunomide is usually self-limited once therapy is stopped, but severe and fatal cases have been reported.[51,140] Because of the enterohepatic circulation and long half-life of leflunomide, therapy with a bile acid resin such as cholestyramine (4 g every 6 hours for 2 weeks) has been recommended to speed up drug clearance. Cholestyramine in association with antihistamines has been reported to accelerate the improvement of chronic cholestasis induced by terbinafine.[322] However, the role for these compounds in hastening recovery or improving liver histology has not been established. Furthermore, there are examples of chronic cholestasis induced by various drugs which can resolve completely in the absence of any treatment.[323,324]

Recommendation

- A short administration of cholestyramine may be used to decrease the course of hepatotoxicity induced by very selected drugs, such as leflunomide and terbinafine. **Grade C**.

Evidence: Level 4 (case series, individual cases)

Carnitine administration

Carnitine appears to be a specific antidote for valproate hepatotoxicity and studies in animal models as well as human studies suggest that prompt administration of carnitine (particularly when given intravenously) improves survival in acute valproate hepatotoxicity.[325–328] The beneficial effect of carnitine against valproate hepatotoxicity is ascribed to its role in regulating mitochondrial acetyl-CoA levels, which leads to enhanced fatty acid uptake and β-oxidation in the mitochondria. Oral carnitine was approved for use in valproate toxicity in 1992 and an intravenous formulation in 1996. The typically recommended dose is 100 mg/kg intravenously over 30 minutes (but less than 6 g), followed by 15 mg/kg every 4 hours until clinical improvement.[140]

Recommendation

- Carnitine may be used to improve the course of valproate hepatotoxicity. **Grade C**.

Evidence: Level 4 (case series, individual cases)

N-acetylcysteine

Beside its use in paracetamol intoxication, N-acetylcysteine (NAC) has been occasionally used as a treatment for other types of DILI.[329] The efficacy of NAC combined with oral prednisolone was analysed in a retrospective cohort of 21 patients with severe idiosyncratic injury ascribed to flupirtine (sFILI).[330] These patients received 10 g of NAC given intravenously over 24 hours for 7 days and an oral dose of 1 mg/kg prednisolone per day, with the dose tapered according to biochemical response. The combined NAC/prednisolone treatment led to significant liver profile improvements within 2 weeks and the cases resolved more rapidly than in untreated sFILI patients.[330] However, the uncontrolled design of the study precluded the ability to draw firm conclusions.

Recommendation

- The efficacy of NAC to reduce the severity of liver injury from drugs other than paracetamol may not be substantiated. **Grade D**.

Evidence: Inconclusive level 4 (case series, individual cases) studies.

Ursodeoxycholic acid

Chronic cholestasis following DILI is often treated with ursodeoxycholic acid (UDCA). However, the effects of UDCA in

DILI are not well documented and contradicting results have been reported.[331–337] No controlled studies have been undertaken that have proven UDCA and steroid efficacy in patients with DILI.

Recommendation

- The efficacy of UDCA to reduce the severity of liver injury may not be substantiated. **Grade D**.

Evidence: Inconclusive level 4 (case series, individual cases) studies.

Management of drug-induced ALF

Treatments non-specific to DILI

Current approaches to treat ALF are aimed at providing temporary replacement of hepatic function and detoxification (extracorporeal devices) while awaiting spontaneous recovery or recovery with therapies that enhance liver regeneration (stem cell and growth factors). Supporting detoxification and synthetic functions of the failing liver is the rationale for using extracorporeal liver support systems, broadly comprising artificial (MARS®) and bioartificial systems (devices based on the use of human hepatocytes). Extracorporeal treatments have, however, failed to show any clear benefits with regards to decreased mortality rate in patients with liver failure. Data from stem cell therapies and liver regeneration enhancement, in particular the use of granulocyte colony-stimulating factor (G-CSF), are still limited.[338] Liver transplantation is still the primary rescue treatment for ALF, with a 1-year survival rate of around 80% in liver transplant recipients with ALF.[338]

Treatments specific to DILI

There are 2 main treatment approaches for drug-induced ALF: a) rapid depuration of the body from the toxic drug to stop further aggression before the agent may reach the liver; b) administration of an antidote to prevent and/or stop the aggression once the drug has reached the liver. Charcoal depuration is mainly used as a treatment for paracetamol toxicity. It is an efficient treatment that prevents further absorption of the drug if administered within 3–4 hours following an acute ingestion.[338]

N-acetylcysteine used early in the course of ALF may prevent progression to more severe encephalopathy and may also exert renal protective effects. The benefit of NAC treatment in patients with ALF caused by idiosyncratic DILI has been debated over the years and recommendations vary. A randomized controlled trial of NAC in adults with non-paracetamol ALF argued for its efficacy in diminishing the number of deaths and transplants in patients with early grade encephalopathy. The spontaneous survival rate increased from 30% to 52% in the coma grade I-II group, although overall and spontaneous survival of all coma grades were not significantly improved. Of note, the idiosyncratic DILI ALF subgroup within this NAC trial showed the most promising beneficial effect with spontaneous or transplant-free survival increasing from 27% to 58% with NAC treatment.[329] However, 2 similar trials in children showed no efficacy.[339,340] Furthermore, a meta-analysis indicated limited benefit with NAC treatment in patients with non-acetaminophen-related ALF, but the data is based on a limited number of trials.[341]

Corticosteroids are often given when all else fails to produce results. Early trials of corticosteroid treatments, for all forms of ALF, demonstrated limited benefits.[342] A retrospective analysis of 361 patients with autoimmune ALF, indeterminate ALF or drug-induced ALF concluded that corticosteroids did not improve overall survival in drug-induced, indeterminate, or autoimmune ALF, and that corticosteroid treatments were associated with lower survival in patients with more severe liver injury. Among the 131 patients with drug-induced ALF, 69% of those who received corticosteroids survived *vs.* 66% of those without corticosteroid treatment.[343] In addition to ALF, corticosteroids can also be used to treat drug-induced cholestatic hepatitis, in particular in DILI associated with hypersensitivity features such as eosinophilia, rash and fever.[336] Liver injury caused by antiepileptic drugs are commonly associated with features of hypersensitivity and may respond to steroids.[344]

Overall, there is no certain or specific treatment for drug-induced ALF except for good care of the critically ill patient, but NAC is commonly used given its impressive safety profile.

Recommendations

- In case of drug-induced ALF, liver transplantation should be considered as a therapeutic option. **Grade B**.

Evidence: Consistent level 2 studies (cohort studies with good follow-up)

- Adults with idiosyncratic drug-induced ALF should receive NAC early in the course (coma grade I-II). **Grade B**.

Evidence: Extrapolation from 1b (individual RCT) study.

- In idiosyncratic DILI, routine use of corticosteroid treatment may not be substantiated. **Grade C**.

Evidence: Level 4 studies (case series and case-control studies with poor reference standards)

Preventing DILI

The value of liver test monitoring

As with other liver diseases, clinical symptoms associated with DILI may occur only when serious injury has already happened. In most cases, the first sign of injury is elevation in liver enzymes.[345] At the same time, as there is no specific treatment for DILI, the only measure to limit risk to patients and avoid further damage after initial injury is to either reduce the dose of, or, in most cases, stop treatment with a suspected causative drug.[345] Table 6 provides an overview of the panel of standard chemistry tests that could be used to monitor and assess liver safety. None of these measures should be interpreted in isolation, but only as a full panel of safety biomarkers. If there is reasonable evidence to suggest a risk for hepatotoxicity with a new drug, it is important to keep monitoring intervals as short as practically feasible. When defining suitable intervals,[346] the level of evidence for a DILI hazard attributable to the drug should be taken into account. However, it is important to highlight that monthly monitoring has not been proven to be effective. In addition to compliance issues, idiosyncratic DILI can have a long latency before manifesting.

One exception is prevention of anti-TBC treatment-associated hepatotoxicity. These drugs, in particular isoniazid, are a leading cause of liver injury and early treatment cessation is important for a better outcome. The American Thoracic Society (ATS) recommends ALT monitoring during treatment of latent TBC infection for those who chronically consume alcohol, take concomitant hepatotoxic drugs, have viral hepatitis or other pre-existing liver disease or abnormal baseline ALT, have experienced prior isoniazid hepatotoxicity, are pregnant or are within 3 months postpartum. According to the ATS guidelines patients should stop isoniazid treatments if ALT >3 × ULN in the presence of symptoms, such as nausea, abdominal pain, jaundice and/or unexpected fatigue, or when ALT >5 × ULN in the absence of symptoms.[347] The most adequate interval for monitoring is not well established. A monthly interval has been suggested with weekly ALT monitoring in case of asymptomatic ALT >3 × ULN until resolution or discontinuation of therapy if the liver condition worsens.[348] Patients receiving multidrug anti-TBC regimens, without underlying liver diseases or risk factors, are also suggested to undergo liver profile monitoring every 2 weeks for the first 8 weeks, and then every 4 weeks until the completion of therapy. In addition to the stopping rules recommended by the ATS, discontinuation of further therapy is advised in cases of serum bilirubin >1.5 × ULN (with ALT >3 × ULN) or prothrombin time >1.5 × ULN, irrespective of the absence or presence of symptoms.[348] A study of isoniazid hepatotoxicity in the US found poor adherence to the ATS guidelines, which was associated with more severe outcomes including hospitalization, death and liver transplantation.[349]

Class effect and cross reactivity

Although almost any drug can in theory elicit idiosyncratic DILI given an individual patient's susceptibility, incidence across different drug classes seems to vary significantly. Certain groups of medical drugs such as antibiotics, NSAIDs, statins, anticonvulsants, antivirals, kinase inhibitors, TNFα antagonists, and checkpoint inhibitors apparently confer a higher risk for hepatotoxicity than others.[14,15,48,350] This may partially be due to the widespread use of certain compound classes such as NSAIDs, or to associations with potential risk factors for DILI in certain patient groups, such as alcohol abuse in patients with seizures being treated with anticonvulsants.[351] It may, however, also be related to a class or family effect across a given drug group, with class effect referring to association with the therapeutic target, and family effect to shared structural features of a group.[352] Although a class effect has been postulated for NSAIDs by the FDA,[353] this is still controversially debated, as is a potential family effect.[352] For other groups, such as TNFα antagonists or checkpoint inhibitors, an association with the underlying mode of action is much more evident.[350,354] In particular for antibiotics it has been speculated that the strong predominance of DILI caused by these agents could be related to their effects on gut microflora and changes in lipopolysaccharide exposure, which are known to play an important role in inducing immune tolerance, resulting in *defective adaptation*.[5] Irrespective of whether or not there is a suspected class or family effect for a given compound, a patient who has just experienced DILI with that drug may have to be put on an alternative therapy immediately to continue treatment of the underlying disease; selecting the drug with the least risk for hepatotoxicity can be a life-saving decision in some cases.

A comprehensive database developed under the auspices of the US National Institutes of Health and the National Institute of Diabetes and Digestive and Kidney Diseases, available online (LiverTox® http://livertox.nlm.nih.gov), provides up-to-date, easily accessed information on the diagnosis, cause, frequency and patterns of liver injury attributable to both prescription and non-prescription medications. LiverTox® currently hosts data on 1,124 different compounds, including 23,000 annotated references, and 400 case descriptions.[355] For selection of alternative treatments replacing a compound that is suspected to have caused DILI in a patient, LiverTox® may be an immensely useful guide, as it also provides a structured overview on key drug classes. A categorisation of drugs leading to DILI based on critical assessment of documented hepatotoxicity in the literature has also been[356] published.

Detecting DILI in clinical trials

Signal detection

Given the evidence that the risk of progression to liver failure and fatal outcome is higher for hepatocellular than cholestatic injury,[357] stringent monitoring and follow-up is required. In a clinical trial, DILI should be suspected if – with liver chemistry results being normal at baseline – aminotransferases exceed 3 × ULN (hepatocellular injury).[345] Elevations of ALT and/or AST less than 3 × ULN are much less specific for DILI, and can also be observed in placebo treated patients or healthy individuals.[358–363] In particular during phase I studies with healthy individuals or patients being kept on a ward for days or weeks, aminotransferase elevations are often confounded by the effects of physical exercise or diets.[364]

With abnormalities being present at baseline already, doubling of baseline values may be considered a threshold warranting close observation.[108] For patients with underlying chronic liver disease, an algorithm for signal detection and treatment discontinuation has been proposed,[365] which may have to be adapted to more conservative thresholds if a drug candidate is already suspected of increasing the risk of liver injury. Key signals for potential DILI are imbalances in aminotransferases, in particular ALT, elevations across treatment *vs.* control groups, and, as an indicator for more serious injury, the combination of aminotransferase and bilirubin elevations.

Hy's law

Hy's law is a sensitive and specific predictor of a drug's potential to cause severe hepatotoxicity.[366] If observed, it indicates hepatocellular injury severe enough to impair hepatic function, which is anticipated to result in patients experiencing liver failure that is fatal or requires liver transplantation in at least 10% of cases.[14,345]

Hy's law consists of 3 components:

1. A statistically significant higher incidence of 3-fold or greater elevations above ULN of ALT or AST compared to (non-hepatotoxic) control or placebo

2. Individuals showing ALT or AST >3 × ULN, combined with elevation of serum TBL >2 × ULN, without initial findings of cholestasis, indicated by elevated ALP

3. Absence of any alternative cause likely to explain the combination of increased ALT or AST and TBL, such as viral hepatitis A, B, C, or E, pre-existing or acute liver disease, or another drug capable of causing the observed injury[345]

Thus, Hy's law refers to a signal in a given trial *population*, with all 3 elements, *i.e.* imbalance of ALT/AST elevations across treatment groups, individual cases with combined elevation of ALT/AST and TBL, and absence of a plausible alternative cause in such cases, required to match the definition. In clinical practice, *individual patients* matching criterion 2 and 3 are referred to as "*Hy's law cases*".

Points to consider:

i) Although the definition of Hy's law refers to TBL, hepatocellular dysfunction is indicated by increased direct, *i.e.* conjugated bilirubin only. Elevations of TBL due to indirect, *i.e.* unconjugated bilirubin, may be caused by haemolysis, or reduced bilirubin glucuronidation via UGT1A1, either due to genetic variation (Gilbert's syndrome) or drug-related enzyme inhibition. Thus, it is important to assess fractionated bilirubin since cases with predominantly unconjugated mild hyperbilirubinemia would not qualify as potential Hy's law cases. ii) Combined elevation of ALT or AST and TBL does not only refer to concurrent elevation. More often, bilirubin elevation follows ALT or AST elevation with a lag time of up to 4 weeks. Thus, any screening for potential Hy's law cases in a development programme needs to factor in that time lag. iii) Even with initially "pure" hepatocellular injury, ALP often shows secondary elevation due to intrahepatic cholestasis. Thus, cases with elevated ALT or AST and TBL, associated with elevated ALP, cannot automatically be discarded as not matching Hy's law criteria; the time course of elevations needs to be assessed carefully. Furthermore, ALP values >2 × ULN were not found to reduce the risk of ALF in patients fulfilling Hy's law in the Spanish DILI registry.[56] iv) Exclusion of cholestatic or mixed type injury needs to factor in both ALP activity and the R ratio (see Section *Clinical-pathological manifestations*). It is not uncommon to observe ALP >2 × ULN at the time of ALT/AST peak, at first glance suggesting cholestatic injury by old standards, but with an R value clearly exceeding 5 it confirms hepatocellular injury. Respective cases may still have to be considered as fulfilling Hy's law, such that even if the criteria of the FDA DILI guidance are not literally met, they still have to be interpreted in this sense.

As the FDA puts it in their guidance document, "finding one Hy's law case in the clinical trial database is worrisome; finding 2 is considered highly predictive that the drug has the potential to cause severe DILI when given to a larger population." The FDA has been using Hy's law rigorously to screen out potentially hepatotoxic drugs for almost 20 years, and "since 1997 did not have to withdraw a single drug approved after 1997 because of post-marketing hepatotoxicity".[367]

Non-Hy's law signals

In order to increase the chances of detecting a risk for DILI as early as possible in a development programme, it is of utmost importance not just to ensure proper identification of Hy's law cases, but to look diligently and systematically for patterns of liver injury across the programme. Any individual elevation of ALT or AST >3 × ULN or ALP >1.5 × ULN in the absence of known bone pathology, as well as respective imbalances between treatment and control groups, need to be followed up carefully.

Signal follow-up

In cases of ALT, AST and/or ALP elevations exceeding the defined thresholds, repeat testing should be done within 48 to 72 hours, including ALT, AST, ALP, GGT, TBL, INR, albumin, creatine kinase, and GLDH. If TBL is elevated >2 × ULN, fractionation into direct and indirect bilirubin is required. If abnormalities are confirmed, close observation and follow-up as per FDA guidance[345] needs to be initiated: i) repeat liver chemistry tests 2 or 3 times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the individual is asymptomatic; ii) full medical history including cardiac disease, blood transfusions, intravenous drug abuse, travel, work, alcohol intake; iii) full clinical examination looking for evidence of acute or chronic liver disease, cardiac disease and infection; iv) history of concomitant drug use (including non-prescription medications and HDS preparations), alcohol use, recreational drug use, special diets, and chemicals administered within 1 month of the onset of liver injury; v) exclusion of underlying liver disease, as specified in Table 7.

Decision to stop drug administration

The final decision to discontinue study medication is up to the judgement of the clinician responsible for the patient. Thresholds for treatment discontinuation in clinical trials (not post-marketing) suggested by the FDA guidance[345] are:

- ALT or AST >8 × ULN
- ALT or AST >5 × ULN for more than 2 weeks
- ALT or AST >3 × ULN and (TBL >2 × ULN or INR >1.5)
- ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

These thresholds may have to be adapted to the specific study indication and protocol. The decision to stop treatment does not mean discontinuation of the patient from the clinical trial; vital status and liver status should continue to be collected where possible. Discontinuing an investigational drug is usually the only available therapy to treat suspected DILI and may not result in an immediate improvement as test values and symptoms can last (sometimes even progress) for days or weeks after the drug has been discontinued. Once discontinued, patients should not be re-exposed to the suspected drug.

Signal assessment

Use of new liver safety biomarkers

Standard liver chemistry tests have some shortcomings, limiting adequately sensitive and specific detection, prediction of clinical outcome, as well as mechanistic assessment of liver safety signals.[241] As outline in the previous section on *Diagnosis and causality assessment*, new liver safety biomarkers have been evaluated by the IMI SAFE-T consortium in collaboration with DILIN and PSTC. This resulted in regulatory support ("Letters of Support") by the FDA and EMA for the exploratory use of several new markers in drug development,[296,297,368] to improve: i) prediction of progression from hepatocellular injury to severe DILI (HMGB1, osteopontin, keratin 18 and MCSFR1); ii) early (within 24 hours) prediction of the occurrence of suspected intrinsic liver injury (HMGB1, keratin 18, miR-122 and GLDH).

Recommendations

- Systematic monitoring of liver tests can be necessary for drugs with known DILI liability in clinical development. In the post-marketing setting, drugs with a relevant risk may have a boxed warning for hepatotoxicity, in which case intensified monitoring and surveillance of liver function is indicated. Grade D.

Evidence: Inconclusive level 2b (individual cohort studies or RCTs with <80% follow-up) studies.

- Hy's law should be considered to identify patients at risk of progressing to severe DILI in the setting of clinical trials. Thresholds for interrupting or stopping treatment with a study drug, as recommended by the FDA, are intended as guidelines for studies in drug development and may be adapted depending on individual risk–benefit assessment. Grade B.

Evidence: Consistent level 2b (Exploratory cohort study with good reference standards) studies.

Post-marketing surveillance for DILI

The likelihood of detecting a drug candidate's potential to cause severe DILI in a drug development programme using Hy's law depends on sample size. For example, if the true incidence of severe injury is 1/10,000 and the rate of Hy's law cases is 1/1,000, about 3,000 exposed individuals would be needed to have a 95% probability of observing at least 1 Hy's law case in the treated population.[369] Thus, even given increasingly large trials in drug development programmes, there is a genuine risk that the first signal for a new drug's potential for hepatotoxicity may only be detected after launch of the product,[345] either during post-marketing surveillance studies, specific DILI registries, or from spontaneous reporting. Hence, Temple's corollary (a background incidence of more instances of ALT >3 $\times$ ULN for the candidate drug compared to placebo) is important, as it is a more sensitive, less specific signal and is not missed in current regulation studies. One exception may be antibiotics, which are administered for 1–2 weeks and ALT elevations may not occur until after treatment cessation.

While dedicated post-marketing surveillance studies and registries help to generate high quality data and structured output, unsolicited spontaneous reports often lack adequate quality and completeness to support timely detection and causality assessment of suspected DILI. Key challenges comprise, on top of a widespread lack of awareness of DILI in clinical practice: i) missing baseline liver chemistry values; ii) absence of regular monitoring, even with products that carry a boxed warning for DILI; iii) lack of adherence to recommended monitoring intervals;[370–372] iv) treatment with multiple drugs, including self-medication *e.g.* with HDS preparations.

To address these challenges, it is helpful to: i) take baseline blood samples in all patients that are prescribed a recently approved new drug; ii) ensure adherence to recommended monitoring intervals for liver tests for products that have DILI in the label; iii) support complete capture of key data for causality assessment, *e.g.* by providing a structured DILI questionnaire, including key elements of the CIOMS/RUCAM score. Such a questionnaire could be offered via a web-based platform accessible from desktop and mobile devices to ease data entry.

To overcome some of the challenges with causality assessment for DILI in a post-marketing setting, a modified CIOMS/RUCAM algorithm, the PV-RUCAM, has been proposed and recently introduced in a proof of concept study.[373] The algorithm may have the potential to address some of the key gaps in DILI causality assessment, limiting subjectivity, and reducing inter-observer variability. Timely detection of DILI signals at the regulatory level, in particular for new compounds, is key to minimizing risks to patients and ensuring adequate translation of hepatotoxicity risks into product labels, *e.g.* in the warnings or precautions section, monitoring recommendation, or restriction to certain patient populations. A crucial prerequisite is proper reporting of suspected DILI cases to regulatory agencies, capturing information on time to onset, clinical course, risk factors, concomitant drugs, relevant medical history, and response to re-administration.[63,374,375] Hy's law cases should be reported to the agencies as a serious adverse event even in the post-marketing setting, and before completion of follow-up assessments.[345]

Unresolved questions and unmet needs

Epidemiology

- Big data analysis incorporating information from health care systems with integrated primary care, secondary/specialist services, diagnostics and pharmacy is needed to estimate the incidence of adverse hepatic reactions among individuals exposed to drugs in general and specific drugs in particular.
- Estimates of socioeconomic burden of DILI and its impact on quality of life are needed so that the risk-benefit ratios of interventions can inform decision making by patients, clinicians, health care providers and regulators.
- Robust case-control or population-based cohort studies are required to evaluate the risk of herbal and complimentary product-related liver injury.
- Botanical identification and chemical analysis of herbal toxic ingredients is paramount for advancing the study of herbal hepatotoxicity, ensuring consumer safety and facilitating a more accurate risk-benefit assessment in clinical practice.

Pathogenesis

- Although identification of genetic susceptibility related to common variants in HLA alleles has highlighted the important role of adaptive immune response in the pathogenesis of DILI, there are still significant gaps in our understanding of other factors that unmask or prevent liver injury as well as the determinants of severity in DILI.
- A shift in the paradigm towards an integrative approach taking into consideration drug and host interactions could enhance the mechanistic comprehension of idiosyncratic DILI.

Diagnosis

- There is an important need for practicing clinicians and medical students to acquire the knowledge to request, receive genomic data and interpret it robustly for their patients in

the era where genomics would be increasingly incorporated into patient care.
- Research should focus on the discovery, evaluation and validation of biomarkers, which can distinguish self-resolving elevation of liver enzymes related to drugs from those with a potential to evolve into symptomatic DILI and alternative diagnoses from DILI.

Outcomes
- Studies on patients with drug-induced jaundice are needed in order to identify those at risk of developing ALF, which could lead to death or the requirement for liver transplantation. New biomarkers that help to predict the clinical outcome of DILI, as well as the mechanism of injury, have yet to be evaluated as diagnostic markers by regulatory agencies.
- More detailed phenotypic data and prolonged follow-up is needed in patients who have persistent elevation in liver tests after the acute and symptomatic phase of DILI. The clinical significance of "chronic" injury is unclear and whether this will lead to significant morbidity and/or mortality is unresolved.

Treatment
- Randomized controlled trials are needed to evaluate the effect of specific interventions on the clinical outcomes of DILI.

Prediction
- Algorithms that reliably predict the DILI liability of a drug have yet to be developed and may need to consider drug-related and host factors as well as mechanistic considerations.

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Conflict of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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