Drug-induced liver injury (DILI):

Current status and future directions for drug development and the post-market setting

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A consensus by a CIOMS Working Group

Council for International Organizations of Medical Sciences (CIOMS)

Geneva 2020

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Geneva, Switzerland, June 2020

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- The supplemental Appendices 5-11 are available online only, and can be freely accessed via the above-mentioned URL or through hyperlinks in the online version of this report. They may be updated with important new information, corrigenda and/or errata after publication of the report.
- **References:** The first occurrence of each reference is preceded by an arrow.[→#] In the online report, arrows can be clicked to navigate between in-text references and the reference list. Subsequent occurrences of the same reference are shown without an arrow.[#] In the online report, the number can be clicked to navigate to the first in-text occurrence of that reference.

ABBREVIATIONS AND ACRONYMS

ADC	Antibody-drug conjugate
AIH	Autoimmune hepatitis
ALD	Alcoholic liver disease
ALF	Acute liver failure
ALI	Acute liver injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMA	Anti-mitochondrial antibody
ANA	Antinuclear antibody
ASMA	Anti-smooth muscle autoantibody
Anti-HBc	Hepatitis B core antibody
Anti-HBs	Hepatitis B surface antibody
APAP	Acetaminophen (paracetamol)
AST	Aspartate aminotransferase
BiTE®	Bi-specific T-cell engager
BMI	Body mass index
BTKi	Bruton's tyrosine kinase inhibitor
CBL	Conjugated bilirubin
ccCK18	caspase-cleaved cytokeratin 18
CD20	B-lymphocyte antigen CD20
CGS	Candidate gene study
CK18	Cytokeratin 18
CMV	Cytomegalovirus
COU	Context of use
CPK	Creatine phosphokinase
CRF	Case report form
CPT® code	Current Procedural Terminology code (United States)
CRS	Cytokine release syndrome
CSF1R	Colony stimulating factor 1 receptor
CT	Computed tomography
CTP score	Child-Turcotte-Pugh score
DIAIH	Drug-induced autoimmune hepatitis
DILI	Drug-induced liver injury
DILIN	Drug-Induced Liver Injury Network (United States)
DNA	Deoxyribonucleic acid
DRESS	Drug reaction with eosinophilia and systemic symptoms
EBV	Epstein-Barr virus
eDISH	Evaluation of Drug-Induced Serious Hepatotoxicity
EMA	European Medicines Agency
EMR	Electronic medical records

ERCP	Endoscopic retrograde cholangiopancreatography
EudraVigilance	European Union Drug Regulating Authorities Pharmacovigilance
EVDAS	EudraVigilance data analysis system
FAERS	FDA Adverse Event Reporting System (United States)
FDA	Food and Drug Administration (United States)
FGFRi	Fibroblast growth factor receptor inhibitor
GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase
GPRD	General practice research database (United Kingdom); since 2012: Clinical Practice Research Datalink (CPRD)
GWAS	Genome-wide association study
HAART	Highly active antiretroviral therapy
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDS	Herbal and dietary supplements
HEV	Hepatitis E virus
HLA	Human leukocyte antigen
HMGB1	High mobility group box 1
HSV	Herpes simplex virus
ICD	International Classification of Diseases (WHO)
ICI	Immune checkpoint inhibitor
ICSR	Individual case safety report
IDILI	Idiosyncratic DILI
iHLCs	iPS-derived hepatocyte-like cells
ILICI	Immune-mediated liver injury caused by immune checkpoint inhibitors
IMI	Innovative Medicines Initiative (European Union)
INR	International normalized ratio
iPS	Induced pluripotent stem cells
IRAE	Immune-related adverse events
LDH	Lactate dehydrogenase
LTT	Lymphocyte transformation test
MCSFR1	Macrophage colony stimulating factor receptor 1
MELD	Model for end-stage liver disease
MHLW	Ministry of Health, Labour and Welfare (Japan)
MID-NET	Medical Information Database Network (Japan).
miR122	microRNA 122
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis

NCI-CTCAE	Common Toxicity Criteria for Adverse Events (National Cancer Institute, NCI)
NIH	National Institutes of Health (United States)
NNH	Number needed to harm
NPV	Negative predictive value
NRH	Nodular regenerative hyperplasia
NSAID	Non-steroidal anti-inflammatory drug
NTRKi	Neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion inhibitor
OR	Odds ratio
PHH	Primary human hepatocytes
PI	Product information
PMDA	Pharmaceuticals and Medical Devices Agency (Japan)
PPV	Positive predictive value
Pro-Euro-DILI NET	Prospective European Drug-Induced Liver Injury Network
PCSK9	Proprotein convertase subtilisin/kexin 9
Pi3Ki	Phosphoinositide 3-kinase inhibitor
PTKi	Protein kinase inhibitors
REMS	Risk Evaluation and Mitigation Strategy (U.S. FDA)
RETi	*RET* proto-oncogene inhibitor
RMP	Risk management plan (EMA)
RNA	Ribonucleic acid
ROC	Receiver-operating characteristic
ROSi	*ROS1* gene inhibitor
RUCAM	Roussel Uclaf Causality Assessment Method
SDH	Sorbitol dehydrogenase
SI	Système international, the international system of units based on the metre, kilogram, second, ampere, kelvin, candela and mole, together with prefixes indicating multiplication of division by powers of ten
SJS	Stevens-Johnson syndrome
SOS	Sinusoidal obstruction syndrome
TBL	Total bilirubin
TEN	Toxic epidermal necrolysis
TNF	Tumour necrosis factor
TransBioLine	Translational Safety Biomarker Pipeline consortium (under the umbrella of IMI)
UGT	Uridine glucuronyl transferase
ULN	Upper limit of the normal range
ULRR	Upper limit of the reference range
WHO	World Health Organization

FOREWORD

Drug-induced liver injury (DILI) is a growing challenge because of the ever-increasing number of drugs used in medical care. After excluding acetaminophen (paracetamol) overdose, DILI caused by all other drugs, biological agents and HDS product is responsible for more than 10% of all cases of acute liver failure, posing a major clinical and regulatory challenge.

The CIOMS Working Group on Drug-Induced Liver Injury was established to provide a balanced and global perspective on DILI detection, susceptibility factors and outcome, advise on causality assessment tools, monitoring and management during the drug development and post-marketing phases, and provide insights into liver safety biomarker development. The report can serve as a reference for regulators, clinicians and companies involved in product development and/or the assessment, communication and management of drug-related risk in a postmarket setting.

The Working Group was composed of expert senior scientists in the field of DILI from academia, the biopharmaceutical industry and regulatory authorities. The members met in five face-to-face meetings in various locations hosted by the organizations where some Working Group members hold positions. A list of members and Working Group meetings is shown in Appendix 4.

This document reflects the consensus opinion of the CIOMS DILI Working Group. The group members are alone responsible, in their capacity as experts, for the views expressed in this publication. These views do not necessarily represent the decisions, policies or views of any specific organization or agency. It is anticipated that this document will prove useful to all stakeholders involved with medicines safety from the time of pre-clinical development through clinical trials to the clinical use of drugs post-marketing.

INTRODUCTION

Drug-induced liver injury (DILI) is an uncommon but potentially lethal adverse drug reaction.[→1–2] In population-based studies using different methodologies and cut-offs, the crude annual incidence of DILI post-marketing ranged from 2.4 to 13.9 per 100000 inhabitants.[→3-6] Nevertheless, DILI is the most frequent cause of acute liver failure in North America and Europe, the main reason why drugs fail to achieve marketing authorization, and a frequent cause for post-marketing restrictions and withdrawals of products.[→7–8] Some life-saving drugs, such as cancer medicines, are used with caution despite their risk of liver injury because there are no therapy alternatives, or because their benefits still outweigh their risks.

DILI may mimic almost any known type of liver disease.[→9–10] There are several well-recognized phenotypes, which are defined based on clinical and pathological criteria.[→11–12] Acute hepatocellular injury has been observed and studied most often, but it is increasingly recognized that other forms of DILI can also be serious and even life-threatening. It is likely that DILI has a worse outcome when it affects a patient with advanced liver disease.[7, →13] DILI events can be categorized as *intrinsic* (predictable, linked to toxic exposure levels of a drug or its metabolites), *idiosyncratic* (rare and unexpected given the drug's pharmacological action, linked to a yet poorly understood interplay of individual host susceptibility-related and other factors), and "*indirect*" (linked to an unwanted biological action of a drug in an individual patient).[→14]. There are currently no biomarkers that can point to an individual DILI risk in humans. Genetic studies have found some HLA alleles that are associated with DILI due to various drugs, but the predictive value of such associations is low.[→15-16]

DILI is difficult to predict during drug development. Its underlying mechanisms are still incompletely understood. Preclinical models and *in vitro* test systems can flag some potential risks, especially for intrinsic DILI, but are of limited use for assessing the risk of idiosyncratic DILI. [→17–18] Because severe DILI is typically rare, many thousands of people from across diverse patient populations may need to be treated to find one such case.[→19] In clinical trials, where limited numbers of specially selected subjects are treated under controlled conditions, the main approach to anticipate a possible DILI risk is through monitoring of standard serum liver tests to detect milder liver injury.[7, →20–21] Since there can be different DILI mechanisms and clinicopathological phenotypes, the optimal evaluation of each potential DILI case in clinical trials requires a systematic collection of adequate diagnostic datasets and a rigorous assessment for causality, performed by individuals with clinical expertise in this field.

With the overall rarity of severe DILI, assessing and managing the risk of liver injury of drugs on the market is essential. However, this requires that health care practitioners are alert to the problem and look for complete clinical, laboratory and serology data enabling them to exclude alternative causes of liver injury. The Roussel Uclaf Causality Assessment Method (RUCAM) [→22]—also known as the "CIOMS scale"—is frequently used to assess suspected DILI cases in the post-marketing setting despite several limitations. Data and cases from post-marketing surveillance, prescription event

monitoring and data mining of electronic medical records can all help to identify DILI signals in the general population. A most valuable tool is the collection of ascertained cases in DILI registries.[→23-27] Data and samples from registries can provide key insights for case detection, DILI characterization and the development of new biomarkers.

Lastly, liver injury can also be caused by herbal and dietary supplements (HDS). These products are a known cause of DILI especially in Asian countries,[→28] and initiatives have been undertaken to gather data in this area throughout Asia.[24, →29–31]. As HDS products are increasingly used alongside conventional medicines all over the world, HDS-induced liver injury is a complex and growing issue that needs to be addressed.

CHAPTER 1.

WHAT IS DILI?

1.1 DILI classification

Summary

- DILI reactions are commonly categorized as intrinsic (*i.e.* predictable following excess drug exposure), idiosyncratic (rare but potentially severe due to unique host susceptibility factors), or "indirect" (unintended injuries due to biological actions of a drug).
- In most instances, the mechanisms and risk factors for DILI are poorly understood. Despite their low incidence, both idiosyncratic and indirect DILI may progress to severe and sometimes fatal liver injury.
- A DILI episode can be characterized as hepatocellular, mixed or cholestatic based upon the R value which is defined as the ratio of serum ALT to alkaline phosphatase elevations (expressed as multiples of upper limit of normal, × ULN) at the onset of DILI. Hepatocellular, cholestatic and mixed episodes of DILI tend to have different outcomes and rates of recovery.
- DILI can mimic almost all known forms of acute and chronic liver disease. A particular drug may be associated with more than a single biochemical pattern of liver injury or clinicopathological phenotype at presentation.

Conclusions / recommendations

1. Determination of the R value is recommended in all patients with suspected DILI to help categorize the type and pattern of liver injury.
2. Clinicians should assess clinical features, laboratory abnormalities, liver histology (if performed) and imaging findings to identify the clinicopathological phenotype and the likelihood of causal association with a suspect drug (see Chapter 2).

1.1.1 General categories of DILI

Drug-induced liver injury can be caused by drugs or chemicals that have direct and predictable toxicity on liver, biliary, sinusoidal endothelial and stellate cells. This category of liver injury, called **intrinsic**, is typically dose-dependent occurring once a threshold dose or exposure level—which may differ between individuals—is reached.

A majority of the hepatotoxic drugs in clinical practice, however, induce liver damage in an unpredictable fashion. This category is termed **idiosyncratic,** as it is largely independent of the dose, route, or duration of medication exposure and mainly related to unique host characteristics. Idiosyncratic DILI refers to a hepatotoxic reaction to a drug that occurs in a small proportion of individuals who are exposed to the drug and is unexpected from its known pharmacological actions. It is believed to be precipitated by the interplay of several critical factors including the toxicological properties of the drug in conjunction with selective host-related factors and environmental conditions.[→32] In a population-based case-control study in the United Kingdom the highest crude incidence rates for idiosyncratic DILI among the users of a subset of drugs associated with acute and clinically relevant hepatoxicity were approximately one out of 1 000 patients;[4] in an Icelandic prospective DILI study the risk was higher,[5] but in this study liver injury was defined using a lower threshold than the current consensus.[20] (see Tables 4 and 5 in Section 1.1.3). Other drugs with a well-documented association with hepatotoxicity have a much lower risk of DILI.[4, 5, →33–34]

More recently a third "**indirect**" category has been proposed [14, →35] that reflects unintended liver injury associated with known actions of a drug. These may exacerbate a pre-existing chronic liver condition such as fatty liver or provoke worsening of an underlying hepatic inflammatory disease. This category also includes liver injury associated with some immunotherapies, as well as the reactivation of hepatitis B viral infection triggered by exposure to certain immunomodulatory or immune-suppressive agents (**Table 1**).

Table 1. General categories of DILI

(Modified from: [14])

	Direct (intrinsic)	Indirect	Idiosyncratic
Dose-related	Yes	No (generally)	No (with some exceptions)
Latency	Short (few days)	Typically delayed (weeks to months)	Variable (days to months), may occur after treatment discontinuation
Rate of occurrence	High	Intermediate	Low
Predictable	Yes	Occasionally	No
Implicated drugs (examples)	Acetaminophen, nicotinic acid, aspirin, cocaine, many cancer chemotherapies, fialuridine, amiodarone, methotrexate (intravenous), plants containing pyrrolizidine alkaloids	High-dose corticosteroids; some antineoplastic agents: immune checkpoint inhibitors, protein kinase inhibitors, monoclonal antibodies (*e.g.*anti-TNF, anti-CD20), daclizumab	Isoniazid, amoxicillin-clavulanate, macrolide antibiotics, fluoroquinolones, statins, flucloxacillin, diclofenac; certain herbal and dietary supplements (HDS), *e.g.* green tea extract, *Polygonum multiflorum*
Pathologic mechanisms	Liver damage occurs if parent drug or metabolite concentrations in liver cells exceed a toxic threshold	Unintended effects of drug actions on the liver (*e.g.* increased drug-induced immune autoreactivity or reduced insulin sensitivity may cause immune-mediated hepatitis and fatty liver, respectively)	Adaptive immune response to a parent drug or drug metabolite may contribute. Mitochondrial damage and hepatic steatosis may also be observed

1.1.2 Biochemical patterns of liver injury and R value

Regardless of the mechanism, drugs or their metabolites can target different hepatic cells leading to a wide variety of acute, subacute or chronic hepatobiliary diseases. Acute DILI is characterized based on the initial standard serum liver test results. The ratio (**R value**) of ALT (or AST when ALT is lacking) activity to ALP activity expressed as multiples of ULN is used to categorize the injury pattern of DILI as hepatocellular, cholestatic or mixed:

$$R = \frac{ALT/ULN}{ALP/ULN}$$

In hepatocellular injury the peak elevation of serum ALT is substantially higher than the ULN with no or minimal elevation of serum ALP. These test results correspond to a R value ≥5. In contrast, with cholestatic injury the peak elevation of ALP is substantially higher than the ULN and the R value is ≤2; with mixed-type injury (hepatocellular and cholestatic) the R value is >2 – <5.

As the ratio of elevated liver enzymes may change over the course of the event,[→36] categorization of DILI is based on the first set of laboratory tests available in relation to the clinical event.[20] A "new R" (nR) value defined as the ratio of either ALT or AST (whichever is higher) to ALP, expressed as multiples of their ULN, has also been used to categorize the type of liver injury in the study of DILI outcomes.[→37]

1.1.3 DILI phenotypes

Drug-induced liver injury can present with different clinicopathological phenotypes (**Table 2**). Some of these DILI phenotypes are associated with one or more characteristic biochemical patterns of injury (described in the previous section). With some phenotypes, progression of injury can lead to life-threatening outcomes. These include acute liver failure, vanishing bile duct syndrome, progression of acute forms of hepatotoxicity to chronic injury and cirrhosis, and hepatic decompensation in patients with cirrhosis due to underlying liver disease.

Table 2. DILI clinicopathological phenotypes and examples of associated drugs

Phenotype	Case definition	Examples of associated drugs	Observations
Acute hepatic necrosis	Hepatocellular pattern of injury; short time to onset (within days); rapid resolution in patients who recover after agent is stopped.	High dose acetaminophen (paracetamol), niacin, aspirin, intravenous amiodarone	Liver biopsy often shows bland centrilobular necrosis similar to ischaemic injury of the liver. May progress to acute liver failure.
Acute hepatitis	Hepatocellular pattern of injury; prodromal symptoms resemble acute viral hepatitis; time to onset often between 2 and 12 weeks.	Isoniazid, flutamide, diclofenac, ketoconazole, ximelagatran	Liver biopsy resembles viral hepatitis; viral infection must be excluded. May progress to acute liver failure.

Phenotype	Case definition	Examples of associated drugs	Observations
Cholestatic and mixed hepatitis	Cholestatic and mixed injuries defined by R≤2 and 2<R<5, respectively. Time to onset typically 2–12 weeks. Jaundice and pruritis may occur.	Amoxicillin-clavulanate, sulfonylureas, macrolides	Course may be prolonged until resolution after drug discontinuation. *HLA-DRB1*1501-DQB1*0602* and *HLA-A*0201* associated with amoxicillin-clavulanate in north-Europeans
Hypersensitivity syndrome with liver involvement	DRESS syndrome. Liver is involved in >50% of cases reaching 10% of mortality.	Carbamazepine, allopurinol, lamotrigine, sulfasalazine, phenobarbital, nevirapine, phenytoin, abacavir, mexiletine, dapsone, minocycline	HLA associations identified for specific syndromes and drugs: *HLA-B*1502* (Asians), *HLA-A*31:01* (Europeans)" and SJS/TEN associated with carbamazepine, *HLA-B*13:01* and DRESS associated with dapsone, *HLA-B*35:02* and minocycline, *HLA-B*5801* and SJS/TEN and DRESS associated with allopurinol. Mostly cholestatic injury. May progress to acute liver failure.
Severe cutaneous adverse reactions	SJS/TEN. High mortality that increases in the presence of DILI (36% to 46%). Positive re-challenge is common.		
Drug-induced autoimmune hepatitis	Presenting as acute or chronic injury resembling autoimmune hepatitis (AIH) serologically and/or histologically.	Nitrofurantoin, minocycline, statins, diclofenac, and anti-TNFα agents.	Responsive to corticosteroid but unlike idiopathic AIH, relapse rarely occurs after steroid discontinuation.
Hepatic steatosis	Evidence of micro- or macrovesicular hepatic steatosis due to the drug with or without inflammation and fibrosis	Amiodarone	Steatohepatitis, fibrosis and cirrhosis
		Methotrexate	Fatty infiltration; fibrosis with potential to progress to cirrhosis after long-term exposure.
		Tamoxifen	Higher risk for fatty liver disease in patients with other risk factors.
		Irinotecan	Fatty liver and steatohepatitis
Sinusoidal obstruction syndrome (SOS)	Hepatic endothelial cell injury with sinusoidal obstruction	Busulfan, Other myeloablative agents, Vinca alkaloids, pyrrolizidine alkaloids	Can present with abdominal pain, fluid retention & ascites. High ALT levels with hepatocellular pattern of injury. Liver biopsy shows obliterative venulitis.
Nodular regenerative hyperplasia (NRH)	Benign small regenerative nodules. NHR may lead to portal hypertension.	Azathioprine, HAART, oxaliplatin, 6-thioguanine, bleomycin, busulfan, cyclophosphamide, cytosine arabinoside, chlorambucil, doxorubicin and carmustine	Oxaliplatin in the treatment of colorectal carcinoma can cause NRH that manifests with evidence of portal hypertension many years after exposure.

Phenotype	Case definition	Examples of associated drugs	Observations
Neoplasia	Adenoma or hepatocellular carcinoma	Oral contraceptives	10 times the incidence of liver cell adenoma in general population.
		Androgens: xymetholone, methyltestosterone, danazol	Hepatic adenomas, hepatocellular carcinomas cholangiocarcinoma and angiosarcoma
Secondary sclerosing cholangitis	Acute presentation. Resembles primary sclerosing cholangitis in imaging and/or histologically	Amoxicillin-clavulanate, amiodarone, atorvastatin, infliximab, 6-mercaptopurine, venlafaxine, sevoflurane, amiodarone	May progress to chronic liver disease detected by MRCP and ERCP.
Granulomatous hepatitis	Central accumulation of macrophages, with a surrounding rim consisting of lymphocytes and fibroblasts	Allopurinol, carbamazepine, phenytoin, quinidine, methyldopa, sulphonamides; herbs, *e.g. Centella asiatica*	Typically mixed liver injury
Acute fatty liver	Acute onset of microvesicular steatosis	Sodium valproate, nucleoside analogue reverse transcriptase inhibitors, amiodarone, salicylates	Salicylates associated with the 'Reye's syndrome' in children.
Vanishing bile duct syndrome [→38]	Unresolving cholestasis associated with loss of intrahepatic bile ducts.	Azathioprine, amoxicillin-clavulanate, carbamazepine, chlorpromazine, erythromycin, flucloxacillin, phenytoin, terbinafine, co-trimoxazole, pexidartinib [→39–40], herbals	Poor outcome requiring liver transplantation in many instances.
Peliosis hepatis	Proliferation of the sinusoidal hepatic capillaries resulting in cystic blood-filled cavities.	Anabolic steroids tamoxifen, and azathioprine	Pain in right upper quadrant, intrahepatic bleeding

DRESS=Drug reaction with eosinophilia and systemic symptoms, **ERCP**=Endoscopic retrograde cholangiopancreatography, **HAART**=highly active antiretroviral therapy, **HLA**=human leukocyte antigen, **MRCP**=Magnetic resonance cholangiopancreatography, **SJS/TEN**=Stevens-Johnson syndrome/toxic epidermal necrolysis.

1.2 DILI case definition and severity grading

Summary

- The presence or absence of clinical symptoms such as fatigue, nausea, abdominal pain and immunoallergic signs such as fever, rash and adenopathy are criteria used in the assessment of DILI.
- Although various approaches have been taken to assess the severity of DILI, there is currently no universally recognized severity scale.
- The model for end-stage liver disease (MELD) scores and other mathematically derived algorithms correlate with clinical outcomes in DILI patients.
- DILI patients with acute hepatocellular injury and an ALT > 3 × ULN with a total bilirubin of > 2 × ULN that meet criteria for "Hy's law" have an approximately 10% likelihood of death during short-term follow-up.
- Clinical trials in oncology patients utilize the National Cancer Institute's grading system of Common Toxicity Criteria for Adverse Events (NCI-CTCAE) criteria to grade organ system related adverse events. The NCI-CTCAE criteria for liver toxicity do not correlate well with other clinically derived prognostic indices or likelihood of an adverse outcome.

Conclusions / recommendations

1. DILI severity grading scales should be defined by specific new onset liver-related biochemical or clinical findings, *e.g.* acute liver failure, coagulopathy, encephalopathy, or other organ dysfunction, hospitalization, death and liver transplant. Tiered severity grades such as those used by the National Institutes of Health (NIH) Drug-Induced Liver Injury Network (DILIN) or International DILI Expert Working Group have proven useful for this purpose (**Table 3**).
2. Isolated descriptive terms such as 'severe' and 'serious' should be qualified by defining their corresponding grades of DILI severity.

1.2.1 DILI case definitions

Patients with normal standard serum liver test results at baseline

An international DILI expert working group [20] has suggested that any of the following laboratory criteria of serum analytes are indicative of DILI once other causes of liver injury have been systematically excluded.

- ALT equal or greater than 5 × ULN
- ALT equal or greater than 3 × ULN, and total bilirubin > 2 × ULN, and no or minimal elevations in ALP
- ALP equal or greater than 2 × ULN when the source of increased ALP levels is the liver

In an ongoing large prospective study of DILI in a post-market setting, the NIH Drug-Induced Liver Injury Network (DILIN) [23] has used the following biochemical criteria to identify potential DILI cases:

- ALT or AST > 5 × ULN or ALP > 2 × ULN on two consecutive occasions
- TBL >2.5 mg/dL and elevated AST, ALT or ALP
- INR > 1.5 and elevated AST, ALT or ALP

Compared with serum AST elevations, increases of serum ALT generally have greater liver tissue specificity. Nonetheless, in some patients with alcoholic liver disease or cirrhosis, peak AST levels may be higher than ALT. Under these circumstances AST can be a more sensitive biomarker of DILI. Increased ALP levels due to liver injury in the absence of bone pathology are typically accompanied by elevations in gamma-glutamyl transferase (GGT), whereas when the source of increased ALP is bone, GGT levels are generally normal.

Patients with standard serum liver test abnormalities at baseline

Since serum ALT, AST and/or ALP levels are often elevated at baseline in patients with a pre-existing liver disease, laboratory criteria for new-onset DILI also include equivalent fold increases above the patient's pre-treatment baseline levels.[20]

In some patients with advanced stages of pre-existing liver disease and cirrhosis, the onset of DILI is marked by worsening of serum indicators of liver function, such as increasing total and direct bilirubin levels and/or a rising INR in the absence of a substantial rise in serum ALT or AST. In patients who have stable cirrhosis, DILI may present with findings of acute hepatic decompensation. The diagnosis of DILI in patients with underlying cirrhosis who have these profiles of worsening liver function relies on the systematic exclusion of other causes of hepatic deterioration or decompensation.

1.2.2 Grading of DILI severity

Various approaches have been used to assess the severity of DILI. An ALT > 8 × ULN, ALT > 3 × ULN and TBL > 2 × ULN, hospitalization for liver injury, and death or liver transplant are categories that have been studied.[→41] The degree of ALT elevation alone may not reflect the severity of liver injury since these levels do not accurately reflect specific clinical outcomes.[20]

One approach for grading DILI severity in patients with a hepatocellular pattern of liver injury is the application of Hy's law (see Section 2.2.6). This is derived from Dr Hyman Zimmerman's clinical observation that drug-induced hepatocellular jaundice is a serious reaction, with substantial mortality.[→42] During drug development, cases of hepatocellular DILI with increases of ALT and bilirubin without a substantial increase of ALP (< 2 × ULN) point to an increased risk of a study drug to cause severe hepatocellular DILI and acute liver failure in a post market setting (see Chapter 3). Cases that conform to a modified Hy's law definition utilizing the "new R" value (see 1.1.2) have been shown by the Spanish DILI group to be associated with an increased risk for drug-induced acute liver failure [37] and by the DILIN study group to have a higher association with liver transplant and/or death outcomes.[→43] A recent analysis from the DILIN prospective registry study

Table 3. DILI severity grading scales

U.S. Drug-Induced Liver Injury Network (DILIN) [11]		International DILI Expert Working Group [20]	
1 Mild	Elevated ALT and/or ALP but TBL <2.5 mg/dL and INR <1.5	**1 Mild**	ALT ≥5×ULN or ALP ≥2 × ULN and TBL <2×ULN
2 Moderate	Elevated ALT and/or ALP and TBL ≧2.5mg/dL or INR ≧1.5	**2 Moderate**	ALT ≥5×ULN or ALP ≥2 × ULN and TBL ≥2×ULN, or symptomatic hepatitis
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 one of the following criteria: - Hepatic failure (INR >1.5, ascites or encephalopathy) - other organ failure due to DILI	**3 Severe***	ALT ≥5×ULN or ALP ≥2 × ULN and TBL ≥2×ULN, or symptomatic hepatitis and at least one of the following criteria: - INR ≥1.5 - ascites and/or encephalopathy, disease duration <26 weeks, and absence of underlying cirrhosis - other organ failure considered to be due to DILI
5 Fatal	Death or liver transplantation due to DILI	**4 Fatal/ transplan-tation**	Death or liver transplantation due to DILI

ALP=alkaline phosphatase, **ALT**=alanine aminotransferase, **DILI**=drug-induced liver injury, **INR**=international normalized ratio, **TBL**=total serum bilirubin, **ULN**=upper limit of normal.

* In FDA guidance [7] the term "severe liver injury" is used to describe irreversible hepatic failure.

demonstrated that MELD scores may also be associated with adverse outcomes in patients with DILI.[43, →44]

In the assessment of post-marketing cases of suspected DILI, an approach that has been used by both the NIH Drug-Induced Liver Injury Network (DILIN) and the United States Food and Drug Administration (U.S. FDA), includes a five-level categorical scale with specified clinical and laboratory test results.[→45–46] Based on these parameters, DILIN has suggested a 5-point severity grading of mild, moderate, moderate-severe, severe, and fatal.[11] Because different criteria for hospitalization may be followed in different countries, a modified four-point scale was proposed by an international DILI Expert Working Group.[20] The two scales are shown in **Table 3**. These grading scales are typically used in the analysis of post-marketing cases.

A commonly used grading system for standard serum liver test results in oncology clinical trials is the National Cancer Institute's grading system of Common Toxicity Criteria for Adverse Events (NCI-CTCAE)[1].[→47] While this grading system may be useful for signal detection and identification of changes in liver tests throughout a clinical trial at the individual and aggregate levels, it does not specifically correlate with hepatocellular function or clinical outcome. It is not designed specifically for DILI, nor are its severity grades stratified by levels of risk.

[1] The NCI-CTCAE Grading System is based on the terms of the Medical Dictionary for Regulatory Activities (MedDRA®), and defines a severity scale for each of approximately 800 terms for adverse events. In version 5.0 under "Investigations" the following terms for liver abnormalities are included: "ALT increased", "AST increased", "ALP increased", "GGT increased" and "Bilirubin increased".

1.3 Estimated DILI incidence and risk factors in the general population

Summary

- Antibiotics were the most commonly implicated cause of DILI in retrospective population-based studies from the United Kingdom in the 1990s. However, variable laboratory criteria and case definitions were used to identify and grade a DILI episode.
- Prospective registry studies in the United States and Europe indicate that antibiotics and herbal and dietary supplements (HDS) are the leading causes of DILI in adults, while a review from China found the leading causes to be HDS products and tuberculosis chemotherapies.
- The leading causes of DILI in children are antibiotics and anti-epileptic agents.
- Currently available data indicate that DILI is also associated with monoclonal antibodies that target specific cell-surface molecules for oncological or non-oncological treatment. (See Section 6.3 for details on liver injury in cancer patients)
- The incidence of DILI varies between countries; this could be due to a variety of factors including prescribing habits of practitioners, population composition, and/or case definition of DILI.

Conclusions / recommendations

1. Subject age, gender, race, medical co-morbidities and genetic factors have been implicated as susceptibility factors to DILI from individual agents.
2. There is an unmet need to determine the true incidence rates of DILI in the general population of patients treated with specific approved agents in different countries or regions. This is particularly important for anti-cancer drugs, for which limited therapy alternatives exist.

1.3.1 Epidemiological research

The true incidence of DILI in the general population (outside of clinical trials) is not well defined. Studies from the last two decades reported a wide range of incidence rates, probably owing to differences in the study populations and significant inconsistencies in criteria used to define DILI.[3, 4, 5, 6] The criteria used to define DILI in some of these studies are no longer accepted by many experts in this field.

The United Kingdom General Practice Research Database

Studies of the risk of liver injury associated with the use of specific drugs started in the early 1990s. Early studies [→48-56] involved retrospective medical data review of a general population registered in a single large general practice research database in the United Kingdom (GPRD, since 2012:

As an example, for "ALT increased" the NCI-CTCAE grades are:
Grade 1: >ULN - 3.0 × ULN if baseline was normal; 1.5 - 3.0 × baseline if baseline was abnormal;
Grade 2: >3.0 - 5.0 × ULN if baseline was normal, >3.0 - 5.0 × ULN if baseline was abnormal;
Grade 3: >5.0 - 20.0 × ULN if baseline was normal, >5.0 - 20.0 × ULN if baseline was abnormal;
Grade 4: >20.0 × ULN if baseline was normal, >20.0 × ULN if baseline was abnormal;
Grade 5: Not applicable to ALT.[47]

Clinical Practice Research Datalink, CPRD) from 1985 to 1993. Three of these studies had a case-control design.[51, 52, 55] The cohorts of patients were selected according to their use of drugs suspected of causing hepatotoxicity, *e.g.* non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and acid-suppressing agents. The specific drugs and the risk of DILI investigated in these studies are shown in **Table 4**.

In a landmark paper from 2004,[4] absolute and relative risks of acute and clinically relevant DILI were provided from a population-based case-control study using the same source as the early studies mentioned above. Instead of looking for users of drugs and linking them with diagnosis of liver disease, the researchers performed a computer search to find idiopathic liver disease cases serious enough to be referred to either a hospital or a consultant. A total of 5 000 controls who had no prescription of drugs of interest were randomly sampled and matched with cases on age, gender and calendar year (**Table 4**).

Table 4. Drugs assessed for hepatotoxicity in the United Kingdom General Practice Research Database (GPRD)

Drug	Crude incidence among users of drug	
	1997 review [56] of studies [48 - 55] (a); per 100 000 users	2004 nested case-control study [4] (b)
Isoniazid	434	ND
Chlorpromazine	133	Approx. 1 per 1 000 users
Azathioprine	ND	Approx. 1 per 1 000 users
Sulfasalazine	ND	Approx. 1 per 1 000 users
Valproic acid	ND	Approx. 1 per 3 000 users
Cimetidine	22.7	ND
Amoxicillin-clavulanate	22.5	Approx. 1 per 10 000 users
Carbamazepine	ND	Approx. 1 per 5 000 users
Ranitidine	8.9	ND
Flucloxacillin (c)	7.6	> 1 per 10 000 users
Sulfamethoxazole/ trimethoprim	5.2	ND
Omeprazole	4.3	ND
NSAIDs, combined (d)	3.7	ND
Erythromycin (e)	3.6	ND
Oxytetracycline (c)	2.1	ND
Betahistine, chlorpheniramine, diclofenac, metoclopramide, tetracylines, macrolides, tricyclic antidepressants	ND	> 1 per 10 000 users

ND: Not determined

(a) The time window for the exposure of the drugs was somewhat variable but was within 90 days from initiation of the drug. Usually it was within 45 days of prescription of the antibiotics,[49, 50, 53, 54] within 60-90 days for NSAIDs,[48, 52] and within 60 days for chlorpromazine and isoniazid.[51]

(b) Crude incidence of acute liver injury among users of drug, for drugs showing a significant association in a nested case-control analysis [4]

(c) Cholestatic disease only

(d) Includes buprofen, mefenemic acid, indomethacin, ketoprofen, fenbufen, diflunisal, tenoxicam, fenoprofen, sulindac, diclofenac, naproxen and piroxicam [56]

In the 2004 case-control study [4] the strongest associations for acute hepatotoxicity, affecting approximately 1 per 1 000 users, were observed for chlorpromazine, azathioprine and sulfasalazine. A risk of approximately 1 per 5 000 users was observed for the antiepileptics carbamazepine and valproic acid. The risk with flucloxacillin was 1 per 39 000 users and that with amoxicillin-clavulanate close to 1 per 10 000 users.[49, 54] Diclofenac was the only NSAID that was associated with an excess risk but with a low incidence of approximately 1 per 15 000 users (6.3 per 100 000 users).

The weaknesses of the case-control study [4] were its retrospective design, lack of systematic registration of over-the-counter drugs and herbal and dietary supplements (HDS), lack of medical records of those who died, and the relatively limited number of cases identified. In the 2004 study from the United Kingdom the crude incidence of non-fatal, clinically relevant, idiopathic, acute DILI was found to be 2.4 cases per 100 000 person-years (95% CI: 2.0, 2.8).[4] A very similar incidence of 2.3 per 100 000 inhabitants annually was reported in another retrospective study from Sweden.[→57]

Prospective population-based studies

The risk of DILI has also been assessed in prospective and population-based studies. Prospective studies in France and Iceland [3, 5] found 7-9 times higher incidence rates of 13.9 and 19.1 per 100 000 person-years respectively, compared to previous retrospective studies.[4, 48-56, →58-61]

In the prospective study conducted in Iceland,[5] the most common cause of DILI was amoxicillin-clavulanate. However, the highest risk of hepatotoxicity was associated with the use of infliximab with one DILI case per 148 patients treated and of azathioprine with one DILI case per 133 patients treated (**Table 5**). Of note, DILI was defined in this study as ALT > 3× ULN or ALP> 2× ULN. A separate study of all patients identified with DILI due to infliximab over a five-year period found an even higher risk with one DILI case out of 120 patients treated with infliximab.[→62] Another study on the risk of azathioprine-induced liver injury among patients with inflammatory bowel disease confirmed the relatively high risk of hepatotoxicity associated with this drug.[→63]

In a Japanese study of 307 prospectively collected DILI cases,[24] the most commonly identified agents were: anti-inflammatory drugs (11%), anti-microbial drugs (11%), anti-cancer drugs (10%), dietary supplements (9%), drugs for the gastrointestinal system (9%), drugs for the psychiatry and neurological system (8%), and Chinese herbal medicines (6%).

Table 5. Number of DILI cases identified during a two-year period in Iceland

Drug	Number of cases [5]*	Proportion (1 out of all patients treated)
Azathioprine	4	1/133
Infliximab	4	1/148
Isotretinoin	3	1/ 732
Nitrofurantoin	4	1/1 369
Amoxicillin-clavulanate	15	1/2 350
Atorvastatin	2	1/3693
Diclofenac	6	1/9148
Doxycycline	2	1/16339

*Cases of liver injury were defined as ALT > 3 × ULN or ALP > 2 × ULN.

In China, data from a nationwide study including 25927 DILI cases suggest that herbal and dietary supplements (HDS) (27%) and anti-TB drugs (22%) were the two leading classes of implicated agents. Other commonly agents included antineoplastics or immunomodulators (8%), anti-infectious agents (6%), psychotropics (5%), non-sex hormones (3%), cardiovascular drugs (3%), digestive drugs (2%), respiratory drugs (1%) and musculoskeletal drugs (1%). NSAIDs were less commonly reported causes of DILI than in Europe and the United States.[→64]

1.3.2 Risk factors

Age

A relationship between the incidence of DILI and older age was observed in a prospective DILI study from Iceland.[5] However, this does not necessarily indicate causal relationship; rather it might reflect an increased number of drugs prescribed with older age. Age may be a risk factor for DILI associated with the use of specific drugs, as has been seen with nitrofurantoin,[→65] isoniazid [→66] and flucloxacillin.[→67] On the other hand children less than 10 years of age had an increased risk of DILI with the antiepileptic drug valproic acid.[→68]

The phenotype of DILI seems to be affected by advanced age, as cholestatic type of DILI has been found to be more common in patients older than 60 years of age.[→69] This might be influenced by decreased hepatic mass, impaired renal function and decreased hepatic blood flow with advanced age.[→70–71] The risk of a fatal outcome from liver injury with valproate is highest before the age of two, potentially due to reduced plasma protein binding and/or differences in the metabolism of the drug.[→72] While cholestatic liver injury is more common in older age, hepatocellular type of DILI is more common in younger patients.[57, 69, →73]

Gender

Females and males appear to have a similar risk of DILI.[5, 23, 69, →74–75] However, females were found to have an increased risk of developing liver injury from nitrofurantoin,[65] flucloxacillin,[67] diclofenac [4] and tetracyclines.[4, →76]

Furthermore, drug-induced autoimmune hepatitis (DIAH) seemed to occur almost exclusively in women.[62, 76, →77–78] Idiosyncratic liver injury of the hepatocellular type has been shown to occur more commonly in females than males.[52, 57, 69, 73, 74]

The severity of DILI has been associated with female sex in the Spanish Registry,[69] where almost 90% of patients with fulminant hepatic failure from DILI were women. Similar results were observed in a U.S. study, where 77% of patients with ALF were women.[→79] A recent study from India on DILI from anti-tuberculosis drugs demonstrated that although this occurred more commonly in men, women developed ALF from these drugs and died more frequently than men.[→80]

Ethnicity

Little is known about the risk for DILI and patient race and ethnicity. The DILIN study group found that chronicity (defined as elevated liver tests 6 months after presentation of DILI) was more common in African Americans than in other races.[2] Furthermore, Asian ethnicity was an independent risk factor

for the need for liver transplantation from DILI.[43] A recent study found that African Americans had higher rates of liver-related death and higher rates of liver transplantation at 6 months compared to Caucasians.[→81]

Medical co-morbidities, including pre-existing liver disease

There is little data to suggest that medical comorbidities have major impact on the risk of developing DILI. Diabetes mellitus does not seem to increase DILI risk in general;[23, 73, 74] however, in conjunction with obesity it has been shown to be risk factor for methotrexate-induced liver injury.[→82–83] Diabetes mellitus has also been associated with increased risk of mortality in patients with DILI,[73] and dyslipidaemia was associated with increased risk of chronic DILI in patients followed up within the Spanish Hepatotoxicity Registry.[→84] A recent study from DILIN however demonstrated that the number of medical co-morbidities appears to be an important determinant of the likelihood of an adverse outcome in DILI patients.[→85]

It is still unsettled whether chronic liver disease increases the risk of developing DILI.[→86–87] Although patients with chronic liver disease may not in general have an increased risk of developing DILI, the consequences of a DILI episode in these patients might be more severe.[42] Patients with abnormal baseline standard serum liver tests are not at increased risk of hepatotoxicity from statins [→88-90] A few studies suggest that patients with fatty liver disease or components of metabolic syndrome are at increased risk of DILI caused by a number of medications.[85, →91] Some studies have suggested that patients with viral hepatitis, mostly those with hepatitis B and C, are at increased risk of hepatotoxicity due to anti-tuberculosis medications,[→92-96] but other studies have failed to show this increased risk.[→97-99]

In patients with cirrhosis from primary biliary cholangitis (PBC) the use of obeticholic acid has been linked to reports of worsening liver disease, hepatic decompensation and liver failure. These reports prompted the manufacturer to add a boxed warning with clinical management recommendations in the United States product label that highlights the importance of dosage adjustment, interruption or discontinuation of obeticholic acid in patients with advanced stages or decompensated forms of cirrhosis.[→100] Cases of worsening liver function or liver failure have also been reported in chronic hepatitis C patients with advanced fibrosis and cirrhosis treated with combination direct-acting antiviral products containing a protease inhibitor.[→101]

Co-infection with HIV and HCV was found to increase the risk of hepatotoxicity of anti-tuberculosis drugs.[→102] Hepatotoxicity associated with the use of antiretroviral drugs has been reported to be higher in patients co-infected with hepatitis B and C.[→103-108] However, causality assessment of the potential DILI has not been vigorously undertaken. Spontaneous fluctuations in viral loads are common in both hepatitis C [→109] and hepatitis B,[→110] and this should be taken into consideration when assessing the etiology of elevated liver tests in these patients.[→111] Furthermore, comparison is needed with control groups of viral hepatitis patients not treated with the drugs.

CHAPTER 2.

ASSESSING DILI CASES

2.1 Standard serum liver tests

Summary

- When serum ALT, AST and ALP values are accompanied by total bilirubin and INR levels, they can provide insight into the pattern of liver injury as well as its severity.
- The interpretation of pre-treatment baseline values depends on what are considered "normal values" in the target population receiving the drug. Certain patient groups may have baseline values outside the normal reference range.

Conclusions / recommendations

1. Serum ALT, AST, ALP, and total bilirubin levels with fractionation are the current recommended and accepted liver damage biomarkers in clinical trials and in post-marketing studies.
2. Serum ALT is more specific than AST for detection and monitoring of liver injury, irrespective of the cause. AST can be used as a substitute when ALT values are not available.
3. Total bilirubin and INR values, and in some cases albumin levels are used to grade the severity of a DILI episode.
4. Clinical signs and symptoms of hepatic encephalopathy, ascites and bleeding, and loss of liver cell function are valuable in assessing cases with clinically serious liver injury.

Benefits and limitations

Serum ALT, AST, ALP, TBL, and GGT are some of the most commonly ordered laboratory tests in clinical practice. They are used to diagnose and evaluate acute and chronic human liver disease, regardless of the etiology.[→112–113] An overview of tests and reference ranges is given in **Table 6**. Since different units are used in different countries for the laboratory parameters, the reference ranges are given in the units used in cited literature and in SI units.

Measures of serum ALT, AST, ALP, TBL, and GGT present some complexities in clinical interpretation that make it difficult for drug makers and regulators to establish the liver safety profile of a drug.[→114] These tests are found to be abnormal in up to 40% of patients in the general population,[113] although few of these patients are actually diagnosed with significant liver disease.[→115] High ALT levels are present in most

Table 6. Commonly used standard serum liver tests

Analyte	Tissue localization	Conditions causing elevation of the analyte in serum	Reference range*	
			Units as in cited publications	SI units
ALT	Liver, skeletal and heart muscle	Hepatocellular necrosis, rhabdomyolysis, muscle injury	29–33 U/L for men 19–25 U/L for women [112]	0.48-0.55 µkat/L for men 0.32-0.42 µkat/L for women
AST	Liver, heart skeletal muscle, kidney, brain, red blood cells	Hepatocellular necrosis, rhabdomyolysis, muscle injury, haemolysis	10 to 34 U/L [→116]	0.17 to 0.57 µkat/L
ALP	Liver, bone, kidney, intestine, placenta	Cholestasis, biliary injury, normal (*e.g.* child growth) and pathological (*e.g.* bone metastasis) conditions associated with bone involvement, pregnancy (3rd trimester)	20–140 U/L Levels increase especially in women over 50 years old [→117]	0.33-2.34 µkat/L
GGT	Liver, prostate, kidneys, pancreas, intestine, and spleen	Cholestasis, biliary injury, obesity, alcohol consumption, medications (phenytoin, phenobarbital, furosemide, heparin) congestive heart failure, smoking	ULN: 51 U/L for men, 33 U/L for women [→118]	ULN: 0.85 µkat/L for men 0.55 µkat/L for women
Total Bilirubin (direct bilirubin+ indirect bilirubin)	Bilirubin circulates unconjugated (indirect bilirubin) and undergoes conjugation in the liver (direct bilirubin)	Direct > indirect: hepatocellular injury, cholestasis Indirect> direct: haemolysis, impairment in conjugation *e.g.* Gilbert's syndrome	Total bilirubin: <1.1 mg/dl [112]	<18.81 µmol

*Notes:
ULN values for ALT and other hepatic biochemical tests may vary among laboratories due to differences in reference populations and analytical variations among commercial assays.[→119].
The values in the table only pertain to adult study subjects.

cases of hepatocellular liver injury (high sensitivity), but they are not specific to DILI.[115] In addition, even large elevations of serum ALT do not always mean that there is a severe DILI event, as seen with heparin and tacrine.[114]

- Aminotransferases catalyze the transfer of the alpha-amino groups from aspartate or alanine to the alpha-keto group of ketoglutaric acid, forming oxaloacetic acid and pyruvic acid, respectively.[→120] AST and ALT are enzymes found throughout different tissues, but their

highest concentrations are in the liver. ALT is largely confined to the liver (hepatocytes), but it is also found in smaller amounts in skeletal and heart muscle. AST is localized in the liver (hepatocytes), heart, brain, kidney, red blood cells, and skeletal muscle.[113, →121]

- Although ALT is thought to be more specific for liver-injury than AST,[→122] neither test is specific for the diagnosis of DILI, or even liver-specific. ALT has little prognostic value since high levels in the serum only indicate probable liver damage that has already occurred.[114]
- Both tests can vary with host factors such as age, gender and body mass index (*BMI*), as well as dynamic factors such as meal intake and recent exercise. AST in particular varies with gender and ethnicity and can significantly increase in rhabdomyolysis and haemolysis.[113] ALT, too, may vary with ethnicity; ALT elevations are also seen in subjects with high alcohol intake and metabolic diseases including metabolic syndrome.[→123] Lastly, ALT levels can be abnormal in healthy subjects, whereas they may remain normal in patients with documented liver disease such as autoimmune hepatitis, nonalcoholic steatohepatitis (NASH) and hepatitis C.[→124]
- **Alkaline phosphatase (ALP)** refers to a group of enzymes that catalyze the hydrolysis of several organic phosphate esters at a neutral pH. Most ALP is found in the liver, bone, and intestine. The level varies by age; age-adjusted reference levels apply to children. ALP can be elevated in pregnant women as it derives from the placenta. Elevation in ALP occurs when the canalicular membrane is damaged such as in cholestasis, but also in conditions involving the bones, *e.g.* metastases.
- **Gamma glutamyl transferase (GGT)** is an enzyme that catalyzes the transfer of the gamma glutamyl group between peptides. It is predominantly present in the liver, but also in the prostate, kidneys, pancreas, intestine, and spleen. Serum GGT is an indicator of injury to biliary epithelium; however, due to its lack of specificity, it can also be elevated in other non-hepatic disorders such as obesity, diabetes, hyperthyroidism and renal failure, alcohol abuse as well as use of certain medications such as barbiturates and phenytoin (a marker of enzyme induction). Its main use is to confirm the hepatic origin of an elevated ALP, since GGT is not elevated in patients with conditions associated with osseous involvement (*e.g.* bone metastases).[115, 120]
- **Bilirubin**, derived from the breakdown of heme-containing products, serves as a diagnostic marker for liver function. **Total bilirubin (TBL)** is the sum of conjugated (direct) bilirubin and unconjugated (indirect) bilirubin.[→125] Liver dysfunction and pathologies can alter the metabolism of bilirubin; however, it only rises once there has been substantial loss of functioning hepatocytes clinically resulting in jaundice.[→126]

ALT standard and the upper limit of normal

Although many factors can influence ALT serum levels, the diagnosis of DILI is based on the presence of elevated liver tests relative to a reference range delimited by a lower and upper limit of normal (ULN). Several challenges have emerged in establishing a standard for ALT ULN:

- The value of ALT ULN has changed over time.[2] [→127-129]
- Different country-based ALT ULN values have been proposed accounting for factors known to modulate ALT activity,[→130] making it challenging to interpret ALT results in global clinical trials.
- Different laboratories may establish different normal ranges and ULN of ALT based on their tests of local populations that may include individuals with liver disease.[130, →131] To avoid the challenges posed by this inter-laboratory variation in global clinical trials, it is preferable to use a central laboratory repository with a unique reference range. This will standardize ALT results by multiples of the ULN.

The American College of Gastroenterology guidelines suggests that a healthy normal ALT level ranges from 29 to 33 U/L (0.48-0.55 µkat/L) for men and 19 to 25 U/L (0.32-0.42 µkat/L) for women among prospectively studied populations without identifiable risk factors for liver disease.[112] It has been proposed that lower cut-off values for ALT ULN may facilitate the detection of subjects with chronic liver disease. On the other hand, if more people in the general population are classified as having abnormally elevated ALT values, health care expenditures may increase and some patients may undergo unnecessary diagnostic procedures such as liver biopsy.[112, 130, →132–133] Questions around the value and practicality of using the "corrected normal ranges" for diagnosing and monitoring DILI are still not fully resolved.

[2] At first the reference range for ALT was based on the mean and standard deviation of an apparent "healthy" population possibly including patients with hepatitis C or metabolic diseases, and a level of 40 U/L was considered the ULN [124]. A study that included 6,835 blood donors with normal viral serologies and BMI under 24.9 kg/m^2, proposed an ULN of 30 U/L in men and 19 U/L in women [127]. Another study of 1,105 liver donors with normal liver biopsies proposed an ULN for ALT of 33 U/L for men and 25 U/L for women [128]. The maximum correct ALT ULN in the United States population calculated from the National Health and Nutrition Examination Survey (NHANES) database in the period between 1999-2002 and 2005-2008 in subjects without viral hepatitis, significant alcohol use, diabetes, BMI>25, or enlarged waist circumference was reported as 29 U/L for men and 22 U/L for women [112, 129].

2.2 Identifying and characterizing DILI in clinical trials

Summary

- The monitoring of serum ALT, AST, ALP and bilirubin plays a central role in the detection, assessment and management of DILI in clinical trials.
- In patients with known pre-existing liver disease, pre-treatment baseline values of each serum liver test result that is outside the "normal" reference range can be used for comparison to treatment-related changes associated with a study drug.
- Cases that fulfil "Hy's law" criteria in a study population have both prognostic as well as predictive value for drug-related hepatotoxic risk. Such cases are defined as hepatocellular injuries caused by the study drug with peak serum ALT or AST > 3 × ULN in conjunction with jaundice and/or increased levels of serum total bilirubin > 2 × ULN. In contrast to cholestatic forms of liver injury, cases consistent with "Hy's law" are marked by peak ALP levels <2 × ULN and/or R values > 5.
- In cases that conform to "Hy's law" there is a 10-50% risk for progression to acute liver failure (ALF) with an outcome of death or liver transplant. Importantly, the presence of even one or two such cases in a clinical trial programme points to an increased risk for idiosyncratic ALF in a similar post-market population treated with the same drug under equivalent conditions.
- In the case of idiosyncratic DILI associated with a specific drug, only a subset of patients are susceptible to progression to clinically serious liver injury, while many patients show adaptation marked by transient liver test abnormalities that are mild and asymptomatic.

Conclusions / recommendations

1. It is extremely important to obtain pre-treatment levels for serum AST, ALT, ALP and TBL if possible, to compare with on-treatment and post-treatment values.
2. Different stopping rules for dose reduction/ discontinuation should be applied in clinical trials for patients with and without known liver disease or abnormal baseline liver biochemistries.
3. In patients with chronic liver disease, incremental or fold increases of standard serum liver tests from pre-treatment baseline or on-treatment nadir levels should be used to detect and define DILI.
4. In addition to pre-treatment standard serum liver test abnormalities in special patient groups (such as those with chronic liver disease or cancer), the underlying condition and stage of disease are key factors that should be considered to establish criteria for study enrolment and the frequency and schedule of laboratory and clinical monitoring.

2.2.1 Inclusion and exclusion criteria for enrolment

Existing regulatory guidelines on assessing DILI [7, →134] generally pertain to patients in clinical trials with normal standard serum liver tests at baseline. A rationale to exclude patients with abnormal liver tests has been to both prevent their being adversely impacted by the drug, and to avoid background liver test abnormalities that may impede the identification and management of DILI in

study subjects. However, a "real-world" treatment population frequently includes patients with chronic liver disease, and some trials are conducted to evaluate new drugs intended specifically to treat pre-existing liver diseases.

The U.S. FDA considers that patients with stable liver disease should be included in at least some phase 3 trials for drugs that are likely to be used in such patients if they are approved. These patients may be included cautiously in late-stage clinical trials if bilirubin excretory and protein synthetic functions are intact as shown by diagnostic screening, or if there is a strong need for treatment.[7]

In the initial pivotal phase 3 studies of many drug development programmes of agents that are not primarily intended for the treatment of end-stage liver disease, it is often useful to exclude patients with defined advanced stages of cirrhosis, high MELD scores or evidence of hepatic decompensation. This approach is taken to mitigate against life-threatening outcomes and deteriorating liver function in a highly vulnerable subset of study subjects who develop DILI when exposed to a study drug.

Inclusion and exclusion criteria in clinical trial design, with detailed recommendations to detect, assess and manage DILI in patients with different underlying chronic liver diseases, have been proposed in peer-reviewed documents by some public-private initiatives.[→135-137] These documents offer a useful framework for the optimization of DILI risk assessment and management; however, they are not officially endorsed by the U.S. FDA or other governmental agencies with regulatory authority in drug development.

2.2.2 Baseline serum liver testing

In clinical trials, particularly those involving study populations with frequent and variable pre-treatment liver test abnormalities, baseline levels should be assessed at least twice (1-4 weeks apart) prior to treatment initiation.[→138] The first value is usually named the screening value.

Patients with chronic liver disease may have an elevated ALT (> 1.5 × ULN) at baseline, potentially affecting the interpretation of subsequent values. Patients with hepatitis B and C, alcoholic hepatitis, non-alcoholic steatohepatitis (NASH), or primary biliary cholangitis (PBC) may have baseline ALT levels exceeding 3 × ULN to 5 × ULN and/or have frequent fluctuating liver enzymes. Patients with underlying cholestatic diseases such as PBC and primary sclerosing cholangitis (PSC) typically have pronounced elevations of serum ALP. Moreover, a rise of total bilirubin levels is a hallmark of cholestatic disease progression.

In patients with underlying chronic liver disease and/or cirrhosis it is essential to measure indicators of liver function before enrolment into a study. These include but are not limited to the total and direct fractions of serum bilirubin, albumin, creatinine, platelets and INR, as well as MELD and Child-Pugh scores in case of severe liver disease. In addition, physical examination and imaging of the hepato-biliary system to assess the liver, biliary tree and portal vein may be necessary. For certain studies to determine effects of a study drug on disease progression, a baseline liver biopsy or portal pressure measurements may be warranted.

Patients with cancer may have abnormal baseline liver tests.[13, →139] due to hepatic metastases, prior treatments, or other hepatotoxic drugs. In a dataset of 3 998 patients enrolled in oncology trials, the prevalence of elevated baseline liver chemistry values (ALT, AST) of ≥3 × ULN was 5% or less among patients with liver metastases and less than 2% among patients without liver metastases. Baseline bilirubin ≥ 2 × ULN affected less than 1% of those with or without liver metastases. The cumulative incidence per 1 000 person-months of new-onset ALT elevations ≥ 3 × ULN was 6.1 in patients with liver metastases and 2.2 in patients without liver metastases.[139]

2.2.3 Routine monitoring of study subjects

The U.S. FDA guidance [7] recommends that for early trials of subjects with normal liver function liver enzymes (ALT, AST, ALP) and bilirubin should be monitored every 2 to 4 weeks for the first few months (*e.g.*, 3 months), and then every 2 to 3 months as long as no signs of liver injury are observed. Later trials can use less frequent liver chemistry monitoring if there is no indication of hepatotoxicity in earlier trials.[7]

Modifications of these recommendations for more frequent and/or prolonged monitoring should be considered when there is a concern that the study agent may be tied to a liability for clinically significant hepatotoxicity or when clinical studies are performed in study subjects with underlying chronic liver diseases or cirrhosis. Pragmatic, more detailed recommendations for suitable monitoring intervals, depending on data and evidence already available for compounds in development, have been published.[→140] As described in Section 2.2.1 above, a number of public-private initiatives have published best practices in clinical trials to treat different liver diseases.[135, 136, 137]

2.2.4 Interpretation of on-treatment results and triggers of increased monitoring

Current guidance recommends close observation and diagnostic workup for causes of hepatic injury other than the study drug when ALT increases to 3 × ULN during a clinical trial.[7] Most drug companies use this threshold. ALT has a higher specificity for liver injury and is the preferred measure, but AST can be used when ALT is unavailable.[→141]

In a real-world setting, a threshold of equal or greater than 5 ×ULN for close monitoring might be more suitable as it is more likely to exclude self-limited and clinically insignificant medication-related liver injury and non-alcoholic steatohepatitis (NASH).[20] Various studies have found that ALT values of 2-3 ×ULN are much more common than ALT values of more than 5 ×ULN.[20, →142–143][3] In special patient groups with elevated baseline values, finding an ALT > 3 × ULN may not mean that there is DILI.[20]

[3] In a study of patients with atrial fibrillation without liver disease who were assessed over two years, 6% to 8% of patients had ALT elevations of >2× ULN, while 1.4% of patients had ALT elevations of 5× ULN or more.[142] The prevalence of abnormal liver chemistries was assessed in a review of clinical trials that included more than 18 000 patients without liver disease. At baseline, the overall prevalence of ALT elevations of 3× ULN and 5× ULN was 0.08% and 0.01%, respectively.[20]

It is reasonable, then, to explore imbalances in ALT of greater than either 3 or 5 times baseline values between drug and comparator groups. This approach has been recently outlined by the IQ DILI Initiative group in patients with NASH.[135] Some drug makers have developed their own systems for identifying DILI during clinical trials; for example, they may adopt more stringent thresholds for phase 1 trials and less stringent ones for phase 2 and 3 trials.

In interpreting on-treatment values, patients exposed to a new drug with a liability to cause idiosyncratic DILI may fall into three categories: tolerators, adapters, and susceptibles.

- **Tolerators** are individuals who do not demonstrate abnormalities in liver tests while receiving the drug and do not develop any biochemical evidence of liver injury. AST or ALT values remain close to their baseline throughout treatment.
- **Susceptibles** are patients who develop overt liver injury while receiving the drug. They show progressive increase in aminotransferases that will continue while the patient is taking the drug. Patients may show fatigue, nausea or vomiting, and may have right upper quadrant pain or tenderness. Decreased liver function will be accompanied by jaundice, elevated direct bilirubin, or coagulopathy and may even progress to liver failure.[→144–145]
- **Adapters** exhibit transient elevations in serum AST or ALT that do not progress beyond the low-level state. Patients who adapt to a drug generally do not have symptoms of liver disease or decreased liver function, such as jaundice, elevated direct bilirubin, prolonged prothrombin time or increased international normalized ratio (INR). Transient elevated AST and ALT values may be quite common during the first few months of exposure to a new drug, and do not predict DILI. While mild and temporary elevations of liver enzymes may indicate mild liver injury or injury that spontaneously resolves, such changes are usually not clinically significant. The reasons for the phenomenon of adaptation have not been entirely elucidated.[144, →146] A good example of adaptation has been seen with tuberculosis chemotherapy, where 5.2% of 1927 study patients developed peak ALT elevations ≥3×ULN, but nevertheless 99% of patients completed their treatment course.[→147]

DILI may be considered **chronic** when liver enzymes do not return to normal or pre-treatment baseline values after the drug is withdrawn and/or signs and symptoms of liver disease persist six months after the onset of DILI.[20, →148] A recent analysis from the Spanish DILI network suggests that unresolved liver test abnormalities one year after DILI onset may be more relevant to define chronic DILI.[84]

2.2.5 Stopping rules

The U.S. FDA guidance recommends that discontinuation of treatment should be considered in pre-marketing clinical studies if any of the following occur:[7]

- 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); or

- 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 stopping rules should be adjusted for trials in patients with pre-existing liver disease and abnormal baseline liver tests.[→149] Modified stopping rules based on fold increases over baseline measures of different liver test indicators have been incorporated in recommendations published by public-private partnership initiatives.[149; 13, 135, 136, 137]

In patients with advanced liver disease or cirrhosis, worsening measures of hepatic function without substantial increases of ALT or AST above baseline values may signify a clinically serious or life-threatening DILI event. Stopping rules in these patients should also include defined increases above baseline levels in the total and direct fractions of serum bilirubin, INR, creatinine or MELD scores.

Patients with underlying liver disease may be prone to develop serious or life-threatening hepatotoxicity when exposed to agents that typically induce reversible cholestatic or mixed phenotypes of DILI in the absence of pre-existing hepatic abnormalities. These events should be carefully assessed and managed in the clinical trials of patients with underlying liver disease and/or cirrhosis.

Dose reduction may be an option to manage DILI attributed to certain drugs whose toxicity may be related to circulating blood levels. Dose reduction or a change in dosing regimen may also be deemed necessary in patients with progressing underlying liver disease in order to avoid toxic drug levels in liver or biliary epithelial cells and subsequent DILI.[136]

(See CHAPTER 6 for more information on the clinical management of DILI.)

2.2.6 Hy's law

In the clinical trial setting, potentially serious DILI may be detected based on identifying cases that conform to "Hy's law".[7, 138] Dr Hyman Zimmerman observed that a patient who shows evidence of jaundice in association with hepatocellular injury caused by a drug has a 10% to 50% chance of mortality (or liver transplant). This has been dubbed "Hy's law" [→150] and points to advanced and potentially severe DILI when other possible causes of liver injury have been excluded.

The FDA has translated Hy's law into the following criteria for individual cases.[7]

- ALT or AST ≥ 3 × ULN and TBL> 2 × ULN
- Without initial findings of cholestasis (elevated serum ALP ≥ 2 × ULN)
- After careful evaluation no other reason has been identified to explain the combination of increased ALT or AST and TBL elevations (such as viral hepatitis, alcohol ingestion, congestive heart failure)

Establishing threshold liver test criteria that define Hy's law cases is sometimes challenging. Using multiples of the ULN for ALT and AST levels can present difficulties in interpretation, because firstly,

different values of ULN are used in different laboratories and reference populations; secondly, initial serum samples may only be acquired after the onset of DILI, thirdly, ancillary tests such as fractionated bilirubin are needed to rule out other conditions, and fourthly, whether cases of drug-induced hepatocellular injury that have an increased risk for progression to acute liver failure must necessarily conform to all the biochemical criteria for Hy's law as listed above has been a subject of recent study. Notably, a few post-market cohort studies have demonstrated that a modified Hy's law definition using a "new R" value > 5 (see 1.1.2) without an absolute upper ALP limit was more sensitive in predicting drug-induced acute liver failure [37], and had a higher positive predictive value for the outcomes of liver transplant or death in patients with acute liver failure [43], compared with criteria that set an ALP limit of < 2 ×ULN. Finally, it may also prove useful to watch out for hypoalbuminaemia or coagulopathy (*i.e.* increased INR), which may be signs that the liver damage has negatively affected hepatocellular synthetic function, as was noted with fialuridine hepatotoxicity.[112]

2.3 DILI case evaluation and minimum required data in clinical trials

Summary

- DILI in clinical trials is often detected by regular monitoring of standard serum liver test results, whereas in the post-marketing setting it typically presents with acute hepatitis or a cholestatic-like syndrome with variable clinical severity.
- Time to onset is quite variable but most instances of DILI occur within 6 months from drug initiation and less frequently with a delay after treatment interruption.
- A thorough clinical, laboratory and imaging assessment is crucial for DILI case ascertainment to help exclude other more common causes of liver injury.

Conclusions / recommendations

1. The time to DILI onset with medication start and stop dates as well as dosing should be thoroughly investigated.
2. Aminotransferases, bilirubin (total and conjugated) andalkaline phosphatases should be determined at screening and baseline and then followed through serial testing over the course of the liver injury once it has been detected and until recovery. When ALP is elevated, GGT should be measured in order to establish the source of the elevation.
3. Exclusion of alternative causes of liver injury is paramount for case ascertainment and should always include viral serology, autoantibodies and liver imaging techniques.
4. A list of minimal required data acquisition should be included in clinical study protocols to enable full assessment of all cases of new-onset or worsening liver injury in a standardized manner. A case report form (CRF) is proposed in **Appendix 2**. Thresholds for use of the CRF should be defined in the study protocol based on the patient population, indication, and other factors as appropriate.

2.3.1 Overview

The diagnosis of DILI relies largely on excluding other possible causes of liver injury. An overview is given in **Table 7.** A protocol for data collection and assessment of potential DILI cases in the paediatric population has been proposed.[→151]

2.3.2 Clinical history

The clinical spectrum of DILI is very broad both in phenotype and severity. In the clinical drug development setting, particular attention should be paid to the silent injury detected by routine serum liver test monitoring. The majority of cases in clinical practice present with an acute viral hepatitis-like syndrome, with or without non-specific symptoms,[71] except in a fraction of instances in which a rash or other cutaneous manifestations reinforce the suspicion of drug toxicity.[→152]

- **Time to onset, course of reaction and time to resolution** are important data required to establish a compatible temporal relationship with the suspected causative agent. The time to onset (or latency) of DILI is typically measured from the first day on which the suspected agent was taken to the day of onset of symptoms, jaundice, or laboratory test abnormalities, whichever is first.[11] Time to onset varies considerably, yet a large proportion of patients experience DILI within the first 6 months of therapy.[→153] Course and time to resolution should be scrutinized through serial aminotransferase measurements.
- **Clinical symptoms** can be useful to identify clinicopathological phenotype(s) typically associated with the study drug ("drug signatures"), establish alternative causes, and predict outcome.
- **Other drugs, supplements, alcohol:** A careful inquiry on prescription medication, herbal and dietary supplements (often not revealed) as well as over-the-counter drugs (*e.g.* **paracetamol**) exposure – recording start and stop dates – is paramount.[18] Alcohol intake should also be recorded, although no evidence exists that alcohol consumption is a risk factor for most drugs implicated in idiosyncratic DILI.[23]

Table 7. Diagnostic work-up to exclude alternative etiologies of DILI

The steps shown below pertain mainly to adult patients. A protocol for data collection and assessment of potential DILI cases in the paediatric population has been proposed.[151]

Causes of liver injury to exclude	Complementary parameter / test	Comments
1st round		
Hepatitis A virus (HAV)	HAV IgM	
Acute hepatitis B	HBsAg Anti-HBc IgM Anti-HBs	
Acute hepatitis C	Anti-HCV HCV RNA	
Acute hepatitis E	HEV IgM HEV RNA	An emerging cause of viral hepatitis in Western countries
Biliary obstruction, focal lesions, vascular liver disease	Ultrasound, MRI, CT	
Autoimmune hepatitis	Anti-nuclear autoantibodies, anti- smooth muscle autoantibodies and serum IgG levels.	Liver biopsy frequently required
Alcoholic hepatitis	History of alcohol abuse, Ratio of AST:ALT > 2, GGT, MCV Serum PEth levels	ALT values usually less than 300 U/L
Sepsis	Blood culture, data of hypotension, fever	
Ischaemic hepatitis	Serum AST and ALT > 500 U/L ultrasound or MRI	History of severe hypotension, congestive heart failure
2nd round		
CMV acute infection	CMV IgM (CMV-DNA)	
EBV acute infection	EBV IgM (EBV-DNA)	
HSV acute infection	HSV IgM (HSV-DNA)	
Acute hepatitis B, flare-up or reactivation of chronic hepatitis B	HBV-DNA	
Sinusoidal obstruction syndrome	Liver biopsy	
Primary biliary cholangitis	Anti-mitochondrial autoantibodies	
Primary sclerosing cholangitis	MRI, ERCP, anti- nuclear autoantibodies, perinuclear anti-neutrophil cytoplasmic antibodies	
Wilson disease	Ceruloplasmin	In acute phase may be normal. Search for: 24 h urine copper, Kayser-Fleischer rings
Haemochromatosis, alpha-1-antitrypsin deficiency	Ferritin, transferrin saturation	Anicteric persistent hepatocellular injury
Hepatic steaosis	Ultrasound, CTor MRI	Metabolic syndrome, diabetes and elevated BMI frequently present

Anti-HBc=Hepatitis B core antibody, **Anti-HBs**=Hepatitis B surface antibody, **Anti-HCV**=Hepatitis C virus antibody, **CMV**=cytomegalovirus, **CT**=computed tomography, **DNA**=deoxyribonucleic acid, **EBV**=Epstein-Barr virus, **ERCP**=endoscopic retrograde cholangiopancreatography, **HBsAg**=Hepatitis B surface antigen, **HBV**=Hepatitis B virus, **HCV**=Hepatitis C virus, **HEV**=Hepatitis E virus, **IgM**=Immunoglobulin M, **MCV**=mean corpuscular volume, **MRI**=magnetic resonance imaging, **PEth**= phosphatidylethanol, **RNA**=ribonucleic acid

2.3.3 Minimal laboratory evaluation

The first blood analysis after DILI recognition is generally the basis for establishing DILI onset and liver injury type (*i.e.* R value). However, it is important to note that the first abnormal liver profile should be interpreted with caution, as it may not represent the true time of liver injury onset. The liver injury may already be advanced or even subsiding when first identified in blood analysis. Serial liver biochemistry analyses are therefore necessary to clarify the stage of liver injury at the time of detection.[→154] Identification of clinical symptoms prior to biochemical testing can also aid in establishing liver injury onset.

Isolated hyperbilirubinaemia does not qualify as DILI.[20] Nevertheless, bilirubin is important in the context of DILI as an indicator of severity in combination with serum aminotransferases. In addition to total bilirubin levels, fractionation (direct/indirect) of bilirubin is strongly recommended to help identify cases of indirect hyperbilirubinaemia due to haemolysis and Gilbert's syndrome that may be present in 5-10% of the general population.[→155]

Testing for creatine phosphokinase (CPK) can help to distinguish between liver- and muscle-derived ALT elevations. This should be considered particularly in cases with a disproportionate increase in AST compared to ALT, as AST tends to be less liver-specific than ALT. Furthermore, elevated lactate dehydrogenase (LDH) accompanying ALT increases can differentiate between ischaemic injury and acetaminophen (paracetamol) hepatotoxicity.[18]

ALP is similarly not organ-specific and can increase during bone pathologies, but it is a good marker of cholestatic damage when accompanied by elevated gamma-glutamyl transferase (GGT). Isolated GGT elevations, however, are not specific to liver injury and consequently not to DILI.

Initial DILI assessment should also include coagulation parameters such as INR and serum albumin.

Liver biochemistry should be routinely tested in patients with DILI until complete normalization. A steady decline of aminotransferases supports the diagnosis, whereas slow or incomplete resolution of biochemical abnormalities suggests competing etiologies [18] although it may occasionally reflect a chronic DILI outcome.[84]

2.3.4 Viral serology

First round: Hepatitis A, B, C, E

The suspicion of DILI demands an extensive diagnostic workup to rule out alternative causes of liver damage. Age, comorbidities and subject's risk behaviour for acquisition of viral hepatitis, as well as the local burden of infectious diseases potentially affecting the liver can also help in guiding the diagnostic workup. In addition, the pattern of injury can aid in the diagnostic approach; except for pure cholestatic pattern viral hepatitis is an obvious cause of exclusion. Hence, hepatitis A (IgM anti-HAV), hepatitis B (IgM anti-Hbc, HBsAg) and hepatitis C (anti-HCV) should be routinely tested. Hepatitis B virus DNA should also be tested in patients who are known to be carriers of HBsAg in order to rule out chronic hepatitis B virus reactivation as the cause of liver injury. In Western countries hepatitis E is an emerging cause of viral hepatitis and can be a masquerader of DILI.[→156–157] Anti-HEV IgM seroprevalence in suspected DILI cases has ranged from 3% to 8% in DILI Registries.[157, →158] However, anti-HEV IgM as a diagnostic test for active HEV infection has been questioned because of poor sensitivity and specificity.[→159] In fact, the definitive proof of HEV infection is the detection of HEV-RNA in blood or faeces. In Japan, anti-HEV IgA alone or together with anti-HEV IgM has been found to be more specific with a longer duration of positivity than RNA.[→160–161]

Acute hepatitis C is a challenging competing cause in suspected DILI since anti-HCV, usually tested to screen for HCV infection, can be initially negative. In fact, in 1.3% of adjudicated DILI cases in the DILIN Registry HCV-RNA tested positive in the first analysis of the DILIN cohort.[74] Hence, to maximize the probability of identifying acute hepatitis C cases during DILI assessment, routine testing of HCV infection with anti-HCV antibodies should be complemented by HCV-RNA in hepatitis-like (hepatocellular) suspected DILI cases at presentation.

Second round: EBV, CMV, HSV

Rare causes of viral hepatitis are cytomegalovirus (CMV), Epstein-Barr virus (EBV) and herpes simplex virus (HSV) infection, which should be ruled out if there are associated manifestations such as rash, lymphadenopathy and atypical lymphocytes. Although hepatitis due to CMV and EBV is thought to be rare, a recent study from Iceland showed a mean annual incidence of 4.0 cases per 100 000 inhabitants for CMV and 7.8 cases per 100 000 inhabitants for EBV.[→162] Sera of patients with serological evidence of active, quiescent or resolved HBV infection should be assayed for HBV-DNA to rule out the new onset or reactivation of hepatitis B.

2.3.5 Serum autoantibodies (ANA, ASMA, IgG)

In the assessment of a suspected acute hepatocellular DILI episode, screening for antinuclear antibodies (ANA), anti-smooth muscle autoantibodies (ASMA) and serum IgG is mandatory to exclude autoimmune hepatitis (AIH). However, several drugs including nitrofurantoin, minocycline, anti-TNF-α and statins can [62, →163–164] induce DILI with typical laboratory and pathological features of AIH. In cholestatic anicteric cases the appropriate exclusion of primary biliary cholangitis

requires anti-mitochondrial antibody (AMA) testing and in AMA-negative cases ANA sp100/gp 210 testing.[→165]

2.3.6 Case report form

The diagnostic appraisal of suspected DILI cases is strongly dependent on patient data and routine laboratory and imaging tests (see Section 4.3.3 for more information about imaging).

Accurate and complete data ascertainment is a crucial aspect of DILI assessment both in drug development and post-marketing patient care. In drug development, instructions about required data acquisition (taking into account study design and enrolment criteria for the individual study) should be included in the clinical study protocols to enable a full assessment of all cases of new onset or worsening liver injury.[138]

A suggested case report form for suspected DILI cases is shown in **Appendix 2** that covers the following areas.

- Liver-related signs or symptoms
- Medical history of liver-related diseases
- Risk factors for conditions associated with liver disease
- Liver imaging studies
- Liver biopsy
- Family history
- Local laboratory tests
- Serology tests
- Concomitant medications and dietary/nutritional supplements

The implementation of clear instructions for required data elements will:

1) decrease the risk of serious outcomes in individual study subjects with DILI based on prognostic considerations (*e.g.* study drug discontinuation in a timely manner, avoidance of re-challenge, performing time-sensitive diagnostic tests and therapeutic interventions);

2) provide a sound basis to predict the risk for clinically serious DILI in post-marketing treatment populations; and

3) open up new opportunities for DILI research across clinical trials once uniform practices in data collection are adopted.[138]

2.4 Causality assessment

Summary

- Formal causality assessment in DILI strongly relies on a high degree of suspicion by physicians, a complete pharmacological history, and exclusion of alternative causes.
- Structured clinical scales, although far from perfect, provide a framework for a more objective evaluation in DILI causality assessment.
- The Roussel Uclaf Causality Assessment Method (RUCAM) (also known as the "CIOMS scale") is an objective instrument with standardized data fields that is frequently used in post-market case assessment.

Conclusions / recommendations

1. A liver-specific causality assessment instrument rather than a generic instrument should be used for the assessment of hepatic adverse events; however, there is no instrument currently available for widespread use which takes into account all the clinicopathological signatures and severity levels of DILI.
2. Expert opinion in an academic environment is frequently used to assess reports of suspected DILI cases in a series.
3. In clinical trials DILI causality assessment is best made by independent expert opinion from trained hepatologists or clinicians who are skilled in the diagnosis of liver diseases and DILI.
4. In the post-marketing setting the RUCAM scale is frequently used for assessing causality in suspected DILI, since it offers a reasonable balance between the demands for scientific objectivity and the necessity of having a simple enough method for practical use.

2.4.1 Introduction

Prompt recognition of DILI and withdrawal of the causative agent are important in order to decrease the risk of progression to acute liver failure or chronic liver injury.[→166–167] In the post-marketing setting, a correct DILI diagnosis is important in order to avoid inadvertent re-exposure to the causative agent and a second episode, which in some cases can be more severe. On the other hand it is important to avoid unnecessary drug withdrawals that could have serious consequences when the indication of the medication is strong.

Causality assessment in clinical practice requires detailed history-taking and extensive laboratory assessment. The DILI diagnosis is usually made once a retrospective review of the patient information pertaining to the whole episode is performed. The final diagnosis is dependent on:

1) establishment of a compatible temporal sequence between drug intake/cessation and liver biochemistry alterations;

2) exclusion of alternative causes;

3) clinical and pathological features associated with hepatotoxicity induced by a specific causative agent (phenotype); and

4) DILI incidence associated with the suspected causative agent.[166, →168]

Evaluation of potential DILI cases in clinical practice is not a homogeneous process, as the available information may vary from case to case depending on differences in clinical approaches and laboratory test facilities. The importance of a complete history should be emphasized, as missing information could lead to incorrect evaluations.

It is recommended that DILI causality assessment during clinical trials be performed using expert (trained hepatologists') opinions, preferably blinded to treatment assignment. Furthermore, independent assessments prior to seeking consensus tend to lead to more reliable results. Careful monitoring and meticulous data collection are likewise paramount for DILI causality assessment during drug development, although information on drug signature and DILI incidence rates (points 3 and 4 above) are rarely available in early clinical trials.[168]

A number of causality assessment scales have been proposed to weigh the available evidence for or against the possibility of an adverse drug reaction.[22, →169-172] The aim of such scales is to provide a user-friendly diagnostic tool that facilitates better reproducibility between evaluators owing to reduced subjective judgments. The number of criteria in an assessment scale must be considered in terms of medical importance, but also with regard to clinical applicability. A scale including a large number of areas/questions is less likely to be useful in a busy clinical environment. The validity of causality assessment scales strongly depends on the weight given to the criteria included in the specific scale and will be reduced if incorrect weights have been allocated. Non-organ specific causality assessment scales have proven to be less reliable for DILI than liver-specific scales.[→173–174]

2.4.2 The RUCAM scale

The Roussel Uclaf Causality Assessment Method (RUCAM)—also referred to as the "CIOMS scale" is the most commonly used post-marketing DILI causality assessment scale based on published literature.[→175–176] It is often used in a post-market setting and sometimes used to support regulatory actions for regarding marketed medications with a hepatotoxic profile.[→177]

The RUCAM scale assigns points for seven different domains that have been tied to an assessment of a potential DILI case. A tally of these points yields an overall assessment score that reflects the likelihood that the hepatic injury is due to a specific medication. The seven domains are: (a) time to onset; (b) time course of liver injury; (c) risk factors (age, alcohol use and pregnancy); (d) concomitant drugs that may be hepatotoxic; (e) exclusion of alternative non-drug causes of liver injury; (f) prior information whether the suspect drug is potentially hepatotoxic; and (g) the development of repeat liver injury after drug re-administration. The overall assessment score, based on the points obtained in each domain, may indicate the plausibility of DILI as excluded (<1 point), unlikely (1-2 points), possible (3-5 points), probable (6-8 points) or highly probable (>8 points).[22]

The RUCAM was primarily developed to provide a more objective DILI diagnostic tool. It was initially validated with an analysis of a gathered set of post-marketing cases of acute liver injury [181]; The domains of the RUCAM described above encompass a valuable checklist for information that supports a diagnosis of idiosyncratic DILI; nonetheless evaluation of scoring practices with this instrument has demonstrated considerable inter- and intraobserver variability.[22, →178–179] This could be the result of ambiguity associated with the interpretation of some of the criteria for scoring that impact the assignment of points in the process of case assessment. In an attempt to address this problem a Manual of Operations for the RUCAM scale has been developed and made available on the LiverTox® website.[→180] Notably, the RUCAM was not devised to assess cases that progress to severe or fatal outcomes after discontinuation of the suspect drug. Moreover, the algorithm imposes criteria for time to onset of liver injury and time to resolution after drug discontinuation that do not accommodate the clinicopathological phenotypes ("drug signatures", see also page 36) associated with certain drugs.

Use in clinical drug development

The RUCAM scale was developed and validated in the post-marketing setting.[→181] It has several limitations in the clinical trials setting with new drugs:[168]

- The RUCAM scale awards points to drugs with previous information on hepatotoxicity potential, specifically those with hepatotoxicity information included in the drug label. Such information is rarely, if ever, available in early clinical trials.
- The RUCAM scale awards points for potential risk factors such as pregnancy and excessive alcohol consumption. However, patients in these categories are often excluded from clinical trials. Moreover, risk factors that may be specific for certain new drugs or biological agents in development, including drug-drug or disease-drug interactions, or genetic markers that signify heightened susceptibility to DILI, are not accounted for in the RUCAM.
- One of the criteria included in the RUCAM scale is the response to re-administration of a suspected causative drug (re-challenge). While re-challenge can be justified in some situations in clinical practice, it is not recommended in most clinical trial settings.[→182]

These limitations were identified after the use of RUCAM in a large cohort of patients treated with ximelagatran in phase II and III clinical trials. However, the authors did acknowledge that use of the method could provide a framework to minimize the level of subjectivity in a causality assessment of DILI with this agent.[→183]

Use in the post-marketing setting

In the post-marketing setting with certain clinical signatures of suspected idiosyncratic DILI the RUCAM scale offers a reasonable balance between the demands for scientific objectivity and the necessity of having a simple enough method for practical use. Nevertheless, when used alone the RUCAM has significant limitations in establishing a DILI diagnosis.[166, →184–185] The scale has three main disadvantages:

- The RUCAM can be cumbersome to use in cases where the patient has been treated with several medications concurrently, since it evaluates the likelihood of causal association with only a single agent. In such situations, the RUCAM scale needs to be applied to each drug individually, which often leads to the same final score for each of the potentially hepatotoxic drugs.
- New drugs and biological agents without adequate post-market exposure to determine whether they have a hepatotoxic profile by default score fewer points. Similarly, herbal and dietary supplements, which do not undergo the same rigorous premarketing safety assessment as conventional drugs in most jurisdictions, do not have a product label outlining their characteristics in detail to prompt recognition of DILI when it occurs.
- Incomplete case information and atypical presentations of DILI can also reduce the total RUCAM score, underestimating the likelihood that the suspect drug has caused the liver injury.

As a rule, the weightings assigned to each DILI criterion incorporated into a causality assessment algorithm have an impact on its reliability. The scoring system of the RUCAM, when it was devised almost 30 years ago, was by and large based on expert opinion. As our understanding of DILI has evolved with more recently acquired scientific information, an update of the domains and their scores is necessary. One current initiative to take on this challenge has been undertaken by the U.S. DILI Network (DILIN) together with members of an international working group. The aim of this initiative is to provide an easier-to-use clinical and research RUCAM tool with clear operation instructions on a computerized application platform.

The use of the RUCAM scale is illustrated in a case narrative showing the causality assessment process in the post-marketing environment (Appendix 3).

2.4.3 Expert opinion

Expert opinion relies on professional judgment on causality after considering all available and relevant data concerning an individual case.

As the manifestations of DILI can vary considerably, and there are currently no biomarkers that can verify its presence, attribution of causality to a specific medication is challenging and different experts may come to different conclusions on a specific case. Experts in the context of DILI should not only understand the concept of DILI but must also have experience in clinical liver injury in general. This improves the probability of a correct verdict after appropriate exclusions of alternative causes with similar manifestations.

Expert opinion is the method most widely used to assess causality when a significant DILI signal appears during drug development. The key advantages of this approach over RUCAM and other scoring algorithms are that experts may (1) have insights into the differential diagnosis of liver injuries that occur in study subjects, (2) take into account different or unusual DILI phenotypes and pathological mechanisms in their analysis; and (3) weigh and synthesize relevant pre-clinical, treatment population and individual case-level data to provide a full picture of risk assessment. Often

this exercise can be leveraged into a comprehensive report by drug developers for review by regulatory agencies.

Both participants in the U.S. DILI Network (DILIN) [179, →186] and U.S. FDA scientists [46, →187] have employed a detailed and standardized expert opinion method that employs a categorical scale of likelihood for causality assessment. Here each potential DILI case is assigned a panel of independent hepatologists or clinical experts for review to determine the likelihood of causal association with a suspect or study drug. Each reviewer scores the case as a whole, and each implicated agent, as *definite* (> 95% likelihood: evidence for DILI beyond a reasonable doubt), *highly likely* (75%-95% likelihood: clear and convincing, but no definite evidence), *probable* (50%-74% likelihood: evidence supports drug-liver injury link), *possible* (25%-49% likelihood: equivocal but present evidence) or *unlikely* (<25% likelihood: evidence of non-drug cause). The scores are then forwarded to one or more additional hepatologists and discussed in order to reach a consensus by email or teleconference or, if still unresolved, by majority vote.[179, 186] These are not exact determinations but best estimates based on the skill and experience of the rater and quality of the information provided.[→188]

Spontaneous reports of post-marketing cases may not include all the data needed for the detailed differentiation between the grades of *probably related, highly likely related* and *definitely related*. In such circumstances it may be possible to use, instead of the 5-category scale, a 3-category scale including *unlikely related* (0<25%), *possibly related* (25-49%), and *probably related* (> 50%).[168]

The DILIN expert opinion method has the advantage of taking into consideration clinical manifestations and mechanisms of liver injury associated with specific drugs, *i.e.* “drug signatures”, rather than general DILI manifestations. For example, a DILI case that is marked by a very long latency to onset after initiation of the suspect drug with a variant clinicopathological phenotype will score fewer points on the RUCAM scale than is warranted, but an expert may recognize this atypical feature as an expected characteristic of DILI. Furthermore, hepatologists with a research interest in DILI are most likely to be up-to-date with the latest findings in the area, and a consensus diagnosis has a higher chance of being correct as it is based on collective wisdom and experience.

Nevertheless, a consensus does not necessarily equal truth.[186] In addition, the expert opinion method can be cumbersome and time-consuming depending on the number of experts included and the various procedures involved. It can also be challenging to use for small research groups or in certain clinical environments with limited resources. Causality assessment to clinically evaluate and manage a study subject with liver injury in real time need not depend on a structured expert opinion method. Such a method is especially valuable at stages of interim or final clinical trial data analysis (see Section 3.2)

2.5 Liver biopsy for assessment of DILI diagnosis and prognosis

Summary

- DILI does not have a singular or specific histopathological correlate
- The liver biopsy from a DILI patient may show a wide variety of histological findings and help differentiate between DILI and a pre-existing chronic liver disease.
- When more than one drug is suspected as a possible cause, histopathological analysis can occasionally help define which drug is more likely to have caused the injury.
- A liver biopsy can also help in predicting the outcome of the injury and determining the prognosis of the patient.

Conclusions / recommendations

1. A liver biopsy is usually not required for evaluation of a patient with suspected DILI, but when performed, it can provide important information on the pattern of injury and its severity.
2. Favourable prognostic factors in liver biopsies from DILI patients include the presence of hepatic eosinophils and granulomas, while patients with necrosis, fibrosis and intrahepatic bile duct loss have a poorer prognosis.
1. A liver biopsy may provide useful information on the mechanism of liver injury (as has been seen *e.g.* with fialuridine), while assessing possible underlying diseases.

A liver biopsy is usually not required for evaluation of a patient with suspected DILI, but when performed, it can provide important and useful information on the pattern of injury and its severity.[→189]

In the U.S. Drug-Induced Liver Injury Network (DILIN), about 50% of patients enrolled in the prospective protocol underwent liver biopsy during the course of their evaluation.[74] Findings from this study and others have shown that DILI does not have a specific histopathological picture, and the biopsy from a DILI patient may show a wide variety of histologic findings, including inflammation, necrosis, cholestasis, fibrosis, nodular regeneration, vascular injury and bile duct destruction.[189, →190] Therefore, the diagnosis of DILI typically cannot be made based on liver histology alone.

Nevertheless, when a liver biopsy is performed, it may occasionally assist in identifying another underlying liver disease (such as alcoholic hepatitis, Wilson's disease, neoplastic infiltration, miliary tuberculosis or congestive hepatopathy), which may have been overlooked and mistaken for DILI. In certain cases, liver histology may assist in the differentiation between DILI and sporadic autoimmune hepatitis.[→191]

When more than one drug is suspected as possible causal agent, histopathology can occasionally help define which drug is more likely to have caused the injury, since some drugs may exhibit typical (although often not diagnostic) histopathological features.

During early drug development, there is often minimal or no data on the possible histological pattern of liver injury related to the investigational drug, which may make interpretation of histological findings more difficult. Given the high prevalence of chronic liver diseases such as viral hepatitis, nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD), it is common for patients with suspected DILI to have a known (or suspected) underlying liver disease. A liver biopsy may reveal findings that are inconsistent with the underlying liver disease and therefore supportive of a diagnosis of DILI,[189] and provide useful information on the mechanism of liver injury. This is illustrated in the case study on acute fatty liver and lactic acidosis caused by fialuridine (FIAU).[→192–193]

If the liver tests normalize during follow-up, a liver biopsy is usually not required. In rare instances, a liver biopsy can help in predicting the outcome of the injury and assessing the prognosis of the patient. In a recent study by the U.S. DILIN, the most predictive factor of poor outcome in patients with cholestatic DILI was the degree of bile duct loss on liver biopsy.[38]

The following are possible reasons or circumstances that may support performing a liver biopsy in suspected DILI during drug development:[189]

1) Slow and incomplete resolution of liver tests after stopping the implicated agent;

2) investigation of alternative competing causes (*e.g.* autoimmune hepatitis, sepsis, graft-versus-host disease) in certain cases with negative serologies;

3) assessment of the severity of injury to enable clinical decision and risk analysis;

4) known underlying liver disease;

5) experimental agent for which there is little prior information regarding liver injury; and

6) multiple candidates as the causal agents.

7) Provide insight into the mechanism of DILI

CHAPTER 3.

EVALUATING DILI RISK IN DRUG DEVELOPMENT

3.1 Role of pre-clinical assays

Summary

- Potential mechanisms of DILI that can be assessed *in vitro* include the formation of reactive metabolites, as well as inhibition of liver cell membrane transport proteins or metabolizing enzymes, toxicity to mitochondria and oxidative cell stress related to the drug or its metabolites.
- While metabolite formation, enzyme and transporter inhibition are measured routinely during drug development, an *in vitro* assessment of study drug toxicity of cultured cells is typically performed when a DILI liability has been suspected based on preclinical or clinical findings.
- Toxicity testing in animal species can yield important insights into the potential DILI risk that a compound may confer. However, no single test system is currently available which reliably predicts liability of the study drug in man.
- While typical flags such as the formation of reactive metabolites with covalent protein adducts may identify a potential DILI risk, the absence of such signals does not completely exclude the possibility that the compound has a DILI risk.

Conclusions / recommendations

1. *In vitro* cell systems that reliably predict idiosyncratic immune responses to a study drug leading to DILI should remain an ongoing focus for future research.
2. Studies of bioengineered human liver chip models may yield a valuable screening tool for drugs in development to assess whether they have a liability for DILI.

In general, the safety of a drug is typically assessed in two pre-clinical animal species before the first dose is given in humans. There has been much debate as to whether results obtained in animal species can be translated to the human situation. Preclinical models are of limited value for predicting the potential for human DILI.[35] While animal toxicity studies can identify compounds with intrinsic toxicity, standard toxicology studies generally do not identify drugs that produce idiosyncratic DILI.

Drug class effects should be taken into account when predicting the risk of DILI. The observation that the daily dose (*i.e.* a dose of ≥ 100 mg/day) as well as the lipophilicity of a drug (cLogP ≥ 3) are risk factors for DILI has been termed the "Rule of 2".[→194]

DILI is the result of a complex interplay of metabolic processes, cell damage and the host's response to these events. Metabolic processes include uptake of the drug into hepatocytes from the portal blood stream, metabolism within hepatocytes in phase I and phase II reactions, and subsequent elimination of drug metabolites back into sinusoidal blood (for subsequent renal elimination) or into bile. These processes are governed by an array of transport proteins at the basolateral and canalicular membrane of the hepatocyte as well as by intracellular enzymes.

Various attempts have been made to simulate these processes of drug metabolism *in vitro* in appropriate hepatocyte cell systems.[→195–196] Human cell systems that have been used for studying drug transport and metabolism include hepatocyte-derived cells such as HepaRG, HepG2, Huh7, hepatocyte-like cells (iHLCs) derived from induced pluripotent stem cells (iPS), primary human hepatocytes (PHH) in 2D monolayer cultures, as well as more long-term stable hepatic culture systems such as 2D sandwich cultures and 3D spheroid cultures of primary human hepatocytes.[→197] Microfluidic devices consisting of hepatocytes, multiple liver cell types or liver-derived cell lines show improved viability and preserved drug-metabolizing enzyme activity and membrane transporter function over several days.[→198] These cell systems can reproduce intrinsic mechanisms of toxicity (**Table 8**).

Table 8. Assessment of DILI mechanisms in preclinical models

DILI mechanism	Examples of implicated drugs	Representative measure	Remarks
Formation of reactive metabolites (a), including acyl glucuronides (b)	Drugs containing a carboxylic acid moiety (c), *e.g.* diclofenac, fasiglifam (TAK-875)	Detection of adducts (qualitative); covalent binding of radiolabeled test drugs to liver proteins (quantitative)	No clear link established between acyl glucuronide formation and DILI (d) No single assay adequately predicts DILI risk. A stepwise approach is recommended (e)
Inhibition of transport proteins or metabolizing enzymes, including inhibition of the bile salt efflux pump (f)	Bosentan, troglitazone, ketoconazole, tolcapone; oral contraceptives; erythromycin estolate	Uptake of a probe substrate into membrane vesicles or sandwich configuration hepatocytes *in vitro*	
Toxicity to mitochondria (g)	Fialuridine, amiodarone, valproate, tetracycline, antiviral nucleoside analogues	Depletion of cellular ATP content In living cells: oxygen consumption rate	.
Oxidative cell stress related to the drug or its metabolites	Anti-tuberculosis drugs, amoxicillin-clavulanate	Immunostaining for lipid peroxidation products (*e.g.* hydroxy-nonenal)	
Loss of immune lymphocyte tolerance	Amodiaquine	Hepatotoxicity markers in a mouse model	Blockage of checkpoint molecules. Unmasks immune-mediated DILI (h)

(a) Reactive metabolites can covalently bind to cellular proteins, and the protein adducts may form immunogenic haptens that trigger a downstream immune response.[196]

(b) The U.S. FDA has identified acyl glucuronides as metabolites of concern.[→199–200]

(c) Carboxylic acid moieties can be metabolized by hepatic and extrahepatic uridine glucuronyl transferases (UGTs) to form acyl glucuronides, both in man and in animal species.

(d) Diclofenac and fasiglifam also have other potentially DILI-inducing mechanisms,[→201–202] suggesting that the cause of DILI may be multi-factorial. On the other hand, dabigatran produces acyl glucuronides but has not been associated with DILI.[→203] Interestingly, dabigatran is not metabolized by CYP450 isoenzymes and does not form oxidative metabolites.

(e) Initially, *in vitro* metabolism of the experimental compound should be conducted using an appropriate test system (*e.g.* liver microsomes, hepatocyte cultures) to evaluate the formation rate of acyl glucuronides in humans and animal toxicology species. If formation of acyl glucuronides is identified, the compound may have a liability for hepatotoxicity that places a burden on further clinical development. Decisions can then be taken depending on the outcome:

- Should the compound form acyl glucuronides, but at levels in humans that are similar to at least one animal toxicology species, it can be assumed that the metabolite's contribution to the overall toxicity assessment has been established.
- If the acyl glucuronide is estimated to be an abundant pathway in humans (accounting for ≥ 10% of metabolism) and is present at disproportionately higher levels in humans than in any of the animal test species, the U.S. FDA Guidance on Safety Testing of Drug Metabolites recommends that the metabolite should be synthesized and tested for chemical reactivity.[199] *In vitro* assays have been developed to classify the risk for covalent binding based on the kinetic rate of intramolecular acyl migration, which could lead to ring opening and covalent protein binding.[200]

The results of both *in vitro* and *in vivo* metabolism studies should be used when selecting the appropriate animal species for toxicology studies.

(f) The bile salt efflux pump (BSEP, ABCB11 gene) is the key efflux transporter for intracellular bile acids into bile. Inhibition of BSEP by a drug or its metabolites is hypothetically one of several mechanisms of drug toxicity.[→204–206]

(g) Various kinds of mitochondrial injury have been observed including lactic acidosis, microvesicular steatosis and hepatic dysfunction (LASH), in which patients show hypoglycaemia, hyperammonaemia and lactic acidosis but only mildly elevated levels of ALT.[192, →207]

(h) For example, treatment of PD-1 (programmed cell death protein-1) knockout mice with a CTLA-4 immune checkpoint inhibitor together with the hepatotoxic drug amodiaquine led to delayed-onset liver injury with characteristic histologic features of idiosyncratic DILI mediated by CD8 T cells.[→208] This impaired immune tolerance model has been linked to the detection of immune-mediated liver damage in animal species.[195]

Conclusion

Drugs and drug metabolites cause idiosyncratic DILI through many different mechanisms, not all of which can be captured in a single pre-clinical model. No single assay can predict a DILI liability. In addition to standard pre-clinical toxicology testing, innovative tailored assays that address specific mechanisms such as the role of the immune system will more accurately identify the hepatotoxic liability of a compound.

3.2 Data analysis in clinical trials

Summary

- Clinical trials of a study drug offer a unique opportunity to identify associated DILI signal(s) prior to its use in a large treatment population.
- The two main types of DILI signals are 1) an imbalance among study subjects with elevations of serum liver enzymes in randomized controlled trials between study drug and placebo (or comparator) and 2) clinically significant DILI cases marked by liver-related symptoms, elevation of bilirubin, jaundice and/or coagulopathy.
- Analysis of liver test abnormalities is useful for trending over time and to assess imbalance between study drug and placebo or active comparator.
- Use of graphic tools to display data, in addition to standard liver test summary tables and case narratives, is important to assess liver safety. Key graphical displays include the eDISH plot and individual patient profiles.

Conclusions / recommendations

1. Whenever possible, analysis of clinical trial data should include individual trial results, as well as pooled data from multiple randomized controlled trials.
2. The severity of liver injury should be characterized for each individual suspected case of clinically significant DILI using a five-point categorical scale, ranging from 1 (mild) to 5 (fatal) that has been utilized by the NIH DILIN group,[11] as well as by U.S. FDA regulatory scientists.[46]
3. A comprehensive assessment of causality for each of these cases should be performed by individuals who have clinical expertise in the assessment of liver injury, using a five-point categorical scale of likelihood.[11]
4. In a clinical trial setting, the use of e-DISH as a graphic tool provides an efficient platform for the comprehensive identification and review of liver injuries of interest associated with a study drug.

3.2.1 Introduction

A drug development programme provides a unique opportunity to systematically evaluate DILI risk for each enrolled patient as well as integrate results obtained across the entire study population at different protocol-defined time periods that include the pre-treatment phase as well as the on-treatment and post-treatment phases of the clinical trials. There are a number of publications in which currently recommended methodology to address liver safety in clinical trials is reviewed.[138, 145, →209] The required critical data elements and best practices for data collection have been described in order to characterize and interpret a DILI signal in clinical trials.[145]

3.2.2 Sources of data

Data can be analyzed from individual clinical trials, but there is an advantage in an analysis of data pooled from a number of clinical trials. When possible, the analyses should focus on randomized

double-blind controlled trials, either with placebo and/or an active comparator. For certain populations (such as cancer patients), studies may be included that may not meet these criteria of clinical trial design. In addition, identification of cases of clinically significant DILI can be identified from the analysis of a clinical trial of any design conducted.

3.2.3 Questions to ask

A thorough analysis of data in clinical trials to assess for DILI should be based on an evaluation of all reported liver-related adverse events as well as standard serum liver test measurements, taking into account concomitant pathologies and medications. In order to address the liver safety of a drug in clinical trials, an attempt should be made to address all of the following key questions.[41, 209]

- Are there any Hy's law cases in the dataset?
- How are changes across different liver tests correlated, and how do those correlations differ between treatment groups?
- What is the timing of elevations of liver tests in active treatment and comparator arms?
- Is there a "window of susceptibility" in the active treatment arm?
- Are shifts from baseline different between treatment groups?
- Is there any evidence for a dose-response-relationship?
- What do time profiles of individual liver tests or liver test panels look like?
- Are liver test changes observed while a patient is on treatment transient or progressing?
- What do time profiles look like after treatment is stopped?
- How does intake of certain concomitant medications or occurrence and/or resolution of certain adverse events relate to time profiles of liver tests?
- Are liver test elevations correlated with the desired therapeutic effect of the drug?
- Are liver test elevations associated with non-liver side effects or other laboratory abnormalities?
- Are liver test elevations associated with pharmacokinetic parameters of the drug (if available)?
- Are there other forms of liver injury present besides hepatocellular, such as cholestatic or mixed liver injury, or is there acute-on-chronic liver failure?

In order to systematically address these questions, a set of standard graph templates should be used and customized as needed. Graphical display of serial laboratory data is currently recommended.[7] Some approaches to graphic and visual analysis are proposed in Section 3.2.6 below.

3.2.4 DILI detection based on adverse events

Current best practices for DILI identification based on reports of adverse events include utilizing search criteria recommended by the U.S. FDA and the CIOMS working group on Standardized MedDRA queries (SMQs).[→210] The broad "Hepatic disorders" SMQ is utilized to capture events that may be manifestations of compromised liver safety.

The quality and quantity of liver safety data obtained in clinical trials is usually better than that obtained in the post-marketing setting, but there are limitations in relying on reporting of hepatic adverse events to evaluate DILI in clinical trials. The hepatic adverse events reported may not

correlate with liver test abnormalities, and there may be an incomplete diagnostic evaluation to assess alternative causes. In this instance, it is important to determine whether the investigator is aware of other useful diagnostic information that has not been captured in the core dataset. Nevertheless, in drug development analysis of adverse events can be useful for trending and to assess for imbalance between the drug and comparator populations or across different treatment populations receiving the same drug.

3.2.5 Assessment of DILI risk based on an integrated analysis of laboratory results and diagnostic findings in the clinical trial database

During drug development, an adverse hepatic reaction is typically suspected based on abnormalities in standard serum liver tests as described above (see Sections 2.2.1 to 2.2.4 on monitoring and diagnosing DILI in clinical trials).

The incidences of ALT and AST elevations > 3 × ULN, > 5 × ULN, > 10 × ULN and > 20 × ULN are typically tallied as well as ALT and AST > 3 × ULN accompanied by changes in TBIL (> 1.5 × ULN and > 2 × ULN) to determine if there is an imbalance between study drug and placebo or active comparator in the randomized clinical trial datasets. A pooled analysis of the incidences of ALP elevations > 1.5 × ULN for all treatment groups is also recommended.[7] Furthermore, a comparative analysis in the strata of study subjects with ALP > 2 × ULN and ALP > 3 × ULN should be considered.

For the clinical trial setting, U.S. FDA guidance [7] suggests the following indicators of a potential for severe DILI:

- An excess of aminotransferases (AT) elevations to >3 × ULN compared to a control group.
- Marked elevations of AT to 5 ×, 10 ×, or 20 × ULN in modest numbers of subjects in the drug-treated versus the control group.
- One or more cases of newly elevated total serum bilirubin to > 2 × ULN in a setting of hepatocellular injury, when other causes of liver injury have been excluded.

Currently "Hy's law" (see Section 2.2.6) is a generally accepted model used to assess risk for significant, acute hepatocellular DILI in clinical trials.[114] It provides a rough estimate of the incidence of drug-induced acute liver failure cases (*i.e.* those resulting in liver transplant or death) that are likely to occur after a drug is approved, namely one-tenth the rate of cases that fulfil all criteria of Hy's law observed during clinical trials.[149] Hy's law has been supported by at least two large cohort studies that affirmed that approximately 10% of patients with suspected DILI plus jaundice or hyperbilirubinaemia died or required a liver transplant.[73, 75] The presence of even one or two cases marked by Hy's law in a clinical development programme signifies that the suspect drug is associated with an increased risk for acute liver failure in a large treatment population. 'The robust nature of this association is exemplified by accurate predictions previously made by the US FDA of an increased risk for liver failure in ximelagatran or lumaricoxib-treated patients, based on the identification of cases conforming with Hy's law in their clinical trial programmes. Although neither agent gained approval in the US, the subsequent identification of post-marketing cases of serious liver injury and liver failure associated with each product led to their withdrawal in many countries in which their marketing was initially approved.[→211–212]

As described above (see 2.2.6), post-market cohort studies have shown that a modified Hy's law definition using a "new R" value > 5 without an absolute upper ALP limit was more sensitive in predicting drug-induced acute liver failure,[37] and had a higher positive predictive value for the outcomes of liver transplant or death in patients with acute liver failure,[43] than criteria that set an ALP limit of < 2 × ULN. Whether the presence in clinical trials of cases that fit all these modified Hy's law criteria without conforming to the ALP limit would reliably predict an increased risk for post-market drug-induced liver failure is an important concern that should be further evaluated.

It should be noted that the absence of a Hy's law case among a few thousand patients exposed to the study drug does not necessarily imply the absence of a small increase in a risk for serious idiosyncratic DILI: A binomial derivation based on this principal typically only allows exclusion of a hypothetical incidence of acute hepatocellular injury consistent with Hy's law that is greater than one case per approximately 1/3 the number of all the subjects in the clinical study programme treated with the same dosing and duration regimen of study drug under interrogation ('**Rule-of-three**').[7, →213] Thus, if the true incidence of ALF associated with a study drug is 1/10 000 and the rate of Hy's law cases is 1/1 000, about 3000 study patients treated with the agent would be needed to have a 95% chance of observing at least one Hy's law case in the study.[→214]

For a drug that has a liability for idiosyncratic DILI, fewer patients on treatment are needed to observe one case of aminotransferase elevation than one case of liver failure. This affects the number needed to harm (NNH), or inverse of the absolute risk increase, which is sometimes used to communicate the risk of DILI to health care professionals post-marketing. A single NNH figure will not capture the full range of liver injury. The NNH will also vary depending on the length of observation and in some cases the number of treatment cycles observed. In practice, there are many limitations to the usefulness of NNH,[→215–216] and it has not found wide use in medical product labelling.

3.2.6 Visual and graphical analysis of liver tests

The use of graphic tools in addition to standard summary tables and narratives can significantly help to improve liver safety assessment.[209] Graphic displays of study drug and comparator placebo groups should be linked to individual graphic timelines that depict serial biochemical measures in all study subjects with acute or worsening liver injury.[138] Useful analyses include evaluation of drug-induced serious hepatotoxicity (eDISH), time profiles of individual patients, timing of hepatic events such as ALT > 3 × ULN using a Kaplan-Meier plot, and shift plots for laboratory parameters.[209]

Risk plot

In addition to a presentation of the incidence of liver test elevations for study drug, placebo and active comparator, the serum liver test data can also be analyzed by using the risk plot of liver safety-related parameters, which presents the risk difference between study drug and both placebo and active comparators.[41] The risk plot can highlight differences of liver test elevations between study drug and placebo or active comparator (see Figure 1 in the supplemental **Appendix 5**).[4]

[4] Online only – freely available on the CIOMS website at https://cioms.ch/publications/product/drug-induced-liver-injury/

eDISH Plot

The eDISH plot (**Figure 1**) is a key graphical representation of an entire clinical trial population to assess the liver safety profile of a drug and identify individual idiosyncratic cases of concern.[→217] The plot is a log/log display of correlation between peak TBL vs. ALT, both in multiples of ULN. The horizontal and vertical lines dividing the graph into quadrants indicate Hy's law thresholds, which are ALT= 3 × ULN and TBL = 2 × ULN.

There are two key areas of the graph. The first is the right upper quadrant, which identifies the trial participants with peak on-treatment ALT and TBL elevations consistent with Hy's law. This has been dubbed the "Hy's law quadrant". Each study subject with values in the right upper quadrant should be evaluated to determine whether all criteria associated with Hy's law are met, including whether the liver injury has a causal association with the study drug.[209] The second is the right lower quadrant, dubbed the 'Temple's Corollary quadrant', that identifies patients with treatment emergent peak ALT > 3 × ULN, but with TBL < 2 × ULN. An ability to associate points representing the each individual's peak liver test elevations shown in the right upper quadrant with all his/her sequential biochemical data obtained over the full period of the study (displayed on a separate timeline graph that is linked and accompanied by a narrative containing pertinent clinical and diagnostic information to diagnose the severity and determine the most likely cause of the liver injury) ensures the comprehensive survey and assessment of all potential Hy's law cases in a clinical trial.[145, 188] In addition, an evaluation of the relative incidences of ALT > 3 × ULN associated with the study drug versus the randomized placebo / comparator drug shown in the right lower quadrant can play an important role in an overall assessment of DILI risk associated with the study drug.[145]

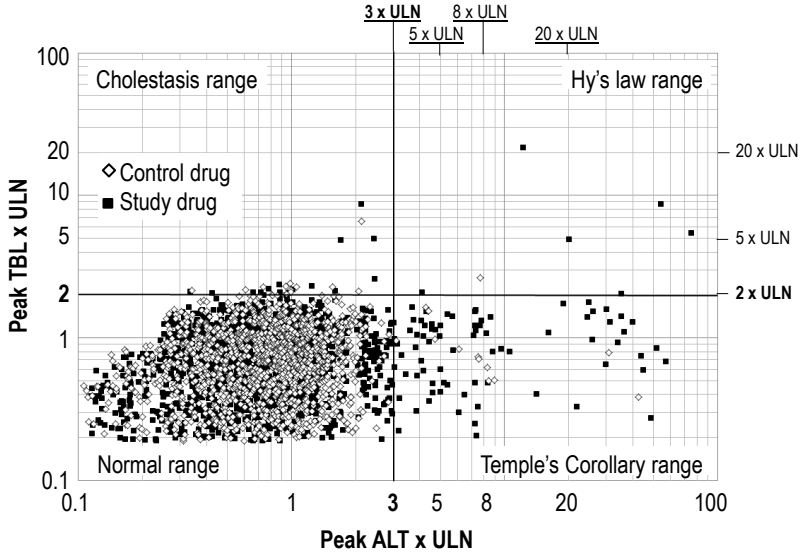
Assessment of respective increases from pre-dose values may be crucial in a range of populations with underlying liver disease, *e.g.* cancer patients with liver metastases, NASH patients etc. Results of comprehensive outlier analyses for ALT and TBL changes from baseline across "healthy" populations and cancer patients have led to recommendations for use of an additional plot, based on multiples of individual ALT and TBL baseline values, instead of multiples of ULN.[→218-220] Visualizing changing ALT and TBL levels as the multiples of each individual's baseline values using eDISH or "mDISH" (a modified eDISH plot) may be useful in combination with an analysis of the same liver test data using plots based on multiples of the ULN. Such a side-by-side graphic comparison can help put outlier cases with liver test abnormalities into perspective and help prioritize an in-depth analysis of potential Hy's law cases.

Time profiles

Time profiles for individual patients of interest with graphic depiction of serial liver tests over time can provide valuable information, including a time-line association of the different liver tests, causal relationship to study drug and underlying pathology.[209] Adding start and stop of study drug, dose levels, associated adverse events and concomitant medications to the profile will result in a more comprehensive analysis and interpretation than if each data element was assessed in isolation (**Figure 2**). Time profiles for individual patients are of particular interest for potential Hy's law cases as well as cases with prolonged recovery of liver tests after study drug discontinuation. A quality case narrative for the individual patient profiles is also important for the evaluation and diagnosis of DILI,

highlighting the advantage of eDISH plots in which such narratives can be linked directly to timeline graphs of each individual's liver test findings.

Figure 1. eDISH plot

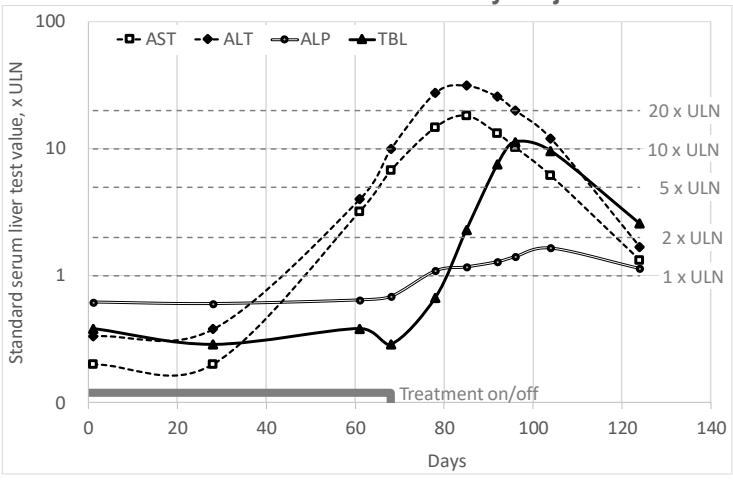


TBL **[**× ULN**]** vs. ALT **[**× ULN**]** on a log/log scale

ALT=alanine aminotransferase, **TBL**=total bilirubin, **ULN**=upper limit of normal

Illustrative data, based on distributions shown in published literature [21, 41, 46].

Figure 2. Time course of serum test results for a study subject of interest



ALT, AST, ALP, and TBL are recorded over time as values x ULN on a log 10 scale.

ALP=alkaline phosphatase, **ALT**=alanine aminotransferase, **AST**=aspartate aminotransferase, **TBL**=Total bilirubin, **ULN**=upper limit of normal

Hypothetical case based on data from several real cases [46]

Kaplan-Meier plot

The Kaplan-Meier plot is a widely used graphical display that shows and compares time to event. Comparing time to elevation of liver test results across treatment groups is important in understanding and interpreting a liver safety signal as well as managing the risk associated with effects of study drug on the liver.[209] The common presentation for the Kaplan-Meier plot is the incidence of ALT elevations over time across treatment groups (see Figure 2 in the supplemental **Appendix 5**).[5] Identification of the window of susceptibility when ALT elevations occur by analysis of the Kaplan-Meier plot can provide important clinical information. For example, the typical time to onset of most cases of idiosyncratic DILI is between 2 weeks and 6 months of treatment.[→221]

Shift plot

Shift plots are a graphical method to compare changes of liver test data from baseline across treatment groups. If control groups (placebo and/or active comparator) are available, a shift plot of peak ALT on y-axis and baseline ALT on x-axis (see Figure 3 in the supplemental **Appendix 5**)[5] can be useful in identifying differences between groups.[41] If no control group is available, plotting only maximum post-baseline values on the y-axis will exaggerate the apparent shift from baseline, and this bias increases with a larger number of post-baseline observations per patient. Alternatives are to plot all post-baseline values per patient, or to display shifts as scatterplots by visit.[209]

It is important to also evaluate the severity of liver injury, which can be defined in a number of different ways based on the liver laboratory test and clinical parameters (see Section 1.2).

The various analyses described are most useful when a large pooled dataset is available that includes unblinded treatment information for study drug, placebo and active comparator groups from a number of different clinical trials. Nevertheless, these analyses should also be carried out as part of the evaluation of liver safety early in clinical development. They can be useful in selected individual clinical trials as well as smaller pooled datasets *e.g.* from studies completed at the time of end of phase 2 clinical development, and can form the background for repeated and additional analyses once a larger population of patients is exposed to the study drug in phase 3 development.

[5] Online only – freely available on the CIOMS website at https://cioms.ch/publications/product/drug-induced-liver-injury/

3.3 Considerations regarding collection and storage of clinical samples

Summary

- It is generally acceptable to collect biological research samples during screening for enrolment in clinical trials to establish a baseline, and at certain time points during the treatment phase and post-treatment follow-up.
- The samples can be stored in an approved biobank for future analyses that are not yet defined in the original study protocol, *e.g.* to identify potential biomarkers of DILI (see CHAPTER 4), or to study efficacy or outcomes.
- Patient-specific data are usually needed to link the biological samples to the clinical outcomes and safety events of interest.
- Several pre-competitive consortia and private-public partnerships are working on best practices for the collection and analysis of biospecimens during the nonclinical and clinical phases of drug development with a goal to develop and qualify reliable DILI biomarkers.

Conclusions / recommendations

1. To enable exploratory investigations beyond the original protocol, a broad consent for the collection of biological samples for future research should be obtained from all participants whenever possible.
2. Storage of clinical samples in biobanks should be compliant with Guideline 11: *Collection, storage and use of biological materials and related data* of the CIOMS ethical guidelines,[→222] the declaration of Taipei,[→223] and national guidelines.
3. It is important to ensure that patient-related data be sufficiently de-identified for privacy purposes while also ensuring that they remain relevant and useful.
4. Relevant guidelines should be applied with regard to patients who lack capacity to give consent, *e.g.* patients with DILI that develop acute liver failure.

3.3.1 Background

The collection of clinical samples, including live cells and genetic materials, before and during clinical trials with new drugs can significantly assist the identification of predictive, diagnostic, prognostic, and safety biomarkers on different organ systems, including liver damage. The number and nature of such samples will be informed by the prior clinical experience with the drug and the results from appropriate nonclinical testing that may identify a potential for liver injury.

To identify individuals or populations who may be at risk from drug-related organ damage, it is necessary to include sufficiently diverse populations and to ensure that interactions with other diseases or medications as well as environmental factors are considered. This diversity may be

based on age, sex, ethnicity, genetic constitution or other factors that may influence the susceptibility to injury.

In the past, the evaluation of DILI biomarkers has mainly relied on cases of acetaminophen (paracetamol) overdose. To evaluate new biomarkers for use in clinical practice, biological samples and data from healthy volunteers, patients with DILI and those with non-drug-induced liver injury are needed. Large prospective studies are being conducted to detect drug induced organ damage. Considering the complexity of such studies, alternative sample collection strategies have been proposed such as retrospective analysis of discarded samples from hospital patients, or prospective collection and storage of samples from drug development trials.[→224]

3.3.2 Areas for consideration

There are ethical issues related to conduct of clinical trials that seek to discover the potential for a new drug (or mode of use) to cause liver injury, as well as those that explore new biomarkers for the diagnosis and management of DILI. The considerations outlined in this section focus on issues specifically related to DILI and have been informed by the 2016 Declaration of Taipei,[223] the Declaration of Helsinki [→225] (particularly Articles 6, 22, 24, 26 and 32), the 2016 CIOMS Ethical Guidelines,[222] ICH Good Clinical Practice guidelines,[→226] and other relevant guidelines.

Anonymization

To interpret the information derived from testing of clinical samples, it is usually necessary to maintain a database of patient-specific information that can be related to the clinical sample. How this material is sufficiently de-identified to protect participants' confidentiality should be considered. The samples and data should be stored in a suitable repository, and may be labelled with the participant's name, or with a coded identifier, or may be totally de-identified, although complete anonymization is becoming difficult as the possibility of cross-matching large datasets improves. The commentary to the CIOMS Guideline 11 [222] provides advice on coding and data anonymization.

Storage and stewardship of samples

Samples may be analysed immediately or – more commonly – stored for some time so that they may be examined at a later time to relate the laboratory parameters to clinical outcomes during the trial.

The conditions of storage have been considered by a number of organizations. The International Society of Biological and Environmental Repositories (ISBER) provides guidance on best practices.[→227] The CIOMS Guideline 11 further states the importance of, and requirements for, material transfer agreements to document the locations, movements and uses of samples.[222]

The collection of additional samples for future research can pose risks to participants, especially when adequate safeguards to protect confidentiality are not in place.[222] Guideline 11 of the CIOMS ethical guidelines [222] provides commentaries on governance systems that should be in place at institutions where biological material and related data are stored or archived for future use.

Informed consent for future use of samples

The CIOMS Guideline 11 describes how broad consent can be given by research participants if the future use of the materials is not known at the time of collection, as well as informed opt-out procedures for research on residual tissue, and withdrawal of consent. The conditions in specific or broad informed consent documents should not be overruled by any regulations that are part of institutional governance systems to ensure good stewardship of stored or archived data.[222]

Capacity to consent

In certain situations participants such as critically ill patients or those with hepatic encephalopathy may be unable to give informed consent. This is relevant in the context of DILI research, where patients that develop acute liver failure may be of particular scientific interest. The CIOMS ethical guidelines [222] provide advice for this situation in its Guideline 16 on *Research involving adults incapable of giving informed consent*. National legislation, such as the Mental Capacity Act 2005 in the United Kingdom [→228] is to be followed in such situations.

3.3.3 Scenarios

There are three scenarios where Ethics Committees may pay particular attention to the above-mentioned ethical issues when samples are collected before and during a clinical trial:

- **Collection and storage of "baseline" samples prior to the study:** Collection of samples may not be directly related to the conduct of the trial, but may aim to establish baseline values to enable comparisons with tests done following treatment-related reactions and eventual resolution. This is particularly relevant to DILI, where standard blood-based tests often need individual interpretation. The above-mentioned ethical considerations apply.
- **Long-term storage of data and clinical samples for future testing as defined in the protocol:** This is required to enable any required analyses in case of subsequent adverse events. The Participant Information Leaflet and Informed Consent documents must detail the numbers of samples, the conditions and the duration of storage based on available knowledge of the medicine, the disease and the population treated. A storage period of five to ten years for samples can be considered, when justified. A separate informed consent should be provided for the aspects of storage related to future research; delineating what may happen to the samples; particularly how they will be stored and who will have access. The content of that consent should be as specific as possible. The participant should be informed of the process for ethics committee approval for future use of stored samples
- **Storage of clinical samples and data for future testing not defined in the protocol**: Where the extent of future testing is not defined, tests may then be used that are not qualified at the time of consenting. Ethical oversight will only be exercised at some future time. However, these tests may enable analysis and understanding of adverse events that will enable safer use of the medicine in the future. New technologies may also enable detection of pre-existing conditions that were unknown or unsuspected at the time of the original study. Lastly, the stored samples may be used to validate newly developed assays. The last two points are specifically relevant to DILI, where a number of new biomarkers are under development.

To address the concern of "unknown" future testing, National Regulatory Authorities (NRAs), Research and Ethics Committees (RECs) and Institutional Review Boards may have developed policies for limiting the storage and use of clinical samples. The guidance provided in the Declaration of Taipei (2016) [223] is helpful in this regard.

It is usual that further studies or analyses will only relate to the objectives of the clinical study. However, when it is envisaged that sample residues (*i.e.* material remaining in the sample container after withdrawal of material for the defined tests) may be made available to other research initiatives, this must be fully explained to the participant. The CIOMS Guideline 11 provides advice on informed consent and opt-out procedures for research on residual tissue.[222]

Several pre-competitive consortia and private-public partnerships [135, 136] are working on best practices for the collection and analysis of biospecimens during the nonclinical and clinical phases of drug development with a goal to develop and qualify reliable DILI biomarkers.[→229–230]

The informed consent document should be designed to include a specific section including full details of the samples and testing as defined in the protocol. In addition, it should include as much information as possible about the future use of the samples.[225][6] The participant's signature may be obtained for each point, or there may be tick boxes with an overall consent signature for the storage and use of clinical samples.

[6] This may include: Description of additional tests that may be conducted and the circumstances for triggering such testing; intention to use the samples for new and undefined tests related to the current trial; potential that the samples may be used for research in a field unrelated to the current trial; how suitable ethics review will be conducted before use of the stored clinical samples for use with undefined tests or in a field outside the current trial; time period over which the clinical samples will be stored; whether the participant will be informed of any results from further testing of samples; the rights of the participant to any discoveries that may be made following use of the stored samples; the rights of the participant in withdrawing consent to use the stored clinical samples at any time, if this is possible; and the place where samples will be stored and the conditions of storage that meet accepted guidelines (*e.g.* ISBER best practice guidelines).

CHAPTER 4.

NEW LIVER SAFETY BIOMARKERS

4.1 Regulatory concepts

Summary

- Biomarkers can be classified into different categories according to their proposed context of use such as diagnostic, monitoring/safety, efficacy, predictive/susceptibility, or prognostic biomarkers.
- The intended role(s) of a biomarker is defined in one or more "context of use" statements.

Conclusions / recommendations

1. Further development of translational biomarkers from animal models and *in vitro* testing is needed.
2. The U.S. FDA framework for the classification and U.S. FDA/EMA "context of use" framework for of biomarkers should be used in the development and validation of DILI biomarkers.
3. New liver safety biomarkers developed in clinical cohorts will be compared to currently available analytes and will require prospective validation in independent patient populations before they can be considered for broader use.

4.1.1 Definition

According to the U.S. FDA, a biomarker is defined as: *"A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions."* [→231] Human biomarkers may measure molecular, histologic, radiographic, or physiologic characteristics.

Although both the EMA and the U.S. FDA have programmes available for the qualification of biomarkers (see Section 4.2.1), only the latter has guidance documents that define the terminology to a sufficiently detailed extent. It is therefore recommended to use the definitions proposed by the U.S. FDA.

4.1.2 Categories of biomarkers

A biomarker may fall into one or more categories, depending on its proposed context(s) of use (see below). This is also the case with the current standard serum liver tests used as safety biomarkers for DILI (see Section 2.1), some of which can be assigned to several classifications. Categories of biomarkers include the following.

Diagnostic biomarker: A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease.

Example: Hepatitis C virus (HCV) RNA detected in the blood by PCR assay, to diagnose a patient with active hepatitis C infection.

Efficacy/ pharmacodynamics biomarker, also called pharmacodynamic/response biomarker [231]: A biomarker used to show that a biological response has occurred in an individual who has been exposed to a medicinal product or an environmental agent.

Example: A biomarker expressing efficacy (and closely related to the above) is HCV RNA negativity 12 weeks after the end of treatment, indicating a sustained viral response (SVR) in the context of HCV drug development.

Monitoring/safety biomarker: A monitoring or safety biomarker is typically measured at baseline and serially during drug therapy to assess the status of a disease or medical condition or for evidence of exposure to (or effect of) a medicinal product or an environmental agent.

Examples: Serum ALT, AST, ALP and total bilirubin levels, when used for the detection and severity grading of DILI.

Predictive / susceptibility biomarker: A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favourable or unfavourable effect or toxicity from exposure to a medicinal product or an environmental agent.

Example: Presence of the HLA-B*5701 *allele is a susceptibility factor for developing flucloxacillin-induced liver injury (see also page 59).*

Prognostic biomarker: A biomarker used to identify the likelihood of a future clinical event or disease recurrence or progression in patients.

Example: The model for end-stage liver disease (MELD) score, serum cytokeratin levels and MCSF-1 levels all showed prognostic value for adverse outcomes (i.e. death, transplant) in a recent study of DILI patients.[229]

Another category of biomarkers are **translational** biomarkers that are initially developed in animal studies to show efficacy and safety in humans. However, these biomarkers are not the focus of this publication.

4.1.3 Context of use

Within the framework of regulatory qualification of biomarkers, context of use statements are required by both the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA).

Definitions

The EMA defines the context of use (COU) as follows: *"Full, clear and concise description of the way a novel methodology is to be used and the medicine development-related purpose of the use. The Context of Use is the critical reference point for the regulatory assessment of any qualification application."*[→232]

According to the U.S. FDA, the COU statement describes the manner and purpose of use for the biomarker in drug development.[→233] It is a concise description of how, when and why a biomarker is to be used in a drug development programme or clinical trial. For instance, the level of serum ALT in combination with total bilirubin (TBL) has been proposed as a prognostic biomarker for patients with acute drug-induced liver injury at risk for severe adverse outcomes.

Examples

There are currently no liver safety biomarkers qualified by regulatory authorities. An example of a COU statement as issued by EMA is given for the GLDH biomarker which received regulatory support in 2017:

> *"(…) elevated serum GLDH enzymatic activity is a measure of hepatocellular injury, and can be used in healthy subjects and patients as an adjunct to alanine aminotransferase (ALT), the current standard biomarker used to assess hepatocellular injury, in all stages of drug development. In a clinical situation when ALT increases are observed, (…) GLDH can lend weight of evidence to confirm or rule out hepatocellular injury." [→234]*

An example for a non-liver-related COU statement qualified by the U.S. FDA, relating to the use of fibrinogen in chronic obstructive pulmonary disease (COPD), is the following:

> *"(…) the use of plasma fibrinogen, measured at baseline, as a prognostic biomarker to select patients with COPD at high risk for exacerbations and/or all-cause mortality for inclusion in interventional clinical trials. This biomarker should be considered with other demographic and clinical characteristics, including a prior history of COPD exacerbations, as an enrichment factor in these trials." [→235]*

Elements of the COU statement

According to the U.S. FDA, a COU statement is comprised of (1) the biomarker category (see Section 4.1.2) and (2) the biomarker's proposed use in drug development, including [→236]:

- The purpose of use in drug development (*e.g.* as a prognostic biomarker for enrichment, as safety biomarker to evaluate organ damage);
- the proposed stage of drug development (*e.g.* phase of clinical studies, nonclinical studies);
- the clinical trial population or model system (*e.g.* healthy volunteers, type of patients, animals, cell culture); and
- the therapeutic mechanism of action for which the biomarker is intended to have value, in case that the mechanism of action is relevant to the biomarker's biology and intended utility.

According to the U.S. FDA, a biomarker category can have a variety of COUs. For example, in clinical trials a prognostic biomarker can be used for patient stratification or for enrichment, *i.e.* to select a study population that is particularly suitable for detection of a potential drug effect.[233]

4.1.4 Biomarker discovery vs qualification

Developing new liver safety biomarkers in general occurs in two steps: discovery and qualification. **Discovery** is finding evidence for performance superior as compared to known valid biomarkers in terms of sensitivity, specificity, or predictive value. Depending on biomarker characteristics, hints for improved performance may be detected in small datasets sometimes, *e.g.* from drug development programmes where a safety signal surfaced in clinical trials and spare samples were still available for biomarker research. Collecting additional samples for future analysis may be most helpful for molecules that have either shown a liver safety signal in preclinical or early human testing or are more likely to be associated with liver toxicity, given mode of action or compound class. However, biomarker sampling can be useful for *any* development programme, considering that many DILI signals may occur very late in the development process or only during post-marketing pharmacovigilance. The potential benefits of routine sampling across all projects however have to be balanced against technical and administrative challenges of long-term biobanking as well as significant additional costs.

Biomarker qualification, as the second step in safety biomarker development, is a process established and defined by EMA and U.S. FDA, and has been published in respective regulatory guidance documents.[232, →237] The U.S. FDA documents define qualification as follows: *"Qualification is a conclusion that within the stated COU, the DDT[7] can be relied on to have a specific interpretation and application in drug development and regulatory review. … Once a DDT has been qualified for a specific COU in drug development, it can be used to produce analytically valid measurements that can be relied on to have a specific use and interpretable meaning. The DDT can be used by drug developers for the qualified context in IND, NDA, and BLA submissions[8] without the relevant CDER[9] review group reconsidering and reconfirming the suitability of the DDT…*.[237] Thus, working with qualified biomarkers has a considerable benefit for drug developers: no new evidence for the validity of the biomarker within its defined context of use has to be generated and presented. That evidence has been generated and agreed upon upfront during the biomarker qualification submission, as outlined in detail in respective guidance.[232, 237, →238]

Further reading

An overview of biomarkers that can be useful in drug development and considerations for qualification was published in 2015.[→239] Coordinated protocols for biomarker qualification have been implemented at regulatory agencies.[→240] The EMA has highlighted some common major challenges and limitations in the qualification of innovative methods, including biomarkers.[232]

[7] DDT: Drug Development Tool, *e.g.* a biomarker

[8] IND: Investigational New Drug; NDA: New Drug Application; BLA: Biologic Licence Application. See the explanations on the U.S. FDA website.

[9] U.S. FDA *Center for Drug Evaluation and Research*

4.2 Biomarker performance and requirements

Summary

- The validation of a biomarker's analytical method and its clinical validation are essential steps for biomarker qualification by regulatory authorities.
- Key features of any DILI biomarker include its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy in identifying susceptible patients.
- In the context of DILI, a biomarker with high specificity may still have limited predictive value due to the low incidence of the event in the population (see the example on page 59).
- The area under the receiver-operating characteristics (ROC) curve is commonly used to compare the performance characteristics of biomarkers to each other.

Conclusions / recommendations

1. All biomarkers should be developed in the setting of predetermined context of use in drug development.
2. The analytical method must be highly reproducible, standardized, and preferably automated to provide accurate results.
3. Clinical validation of DILI biomarkers requires the testing and collection of samples from a large number of exposed patients due to the generally low incidence of idiosyncratic DILI with most drugs and the diverse phenotype presentations. Samples should therefore be collected from all patients enrolled in a clinical trial.

4.2.1 Qualification

The qualification of a biomarker is *"a conclusion, based on formal regulatory process, that within the stated context of use (COU), a medical product development tool can be relied upon to have a specific interpretation and application in medical product development and regulatory review"*.[231] The analytical validation of a biomarker assay performance as well as the clinical validation of the biomarker are key components of a biomarker qualification programme.[→241]

Analytical validation refers to the correct measurement of the biomarker and that the test will provide accurate and reproducible results. It is the process of *"establishing that the performance characteristics of a test, tool or instrument are acceptable in terms of its sensitivity, specificity, accuracy, precision, and other relevant performance characteristics using a specified technical protocol (which may include specimen collection, handling and storage procedures). This is validation of the test, tools, or instrument's technical performance, but is not validation of the item's usefulness"*.[231]

The fit-for purpose expectation defines that the biomarker assay is neither too simplistic nor too rigorous for the goals of the investigation. It is *"a conclusion that the level of validation associated with a medical product development tool (assay) is sufficient to support its context of use"*.[231] Therefore, the

criteria used for assay validation of an exploratory biomarker would be less rigorous than the assay validation of a well-qualified biomarker with clinical application. *"The process from exploratory to advanced validation is continuous and iterative with increasing rigour for all the validation elements,…"*[→242]

Clinical validation refers to the correlation of a biomarker test result with a biological process or a clinical outcome. It is the process of *"establishing that the test, tool, or instrument acceptably identifies, measures, or predicts the concept of interest"*.[231] To establish the clinical validity of a biomarker for a specific COU, data supporting the relationship between the biomarker and a clinical outcome are needed.[236] Due to the generally low incidence of idiosyncratic DILI with most drugs, the clinical validation of DILI biomarkers requires the testing and collection of samples from a large number of exposed patients. Therefore, it is recommended that biological samples should be collected from all patients enrolled in a clinical trial.

4.2.2 Performance characteristics

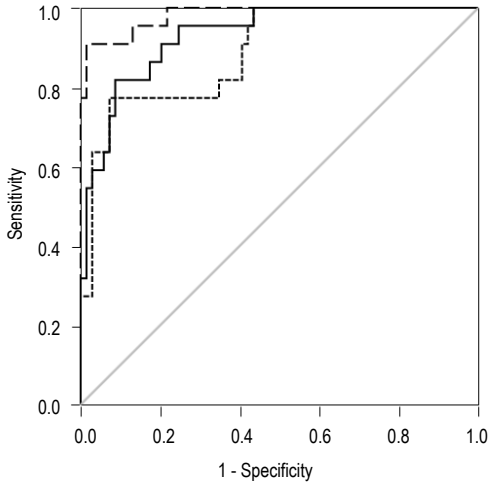
The following parameters should be considered in clinical validation of a biomarker:[→243–244]

- **Sensitivity:** Identifying true positives
  = *true positives / (true positives + false negatives)*
- **Specificity:** Identifying true negatives
  = *true negatives / (true negatives + false positives)*
- **Positive predictive value** (***PPV***): Probability that a person testing positive has the condition
  = *true positives / all positives*
- **Negative predictive value** (***NPV***): Probability that a person testing negative does not have the condition
  = *true negatives / all negatives*
- **Accuracy:** Proportion of tests with correct result
  = *(true positives + true negatives) / all tests performed*
- **Likelihood ratio:** The positive likelihood ratio indicates the likelihood that a positive result will be found in a person with a condition compared to a person without the condition. Values >10 indicate a high post-test probability that a person testing positive has the condition.
- **Receiver-operating characteristic (ROC) curve:** This is a statistical method to assess the accuracy of a biomarker test that is reported out as a continuous value. *"The ROC curve is a graphical display of the trade-offs of the true-positive rate (sensitivity) and false-positive rate (1-specificity) corresponding to all possible binary tests that can be performed from this continuous biomarker"*.[244] The area under the ROC curve indicates the biomarker performance across the whole spectrum of possible results and cut-off values. A perfect correlation with the reference standard is indicated by a value of 1.0. If the biomarker is not better than chance, this is indicated by a value of 0.5. An example is shown in **Figure 3**.

Limitations

In addition to the performance characteristics of a biomarker, some factors should be considered that may limit its usefulness in practice:

Figure 3. ROC curve analysis of emerging biomarkers and liver injury



In this example three emerging biomarkers were assessed against the clinical chemistry criterion of liver injury, defined as the manifestation of one or more of the following: ≥ 5 × ALT or ≥ 2× ALP or (≥ 3 × ALT and ≥ 2 × TBL).[20] Areas under the ROC curve (AUC) for Biomarker 1, 2 and 3 were 0.89, 0.93 and 0.98 respectively; Biomarker 3 has the highest diagnostic power in this example.

- Binary test outcomes for biomarkers that are measured on a continuous scale are heavily influenced by the chosen **cut-off value** for a "positive" or "negative" outcome of the test.
- The **reference standard** ("true disease state") may not always be established with certainty and may be negatively impacted by measurement error or verification bias.
- It is important to note that positive and negative predictive values do not only depend on the specificity and sensitivity of a given biomarker, but also on the **prevalence** of a condition in a given population.[→245][10] Even an almost perfect marker of *e.g.* 95% specificity and 95% sensitivity will not be useful on its own to screen an unselected cohort for a rare condition, because most of the positive test results will be false positives.

By extension, this is also relevant in the case of idiosyncratic DILI, which has an incidence of only 0.1–0.01% or less of all patients taking a specific medicine. An example is given below.

***Example**:*

DILI secondary to flucloxacillin is strongly associated with the MHC class 1 allele HLA-B*5701. *People with this allele are about 36 times more likely to develop DILI than those without it.[→246] The prevalence of* HLA-B*5701 *in the population is about 7%. The genetic test for* HLA-B*5701 *has a sensitivity of 0.84 and specificity of 0.94,[→247] so the PPV for detecting the risk allele is rather low at 0.513 (51.3%) and the NPV is high at 0.987 (98.7%).*

[10] In addition to the formulae given on page 58, PPV and NPV can also be calculated as follows:
PPV = Sensitivity × prevalence / (sensitivity × prevalence + (1–specificity)×(1–prevalence))
NPV = Specificity × (1–prevalence) / (specificity × (1–prevalence) + (1–sensitivity)×prevalence)

However, DILI occurs in only 8.5 per 100 000 people prescribed flucloxacillin, increasing to 110.5 per 100 000 in those aged >70 years who have received two or more prescriptions.[→248] This means that even in the highest risk group one would need to test about 1000 individuals for HLA-B*5701 *to prevent occurrence of one clinical DILI event, i.e. the test has a* ***low predictive utility****.*

In contrast, when a patient presents with acute unexplained liver injury with recent exposure to flucloxacillin, detecting HLA-B*5701 *carrier status would favour the* ***diagnosis*** *of DILI over seronegative hepatitis.[→249]*

Given the complexities of DILI, even if new biomarkers achieve regulatory qualification, a combination of biomarker assessments will still be needed to confidently prevent or diagnose DILI.

4.2.3 Requirements for new serum liver safety biomarkers in DILI

Current standard serum liver tests are less than optimal for the diagnosis and assessment of DILI (see Section 2.1). The increased use of drugs such as checkpoint inhibitors that have potential hepatotoxicity with unusual phenotypes, as well as the increasing numbers of people who have other risk factors for liver disease, make the development of improved liver safety biomarkers a high priority. It is vital for the health community to have precise standards for identifying and assessing DILI in clinical practice and clinical trials, in order to guarantee patient safety and accurately diagnose hepatic toxicity.

Biomarker characteristics

To improve upon current standard serum liver tests, new biomarkers should fulfil at least one of the following criteria:

- Be more specific than ALT in terms of indicating liver injury;
- be more sensitive than TBL in indicating altered hepatocellular function;
- be more informative in terms of mechanisms underlying liver injury;
- be more predictive with respect to translating preclinical findings into potential clinical liver injury, or identifying patients at increased risk or vice versa (reverse translation); or
- have improved prognostic value compared to currently available analytes for clinical outcomes in DILI patients.

They should also be qualified by regulators for defined contexts of use (see Sections 4.1.3 and 4.2.1).

Analytical assays

To enable implementation of new biomarkers both in drug development and in post-marketing surveillance, available assays should preferably:

- Support high throughput analysis,
- be reasonably priced, and
- be technically straightforward to allow routine use in clinical practice.

Assays should also be validated according to applicable Good Laboratory Practice (GLP) standards [→250]—*e.g.* the CFR 21 Part 58 [→251] in the U.S., or Directives 2004/9/EC and 2004/10/EC [→252] in Europe—and have regulatory approval for use in drug development and clinical practice.

4.3 New exploratory biomarkers and approaches

Summary

- Several investigational serum liver safety biomarkers have received regulatory support for application in an exploratory drug development setting.[→253]
- Genetic polymorphisms in human leukocyte antigens (HLA) and other genetic variants show promise as useful susceptibility biomarkers for DILI but require further validation in larger studies.
- Liver imaging studies may also be useful to detect specific forms of DILI including hepatic steatosis, secondary sclerosing cholangitis, and accumulation of iodine in the liver.

Conclusions / recommendations

1. For drug development projects that have shown liver liability, additional serum sampling and biobanking for new liver safety biomarkers, along with standard serum liver tests, is recommended.[230]
2. Total cytokeratin 18 (CK18) and caspase cleaved CK18 (ccCK18), microRNA 122 (miR122), total high mobility group box 1 (HMGB1), glutamate dehydrogenase (GLDH), macrophage colony stimulating factor receptor 1 (MCSFR1), and osteopontin are worthy of further exploration as future DILI biomarkers.[229, 253]
3. Collection of DNA samples for testing of genetic polymorphisms is recommended to identify high risk patients for DILI in future clinical trials.
4. Additional studies using proteomic, transcriptomic, and metabolomics approaches may identify new liver safety biomarkers.
5. Although lymphocyte proliferation data may assist in DILI diagnosis, further development and research is needed.[→254–260]

4.3.1 Soluble markers

Recently, three major initiatives have jointly been working on qualification of a range of promising new soluble liver biomarkers, primarily protein markers, and some genomic markers: the Innovative Medicines Initiative's (IMI) Safer and Faster Evidence-based Translation (SAFE-T) Consortium in Europe, the Critical Path Institute's Predictive Safety Testing Consortium (PSTC), and the NIH-funded Drug-Induced Liver Injury Network (DILIN), both in the United States. **Table 9** provides an overview on the consortia's set of exploratory liver safety biomarkers having shown encouraging performance, along with main areas of application and contexts of use.

Table 9. Exploratory liver safety biomarkers investigated by IMI SAFE-T, C-Path PSTC, and DILIN

(Adapted from [→261])

Exploratory marker	Localisation and description	Application and proposed context of use
Total cytokeratin 18 (CK18) **(a)**	Epithelial cells; the full-length protein is released from necrotic cells. Significantly elevated in acetaminophen (paracetamol) overdose patients that die/require a liver transplant compared to spontaneous survivors.[→262–263]	• Mechanistic assessment (hepatocyte necrosis) • Early diagnosis • Risk of progression
caspase-cleaved cytokeratin 18 (ccCK18)	Epithelial cells; released from apoptotic cells and helps define the type of cytotoxicity. Predicts disease severity in NASH and in hepatitis C.[262, 263, →264]	• Mechanistic assessment (hepatocyte apoptosis) • Early diagnosis
microRNA 122 (miR122)	Hepatocyte-specific. Early marker of hepatocellular injury. Reported as a sensitive DILI marker in multiple clinical studies [262, 263, →265-267] High variability in healthy subjects.	• Mechanistic assessment (hepatocyte necrosis) • Early diagnosis
Total high mobility group box 1 (HMGB1) **(a)**	Detectable in almost all tissues. Marker of acute liver injury.[→268]	• Mechanistic assessment (hepatocyte necrosis) • Early diagnosis
glutamate dehydrogen-ase (GLDH) **(b)**	Mitochondrial matrix; primarily in the centrilobular region of the liver; lower levels in the kidney and brain. A sensitive biomarker of liver toxicity with hepatocellular damage in preclinical species; shown to be elevated in humans with hepatic ischaemia or hepatitis; shown to correlate with ALT in patients with a broad range of clinically demonstrated liver injuries, including acetaminophen (paracetamol)-induced liver injury, and to detect mild hepatocyte necrosis in patients treated with heparin. Marker for mitochondrial injury or cellular injury in multiple clinical DILI and acute liver failure studies.[20, 262, →269]	• Mechanistic assessment (hepatocyte necrosis, mitochondrial injury) • Early diagnosis • Exclusion of extrahepatic sources of ALT increase
sorbitol dihydrogenase (SDH)	Multiple tissue and cell types including liver. Sensitive enzymatic serum marker of liver toxicity increasing with hepatocellular damage in preclinical species. Shown to be elevated in humans with various liver diseases, and to detect mild hepatocyte necrosis in patients treated with heparin.[269]	• Mechanistic assessment (hepatocyte necrosis)
macrophage colony stimulating factor receptor 1 (MCSFR1) **(a)**	Cytokine receptor on macrophages/ monocytes. Data from the ximelagatran biomarker discovery study suggest that MCSFR1 is shed from macrophages during DILI. MCSFR1 serum/plasma levels may have value as a prognostic marker for liver disease associated with inflammation.[→270]	• Mechanistic assessment (immune activation) • Risk of progression
Osteopontin **(a)**	Multiple tissue and cell types including liver. Elevated serum levels detectable in patients with severe liver damage. associated with a poor prognosis compared to acute hepatitis patients and controls. Associated with inflammatory cell activation, and with liver regeneration due to activation of hepatic stem cells [→271]	• Risk of progression

IMI SAFE-T=Innovative Medicines Initiative's Safer and Faster Evidence-based Translation Consortium (Europe),
C-PATH PSTC=Critical Path Institute's Predictive Safety Testing Consortium (U.S.)
DILIN=Drug-Induced Liver Injury Network (U.S.)

(a) Supported by U.S. FDA Letter of Support [253]

(b) Supported by EMA Letter of Support [234]

A subset of the biomarkers shown in Table 9 (marked "(a)") obtained regulatory support from the U.S. FDA in 2016 for a context of use in assessing risk of progression of hepatocellular injury to severe DILI. The agency issued a Letter of Support,[253] encouraging sponsors to apply the new biomarkers in an exploratory setting in drug development and generate more data as a prerequisite for future regulatory qualification. The GLDH biomarker received a letter of support from the European Medicines Agency (EMA).

Although these markers are not yet qualified and hence still have to be considered exploratory, their application can help to strengthen detection and assessment of liver safety signals in clinical trials. Therefore, for projects that have shown a potential for liver toxicity or in instances where liver safety signals are considered more likely due to the mechanism of action of the drug, adding sampling for the new markers, along with standard serum liver tests and detailed phenotype data, for future analysis is recommended. New DILI biomarkers may be particularly useful for individual case assessment. If used across subsets of patients in a trial, measurement in proper controls should be ensured. New DILI biomarkers will also need to be tested in healthy controls and individuals of varying age, race, gender and BMI.

Of note, none of the new biomarkers are meant to replace standard serum liver tests, but rather to be applied as supplement to currently available markers, providing additional insight into injury phenotype, mechanism, or risk of progression. If used for decision-making in a clinical development programme, application and interpretation of new biomarkers should be discussed with regulatory agencies proactively in a safe harbour venue.

Additional markers that have been evaluated, but as yet did not demonstrate superior performance as compared to standard liver markers, include cadherin 5 (CDH5), liver fatty acid binding protein (L-FABP), glutathion S-transferase alpha (GST-alpha), alpha-fetoprotein (AFP), arginase-1, paraoxonase 1 (PON1), leukocyte cell-derived chemotaxin 2 (LECT2), as well as conjugated/ unconjugated bile acids.[Merz M, personal communication]

While standard tests provide information only across two domains, *i.e.* injury (ALT, AST, ALP), and function (bilirubin, INR, albumin), the new markers likely will offer additional insight into mechanisms of liver injury (CK18, ccCK18, GLDH, MCSFR1, HMGB1) and prediction of progression (osteopontin, CK18, MCSFR1, HMGB1). Thus, the new markers may be particularly useful if they are not just applied as individual markers, but as multidimensional marker panels.

Since qualification efforts are ongoing across different collaborations, new incoming data may strengthen or weaken current supportive evidence for some or all exploratory liver safety biomarkers. The online supplemental appendices to this report[11] may be updated with new information as it emerges.

[11] Both the report itself and the online supplemental appendices are freely available on the CIOMS website at https://cioms.ch/publications/product/drug-induced-liver-injury/

4.3.2 *Ex vivo* and *in vitro* tests: "biomarkers" supporting causality assessment

In addition to measurement of soluble biomarkers, use of dedicated *ex vivo* or *in vitro* tests may help to improve diagnosis and management of DILI in specific cases.

Lymphocyte Transformation Test (LTT)

An *ex vivo* test developed in the 1960s and repeatedly modified since then was applied across a range of hypersensitivity reactions such as erythema exsudativum multiforme, maculopapular exanthema, anaphylaxis, Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) Syndrome, and drug-induced hepatitis. The test uses *in vitro* proliferation of patients' T cells exposed to a drug suspected to have caused a prior reaction in the same subject. It has been reported to have a sensitivity of 60–70% and specificity of 85–99% in the hands of an experienced expert.[254] Several studies showed up to 12–56% positive LTT responses for various drugs,[255, 256, 257, 258] and up to 95% for DILI associated with isoniazid.[259]

However, in a study by the U.S. Drug-Induced Liver Injury Network (DILIN) using a modified LTT (mLTT), positive results for various drugs were not reproducible. A robust positive response was observed in the study for two of four samples from three DILIN subjects with isoniazid-related hepatitis. The study found that the mLTT is not a reliable test for diagnosing DILI caused by all drugs, but it may be useful for confirming an adaptive immune response in DILI ascribed to isoniazid.[260]

Neither the LTT nor the mLTT are currently recommended for routine application but they may have some value in specific settings.

Measurement of drug-protein adducts

Formation of reactive metabolites, subsequent covalent binding to cellular proteins, and drug-protein adducts then triggering an immune response leading to hepatocyte apoptosis and/or necrosis is a key mechanism of DILI.[17, →272] Thus, detection of drug-specific protein adducts may support early DILI diagnosis and facilitate more robust causality assessment. A promising point-of-care immune assay has been introduced recently for detection of acetaminophen (paracetamol) adducts,[→273] serving as a marker of exposure and potential toxicity,[→274] and helping to improve DILI diagnosis and management of patients with acetaminophen overdose, the leading cause of acute liver failure in the U.S.[17]

Investigation and detection of specific adducts of other drugs having a potential for serious DILI, together with development of respective robust assays, may help to improve DILI risk management and treatment, as well as mechanistic understanding on a broader scale.

Genetic markers

Genetic markers are primarily predictive, *i.e.* indicating a patient's increased (or decreased) risk of experiencing DILI, but they may also contribute mechanistic or diagnostic information to case assessment. Currently, the only genetic marker indicating a generic risk for DILI across multiple drugs is a polymorphism in the PTPN22 gene.[→275] Other published variants associated with an increased risk for liver toxicity are drug-specific, and include genes related to drug metabolism, hepatobiliary transporters, cytokines, and oxidative stress, as well as, to a significant extent, human leukocyte

antigens (HLAs). Given their central role in immune-mediated adverse drug reactions including DILI, HLA-associated variants have been the focus of large candidate gene studies (CGS) and genome-wide association studies (GWAS), showing some significant links between hepatotoxicity and *e.g.* HLA haplotypes *DRB1*1501–DRB5*0101–DQB1*0602* for amoxicillin-clavulanate, *DRB1*1501–DQA1*0102–DQB1*0602-DRB5*0101* for lumiracoxib, and alleles *B*5701* for flucloxacillin, or *DRB1*07* for ximelagatran.[→276-278] An updated list of genetic factors increasing the susceptibility for DILI in relation to a variety of drugs is provided in the supplemental **Appendix 6**.[249, 246, 275, →279][12] However, the predictive value of such associations is still too low to support their use in clinical development or clinical practice to predict or prevent idiosyncratic DILI.[15, 16]

As with many complex traits a wave of GWAS have brought about a step change in our understanding of the genetic basis of DILI. Most of these have a case-control design. Some specific issues related to DILI include phenotypic heterogeneity which require strict inclusion criteria and rigorous causality assessment and case adjudication processes. In addition, initial studies have focussed on DILI related to individual drugs such as flucloxacillin and amoxicillin-clavulanate.[→280] In general, to detect modest effect size resulting from common polymorphism, sample sizes in the thousands are considered necessary.[→281] However, DILI has been an exception in this regard: a first GWAS with 51 cases and 282 controls demonstrated an association with an odds ratio of 80 between flucloxacillin-induced liver injury and *HLA-B*5701*.[→282] Therefore, it is desirable to have provision to collect DNA samples from cases and controls when DILI potential is suspected, so that the risk allele can be identified following the completion of the clinical trial as in the case of lumiracoxib [212, →283] and lapatinib.[→284–285] It should also be noted that only when 2048 cases were accumulated, GWAS identified a nonsynonymous polymorphism in the protein tyrosine phosphatase non-receptor type 22 gene (*PTPN22*) as a risk allele across multiple drugs.[275] As long as cases and controls are well matched for broad ethnic background, and individuals with genome-wide association data revealing substantial differences in genetic background are excluded, spurious association due to population stratification and cryptic relatedness are avoided. The choice of array platform is influenced by a number of factors such as ever-increasing array density, capability to impute genotypes at untyped loci, sample numbers, ethnicity as well as cost.

In the context of drug development, prospectively collecting and storing baseline whole blood samples for potential future GWAS analyses in case of liver safety signals observed in a study has helped to identify significant genetic risk factors *e.g.* for ximelagatran or lumiracoxib. Genetic baseline sampling is recommended, across all patients, in particular in phase 2 and 3 trials, for ad hoc assessment and guidance of future analyses.

MetaHeps™

The MetaHeps™ test uses hepatocyte-like cells derived from individual patients' monocytes, cultured for 48 hours, and to measure LDH release as primary cytotoxicity endpoint.[→286] A study in 54 patients with acute liver injury of whom 31 had been diagnosed with idiosyncratic DILI compared

[12] Supplemental Appendices are available online only and can be freely downloaded from the CIOMS website at https://cioms.ch/publications/product/drug-induced-liver-injury/

outcomes of the MetaHeps™ assay with RUCAM scores.[→287] Patients had been exposed to NSAIDs, oral anticoagulants, anti-infectives, immunomodulators and antithyroid medications. MetaHeps™ was able to diagnose DILI correctly in 29 of 31 patients. The assay will undergo further validation in the *Trans*lational *S*afety *Bio*marker Pipe*line* (TransBioLine) consortium under the umbrella of the EU's Innovative Medicines Initiative.

4.3.3 Imaging as liver safety biomarker

While imaging methods such as abdominal ultrasound or cross-sectional imaging using CT or MRI have a well-established role in diagnostics of clinical liver disease, their role in confirming a diagnosis of DILI is rather limited to addressing specific questions, *e.g.* assessment of liver fat content and fibrosis. Nevertheless, liver imaging studies are important to support exclusion of potential alternative causes of liver injury such as focal lesions, vascular diseases of the liver and pancreaticobiliary disease and malignancy.

Table 10 provides an overview of imaging methods used in DILI with well-defined clinical application. Technologies considered still at research level with respect to DILI are not included, although some of them, such as positron emission tomography (PET),[→288] hybrid PET-MRI,[→289] magnetic resonance spectroscopy (MRS),[→290] and single photon emission computed tomography (SPECT) [→291] methods may advance to more routine clinical application in the foreseeable future.

Table 10. Imaging methods with application in DILI diagnosis and assessment

Method	Key application	Comment
Ultrasound (US) and contrast-enhanced ultrasound (CEUS)	Detection of focal lesions, cholestasis. Assessment of liver fat content, liver fibrosis, and biliary tract disease [→292]	Low resolution, limited assessment of diffuse liver disease. Established use *e.g.* as transient elastography (FibroScan®) in assessing liver steatosis and fibrosis.[→293]
Computed tomography (CT)	Detection and texture analysis of focal lesions, assessment of hepatic steatosis, exploration of hepatic vascular and biliary system	CT has been used *e.g.* to demonstrate increase in liver density associated with Amiodarone, indicating hepatic steatosis/phospholipidosis [→294–295]
Magnetic resonance imaging (MRI)	Detection of focal lesions, quantification of fat accumulation and advanced fibrosis and biliary tract disease [→296–297]	Compared to CT, higher contrast-to-noise ratio, lack of ionizing radiation exposure. Application of contrast agents with capability to explore both extracellular and hepatocellular compartments.[→298] Not able to definitively differentiate simple steatosis from NASH or accurately identify small changes in the degree of liver fibrosis.[296] Magnetic resonance elastography (MRE) may be superior to FibroScan® in diagnosing significant and advanced liver fibrosis,[297, →299]
Diffusion-weighted (DW) and perfusion-weighted (PW) MRI	Improved liver lesion detection and characterization. Diagnosis and assessment of liver fibrosis and cirrhosis.[→300–301]	Performance and added value for assessment of fibrosis to be investigated further.[300, 301]
Magnetic resonance cholangiopancreatography (MRCP)	Identification of sclerosing cholangitis–like changes in patients with cholestatic DILI.[→302–303]	Further studies of follow-up imaging in patients with chronic cholestatic DILI needed.[302]

CHAPTER 5.

POST-MARKETING SURVEILLANCE FOR DILI

Summary

- Collection of data and DILI cases from post-marketing surveillance can provide key insights into susceptibility factors and signal detection for idiosyncratic DILI in the general population.
- Approaches used to assess the safety of drugs in the general population can be broadly categorized into active and passive surveillance.
- Most countries use a passive approach of voluntary post-marketing reporting. In those countries that have mandatory reporting the rates of adverse event detection tend to be higher than those without this requirement.
- Many reports of post-marketing hepatotoxicity have incomplete or missing, baseline data and lack other key elements of evaluation such as concomitant medications and co-morbidities, making causality assessment challenging.
- Data sets comprised of electronic medical records (EMRs) may lack useful data elements but can help to identify novel risk factors and features of DILI (*e.g.* drug-drug interactions, time to onset).

Conclusions / recommendations

1. Pharmacoepidemiology studies such as those that monitor prescription drug-event pairs, utilize ICD-10 codes in administrative databases, or perform data mining of the U.S. FDA's adverse event reporting system (FAERS) and WHO's VigiBase are strongly recommended for signal detection of potentially hepatotoxic drugs and herbal and dietary supplement (HDS) products in the marketplace.
2. Minimal data elements should be interrogated and reported in all DILI case series publications to facilitate interstudy comparisons and support the education of clinicians.
3. Safety signals arising from spontaneous datasets should not automatically generate alarm, but should trigger an in-depth investigation for a drug of concern including comparative population-based studies to characterize and quantify the post-market DILI risk.[→304]

5.1 Rationale and challenges

Clinical trial and post-marketing data play different roles in providing information on DILI events. While clinical trial data provides more precise data on incidence (at least over the period of observation in the trial) and severity of relatively common events, post-marketing reports can help in detecting signals of DILI events during use of the drug under real-world circumstances.

In a study conducted by the U.S. FDA study it was reported that even when drugs have been studied in large clinical trials and undergone extensive regulatory review, post-marketing boxed warnings or safety withdrawals may still occur. Slightly more than 20% of safety issues leading to post-marketing withdrawals or boxed warnings were related to rare adverse events that are difficult to detect in preapproval clinical trials (*e.g.,* hepatotoxicity, hypersensitivity, and serious skin reactions).[→305] Since idiosyncratic DILI is typically rare, clinical studies are generally underpowered to detect DILI risk. (See also the explanation on the "Rule-of-Three" on page 45).

Assessing and managing the risk of liver injury in the post-market phase is therefore essential. This would ideally be based upon data derived from prospective monitoring of patients in routine clinical practice for hepatotoxicity using widely accessible tools and assays, accompanied by complete and prompt reporting of the cases to marketing authorization holders and regulatory authorities. This information will support both individual patient care and ongoing population level monitoring for potential regulatory action.

In practice, the monitoring for hepatotoxicity and prompt reporting of adverse events are not always adequate, for several reasons.

- Patients in routine clinical practice are typically monitored less intensively than are research participants since the drugs that they are taking have been approved on the basis of a favourable risk/benefit profile.
- Many providers are inexperienced in recognizing and assessing hepatotoxicity since it is typically a rare event.
- Where clinical guidelines or product labels recommend monitoring of hepatoxicity, providers may either be unaware of them or may not comply, resulting in poor adherence. For example, suboptimal adherence has been demonstrated with the extensive guidelines for liver monitoring and DILI risk management for isoniazid,[→306–307] and with the product label recommendations for troglitazone.[→308–309]

5.2 Sources of data

The Report of the CIOMS Working Group VIII, Practical Aspects of Signal Detection in Pharmacovigilance [→310] and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E2E guideline on planning pharmacovigilance activities [→311] both provide an overview of the data sources and analytical approaches that can be used in post-marketing surveillance. Approaches used to assess the safety of drugs in the general population can be broadly categorized into passive surveillance, which relies

on voluntary, spontaneous reporting, and active surveillance, which seeks to ascertain the number of adverse events via a continuous pre-organized process.

5.2.1 Spontaneous reporting

Spontaneous adverse reaction reporting is one of the core pharmacovigilance activities allowing regulatory authorities to assess and potentially introduce additional safety measures (such as revised labelling) following licensure. Large data sets of spontaneous reports of adverse drug reactions provide a unique opportunity for early identification of safety signals, especially for rare events such as DILI.[→312-313]

Many countries have pharmacovigilance systems in place that enable clinicians and other individuals to spontaneously report suspected adverse events associated with the use of a drug or device. In some countries, *e.g.* France and Spain, the reporting of adverse events is mandatory while others have adopted a passive approach to adverse event reporting.

A well-known example of a spontaneous report programme is MedWatch, developed by the U.S. FDA. It is among the largest regulatory reporting systems for adverse events. [→314–315] Currently, over 2 million individual case safety reports (ICSRs) are submitted each year into the FDA Adverse Event Reporting System (FAERS) through MedWatch.[→316] These reports have repeatedly been shown to be very valuable in the detection of liver safety signals at an early time point after a drug has been approved. For example bromfenac, a non-steroidal anti-inflammatory drug, was withdrawn from the market following numerous reports of hepatotoxicity in patients who had taken the medication for longer than the recommended 10-day period,[→317] and the anti-diabetic troglitazone was withdrawn three years later based on the reports of liver injury including acute liver failure associated with its use.[→318] More recently, MEDWATCH reporting has also led to boxed warnings and restrictions for drugs that were recently approved for patients with underlying liver disease, including obeticholic acid.[100] and the combination of paritaprevir, ritonavir, ombitasvir and dasabuvir (PROD regimen) for patients with hepatitis C [101, →319] (see also page 15).

In Europe the EudraVigilance data analysis system (EVDAS) is used by the European Medicines Agency's Pharmacovigilance Risk Assessment Committee (PRAC) to detect and assess safety signals. At the end of 2018 the database held over 14.5 million ICSRs referring to over 8.3 million cases.[→320] An updated list of safety signals discussed by the PRAC since September 2012 is available online.[→321]

At the global level, Uppsala Monitoring Centre maintains the VigiBase database on behalf of WHO. Reports of suspected adverse effects of medicines are submitted from over 130 countries that are members of the WHO Programme for International Drug Monitoring. This database has wide global coverage, although reports from some countries are disproportionately represented. As of May 2019 VigiBase contained over 20 million ICSRs, of which about 2.5 million came from low- and middle-income countries.[→322]

Limitations

Spontaneous ADR reporting has several limitations. The reports submitted to regulatory authorities or pharmaceutical companies often have incomplete or missing data about the timing and dose of medication, concomitant medication use, lack of diagnostic serologies, and incomplete follow-up. Furthermore, regulators and pharmaceutical companies frequently have limited ability to get additional information from the patient or to review source documents directly. Lastly, there can be considerable delays in reporting of adverse events to health authorities and overall there is likely substantial under-reporting.[315] Underreporting is a well-recognized phenomenon in post-licensure spontaneous reporting *e.g.* to VigiBase, EVDAS and FAERS. It has been estimated that only about 4-6% of DILI cases eligible for reporting to the health authorities are in fact spontaneously reported.[3, 306] Even in countries with mandatory reporting, it has been estimated that less than 6% of hepatic adverse events are reported.[3]

To improve the ease of reporting and quality of data received, many regulatory agencies have moved towards electronic data submission. However, this approach also has certain limitations. The MEDWATCH system does not have a causality assessment tool built into it, and its available forms are meant to ascertain adverse events associated with all organ systems. As such, MEDWATCH forms do not contain data fields designed specifically to adjudicate suspect cases of DILI.

Some of these limitations can lead to detection of false safety signals. A study of spontaneous reports in the U.S. FDA's publicly available FAERS detected a disproportionality signal of DILI for rivaroxaban, a direct Factor Xa inhibitor. However, there has been no such association with other drugs within the same therapeutic class. The authors found that a considerable proportion of drug-associated liver injury reports of rivaroxaban (42%) and dabigatran (37%) without a case level filter for causality included possible concomitant treatment with hepatotoxic and/or interacting drugs.[→323] Measuring cumulative proportional reporting ratios (PRRs) using spontaneous reporting databases such as FAERS may be an effective signal detection tool, but it has limited utility for signal evaluation since it does not quantitatively measure drug-related risk.[314] Risk analysis of the suspect drug also requires causality assessment of reports as a subsequent step to signal detection.

5.2.2 Electronic medical records

Electronic medical records (EMRs) included in healthcare datasets may address certain limitations of the spontaneous reporting systems.

In recent years, there has been increased interest by regulatory authorities to utilize large electronic databases for the purpose of post-licensure safety assessment. For the purpose of safety assessment, these data sources are typically derived from data collected in the course of clinical care, and consist of either medical claims data (*e.g.* based on ICD-10 or CPT codes, used for billing) and/or electronic medical record data. Although these data were not collected for the purpose of pharmacovigilance, several regulatory authorities have introduced systems to utilize such data to characterize emerging safety topics following drug licensure. In the U.S., many patients are enrolled

in health insurance plans that contain detailed data regarding medication use, laboratory data, and diagnostic codes. Use of databases such as the Kaiser Permanente EMR database has proven valuable for the detection of DILI.[→324] Two other examples—the U.S. FDA-developed Sentinel Initiative, a national electronic monitoring system for post-marketing product safety,[→325] and the Medical Information Database Network (MID-NET) of Japan, which started full operation in 2018—are described below.

Examples of large-scale databases used for safety monitoring

Sentinel Initiative (U.S.): *In 2016, the U.S. FDA activated the Sentinel System and officially integrated it into the agency's routine drug safety operations. As of July 2019, the Sentinel System contains data for more than 300 million people, collected through a distributed data network consisting of 18 data partners and many more collaborating institutions.[→326] The results of pharmacoepidemiological analyses by the Sentinel Initiative, such as an assessment of intussusception risk after rotavirus vaccination in U.S. Infants [→327] and risk analysis of gastrointestinal (GIH) or intracerebral haemorrhage (ICH) events following new use of dabigatran or warfarin, have validated some of the research methods utilized by the Sentinel Initiative. Currently there are no algorithms that have been validated to interrogate DILI signals in Sentinel. The testing of one proposed strategy to reliably identify all cases of severe acute liver injury within the Mini-Sentinel pilot of the Sentinel database, irrespective of aetiology, demonstrated poor performance with a low positive predictive value.[→328]*

MID-NET (Japan): *The Pharmaceuticals and Medical Devices Agency (PMDA) started full operation of the MID-NET (Medical Information Database Network) in April 2018.[→329] MID-NET consists of claims and electronic medical record data collected from 10 medical institutions and enables the near real-time assessment of drug safety using large quantities of data (currently representing 4 million patients). Data is generally updated on a weekly basis. In addition to insurance claims data the system contains a variety of information, including laboratory test data. Approximately 200 laboratory tests are currently available for analysis.*

Pilot studies have confirmed that MID-NET could monitor laboratory test data such as ALT, AST or ALP etc. and compare the incidence rate of abnormal laboratory results between drugs. In DILI causality assessment methods such as RUCAM, some important inputs include liver test results, drug administration timing, ADR occurrence date, concomitant treatments, etc. This information is available in MID-NET. It is thus expected that useful information for DILI will be obtained easily and widely by using a large-scale electronic medical record (EMR) database which includes laboratory test data. The outcome definition to assess DILI by databases should be validated for its sensitivity and specificity.

Advantages and limitations

Electronic medical records have the advantage of being "real world" data, *i.e.* they record actual patient experiences observed in the setting of routine clinical care. In addition, it is possible to aggregate a large amount of data from millions of patients. On the other hand, the databases have the potential limitations of:

1) incomplete characterization of patients as the databases may not contain all data forming an episode of care;
2) lack of systematic data collection (compared with clinical trials); and
3) lack of representativeness (*e.g.* only insured patients may be covered).

Nonetheless such data sources may serve as an important evidentiary bridge between data collected through spontaneous reporting, and formal hypothesis-driven epidemiologic research (which is able to collect data prospectively and systematically).

Approaches to data mining

International Classification of Diseases Clinical Modification (ICD-CM) coding systems

EMRs include extensive patient-level medical information that can be accessed using diagnostic codes entered through the International Classification of Diseases Clinical Modification (ICD-CM) coding systems. ICD-CM-based phenotyping algorithms have been applied to EMRs to characterize DILI in several studies.[→330–331] These algorithms should be evaluated for precision and accuracy through measures such as positive predictive value (PPV) and negative predictive value (NPV).[→332] The performance of detection algorithms may vary because of inconsistent DILI definition and detection criteria. It should be noted that ICD-9 does not include DILI-specific codes, and the accuracy and reliability of ICD-10 codes that relate to drug-induced hepatitis and hepatotoxicity have not been established.

A recent report from the Mini-Sentinel pilot project assessed the utility of various ICD-9-CM codes to identify patients with severe acute liver injury with a positive predictive value of 24.7%.[328] A meta-analysis of studies using ICD-9 codes for DILI detection indicates a highly variable positive predictive value that was generally low with a pooled estimate of 14% and a range of 1-40%.[→333]

A limitation of this approach is that the accuracy of ICD-CM codes cannot be assumed since they are frequently entered by billing coders rather than clinicians. This approach to signal detection leads to under-detecting and is laborious and time-consuming.

Natural Language Processing (NLP)

Other studies have used natural language processing (NLP) algorithms to improve the sensitivity and specificity of electronic medical record mining. In one study, words associated with hepatotoxicity in the EMR were electronically searched in patient records with predefined ICD-9 codes that was highly specific but not sensitive for idiosyncratic DILI with a PPV of 4%.[→334] Other investigators have used a combination of word searching with objective laboratory criteria to retrospectively and prospectively identify DILI cases.[331]

Limitations of this approach include the need for computerized algorithms, case verification, and review of data from other sources. Furthermore, a causality assessment tool needs to be applied to potential DILI cases.

5.3 DILI registries

Summary

- A registry is an organised system that uses observational methods to collect uniform data on specified outcomes in a population defined by a particular disease, condition or exposure.
- DILI registries can augment case detection and enhance signal detection in the general population.
- Samples collected by DILI registries can be used to develop new diagnostic and prognostic biomarkers.

Conclusions / recommendations

1. Comparison of DILI registries from different countries can identify different causal agents and varying causes of DILI, outcomes, and risk factors. [→335]
2. Expanded use of DILI registries is encouraged, and may be especially useful to collect biological samples for mechanistic studies, including genetic studies (GWAS) and other "omics" -based approaches.[13] Registries are also a source of data for studies, *e.g.* case-control studies.
3. To enable future study of candidate biomarkers from collected biological samples in a DILI registry, complete structured sets of demographic, drug exposure, clinical, diagnostic and laboratory data for each patient enrolled in the registry should be obtained and entered into the registry database.

A registry is an organized systems that uses observational methods to collect uniform data on specified outcomes in a population defined by a particular disease, condition or exposure.[→336] Registries provide important data on relative prevalence of agents and may be suited to characterize and detect rare DILI signals in the post-marketing phase.

5.3.1 Advantages

DILI registries have the advantage of capturing demographic, drug exposure, clinical, diagnostic and laboratory data in a structured fashion shortly after DILI onset. The collected information is likely to be more complete and accurate than retrospective data that can be obtained through an EMR review. Furthermore, questionnaires on environmental factors such as smoking, alcohol consumption, diet, concomitant medications, and underlying diseases can be administered in a protocolized and systematic manner.

Registries also allow for the prospective collection of biological samples at DILI onset and periodically thereafter for future mechanistic and prognostic studies. Although pre-treatment samples are not available, analysis of available samples has already proven valuable for the identification of DILI biomarkers and genetic susceptibility factors.[15, 16, →337-339] Furthermore, the ability to gather liver pathology slides and have them read and grouped together is of great value.[190]

[13] For example, approaches based on genomics, epigenomics, proteomics, or metabolomics (see Glossary).

Certain registries such as the U.S. Drug Induced Liver Injury Network (DILIN), the Spanish DILI Registry and the ProEURO DILI Net have the resources to exclude competing causes of liver injury—if not already done—and identify variable patterns of liver injury associated with certain drugs and therapeutic classes. For example, DILIN and the Spanish DILI Registry have provided important insights into the clinicopathological DILI signatures associated with certain drugs, including amoxicillin-clavulanate [→340–341] cefazolin [→342] and statins.[164, →343]

Registries can also offer insights into differences between countries.[5, 75, →344] The analysis of the crude annual incidence rate of DILI per 100 000 inhabitants was estimated at 19.1 cases in Iceland,[5] 13.9 in France,[3] and 2.4 in the United Kingdom.[4] In Sweden, a systematic monitoring system of DILI has been in use since 1966, with regular causality assessment offering the opportunity to evaluate a large number of patients with DILI. In the reports of suspected hepatic adverse drug reactions received by the Swedish Adverse Drug Reactions Advisory Committee erythromycin ranked among the top five implicated drugs.[75]

Perhaps the greatest value that registries provide is the collection of biological samples to help develop improved liver safety biomarkers. DILIN in collaboration with others has recently published data on the potential utility of a series of serum biomarkers from patients with bona fide DILI that may provide diagnostic and prognostic value.[229] DILIN has also recently completed studies exploring the utility of a modified lymphocyte transformation test for patients with suspected DILI.[260] Other studies have explored the prognostic value of serum osteopontin in patients with idiosyncratic acute liver failure (ALF) and serum acetaminophen (paracetamol)-protein adducts for patients with suspected or confirmed acetaminophen overdose,[274, 273] and the utility of serum proteomics and metabolomics in patients with acute DILI.[→345–346]. Registries can also serve as sources of cases for case-control studies, although it can be challenging to identify suitable matched controls.

5.3.2 Current DILI registries

There are a number of DILI registries worldwide currently enrolling patients with DILI (**Table 11**). As can be seen in the table, the entry criteria for each study and time from DILI onset vary as well as the number of study visits and duration of follow-up. In regard to the suspect agents, patient features, and outcomes one can see variation worldwide. Interestingly, studies from China and Korea demonstrate a high rate of herbal and dietary supplements (HDS) hepatotoxicity. However, the incidence of HDS hepatotoxicity also appears to be increasing in the U.S. DILIN and the Spanish registry. The individual drugs causing DILI differ between Europe and the United States. This may in part be due to varying availability of different drugs worldwide as well as differences in medical practice and preferred agents.

In addition to DILI registries, cohorts can also be found in the literature, in which DILI cases have been collected for the purpose of epidemiological studies. Here the case collections are limited to a predetermined time period and rarely involve biological sample collections.[3, 5, →347-350]

For details on DILI registries and epidemiological studies see the supplemental **Appendix 7**.[14]

[14] Online only – freely downloadable via the CIOMS website, https://cioms.ch/publications/

Table 11. Prospective DILI registries operating at the time of writing

Note: The data shown in Table 11 reflect available information at the time of writing.

Name:	Spanish DILI registry [25]	ALFSG [26]	DILIN [23]	Japanese DILI registry [24]	LATINDILIN [27]	Pro-Euro DILI Net	DILI-P [→351]
Type:	National	National	National	National	International	International	National
Country:	Spain	U.S.	U.S.	Japan			China
Initiation:	1994	1998	2003	2010	2011	2014	2016
Inclusion criteria:	**(a)** (1994-2010) **(b)** (from 2011)	**(c)**	Age >2 years, **(d)**	ALT≥ 150 U/L or ALP≥ 2 × ULN	**(b)**	Age >18 years, **(b)**	**(e)** (2016-2018) **(b)** (from 2019)
Exclusion criteria:	APAP overdose		APAP overdose		APAP overdose	APAP overdose	
Causality assessment tool:	RUCAM	none	DILIN expert opinion, RUCAM	RUCAM scale and DDW-J 2004 score [172] (a modified RUCAM)	RUCAM	RUCAM	RUCAM, expert opinion
Case enrolments:	946* (915‡)	2626* (251‡)	1257* (899‡)	307‡	280* (200‡)	44*	6663*
Leading causes of DILI: (according to referenced studies)	Anti-infectives (37%), nervous system drugs (14%), musculo-skeletal system drugs (11%)	Antimicrobials and antivirals (41%), herbal medications (10%), neurologic medications (8%)	Antimicrobials (45%), herbal agents/dietary supplements (16%), cardiovascular agents (10%)	Anti-inflammatory drugs (11%), antimicrobials (11%), anti-cancer drugs (10%)	Anti-infectives (24%), musculo-skeletal system drugs (11%), herbal and dietary supplements (10%)	Not yet available	Not yet available
Biological sample collection:	Yes	Yes	Yes	Yes	Yes	Yes	Yes

ALF=acute liver failure, **ALFSG**=Acute Liver failure Study Group, **ALI**=acute liver injury, **ALT**=alanine aminotransferase; **ALP**=alkaline phosphatase, **APAP**=acetaminophen (paracetamol), **AST**=aspartate aminotransferase, **CBL**=conjugated bilirubin, **DILI-P**=A Prospective Cohort Study on Drug-induced Liver Injury in China, **DILIN**=Drug-Induced Liver Injury Network, HDS=herbal and dietary supplements, **INR**=international normalized ratio, **LATINDILIN**= Latin American DILI Network, **ND**=no data, **Pro-Euro-DILI-Net**=Prospective European Drug-Induced Liver Injury Network, **TBL**=total bilirubin, **ULN**=upper limit of normal.

Inclusion criteria:

(a) CIOMS consensus criteria: 1) ALT or CBL >2 × ULN or 2) a combined increase in AST, ALP or TBL provided one of them is >2 × ULN.[→352]

(b) New consensus criteria: 1) ALT ≥5 × ULN, 2) ALP ≥2 × ULN or 3) ALT ≥3 × ULN + TBL >2 × ULN (see reference [20])

(c) Acute liver failure: Encephalopathy, INR ≥1.5, and acute onset of illness <26 weeks,
Acute liver injury: acute illness <2 weeks (APAP), INR ≥2, ALT ≥10 × ULN or acute illness <26 weeks (non-APAP), INR ≥2, ALT ≥10 × ULN and TBL ≥3 mg/dL.

(d) i) ALT or AST >5 × ULN or ALP >2 × ULN on two consecutive occasions; ii) TBL >2.5 mg/dL and elevated AST, ALT or ALP; iii) INR >1.5 and elevated AST, ALT or ALP.

(e) ALT or AST>2×ULN or ALP>2×ULN

Case enrolments: *=Unpublished results: personal communication or departmental results, ‡=Number of cases included in referenced study

Leading causes of DILI: Note: In the Japanese DILI registry 546 causative agents were determined for the 307 cases.[24] The percentages are based on the total number of agents.

CHAPTER 6.

CLINICAL CARE

6.1 Monitoring standard serum liver tests in patients on hepatotoxic drugs

Summary

- There is very little data on which to base recommendations for standard serum liver test monitoring when prescribing potentially hepatotoxic drugs.
- Lack of adherence to stopping rules of anti-TB therapy has been shown to increase the risk of morbidity of mortality.

Conclusions / recommendations

1. Systematic studies to document the value of routine liver test monitoring in the post-marketing setting are needed.
2. Adherence to stopping rules recommended in product labels is suboptimal in some cases and should be improved.
3. Compliance with recommendations for monitoring should be studied.

Liver test monitoring to detect and prevent hepatotoxicity is recommended in the labelling of many drugs.[→353] Examples are anti-tuberculosis drugs such as isoniazid and the anti-fungal agent terbinafine. For some products the monitoring recommendations in approved labelling differ between jurisdictions. This is because the labelling is decided based on various factors such as available safety information, treatment guidelines and insurance systems in each country. Clinical monitoring and patient education for signs and manifestations of hepatic injury are very important in all patients.

However, it seems that compliance with monitoring recommendations is generally poor.[353, →354] Possible reasons are the questionable cost-effectiveness of frequent testing for rare side effects such as DILI and a lack of documented effect of monitoring for most drugs, moreover the interval of monitoring is often unclear. Furthermore, hepatic adaptation is common in drugs with documented hepatotoxicity.[→355–356] Examples are isoniazid, the low molecular weight heparins tacrine and ximelagatran, which cause ALT elevations in a significant proportion of patients. In most of these patients ALT will normalize despite continuation of treatment.[211, →357–358] However, some

patients will fail to adapt, and in some of them initial ALT elevation can progress to fatal liver injury. Bromfenac, troglitazone, tacrine and ximelagatran are examples of drugs that have been removed from at least one national market after drug approval due to serious hepatotoxicity, whereas isoniazid is still used in the treatment and prevention of tuberculosis because of its known benefit in a medically vulnerable population.

Examples exist where hepatotoxicity associated with some drugs has developed and progressed to severe and sometimes irreversible liver injury between testing intervals. This was observed in clinical trial and/or post-marketing cases associated with troglitazone and ximelagatran.[211] The phenomenon of a rapid acceleration of hepatotoxicity in some patients reinforces an unmet need for new biomarker(s) that would predict a likely course of progression in the severity of hepatotoxicity to reduce the risk for a fatal outcome. Moreover, ALT >3 × upper limit of normal (ULN) or even up to >5 × ULN in monitoring is associated with a high proportion of false positives, leading to high costs of additional studies to exclude non-drug related causes.

It is not clear whether routine liver test monitoring could reliably prevent the occurrence of serious or fatal liver injury by the timely discontinuation of a hepatotoxic drug. It was pointed out a decade ago that there was very little data on the course of hepatotoxicity in patients with DILI in clinical trials on which to base recommendations in product labels for liver test monitoring.[353] This seems largely unchanged, although there is now some evidence to suggest that regular monitoring of liver tests can prevent serious hepatotoxicity with certain potentially hepatotoxic drugs.[→359–360] However, systematic studies to measure the effectiveness of ALT monitoring protocols with specified intervals of serum testing are largely lacking.

6.2 Clinical management of DILI

Summary

- Discontinuation of the suspect drugs or herbal and dietary supplements is important in patients with suspected DILI, although care should be taken not to stop needed treatment unnecessarily.
- Patients with drug-induced jaundice need careful follow-up, and frequent liver testing should be undertaken.
- DILI with prominent autoimmune features may benefit from corticosteroids although doses and duration of therapy are unclear.
- Patients with acute liver failure (early stages) due to idiosyncratic DILI have a <30% likelihood of transplant-free survival and have been shown to benefit from early treatment with N-acetyl-cysteine (NAC).

Conclusions / recommendations

1. Studies in moderate and severe DILI using a safe and effective hepatoprotective agent are needed to improve the outcomes in patients with DILI.
2. In conjunction with discontinuation of the suspect drug, it is unclear what kind of therapy should be undertaken to modify the natural course of DILI. Options under consideration that require further study include ursodeoxycholic acid and corticosteroids for the treatment of certain immunoallergic and autoimmune forms of DILI.
3. Patients with severe idiosyncratic DILI with evidence of acute liver failure should be urgently referred to a liver transplant center.

In a patient with acute liver injury who has recently started a drug that has well documented hepatotoxicity, it is important to discontinue the implicated agent as soon as possible. Although this seems obvious, there are several examples when patients have continued treatment after detection of abnormal liver tests. Long-term follow-up of patients with drug-induced jaundice revealed that most patients recovered completely before discharge from hospital, and that those who did not had been treated significantly longer with the suspect drug on average.[167] In the vast majority of patients with DILI, the patient recovers soon after cessation of the implicated agent and liver tests normalize within weeks or months. Patients with drug-induced jaundice without coagulopathy require close observation. In such cases liver tests should be repeated frequently in order to assess whether the liver function is deteriorating or improving. Several papers have dealt with the important issue of management of DILI [20, 141, 115, 221, 167, →361].

Patients with hepatocellular DILI who present with jaundice and/or coagulopathy often require hospitalization as they are at a high risk of developing acute liver failure (ALF) and may require liver transplantation. There has been an ongoing interest to develop effective treatments that reduce life-threatening outcomes in these patients. In a placebo-controlled trial, the majority of patients with non-acetaminophen (paracetamol)-related ALF that was causally associated with a drug, hepatitis B

infection, idiopathic autoimmune hepatitis or indeterminate aetiologies were shown to benefit from early treatment with intravenous N-acetylcysteine (NAC).[→362] In patients with grade I–II hepatic encephalopathy significant improvement in transplant-free survival was observed compared with placebo (52% vs. 30%, p=0.010). In the subset of ALF patients with DILI (n=45) there was a promising trend for a NAC-associated increase in transplant-free survival from 27% to 58%.[362] In most transplant centres NAC is recommended as the standard of care in ALF patients, particularly early in the course of the disease. Unfortunately, similar trials in children did not show benefit in non-acetaminophen (paracetamol)-induced acute liver failure.[→363–364]

In unselected patients with DILI, corticosteroids have limited value. In a retrospective analysis of patients with ALF of whom 131 had DILI, 69% of those who received corticosteroids survived, compared to 66% who did not receive steroids.[→365] Corticosteroids do not have a documented therapeutic benefit in drug-induced ALF and cannot be recommended in unselected patients. However, patients with DILI who manifest prominent immunoallergic features (*e.g.* eosinophilia, rash and fever) may benefit from a short course of corticosteroids although, so far, no placebo-controlled trials have documented their efficacy.

Drug-induced autoimmune hepatitis (DIAIH) is another important clinicopathological phenotype of DILI.[62, 77, 78, 163, →366–367] Corticosteroids are often used to treat DILI with autoimmune features. They may alleviate symptoms and shorten the course of injury, but their ultimate efficacy has not been established. The liver injury can sometimes resolve without corticosteroid or immunosuppressive therapy. In a recent study of unselected patients with drug-induced autoimmune hepatitis, six of 15 patients recovered spontaneously, whereas nine required corticosteroids.[78]

An optimal protocol for the dosing and duration of corticosteroid therapy to treat DIAIH has not been established. Nonetheless, corticosteroid exposure should generally be kept at a minimum. Most patients with DIAIH can be successfully treated for just a few weeks with 30-40 mg of prednisolone.[62, 77, 78] Patients should be followed thereafter for evidence of relapse. Acute liver injury resembling autoimmune hepatitis associated with immune checkpoint inhibitors is usually treated with systemic corticosteroids at high doses with subsequent tapering to lower oral doses.[366, 367]

Chronic cholestasis following DILI is often treated with ursodeoxycholic acid; however, there is inconclusive evidence supporting the efficacy of this treatment to reduce the severity of liver injury.[141]

In China, magnesium isoglycyrrhizinate (MgIG) which is the magnesium salt form of the saponin, a derivative of glycyrrhizic acid, is the only agent approved for the treatment of acute DILI. In a randomized, double-blind multi-center trial, low dose and high dose MgIG was compared with tiopronin, a standard therapy for DILI in China. The proportions of ALT normalization at Week 4 were significantly greater in the low dose and high dose MgIG groups (85%, and 86% respectively) than in the control group (61%).[→368]

6.3 Liver injury in cancer patients

Summary

- Cytotoxic chemotherapy is often associated with self-limited serum aminotransferase elevations that may abate with continued therapy. However, rare instances of jaundice and liver failure have been reported in association with many of these agents.
- Risk management strategies for potentially hepatotoxic anti-cancer drugs include the monitoring for clinical manifestations of liver injury and abnormal standard serum liver tests at baseline, during therapy at regular pre-specified intervals, and after treatment discontinuation.
- The NCI-CTCAE grading system(see footnote on page 10), is often used to grade the severity of liver test abnormalities for suspected DILI cases in oncology clinical trials, and product labels may refer to these severity grades to recommend risk management actions.
- Hepatotoxic immunotherapy drugs and/or biological agents used in combination with certain other cancer treatments may result in more severe levels of hepatotoxicity than therapy with each single agent.
- Immunotherapy for various metastatic solid organ tumours has significantly improved patient survival, but frequently leads to immune-related adverse events (IRAEs) including hepatotoxicity both in clinical trials and in the post-marketing setting.
- Additional studies of risk factors, prognostic factors, and optimal management strategies are needed for cancer patients receiving potentially hepatotoxic chemotherapy or immunotherapy alone or in combination with other anti-tumour agents.
- High NCI-CTCAE grades associated with increased serum ALT and/or AST, without an abnormal elevation of total bilirubin, do not necessarily signify a more clinically severe form of liver injury than lower grades.

Conclusions / recommendations

1. All oncology patients should undergo standard serum liver testing before, during and after treatment with chemotherapy, immunotherapy or new targeted treatments that cause liver injury.
2. Patients receiving intravenous infusions of potentially hepatotoxic immunotherapy should undergo standard serum liver testing before each infusion; administration should be delayed or discontinued based on clinical findings and/or prespecified abnormal serum biochemical test results.
3. Pre-treatment assessment for hepatic metastases with contrast-enhanced magnetic resonance imaging (MRI) or computed tomography (CT) imaging is recommended prior to the administration of chemotherapy, immunotherapy, or new agents that are potentially hepatotoxic in all oncology patients who are at increased risk for hepatic spread of their tumour.
4. Patients with advanced NCI-CTCAE grades of hepatotoxicity associated with immunotherapy can frequently be managed with short courses of intravenous or oral corticosteroids along with interruption of immunotherapy. Patients with clinically serious liver injury that does not improve with steroids may require additional treatment with an immunosuppressive agent, although standard algorithms and approaches for this adverse event have not yet been established.

6.3.1 Chemotherapeutic agents

Almost all antineoplastic agents are associated with some degree of hepatotoxicity, as well as toxicity in other organs. The hepatotoxicity associated with standard chemotherapeutic drugs is often direct and dose-dependent.[→369–370] However, idiosyncratic mechanisms also frequently contribute to serious liver injury with many agents in this class.[→371] Moreover, hepatotoxicity with these agents can manifest a variety of abnormal histological patterns and clinicopathological phenotypes (**Table 12**).

6.3.2 Newer classes of cancer therapies

Immunotherapy

Immunotherapy represents a broad category of treatments in tumour management. As a general principle, agents used for this purpose are intended to activate or increase immunological activity directed against a patient's neoplasm.

Immune checkpoint inhibitors (ICIs)

While use of ICIs has shown a substantial therapeutic benefit marked by a significant improvement in patient survival in the treatment of a number of different tumour types, the corollary reduction of immune self-tolerance caused by these agents has also led to a set of safety concerns represented by an increase in immune-related adverse events (IRAEs), including hepatotoxicity.[→372-374]

Indeed, treatment-emergent hepatotoxicity, also known as immune-mediated liver injury caused by ICIs (ILICI), is being detected in clinical trials powered for efficacy.[→375] Hepatotoxicity has been reported in up to 6% or 7% of patients due to anti-CTLA-4 or PD1 agents, whereas the combination of both ipilimumab and nivolumab increased the rate to 30%.[→376–377] A recent meta-analysis of published data found that CTLA-4 inhibitors were associated with a higher rate of all-grade and high-grade hepatotoxicity than PD-1 inhibitors.[→378] In general, anti-PD-1 therapy appears to be associated with less severe toxicity than ipilimumab.[→379]

Patients may present with abnormalities ranging from asymptomatic increases in aminotransferases to acute hepatitis leading to fulminant liver failure. Time to onset is typically 6 to 14 weeks after treatment initiation, although liver injury can occur after longer periods of therapy and even after discontinuation of the agent.[379, 367] In the largest series of 17 melanoma patients developing ILICI, a mainly hepatocellular pattern of injury was seen, with concurrent IRAEs in 47% of the cases (gastrointestinal, endocrine, dermatological and lung disorders). The median time to resolution after initiation of immunosuppression was 31 days (range 6–56 days) with a median of 42 days on corticosteroids (range 7–78 days).[367]

Table 12. Liver injury associated with cancer chemotherapies

Notes: (1) The table may not include every new therapeutic class of oncologic treatments and only lists some products as examples. (2) Risk data can evolve with new patient populations, combined treatments and other factors. Sources such as updated product labels, regulatory agency websites and the LiverTox® database should be consulted if detailed information for a product is required.

Type	Mechanism(s) of hepatotoxicity (known or suspected)	Examples of associated drugs	Phenotype of liver injury
Direct			
	Alkylation of DNA leads to damage to small blood vessels in the liver	busulfan (when given for prolonged periods)	Nodular regenerative hyperplasia (NRH)
	Alkylation of DNA leads to damage to hepatic sinusoidal lining cells	busulfan, cyclophosphamide melphalan (at high doses used in bone marrow transplant conditioning regimens); dacarbazine	Sinusoidal obstruction syndrome (SOS)
		oxaliplatin	SOS; histological changes that may progress to NRH
	Inhibition of mitochondrial function	fluorouracil	Macrovesicular steatosis, portal inflammation Hepatic dysfunction that can induce rapid onset of hyperammonaemia and coma
	Inhibition of thymidine triphosphate, necessary for DNA synthesis	floxuridine, fluorouracil	Sclerosing cholangitis-like syndrome (higher risk with floxuridine but combination of floxuridine and fluorouracil has additive effects)
	Inhibition of methionine synthesis leading to endoplasmic reticulum (ER) stress and activation of stellate cells by excess of homocysteine	methotrexate	Steatohepatitis, fibrosis, cirrhosis
	Production of toxic intermediates during drug metabolism in the liver	fluorouracil, cytarabine	Hepatocellular or cholestatic injury
		doxorubicin	Acute liver injury with jaundice (rare)
		irinotecan, etoposide	Steatosis and steatohepatitis
	Inhibition of protein synthesis and lipoprotein export	l-asparaginase, pegaspargase	Hepatic dysfunction sometimes leading to coma accompanied by hepatic steatosis
	Hypersensitivity	hydroxyurea	Syndrome of fever and acute hepatitis arising 1 to 3 weeks after starting the drug
Indirect			
	Immunomodulatory actions	romidepsin rituximab	Reactivation of hepatitis B or Epstein-Barr virus infection
	Secondary to effects on gastrointestinal motility, gut microbiome and bile acid levels	octreotide	Acute liver injury
	Estrogenic effects on fat metabolism in the liver	tamoxifen	Fatty liver and steatohepatitis
	Reduction of TNF-α production affecting liver regeneration; or worsening preexisting hepatic conditions	thalidomide, lenalidomide	Acute liver injury, which can be severe
Idiosyncratic			
	Unclear	temozolomide, cyclophosphamide, melphalan, chlorambucil, azathioprine, mercaptopurine, tamoxifen	Acute liver injury (mostly cholestatic)

Source of information: [369] and subpages

Potential host factors for ILICI are not yet well characterized. Pre-existing liver diseases which are associated with increased expression of PD-L1 and PD-L2 can be predisposing factors for ILICI.[375] Likewise, silent hepatic metastases, by promoting the expression of liver self-antigens and the release of pro-necrotic cytokines, may facilitate pro-inflammatory pathways that can synergize with ICI-activated T cells.[375]

Strategies for effectively managing ILICI have been proposed by several societal groups, including the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), and the Society for Immunotherapy of Cancer (SITC).[211, 374, →380] Risk management measures include routine liver test monitoring during therapy and after treatment discontinuation. Once significant liver injury is detected, management includes drug discontinuation and frequent use of corticosteroids or an immunosuppressive agent for persistent or worsening liver injury.[375] However, optimal data-based criteria for when to start corticosteroids may be lacking since product label recommendations often follow the protocols used in their corresponding clinical trials. A recent study based the decision on laboratory (bilirubin > 2.5 mg/dL and/or INR > 1.5) or histological indicators of severity. Six out of 16 patients assessed according to these pre-established guidelines did not receive corticosteroids and spontaneously improved.[404]

Other immunotherapies

In addition to the ICIs, other agents that stimulate immunity against tumours are under development. These include antibody-drug conjugates (ADCs). Although hepatotoxicity has been observed with this class of agents, the potential of liver injury differs depending on the toxophore. Direct hepatocellular injury [→381] and fatal liver injury have been reported.

There are many ongoing clinical trials investigating bi-specific T cell engager (BiTE®) technology, and hepatotoxicity has also been observed with BiTE® inhibitors. BiTE® antibody is a type of fusion protein engineered from two flexibly linked, single-chain antibodies, with one that has binding specificity for a selected tumour antigen and the other for CD3 expressed on T cell surface membrane.[→382] Blinatumomab is the first member of this class of agents approved by the U.S. FDA for relapsed or refractory B-cell precursor acute lymphoblastic leukaemia in adults and children. The majority of liver enzyme elevations observed in the clinical trials for blinatumomab were in the setting of cytokine release syndrome (CRS) in which the median time to onset of transient elevated liver enzymes was 3 days. For events that were observed outside the setting of CRS the median time to onset was much longer, 19 days, and few patients discontinued the agent due to elevation of liver enzymes.[→383]

Protein kinase inhibitors

This large group of antineoplastic agents specifically targets protein kinases whose activities are altered in cancer cells accounting for some of their abnormal growth. Many protein kinase inhibitors have been associated with related to clinically apparent liver injury that can be hepatocellular or cholestatic. The liver injury is generally self-limited but may be fatal with some agents. In some cases of protein kinase-induced DILI there were features of autoimmunity, suggesting that the liver injury

may be caused by an immunological autoreactivereaction. At least two protein kinase inhibitors (imatinib and nilotinib) have been linked to the reactivation of hepatitis B that may be due to their immunosuppressive effects or the potentiation of hepatitis B virus replication. [→384]

An understanding of why protein kinase inhibitors are hepatotoxic is currently still limited. Notably, many of these products have only been on the market for just a few years. In a recent review [→385] among the product labels of 53 kinase inhibitors approved by the U.S. FDA as of October 2019, 35 contained warnings for liver injury, including seven with boxed warnings. Although a number of mechanisms may explain why so many of these agents are associated with hepatotoxicity, sufficient scientific data are lacking in this area to draw firm conclusions.[→386–389]

Some of the newly approved precision (targeted) therapies are associated with elevation of liver enzymes. Whether these findings with some products in this category represent an increased risk for clinically significant liver injury will require further study.[→390] The *NTRK* gene fusion inhibitors (*e.g.* larotrectinib and entrectinib) are a new class of drugs that have been approved for tumour-agnostic indications, *i.e.* their indications of use are based on the cancer's genetic and molecular features without regard to the tumour type or location. Entrectinib also functions as a *ROS/ALK* inhibitor. Crizotinib, an *ALK/ROS1/MET* inhibitor, has known hepatotoxicity with fatal cases.[→391] Several *RET* inhibitors (selpercatinib, pralsetinib) have also shown elevated liver enzymes in clinical development, [→392-393] prompting a warning for hepatotoxicity in the product label of selpercatinib after its recent approval in the U.S.[→394]

Pexidartinib is a CSF1R inhibitor approved in the U.S. for the treatment of adults with symptomatic tenosynovial giant cell tumour not amenable to surgical intervention. Because of the liability for serious liver injury of this kinase inhibitor the prescribing information has a boxed warning for fatal hepatotoxicity. Pexidartinib can only be distributed under a strict Risk Evaluation and Mitigation Strategy (REMS) programme in the U.S., in which both prescribers and the dispensing pharmacies must be trained and certified in its correct use. Across clinical trials, there were pexidartinib-induced cases of biliary ductopenia associated with vanishing bile duct syndrome, an irreversible and potentially fatal condition.[→395]

An overview of some newer cancer therapies and their currently known hepatotoxic potential is provided in **Table 13**.

Table 13. New classes of cancer therapies and their hepatotoxic potential

Notes: (1) The table may not include every new therapeutic class of oncologic treatments and only lists some products as examples. (2) Risk data can evolve with new patient populations, combined treatments and other factors. Sources such as updated product labels, regulatory agency websites and the LiverTox® database should be consulted if detailed information for a product is required.

Drug class	Mechanism of action	Drug examples	Liver injury potential (as currently observed)
Immune checkpoint inhibitors (ICIs)	ICIs block the cell surface activities of CTLA-4, PD-1 protein or PD-L1 in order to stimulate anti-tumour immune responses	*CTLA-4 inhibitor:* ipilimumab *PD-1 inhibitors:* nivolumab [→396], pembrolizumab. [→397-398] *PD-L1 inhibitors:* atezolizumab, avelumab, durvalumab, cemiplimab [→399], sintilimab [→400-401])	Immune-mediated liver injury including hepatitis (a) with some distinct histological patterns (b)
Antibody-drug conjugates (ADCs) [→402]	Cytotoxic drugs covalently linked to monoclonal antibodies directed to antigens differentially overexpressed in tumour cells	brentuximab vedotin polatuzumab vedotin inotuzumab ozogamicin gemtuzumab ozogamicin trastuzumab deruxtecan trastuzumab emtansine	Most but not all ADCs are associated with liver toxicity, including fatal liver failure. Pattern of liver injury differs depending on the toxophore
Bispecific T cell Engager (BiTE®) antibody inhibitor	BiTE® molecules are a type of fusion protein engineered from two flexibly linked, single-chain antibodies, with one specifically for a selected tumour antigen and the other specifically for CD3 found on T cells.	blinatumomab	Elevations in in liver enzymes, especially in the context of CRS
NTRK fusion inhibitors (NTRKi)	Used in patients with *NTRK* gene fusion-positive tumours. Trk fusions may activate signal transduction leading to oncogenesis.	larotrectinib entrectinib	Mild, elevations in liver enzymes
RET inhibitors (RETi)	The *RET* gene codes for a transmembrane receptor tyrosine kinasewith proto-oncogene properties. *RET* fusions or rearrangements are somatic juxtapositions of 5' sequences from other genes with 3' *RET* sequences encoding tyrosine kinase.	selpercatinib pralsetinib (not approved for marketing at the time of writing)	Transient elevations in liver enzymes. Some CTCAE grades 3-4 adverse liver events observed with selpercatinib
FGFR inhibitors	FGFRs are a subset of tyrosine kinases which are unregulated in some tumours and influence tumour cell differentiation, proliferation, angiogenesis, and cell survival.	erdafitinib	Mild, transient elevations in liver enzymes
Pi3Ki	Inhibitors of phosphoinositide 3-kinase enzymes, whose activities regulate the PI3K/AKT/mTOR pathway	idelalisib duvelisib copanlisib alpelisib	Idelalisib can cause severe acute hepatocellular injury and acute liver failure. Mild elevations in liver enzymes observed with some other Pi3Kis
BTK inhibitors	BTK is a non-receptor kinase that plays a crucial role in oncogenic signalling, which is critical for proliferation and survival of leukaemic cells in many B cell malignancies.	ibrutinib zanubrutinib acalabrutinib	Clinically serious liver injury is only a rare occurrence with some of these agents. Reactivation of type B hepatitis has been reported.
CSF1R antagonist	CSF1 is a cytokine that is is produced at high levels by tenosynovial giant cell tumour (TSGCT) and some other neoplasms. The cytokine activates tumour-associated macrophages and Kupffer cells which abundantly express CSF1R.	pexidartinib	Can cause serious and potentially fatal liver injury. Occurrence of hepatotoxicity with ductopenia and cholestasis
Alpha-specific VEGF inhibitor and PD1/PDL-1	*(in combination):* VEGF inhibitors may potentiate the effect of PD1/PDL-1	avelumab plus axitinib pembrolizumab plus axitinib	These combined treatments with axitinib increase the frequencies of higher CTCAE grades of hepatoxicity

ADC=antibody-drug conjugate, **BTK**=Bruton's tyrosine kinase, **CSF1R**=Colony stimulating factor 1 receptor, **CTLA-4**=cytotoxic T-lymphocyte antigen-4, **FGFR**=fibroblast growth factor receptor, ***NTRK***=neurotrophic tyrosine receptor kinase, **PD-1**= programmed cell death-1, **PD-L1**=programmed cell death ligand 1, **PI3K**=phosphoinositide 3-kinase, **Trk**=tropomyosin receptor kinase

(a) In contrast to idiopathic autoimmune hepatitis (AIH), ICI-related hepatitis is typically "seronegative", not presenting with antinuclear antibodies (ANA), anti-smooth muscle autoantibodies (ASMA) or other AIH-associated autoantibodies and—upon ICI discontinuation—responds to a course of immunosuppressive therapy with no recurrence.[375]

(b) Histologically, in a series of five cases of severe hepatitis related to ipilimumab there was a prominence of portal and periportal inflammation and hepatocyte necrosis with infiltrating lymphocytes, plasma cells and eosinophils similar to what is observed with acute viral and autoimmune hepatitis.[→403] A single-centre large-scale study including a per protocol-liver biopsy for patients with hepatotoxicity grade ≥3 has defined distinct patterns of liver damage for anti-CTLA-4 and anti-PD-1/PD-L1 agents, with anti-CTLA-4 drugs inducing a specific pattern of granulomatous hepatitis associated with severe lobular necrotic and inflammatory activity, fibrin deposits and central vein endothelitis. A more heterogeneous histological pattern of injury has been observed with anti-PD-1/PD-L1 agents alone. It is characterized by active hepatitis with spotty or confluent necrosis and mild to moderate periportal activity, which were not associated with granulomatous inflammation.[→404]

6.3.3 Labelling recommendations for anti-cancer therapies

Summary

- Information on liver toxicity in the labels for oncology products is mostly based on hepatotoxicity identified during clinical development rather than post-marketing data.
- If there is evidence of potential liver injury, the EU Summary of Product Characteristics (SmPC) and U.S. product information are generally consistent in including information of hepatotoxicity in the respective labels.
- There is a lack of harmonization between product labels regarding the format, location, and level of detail of information on hepatotoxicity. Guidance on liver monitoring is often included, however time to DILI onset, biochemical profile, dose modification tables or information on re-challenge are not always provided.
- Some oncology products include a "boxed warning" on hepatotoxicity in the product label and/or require additional risk evaluation mitigation strategies (*e.g.*, REMS in the U.S.).

Conclusions / recommendations

1. Information collected during drug development of oncological agents relating to liver toxicity (time of onset, pattern of injury) should be standardized. This would also help to standardize the information in the label (see Point 3 below).
2. Information on individual product liver toxicity (*i.e.*, mechanism, pattern of injury, time of onset) should be used for assessing overlapping liver toxicities with combination therapies.
3. Consistency of information on liver toxicity in drug labels in terms of location (sections within the label), format, and level of detail (*e.g.* monitoring schedule, dose modification table) is highly recommended.

Introduction

Given the poor prognosis of many cancer patients and the limited treatment options for this population, many oncology drugs have been approved despite severe or fatal hepatotoxicity, as the benefit of the drug is considered to outweigh the risk of hepatotoxicity. The goal of including guidance on monitoring in the label is to help detect patients at risk of liver injury and to help manage the risk in those patients with elevated liver enzymes. Information about liver toxicity is often included in the sections on warnings and precautions, dose modification, adverse drug reactions (ADRs), in information on laboratory abnormalities, and/or in the section on the use of the drug in specific populations. Some products have a boxed warning, the strongest warning in U.S. FDA-approved product labels.

Review of labelling information for selected anti-cancer products

Product information of 40 selected oncology compounds across different classes of anti-cancer drugs was reviewed for this report to highlight the differences and similarities of U.S. FDA-approved product labels that describe and provide information to mitigate the risk of liver injury caused by each agent. The findings are summarized in the supplemental **Appendix 8.**[15] In most instances, this risk was identified and characterized during clinical development and was not based on post-marketing data. Most of these product labels provide some instruction or recommendation to guide monitoring practices for liver abnormalities in the label, but only a few have a boxed warning for hepatotoxicity. Risk evaluation and mitigation strategies (REMS) were instituted in the U.S. in 2007 to develop and implement tools to minimize risks while preserving benefits for some newly marketed products with demonstrated safety concerns.[→405] Based on the nature of the identified product-related risks and benefits each REMS programme incorporates risk mitigation tools that are determined to be most appropriate for the individual drug and indicated patient population. Among the 30 products listed in the supplemental Appendix 8 that were approved after 2007, some have an associated REMS programme.[→406]

Product labels for the older drugs were found to have very little information related to liver toxicity. The newer chemotherapies, such as calaspargase pegol, listed severe hepatic impairment as a contraindication and recommended discontinuation when appropriate. The clinicopathological phenotype of injury is not always described in the labels, with the exception of a few protein kinase inhibitors. In their approved product labels, the hepatotoxicity of imatinib observed in an animal study is described to be based on a finding of hepatocellular necrosis, elevated liver enzymes, bile duct necrosis, and bile duct hyperplasia,[→407] and that of regorafenib as *"manifested by elevated liver function tests or hepatocellular necrosis, depending upon severity and persistence."*[→408] The time of onset (latency) of hepatotoxicity is often not described in the labels.

Monitoring recommendations

For standard chemotherapies the recommendations often vary depending on the specific drug (*i.e.*, mode of action, dosing, indication, underlying disease) and the severity of liver injury.

[15] Supplemental Appendices are available online only and can be freely downloaded from the CIOMS website at https://cioms.ch/publications/product/drug-induced-liver-injury/

The recommended monitoring is usually more frequent at the beginning of the treatment. The U.S. product labels for idelalisib,[→409] pazopanib [→410] and regorafenib [408] all recommend weekly monitoring in patients experiencing elevated liver tests above 3 × ULN until improvement to less than 3 times the ULN or baseline, regardless of the duration on therapy.

For immune checkpoint inhibitors, the onset of liver toxicity may be delayed as compared with other types of cancer therapies. The frequency of monitoring and details of dose modification were not well defined in the labels reviewed. For example the label of pembrolizumab [→411] states: *"Monitor for changes in hepatic function. Administer corticosteroids (…) and, based on severity of liver enzyme elevations,* withhold or discontinue*"*, and the label of nivolumab recommends to *"Monitor patients for abnormal liver tests prior to and periodically during treatment."*[→412] The recommendation to monitor "periodically" may not provide sufficient guidance for treating physicians who are not familiar with the drug. More detailed guidance on monitoring and dose modification for immunotherapies may need to be included in the product labels as further data accumulate for this class of therapies. On the other hand, the ipilimumab product label is one of the few reviewed that have an attached REMS. In addition it contains recommendations for themonitoring and management of the hepatotoxicity associated with the product.[→413]

For anti-drug conjugates, which can cause different patterns of liver injury depending on the molecule that causes the toxicity, the labels reviewed include very specific frequencies of monitoring.

Literature on the compliance with liver testing and monitoring requirements in the oncology population is scarce. A recent study conducted for pazopanib as part of post-approval regulatory commitments [→414] found that liver monitoring by prescribers was less than recommended on the label. Compliance was highest for baseline testing with 73–74% and decreased to 37–39% for 4-weekly on-treatment testing. Among patients who should have had weekly testing, the compliance was 56%.

Dose modification and re-challenge

Many of the 40 oncology compounds reviewed include recommendations for dose modification in the label. This is even true for products with boxed warnings on hepatotoxicity in the U.S. product labels, for example idelalisib.[409] The labels of trastuzumab emtansine, regorafenib and pazopanib all recommend permanent discontinuation if AST or ALT increases to > 20 × ULN (NCI-CTCAE Grade 4) or bilirubin to >10 × ULN (NCI-CTCAE Grade 4). In contrast, the labels for pembrolizumab and nivolumab appear to be more conservative as they recommend permanent drug discontinuation in patients with AST or ALT > 5 × ULN (NCI-CTCAE Grade 3) or total bilirubin > 3 × ULN (NCI-CTCAE Grade 3). All of the above drug labels recommend a permanent discontinuation of drug treatment in patients who develop serum aminotransferases > 3 ×ULN and concomitant total bilirubin > 2 × ULN.

Reexposure or re-challenge of a patient to a medication or agent thought to be responsible for DILI is usually not advisable. However, in oncology deliberate re-challenge may be warranted if the medication is considered life-saving and the initial injury was mild and rapidly reversed upon stopping treatment. Therefore, recommendation for resuming treatment (or re-challenge) can be seen as part of the dose modification guidelines in the labels.

Conclusions

The recommended frequency of liver enzyme monitoring was found to vary in the product information reviewed, but a higher frequency is usually recommended at the beginning of treatment and based on the severity of the liver enzyme elevation. Measures to manage DILI include dose interruption, dose reduction, and permanent discontinuation when the risk of hepatotoxicity is considered to outweigh the benefit of the drug. The frequency of recommended liver toxicity monitoring appears to be more dependent on the nature and severity of the hepatotoxicity than on the route of administration of the drug.

One should be mindful that initial labels are based on limited data obtained during drug development, and that the information collected in clinical trials may differ between development programmes. Dose modification tables are not always included in the product labels, and the amount of information on liver toxicity and the location of the information within the label also varies. Consequently, the labels are sometimes vague, allowing for specific medical decisions to be made between the individual physician and the patient. Once a drug is approved, however, labels may be updated in line with additional data from post-approval clinical trials and/or spontaneous post-market reports.

The format and the granularity of the information on hepatotoxicity varies between product labels. Moreover, details on the hepatotoxicity of a product are often scattered in multiple sections of the label (*i.e.* Dose modification, Warnings and Precautions, Adverse Reactions, or Use in Specific Populations – Hepatic Impairment). It is worthwhile to consider more consistent ways of presenting information on hepatotoxicity and other adverse events in product labels in the future.

6.4 Liver injury in patients treated with anti-tuberculosis chemotherapies

Summary

- Many antibiotics and chemotherapeutic agents have been used for the treatment of tuberculosis.
- Due to the nature of the disease and potential for development of antimicrobial resistance, the use of combinations of different classes of anti- tuberculosis drugs for an extended time increases the potential for development of adverse events including DILI.
- DILI during treatment for tuberculosis is usually detected as elevated serum aminotransferase levels. An increased risk of liver failure has been reported with several treatment combinations.
- Combinations with drugs used to treat other concomitant diseases, particularly HIV/AIDS, exacerbate the risks and/or severity of DILI during treatment of tuberculosis.
- Clinical risk factors for hepatotoxicity of anti- tuberculosis chemotherapy include older patient age, HIV infection, duration of treatment, and underlying viral hepatitis.

Conclusions / recommendations

1. Clinicians should be aware of :
   (a) the increased risks of DILI during treatment for tuberculosis, and
   (b) the fact that treatment of concomitant diseases such as HIV/AIDS can further increase the risks and/or severity of DILI.
2. The prescribing information in the product labels of anti-tuberculosis chemotherapies should be followed to optimize the selection and use of appropriate agents
3. Practice guidelines for serum liver test monitoring should be adhered to during treatment with anti-tuberculosis chemotherapies.
4. Stopping rules in practice guidelines should be followed when patients show either clinical or prespecified laboratory evidence of DILI.

Background

Tuberculosis (TB) is a major health problem in both developing and developed countries due to persistent poverty and its resurgence in immunosuppressed patients. The World Health Organization (WHO) in 1993 declared tuberculosis to be a "global emergency". About a quarter of the world's population are infected with *M. tuberculosis* and thus at risk of developing tuberculosis disease; about 10 million new cases of tuberculosis occurred in 2018 globally.[→415]

Treatment of *Mycobacterium tuberculosis* disease has employed more than twenty antibiotics, semi-synthetic antibiotics and chemotherapeutic agents over the years since the 1940s.[→416-417] The propensity of *M. tuberculosis* to develop resistance to these agents has required that combinations of agents are administered in order to limit the development of resistance during treatment. As

treatment regimens may be for an extended time (6 months to 2 years) the potential for adverse events to manifest themselves is worsened relative to short term treatments.

Treatment of multidrug-resistant tuberculosis requires a combination of second-line drugs. These are generally more costly and have a higher risk of side effects. As patients will receive these drugs in combinations, the influences of the second-line and add-on drugs on liver injury are difficult to ascertain. As a result most will include a warning on risk of liver injury when used in combination with the first-line drugs. The classification of anti- tuberculosis drugs helps the clinician to build an appropriate regimen for multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis cases that do not fulfil the criteria for the shorter MDR-TB regimen.[→418]

DILI risk and the value of monitoring

DILI has been a concern in the treatment of tuberculosis for over three decades. Liver injury associated with isoniazid can occur in up to 20% of patients treated.[→419-422] Despite this there is limited documentation of the impact of monitoring with liver tests to detect and prevent DILI. A non-randomized study suggested that monitoring decreased the severity of pyrazinamide-induced liver injury.[359] Interestingly, according to studies from the 1970s as well as more recent studies, isoniazid-related hospitalization rates declined from 5 to 0.1 to 0.2 per 1 000 treatment initiations, and mortality was reduced from 1 to 0-0.3 per 1 000 treatment initiations.[→423-426] The reasons behind this progress is not clear. It is conceivable that active monitoring for adverse reactions to isoniazid may be at least partly responsible, but education and better patient selection might also play a role.

Isoniazid monotherapy to prevent the reactivation of latent tuberculosis was the subject of two large clinical trials in similar populations. The earlier surveillance study [424] included monthly monitoring for signs and symptoms of liver injury in approximately 13 800 patients who received isoniazid for one year; eight of these patients died from hepatic failure. In the more recent study [→427] approximately 11 000 patients were educated to discontinue treatment if they had mild symptoms suggestive of liver injury. This monitoring led to permanent withdrawal of isoniazid therapy in only 11 patients, and no patient died from liver failure during the trial.

A recent report from the DILIN group found isoniazid to be the second most commonly reported agent associated with DILI in the DILIN study (after amoxicillin-clavulanate).[74] Interestingly, in these patients there was poor adherence to monitoring guidelines for isoniazid-induced hepatotoxicity and for reporting of hepatotoxicity.[306] It took patients a median time of 9 days to stop taking isoniazid after developing stigmata of liver injury (range, 0-99 days). Thirty-three patients (55%) continued taking isoniazid for more than seven days after the American Thoracic Society (ATS) stopping criteria [→428] were met, and 24 patients (40%) continued for more than 14 days after meeting stopping criteria. A delay in drug discontinuation was associated with significantly more severe liver injury. Out of 13 patients who died or underwent liver transplantation, 9 (70%) had continued taking isoniazid for more than seven days after meeting stopping criteria. Of 25 cases of isoniazid hepatotoxicity eligible for reporting to the CDC only one was actually reported.[306]

A recent study [→429] of HIV-uninfected tuberculosis patients receiving first-line treatment showed that 25% developed evidence of DILI (defined as ALT >3 × ULN with clinical symptoms, or asymptomatic ALT elevation>5 × ULN), and 25% developed severe hepatotoxicity (ALT>10 × ULN).

In a 2013 study of patients that experienced liver injury on anti-TB combination therapy [→430] about 70% developed jaundice, and 25% developed acute liver failure; increased mortality was associated with DILI accompanied by jaundice, encephalopathy or ascites.

In a study of children with DILI, those who had hypersensitivity reactions had a more benign outcome than those who did not, and the fatalities observed in the study were largely the result of chemotherapy for tuberculosis.[→431]

Results of a few studies have suggested that risk factors of hepatotoxicity due to anti-tuberculosis medications include chronic hepatitis B and C infection,[→432] but other studies have failed to show this increased risk with hepatitis B infection.[→433]

Recommendations for monitoring

According to an ATS statement,[357] serum ALT monitoring is recommended during treatment of latent tuberculosis 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 hepatitis, are pregnant or are within three months postpartum. During treatment of tuberculosis disease, in addition to these individuals, patients with HIV should receive monitoring and some experts recommend it in those older than 35 years of age. Treatment should be interrupted and, generally, a modified or alternative regimen should be used for those who have ALT elevation $>3 \times$ ULN in the presence of hepatitis symptoms and/or jaundice, or $5 \times$ ULN in the absence of symptoms.[357]

Product labelling

The approved product information of agents used for tuberculosis treatment attempts to mitigate the risk of hepatotoxicity. Product information of 23 tuberculosis chemotherapies was reviewed for this publication (supplemental **Appendix 8**).[16] While warnings about DILI risk was found to be included for 19 products and monitoring recommendations for 12, a boxed warning for DILI is only included in one leaflet, that for isoniazid. This is the most commonly used first-line treatment and is also included in many combinations for second-line and MDR treatments.

[16] Online only – freely available via the CIOMS website, https://cioms.ch/publications/

6.5 Liver injury in patients treated with antiretrovirals

Summary

- Antiretroviral drugs have been used for more than 30 years for prevention and treatment of HIV infection.
- These drugs belong to different classes with specific modes of action and are commonly employed in combinations.
- The potential for adverse effects with highly active antiretroviral therapy (HAART) is increased by many concomitant and diseases and opportunistic infections in HIV patients that may require treatment with other medicines.
- Infection with HIV may result in liver damage and/or may accelerate liver damage caused by hepatitis B virus or hepatitis C virus infection.
- Antiretroviral treatment may also result in immune reconstitution inflammatory syndrome (IRIS) that may cause liver damage.

Conclusions / recommendations

1. Clinicians should be aware of the increased risks of DILI during treatment for HIV/AIDS.
2. The recommendations in product labels supplied with antiretroviral medicines regarding standard serum liver testing should be followed.

Despite vast expenditure on research efforts, human immunodeficiency virus (HIV) remains uncontrolled by vaccines. Novel antiviral drugs have been used for more than 30 years as prophylaxis in uninfected persons, and to treat symptoms, restore and preserve immune function, prolong life-expectancy, reduce viral load and prevent onward transmission of the virus from infected persons.[→434] Antiretrovirals act on basic viral-cellular functions and have the potential for adverse events. Certain classes of antiretrovirals have documented evidence of association with liver injury and these are set out in the approved labelling together with advice on minimizing the effects of treatment.

Antiretroviral drugs have been licensed based on a positive benefit-risk profile in clinical trials and advice on risk mitigation is included in the product labelling and professional information. Many of these drugs have novel modes of action, and the potential for adverse effects is high. These drugs belong to different classes with defined modes of action and are commonly employed in combinations, intended to minimize harms and limit the development of viral resistance mutations that may occur during treatment.

HIV patients often have concomitant diseases and opportunistic infections such as hepatitis B, hepatitis C and tuberculosis that may require treatment with other medicines, increasing the potential for liver toxicity, or interactions leading to DILI.

Even without HAART, infection with HIV may result in liver damage.[→435] Chronic liver disease is common among HIV-infected patients and HIV infection may accelerate liver damage caused by hepatitis B virus (HBV) or hepatitis C virus (HCV) infection.

Co-infection with HIV and HCV and HBV has been shown to increase the risk of hepatotoxicity associated with the use of antiretroviral drugs [102, 103, 104, 105, 106, 107] However, the relative contributions to DILI of HIV infection, HAART and the spontaneous fluctuations of hepatitis B/C viral loads, have not been well described.[108, 109, 110] (See also Section 1.3.2)

In addition, antiretroviral treatment may result in immune reconstitution inflammatory syndrome (IRIS) which can make the symptoms of co-infections such as hepatitis B and tuberculosis worse in HIV patients. There is also a risk of auto-immune-IRIS that has been reported to cause liver injury in HIV patients without pre-existing liver disease.[111]

Product information of seven antiretroviral products from different drug classes was reviewed for this report (supplemental **Appendix 8**).[17] Warnings related to hepatotoxicity and DILI are included in the product information of five of the seven products. As patients receive antiretrovirals in combinations, the influences of individual drugs in liver injury are difficult to ascertain, and most will therefore include a warning on risk of liver injury when used in combination with other drugs. Boxed warnings are required for two of the products, and reported fatal cases of liver injury are mentioned for five products.

[17] Online only – freely available via the CIOMS website, https://cioms.ch/publications/

CHAPTER 7.

DILI RISK MANAGEMENT AND COMMUNICATION

7.1 DILI risk management

Summary

- Risk stratification is a tool used to identify and predict the likelihood of a specific outcome among individuals that may be exposed to a particular drug(s). Risk stratification typically relies upon the product label to communicate known product risks in patient populations that may be prescribed the product.
- Currently available regulatory recommendations to detect and mitigate DILI risk were developed for the setting of closely monitored clinical studies. They are also typically used in product labelling, but might not suffice in the more heterogeneous setting of real-world use of the products.
- Risk mitigation in special patient populations (*e.g.* those with preexisting liver disease or malignancies) remains an unfulfilled goal despite previous international efforts and clinical society consensus statements on this issue.
- The information provided in the product labels of drugs marked by similar risk often varies, even among drugs within the same class, as a result of differences in regulatory, medical and insurances strategies and requirements.

Conclusions / recommendations

1. Descriptions in product labels of risk for DILI (*e.g.*, time course, clinical pattern and mechanism of liver injury) and recommendations for liver monitoring should be informed by available product-related data.
2. Peer-reviewed publications can often provide a rich source of developing information on risk for DILI that is associated with a suspect marketed drug or class of drugs.
3. Regulatory agencies' websites and the LiverTox® database provide useful additional information for clinicians to guide them in managing hepatotoxicity risk.
4. Medical professional societies and practice guidelines generally provide valuable recommendations on the optimal use of potentially hepatotoxic drugs in patients.

7.1.1 Principles

Post-marketing risk management is essential to manage DILI risk in a real-world setting. It is a structured process marked by periodic reviews and updates to calibrate the safety and effectiveness profile of a product, in conjunction with actions to minimize its known associated risks and maximize its benefits for patients. In recent years, there has been a fundamental shift towards the benefit-risk paradigm, focusing on proactive signal detection and periodic benefit-risk evaluation. This shift is well reflected in WHO recommendations [→436] and regulatory requirements.[311, →437-438]

The product label constitutes the basis of information for healthcare professionals on how to use a medicinal product safely and effectively. It is a document with legal standing in alignment with a requirement to communicate structured information to support optimal use of the approved product in a post-marketing setting. The product label is based on preclinical and clinical data reviewed by the regulatory authority. In the U.S., many approved products also have an appended medication guide which has been developed as a communication tool for patients. In Europe the Patient Information Leaflet (PIL) is available in the package and on the internet for all approved medicines. In addition, there are patient alert cards if there are some significant safety issues for the patient to be aware of. The product label describes known product risks (as well as those considered potential, or where there is missing information) in different patient populations that may potentially be prescribed the medicinal product. It is not in the remit of the product label to give general advice on the treatment of particular medical conditions or on monitoring procedures.

Risk stratification is used to identify and predict the likelihood of a specific outcome among individuals with identifiable characteristics that may be exposed to particular drugs.[→439] Following licensure, risk tools for DILI would ideally be based upon data derived from effective monitoring of patients in routine clinical practice for hepatotoxicity using widely accessible tools and assays, accompanied by complete and prompt reporting of the cases to marketing authorization holders and regulatory authorities. To characterize and manage the risk of DILI, a risk management plan may be effective if the following criteria are fulfilled:

- risk factors are well characterized;
- the known features of liver injury due to a drug (the "drug's signature") are well described;
- risk monitoring and management are based on reliable measures that can predict the outcome; and
- the pattern and course of the injury are well defined, accounting for possible variability related to different features of patient groups.

In practice, characterizing DILI risk can be difficult due to inconsistency in nomenclature, uncertainties in pathogenesis, limited data, and low event rates.

The U.S. FDA DILI guidance on the premarketing evaluation of DILI published in July 2009 [7] remains the only available regulatory guidance that specifically addresses risk of drug-associated hepatotoxicity at the time of writing this document. A number of data streams and analytic tools are routinely used by regulatory scientists, drug manufacturers and academic investigators for DILI surveillance and characterization in the post-market phase.[46, 214, →440-441] Standard threshold levels of liver tests

and clinical symptoms have been identified for pre-marketing assessment,[7] and these are typically recommended in the labels of products suspected to cause DILI.

The U.S. FDA guidance [7] acknowledges that DILI risk management recommendations for specific products should be modified in light of accumulating data. There is also agreement that they should be adapted for special populations, *e.g.* patients with preexisting liver disease or malignancies.[7, 20, 375, →442] Yet, despite international efforts and consensus statements from an international expert working group,[20, →443] gaps in effective risk mitigation in these patient populations remain unresolved.[→444]

7.1.2 Challenges in communicating DILI risk in product labelling

Unclear terminology

DILI can be characterized in many different ways, and the terminology chosen in product labelling requires careful consideration.

The terms used to describe DILI in product labelling is often based on the terminology used in adverse event reporting. Ideally, reporters should describe the case by recording the most appropriate diagnosis or medical description of the event. However, terms such as hepatocellular, cholestatic and mixed liver injury are rarely used in adverse event reports, including those from investigators in clinical trials. This is sometimes the case even though the reports do provide laboratory testing information that allows this kind of classification. There is even more imprecise use of specialist terms such as "autoimmune hepatitis" or "steatohepatitis". Even broad classifications and definitions of categories of liver failure such as acute, subacute, acute-on-chronic and chronic liver failure differ internationally, and suggestions have been made to help achieve more uniformity between the East and the West.[→445-446]

Confusion between 'liver function' and 'liver injury'

The severity of liver injury (see Section 1.2.2) and the degree of impaired liver function are two separate concepts, although the terminology and laboratory tests used to discuss these concepts overlap in sometimes confusing ways: for instance, serum ALT and AST are erroneously called "Liver Function Tests" (LFT). These aminotransferases are not measures of liver function, but rather are indicators for the presence of damaged cells, irrespective of whether hepatic function has or has not been compromised. In contrast, markers that are consistent with worsening liver function include increases above normal levels in serum total and direct bilirubin and INR.

To align with inclusion and exclusion protocol criteria in clinical trials that enrol cirrhotic patients, the severity of compromised liver function in cirrhosis is commonly graded by means of the Child-Turcotte-Pugh (CTP) score. CTP is based on a composite of three blood test measures linked to liver function (total serum bilirubin, serum albumin and prothrombin time or INR) and the presence or absence of clinical signs of ascites and hepatic encephalopathy. The scoring system was originally developed as a prognostic indicator of surgical mortality in patients with liver cirrhosis.[→447]

As described in the U.S. FDA guidance on the study of pharmacokinetics in patients with impaired hepatic function, CTP has proven useful in Phase I studies measuring the pharmacokinetics of medications in subjects with liver dysfunction.[→448] Cirrhotic patients can manifest a broad range of changes in the uptake of a drug, its metabolism and/or clearance by the liver. These changes can have a strong impact on the dosing requirements of the drug or whether it can be used safely in patients with defined stages of cirrhosis.[→449-450] It is not unusual for different pharmacokinetic profiles in subjects who have CTP scores of B (moderate impairment) or C (severe impairment) to lead to lower recommended doses in those populations in approved product labels.

However, there are a number of criticisms of CTP scoring. It has only been validated in cirrhosis, and the grading of ascites, which often needs abdominal ultrasound—particularly in suspected new-onset ascites—and hepatic encephalopathy are subjective and may vary over time.[→451] Comparisons of CTP scores to model for end-stage liver disease (MELD) scores have shown varying correlations with prognosis of liver disease in cirrhotic patients awaiting liver transplantation.[→452-453] Currently there is limited literature comparing the two scoring systems as measures of liver function in relationship with drug pharmacokinetics.[→454]

Inconsistent product labelling

Although the Ministry of Health, Labour and Welfare (MHLW) of Japan, the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) guidelines for product labelling follow the same general principles,[→455-456] the information provided in labelling for the same risk often varies even across products of the same class as a result of a variety of factors.[→457–458]

Each actual label sets out the agreed position on the product's benefit-risk profile as distilled during the assessment process by the competent regulatory authority. In addition, the variability in risk communication might be due to regulatory review division strategies, changes in regulatory requirements over time, time lag in adopting new scientific evidence, differences in medical treatment practice and guidelines, and differences between medical insurance systems.

Some discrepancies might be explained at least in part by the perceived level of risk. A study of U.S. FDA-approved labels [→459] showed that the label informativeness score across 95 drugs with labelled hepatic failure or necrosis was highly correlated with the level of perceived hepatotoxicity risk[18] ($p<0.001$); also there was rarely a detailed and specific liver enzyme monitoring schedule, and treatment stopping rules differed between products. Moreover, because the wording of safety-related information may be flexible,[457] heterogeneous semantic descriptions may be proposed by companies and included in approved labels to communicate the risks and their recommended management.[→460]

Discrepancies in the product information about the risk of hepatotoxicity across regions have been described.[459, →461] Hepatic adverse effects and recommendations to manage them may indeed

[18] The section of product labelling that first referenced the possible risk of hepatotoxicity was used as a surrogate for the level of the perceived risk. A drug with hepatotoxicity information listed in the Black Boxed Warning was perceived as having a high level of risk, a drug with information first listed in the Warnings or Precautions section was perceived as having medium level of risk and a drug with only an Adverse Reaction statement as a low level of risk.

be worded differently for drugs with different indications and/or benefit-risk profiles, despite similar risks of hepatoxicity. Examples are found in the supplemental **Appendix 9**.

7.1.3 Examples of risk management failure

Product labelling and additional risk minimization measures are not always optimal to manage DILI risk effectively for specific products on the market. This can occur for a number of reasons:

- Legacy of risks for the first product in a class can affect the risk management strategies of follow-up products in the same class. A U.S. FDA study showed that 20% of boxed warnings for safety issues were related to broader class warnings.[305] Not all such legacy recommendations applied to a new member in a class reflect the subsequently measured risk associated with the newly marketed product.

  Example: The product label of the PPAR-gamma agonist pioglitazone, an antidiabetic of the thiazolidinedione class, incorporated a recommendation to routinely monitor serum liver safety biomarkers that was previously included in the label of troglitazone, the first marketed member of this class of drugs. Ten years later, after post-market risk assessments performed by the sponsor and the U.S. FDA revealed that the pioglitazone-associated risk for serious DILI is significantly lower than that of troglitazone, pioglitazone's label was updated with a removal of the earlier recommendation for routine liver test monitoring.

- Another form of "legacy" is the history of a product itself. The U.S. FDA found that new drugs approved with a boxed warning were almost four times more likely to receive additional post-marketing boxed warnings,[305] and that boxed warnings were associated with increased reporting to the FDA Adverse Event Reporting System (FAERS).[→462]

- Additional risk management measures may unduly limit patients' access to needed products.

  Example: Daclizumab [→463] was approved in the U.S. for access through restricted distribution programmes under a Risk Evaluation and Mitigation Strategy (REMS) to mitigate the risk of liver toxicity. Despite this, cases of acute liver failure occurred with daclizumab, which was subsequently withdrawn from the market following reports of treatment-associated meningo-encephalitis. The effectiveness of the programme in minimizing DILI risk has not been analysed to date.

- Monitoring recommendations in product labels are often patterned after preapproval clinical trial protocols and not driven by data from post-market studies to determine their effectiveness in risk mitigation.

  Examples: The product labels of the immunosuppressants infliximab and natalizumab do not provide detailed instructions when to start monitoring liver laboratory tests and other clinical signs, and how frequently to do this, despite the well described time course and outcomes of liver injury of these two products.

  Infliximab is associated with the risk of HBV reactivation as well as at least three forms of liver injury with separate causes and different clinical outcomes;[→464] Both the EU and U.S. labels simply indicate that patients with symptoms or signs of liver dysfunction should be evaluated for evidence of liver injury.[→465–466]

Natalizumab has been associated with serum aminotransferase elevations in large clinical trials.[→467] Subsequently, a warning for hepatotoxicity was incorporated into the U.S. product label of natalizumab when a number of post-marketing cases of clinically significant DILI were identified.[→468] For natalizumab the U.S. FDA-approved label states that natalizumab "should be discontinued in patients with jaundice or other evidence of significant liver injury (e.g., laboratory evidence)"[→469], while the EU SmPC states that "patients should be monitored as appropriate for impaired liver function […]"[→470] The type or frequency of monitoring is not specified.

- An additional challenge is that even if labelling recommendations are appropriate and up-to-date, there may still be low adherence by health care providers. In clinical practice, providers typically apply flexible criteria of risk stratification that do not precisely follow those described in the product label, as they make therapeutic choices based in part upon a clinical sense of the benefit-risk balance of a medicinal product for an individual patient.

- Risk management failures for specific products may reflect a combination of root causes.
  Example: Despite detailed monitoring recommendations in the product information of the anti-diabetic troglitazone, which were reiterated several times in "Dear Health Care Professional" communications, cases of severe liver injury and ALF continued to occur in patients treated with this product and ultimately led to its withdrawal from the market.[→471] The risk management strategy may have failed because adherence by health care providers to the strict recommendations was poor,[354] and/or because physicians relied only on ALT monitoring. In some cases of troglitazone-induced ALF it was too late to prevent life-threatening liver injury once clinical symptoms or signs of hepatic dysfunction set in.[→472].

7.2 Publicly available information on medicines and DILI risk

In recent years many regulatory authorities have been developing public websites that contain extensive background information for medicines. Some examples are referenced here.[→473-480] Pre-approval clinical trial reviews by regulatory scientists are found on the regulatory websites of countries using the ICH harmonized regulatory requirements, where most innovative medicines are first approved,[→481] while national sites provide information on marketed products for health care professionals and patients, post-market assessments, and safety communications. Regulatory websites include information on hepatotoxicity when this is an emerging concern that can impact overall benefits and risks of a product.

Another useful source of information on hepatotoxicity is the LiverTox® database,[→482] a collaborative project between the U.S. National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the U.S. National Library of Medicine (NLM). It provides valuable up-to-date information on liver injury attributable to prescription and non-prescription medications, herbals and dietary supplements. The information provided in this database is generally complementary with that published in U.S. FDA-approved product labels. LiverTox® provides agent-specific clinical and diagnostic summaries with laboratory test findings related to hepatotoxicity that have been identified in premarket clinical trials and post-market case reports. In the summaries, clinicopathological phenotypes and mechanisms of liver injury are provided when this information is available.

CHAPTER 8.

LIVER INJURY ATTRIBUTED TO HERBAL AND DIETARY SUPPLEMENTS

Summary

- Risk factors for liver injury induced by herbal and dietary supplements (HDS) may be related to HDS products themselves, patient characteristics, and inappropriate use of the HDS products.
- Factors to consider in the assessment of HDS-induced liver injury include different plant species, their geographic origin, the parts of the plant used, as well as harvesting time and processing methods.
- Studies of selected HDS products have demonstrated that certain ingredients stated on product labels may not be present and that other ingredients not listed on the label, including potential hepatotoxicants, may be present.
- HDS are often used concurrently with conventional medicines, making it difficult to judge causality and to identify risks.
- An integrated evidence-based method is essential for causality assessment of HDS-induced liver injury. An example is the 5-tier 6-point approach recommended in China.

Conclusions / recommendations

1. The potential for liver injury with HDS should be considered during their development (although in many cases there is no formal development process) as well as in post-marketing pharmaco-vigilance and when assessing liver injury cases in clinical practice.
2. Little is known about the role of dose, duration of exposure, route and host factor effects such as immunity, heredity, metabolism, and underlying diseases, on the susceptibility to HDS-induced liver injury. Future studies should address these parameters.
3. Thorough collection of risk signals for HDS-induced liver injury from the literature, preclinical safety evaluation, clinical trials and post-marketing evaluation are recommended to understand any underlying safety risks.
4. Risk management measures for HDS-induced liver injury include close observation, adjusting or discontinuing treatment for the individual patient, halting clinical trials, amending the product label (where one exists), restricting commercial circulation and withdrawing products.
5. Enrolment of patients with suspected HDS hepatotoxicity into DILI registries is recommended to characterize the clinical features, risk factors, liver histopathology and outcomes.
6. Further studies of HDS products associated with liver injury using analytical chemistry, mass spectroscopy and toxicological assays are needed to better define the mechanisms and ingredients responsible for the observed hepatotoxicity.

8.1 Introduction

Throughout human history, traditional medicines have significantly contributed to the prevention and treatment of diseases in different countries and regions. The efficacy and safety of many traditional medicines have been shown empirically, although they have not been proven as systematically as for conventional drugs. According to figures from the World Health Organization (WHO), 40% to 80% of the population in developing countries have experienced some form of traditional medicine therapy.[→483–484] In China, traditional medicine accounts for around 40% of all health care delivered, and is used to treat roughly 200 million patients annually.[→485]

Meanwhile, in many developed countries HDS products are becoming more and more popular. The percentage of the population that has used complementary and alternative medicine at least once is 48% in Australia, 70% in Canada, 42% in the USA, 38% in Belgium and 75% in France;[483] and 84% of Japanese doctors are using complementary medicines in their daily practice.[→486] In a prospective study of 307 DILI cases in Japan,[24] dietary supplements and Chinese herbal medicines accounted for 9% and 6% of cases respectively. In Switzerland, approximately half of the population has used complementary medicines according to a variety of surveys conducted since 1990.[→487] In 2008 WHO introduced a classification of traditional medicine into the eleventh edition of its International Statistical Classification of Diseases and Related Health Problems (ICD) for the first time [→488]. However, considering the growing demand for HDS, the attention to these products and the research on their safety have lagged far behind those for conventional drugs.

With increasingly widespread use of HDS all over the world, as well as constantly improving drug administration systems, new challenges for the safe use of HDS are arising. In recent years, HDS-related adverse events including suspected HDS-induced liver injury have occurred more frequently. Because of the complexity of HDS products themselves and a lack research about them, our knowledge and understanding of potential causes of HDS-induced liver injury is very limited. HDS are often used concurrently with conventional medicines, making it difficult to judge causality and to identify risks. In addition, a widespread misconception among the general public that HDS are "natural, without toxic and side effects" poses challenges for risk prevention and control. And importantly, HDS are not subject to drug regulation in most countries, making the prevention and control of HDS safety risks extremely challenging.

8.2 Potential risk factors for HDS-induced liver injury

8.2.1 HDS product-related risk factors

When evaluating HDS-induced liver injury risk, the following interfering factors should be considered:

1) Erroneous substitution of plants can occur due to ambiguous or wrongly translated plant names or aliases used in different countries or regions.[→489] For example, some patients erroneously take *Gynura segetum* (Lour.) Merr instead of *Panax notoginseng* (Burk.) F.H.Chen, or *Aristolochia manshuriensis* Kom. instead of *Akebia quinata (Thunb.)* Decne. The free online

Kew's Medicinal Plant Names Services (MPNS) portal [19]is a very useful resource to validate plant names.

2) The place of origin, plant parts used, harvesting time, processing and formulation method, and exogenous contaminants such as impurities, agricultural and farm chemicals and heavy metal residues, can increase HDS-related risks.

3) Intentional adulteration by adding chemicals or synthetic drugs increases the risk to HDS safety.

Examples:

In Denmark, Shen-fu-cao, a Chinese herbal ointment, was illegally supplemented with a potent corticosteroid (clobetasol propionate) and antifungals (ketoconazole and miconazole), and the clobetasol propionate caused adverse reactions to patients.[→490]

In China, different types of adulterants were detected in traditional Chinese medicines and herbal products, drawing attention to a global concern since traditional Chinese medicines are exported worldwide.[→491]

In the U.S., a recent study from DILIN identified that the majority of HDS products implicated in causing human liver injury were mislabelled. A total of 272 products were analysed using mass spectroscopy, and the ingredients that were chemically identified were compared to the product labels. A high rate of under-reporting of detected ingredients identified in the products was noted compared to the label, and conversely many of the ingredients listed on the label could not be found. Additional studies using analytical chemistry and mass spectroscopy are recommended in future for HDS products suspected of causing liver injury.[→492]

8.2.2 Host-related risk factors

Since HDS-induced liver injury can be idiosyncratic, possible host-related factors such as immunity, heredity, metabolism, underlying diseases and body constitution type should be considered. For example, abnormal immune activation or defects in immune tolerance may increase the susceptibility of the liver to drug toxicity, increasing the risk. Although there are few studies on the susceptibility genes of HDS-induced liver injury, a recent report showed that *HLA-B*35:01* was linked to *Polygonum multiflorum*-induced liver injury,[→493] and another study describes a metabolomic biomarker panel that characterized the risk profiles of patients who had abnormal liver tests after ingestion of *Polygonum multiflorum,* as compared with matched controls.[→494] Determination of such susceptibility biomarkers may prove useful to identify susceptible individuals and thus be helpful for the risk management of HDS-induced liver injury.

8.2.3 Risk factors related to HDS product use

Changes in the route of administration and dosage form of HDS products may increase their safety risks, especially if there is a change from external use to internal administration, from topical to systemic use, or from oral administration to injection. Changes in the dosage and duration of treatment may also significantly increase the safety risk of HDS.

[19] https://mpns.science.kew.org/mpns-portal/version

Changes in indications, which are often accompanied by changes of dosage, duration, or administration route, can also lead to liver injury risks of HDS. For instance, while artemisinin is mainly used for treatment of malaria in China, its use in New Zealand as the main component of dietary supplements to maintain and support joint health and movement recently led to a series of liver injury cases.[→495]

In most countries it is common to find combination use of HDS with conventional medicines, with a potential for interactions.[→496] Since HDS is typically accessible without prescription in most countries there may be drug combinations that physicians are not aware of, and at the same time there is no professional guidance from traditional practitioners. This all poses increased challenges for safe use of HDS.

8.3 Improving detection and management of HDS-induced liver injury

Some examples HDS that have been linked to hepatotoxicity are listed in **Table 14**. However the body of research about HDS DILI is currently limited. Scientists and health care professionals should collect information about HDS DILI risk signals from the literature, preclinical safety evaluations, clinical trials and post-marketing evaluations.

From literature and traditional experiences

If an HDS product has been associated with liver injury in published literature, further evidence should be collected. A potential risk of liver injury should also be considered if a closely related variety of an HDS, or an HDS containing an identical or similar structure, has been associated with liver injury in the literature or a known database. The LiverTox® database currently contains more than 40 chapters summarizing the world's literature on human hepatotoxicity attributed to various HDS products.[→497] This resource may prove useful to clinicians, scientists and regulators interested in HDS hepatotoxicity. In the absence of scientific literature on HDS-related liver injury, experiences documented in ancient classics or public media may also have reference value for identifying potential risks of an HDS.

Preclinical safety evaluation

Preclinical safety evaluation studies would provide useful information for HDS products. However, in many countries HDS are not as strictly regulated as medicines. In China, herbal and traditional medicines are subject to strengthened regulatory requirements [→498] that include an element of preclinical safety evaluation corresponding to the toxicity evaluation requirements by the International Council on Harmonization (ICH), investigating the general toxicity, target organ, and the toxicity mechanism and pharmacokinetics (toxicokinetics), with particular attention to the close monitoring of liver function-related biochemical indicators and pathological changes. These assessments are generating data that would be helpful for regulators in other countries in evaluating HDS products containing the same or similar substances.

For the HDS products that have been associated with a risk of liver injury, research data should be collected on the substances causing the risk, the types of liver injury, the mechanism and the

Table 14. Examples of plants and HDS products linked to hepatotoxicity

Plant or HDS product	Reference source	Research type	Re-challenge
Aloe [→499]	Korea	Case report	Yes
Anabasis articulata extract [→500]	Egypt	Basic research	N/A
Artichoke [→501]	Portugal	Case report	No
Bavachinin (*Fructus Psoraleae*) [→502]	China	Basic research	N/A
Chelidonium majus L. [→503]	Portugal	Review	N/A
Chinese skullcap [→504]	U.S	Case report	Yes
Dictamnine (Cortex Dictamni) [→505]	China	Basic research	N/A
" [→506]	Germany	Review	N/A
Euphorbia hirta [→507]	Malaysia	Basic research	N/A
Fructus Meliae Toosendan [→508]	China	Basic research	N/A
Garcinia cambogia [→509]	Italy	Case series & literature review	No
Germander, black cohosh, Kava extract, and green tea extract [→510]	U.S.	Review	N/A
Ginseng [→511]	U.S.	Case report	No
Green tea extract [→512–513]	U.S.	Case reports	No
Gynura japonica [489, →514]	China	Basic research	N/A
Kava, Kratom and Khat [→515]	Germany	Review	N/A
Nigella sativa [→516]	Malaysia	Basic research	N/A
Noni (*Morinda citrifolia*) juice and phenobarbital [→517]	Croatia	Case report	No
Olive and rosemary leaves extracts [→518]	Saudi Arabia	Basic research	N/A
Petroselinum crispum (Mill) Nyman ex A.W. Hill (Parsley) [→519]	Nigeria	Basic research	N/A
Polygonum multiflorum [→520]	China	Basic research	N/A
[→521]	China	Clinical research	No
[→522]	China	Case report	No
[→523]	China	Clinical research	Yes
Safe Lean™ (*Garcinia cambogia, Trigonella foenum graecum*) [→524]	India	Case report	No
Triptolide (*Tripterygium wilfordii* Hook f.) [→525]	China	Basic research	N/A
Za ga-do Kowa, Ohta-Isan (Cinnamon) [→526]	Japan	Case report	No

relationships among the drug dosage, exposure time, toxicity and efficacy. Attention should also be paid to individual and species differences as well as to differences in the liver injury risk of different disease models in experimental animals.

Case assessment of HDS-induced liver injury in clinical practice

Risk signals of HDS-induced liver injury are the usual liver injury or dysfunction indicators, including clinical manifestations, signs, biochemical indicators, liver pathological and imaging changes and biomarkers. These should be assessed in accordance with the diagnostic criteria of DILI. The pathological and imaging changes induced by HDS in the liver resemble those caused by conventional medicines. A few known herbal products cause some specific changes, for instance *Gynura segetum* (Lour.) Merr and *Senecio vulgaris* L., which may cause hepatic sinusoidal occlusion syndrome (HSOS)/ hepatic veno-occlusive disease (HVOD).[514] This is similar to the liver injury induced by azathioprine and thioguanine leading to HSOS/HVOD.

Research on diagnostic biomarkers of HDS-induced liver injury is scarce, and it is worthwhile to screen specific biomarkers and explore their clinical application. For example a method has been devised to quantify pyrrole-protein adducts present in the blood as a result of ingestion of pyrrolizidine alkaloids contained in *Gynura segetum* (Lour.) Merr and other plant species, potentially serving as hepatotoxicity-specific biomarkers for clinical diagnosis.[→527] 'Whether genetic risk factors play a role in DILI susceptibility to certain HDS products is an area of ongoing interest.[493]

Analysis of HDS product ingredients

Studies by DILIN have demonstrated discrepancies between product labels and chemical analysis in over 50% of potentially hepatotoxic HDS products tested.[492] Therefore one critical avenue to evaluate HDS products that have been associated with a liver injury signal is an analysis of the chemical content of a formulation. Programmes and tools for such analyses exist in the U.S.[→528] In Europe, regulatory approaches introduced in recent years do not currently require testing for pre-market safety;[→529] a reflection paper on new analytical methods and technologies in the quality control of herbal medicinal products is being developed.[→530]

8.4 Causality assessment of HDS-induced liver injury

RUCAM scoring is commonly used for DILI causality assessment, but is not well suited for evaluating the complexities of HDS use and risks. An example of an alternative approach is the evidence-chained method recommended in the *Guidance for the clinical evaluation of traditional Chinese medicine-induced liver injury* drafted by the China Food and Drug Administration.[498] (supplemental Appendix 10).[20]

8.5 Risk prevention and management of HDS-induced liver injury

An important element of preventing and managing HDS-induced liver injury is to raise public awareness of this potential risk. In addition, the gaps in HDS regulation should be objectively recognized, and two long-standing misconceptions eliminated: Firstly, the widespread belief that traditional medicines are natural and inherently safe, that more HDS products are better than less and that the products must be safe and standardized since they are available for purchase without a prescription;[528] and secondly, the misperception that a given type of HDS has no benefits when a specific product or preparation is reported to have toxic and adverse effects. To prevent HDS-induced liver injury care should be taken to reduce or avoid overuse of HDS as well as irrational uses (*i.e.* overdose, excessive duration of treatment, off-label use and unreasonable combinations).

HDS products have a long history of use. Abundant experience has accumulated to guide their safe use, and modern technologies are now used in clinical and laboratory research for early detection of risk signals. To promote an information exchange between countries and regions with different languages and cultures, it would be useful to establish an international database with information on assessment and diagnosis of cases of HDS-induced liver injury. Such a database would provide a platform for

[20] Online only – freely available on the CIOMS website at https://cioms.ch/publications/product/drug-induced-liver-injury/

monitoring, early warning and clinical evaluation of reported cases. In 2019 CIOMS initiated collaborative efforts to promote the sharing of HDS risk information around the world to make the best possible use of existing experience in the prevention and management of HDS-induced liver injury.

Since HDS DILI is difficult to detect, it may be useful to periodically monitor standard serum liver tests in order to identify potential risks in a timely manner. A recently developed paper-based serum aminotransferase activity test for use at home could support such testing in a cost-effective way.[→531] Generally, ALT ≥ 3 × ULN could be the limit for early warning. When it is accompanied by an increase in TBL, INR or obvious clinical symptoms, further monitoring of standard serum liver tests is recommended.

Considering the complexity of HDS-induced liver injury, in 2018 the regulatory authority of China issued its *Guidance for the clinical evaluation of traditional Chinese medicine-induced liver injury*,[498] which elaborates on the recognition and collection of risk signals as well as causality assessment. Aiming to ensure regulatory oversight during the entire life cycle of HDS products and effectively improve the risk prevention and control, this guidance applies the same processes of approval, product use and post-market evaluation to traditional Chinese medicines and ethnic remedies as is the case for conventional medicines. Based on the management experience of China's regulatory authority, consensus has been reached to include HDS as a regulated group of products in its drug administration system.

In the U.S. HDS products are regulated separately from pharmaceutical drugs and biological agents under the Dietary Supplement Health and Education Act (DSHEA) of 1994. This congressional law has given FDA authority over the safety of these products, places some constraints on their manufacturing and labelling, and prohibits their misbranding and/or contamination with adulterants. Recently, FDA announced an intention to strengthen its operations under DSHEA to ensure the effective evaluation of product safety issues and rapid public communication of safety concerns surrounding HDS products.[→532]

With this background, as manufacturers are required to monitor the safety of their products, if an HDS-associated risk of liver injury is suspected they should report and disclose the findings, as well as further investigate the liver injury concern. Based on findings surrounding evidence of hepatotoxicity caused by an HDS product, a national regulatory authority may consider instituting different measures including the issuance of one or more safety communications, the initiation of close observation, the discontinuation of clinical trials, an amendment of the product label, a restriction of its commercial circulation and/or a discontinuation of its marketing. If hepatotoxicity in a patient or consumer is identified by a health care professional, use of the product should be discontinued.

8.6 Conclusion

Further research is needed on how best to prevent and manage the risks of HDS-induced liver injury and other organ toxicities in a systematic manner. Some recommendations are outlined at the start of

this Chapter. With the increasing use of traditional and complementary medicines globally, reliable information to guide their safe use could have a significant public health impact.

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APPENDIX 1.

GLOSSARY

Some of the entries below are sourced from the following past CIOMS pharmacovigilance working group reports:

CIOMS VI: *Management of Safety Information from Clinical Trials. Report of CIOMS Working Group VI. Geneva: CIOMS; 2005.*

CIOMS VII: *Development Safety Update Reports (DSUR): Harmonizing the Format and Content for Periodic Safety Report during Clinical Trials. Report of CIOMS Working Group VII. Geneva: CIOMS; 2006.*

CIOMS VIII: *Practical Aspects of Signal Detection in Pharmacovigilance. Report of CIOMS Working Group VIII. Geneva: CIOMS; 2010.*

CIOMS IX: *Practical Approaches to Risk Minimisation for Medicinal Products. Report of CIOMS Working Group IX. Geneva: CIOMS; 2014.*

CIOMS X: *Evidence Synthesis and Meta-Analysis for Drug Safety. Report of CIOMS Working Group X. Geneva: CIOMS; 2016.*

Active surveillance: A system for the collection of case safety information as a continuous pre-organized process.[→1]

> Active surveillance can be: 1. Drug based: identifying adverse events in patients taking certain products; 2. identifying adverse events in certain healthcare settings where they are likely to present for treatment 3. Event based: identifying adverse events that are likely to be associated with medicinal products, *e.g.*, liver failure.[→2] *Source: CIOMS VIII*

Baseline characteristics: Factors that describe study participants at the beginning of the study (*e.g.*, age, sex, disease severity). In comparison studies, it is important that these characteristics be initially similar between groups; if not balanced or if the imbalance is not statistically adjusted, these characteristics can cause confounding and can bias study results. *Source:* [→3]

Biomarker: A measured characteristic of either normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives. *Source:* [→4]

Boxed warning ("black box warning"): A warning that appears on a prescription drug's label and is designed to call attention to serious or life-threatening risks. Not all health authorities implement boxed warnings in the label, however some health authorities do (*e.g.* those of the U.S., the United Kingdom and Japan). In the U.S., boxed warnings are ordinarily used to highlight for

prescribers one of the following situations: (1) There is an adverse reaction so serious in proportion to the potential benefit from the drug (*e.g.*, a fatal, life-threatening or permanently disabling adverse reaction) that it is essential it be considered in assessing the risks and benefits of using the drug, ***OR*** (2) There is a serious adverse reaction that can be prevented or reduced in severity by appropriate use of the drug (*e.g.*, patient selection, careful monitoring, avoid certain concomitant therapy, addition of another drug or managing patient in a specific manner, avoiding use in a specific clinical situation), ***OR*** (3) FDA approved the drug with restrictions to ensure safe use because FDA concluded that the drug can be safely used only if distribution or use is restricted (…) Infrequently, a boxed warning can also be used in other situations to highlight warning information that is especially important to the prescriber (e.g., reduced effectiveness in certain patient populations). Infrequently, a boxed warning can also be used in other situations to highlight warning information that is especially important to the prescriber (e.g., reduced effectiveness in certain patient populations).

Proposed by CIOMS DILI Working Group, adapted from: [→5]

Candidate gene study: A study that evaluates the association of specific genetic variants with outcomes or traits of interest, selecting the variants to be tested according to explicit considerations (known or postulated biology or function, previous studies, etc).

Source: [3]

Case Report Form (CRF): A printed, optical, or electronic document designed to record all of the protocol-required information to be reported to the sponsor on each trial subject.[→6]

Source: CIOMS VI

Causality assessment: The evaluation of the likelihood that a medicine was the causative agent of an observed adverse event in a specific individual. Causality assessment is usually made according to established algorithms. [Adapted from:→7] *Source: CIOMS VIII*

Context of use (COU):

(EMA) Full, clear and concise description of the way a novel methodology is to be used and the medicine development related purpose of the use. The Context of Use is the critical reference point for the regulatory assessment of any qualification application.

Source: [→8]

(U.S. FDA) A statement that fully and clearly describes the way the medical product development tool is to be used and the medical product development-related purpose of the use.

Source: [4]

Data mining: Any computational method used to automatically extract useful information from a large amount of data. Data mining is a form of exploratory data analysis. [Adapted from:→9]

Source: CIOMS VIII

Disproportionality analysis: The application of computer-assisted computational and statistical methods to large safety databases for the purpose of systematically identifying drug-event pairs

reported at disproportionately higher frequencies relative to what a statistical independence model would predict.[→10] *Source: CIOMS VIII*

Endpoint: A precisely defined variable intended to reflect an outcome of interest that is statistically analyzed to address a particular research question. A precise definition of an endpoint typically specifies the type of assessments made, the timing of those assessments, the assessment tools used, and possibly other details, as applicable, such as how multiple assessments within an individual are to be combined. *Source:* [4]

Epigenomics: The study of all of the epigenetic changes in a cell. Epigenetic changes are changes in the way genes are switched on and off without changing the actual DNA sequence. They may be caused by age and exposure to environmental factors, such as diet, exercise, drugs, and chemicals. Epigenetic changes can affect a person's risk of disease and may be passed from parents to their children. *Source: [→11]*

Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) plot: A log/log display of correlation between peak TBL vs. ALT, both in multiples of ULN, with horizontal and vertical lines indicating Hy's law thresholds, *i.e.* ALT = 3 × ULN and total bilirubin = 2 × ULN. The eDISH plot makes immediately evident subjects potentially matching Hy's law laboratory criteria, all located in the upper right quadrant of the graph.

Proposed by CIOMS DILI Working Group, adapted from: [→12]

Genome-wide association study (GWAS): A study that evaluates the association of genetic variation with outcomes or traits of interest by using 100 000 to 1 000 000 or more markers across the genome. *Source:* [3]

Genomics: The study of the complete set of DNA (including all of its genes) in a person or other organism. Almost every cell in a person's body contains a complete copy of the genome. The genome contains all the information needed for a person to develop and grow. Studying the genome may help researchers understand how genes interact with each other and with the environment and how certain diseases, such as cancer, diabetes, and heart disease, form. This may lead to new ways to diagnose, treat, and prevent disease. *Source:* [11]

Hy's law: A term based on the observation by Dr Hyman Zimmerman that "*drug-induced hepatocellular jaundice is a serious lesion*"**,** with mortality ranging from 10 to 50%. The term applies to patients who develop hepatocellular liver injury attributed to the suspect drug with an AST or ALT level >3 x upper limit of normal (or baseline levels if elevated) and have a total bilirubin > 2 x ULN, without significant initial cholestasis. This observation formed a basis for the development of the e-DISH plot by the U.S. FDA.

Proposed by CIOMS DILI Working Group, adapted from: [→13]

Idiosyncratic DILI (IDILI): A hepatic reaction to drugs that occurs in a small proportion of individuals exposed to a drug and is unexpected from the drugs pharmacodynamic and pharmacokinetic profile in humans. It 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 and less frequently many months. *Proposed by CIOMS DILI Working Group, adapted from:* [→14]

Intrinsic DILI: Intrinsic DILI is typically dose-related and occurs in a large proportion of individuals exposed to the drug (predictable). Its onset is within a short time span (hours to days). *Proposed by CIOMS DILI Working Group, adapted from:* [14]

Labelling: The definition of this term varies by regulatory jurisdiction. In EU legislation the term refers to the information given on the immediate or outer packaging.[→15] In other medicinal product legislation, including that of the United States, labelling may refer more broadly to theapproved content of product information (see *Product information*). *Source: CIOMS IX*

Metabolomics: The study of substances called metabolites in cells and tissues. Metabolites are small molecules that are made when the body breaks down food, drugs, chemicals, or its own tissue. They can be measured in blood, urine, and other body fluids. Disease and environmental factors, such as diet, drugs, and chemicals, can affect how metabolites are made and used in the body. Metabolomics may help find new ways to diagnose and treat diseases, such as cancer. *Source:* [11]

Model for End-Stage Liver Disease (MELD): A numerical scale that is currently used by United Network for Organ Sharing for allocation of livers for transplantation. It is based on objective and verifiable medical data (international normalized ratio, serum total bilirubin level, and serum creatinine level [or dialysis]) that summarize a patient's risk of dying with cirrhosis while awaiting liver transplantation. The MELD-Na score also incorporates the patient's serum sodium level. *Proposed by CIOMS DILI Working Group, adapted from:* [3]

Negative predictive value (NPV): The proportion of those who tested negative who actually do not have a disease or condition. *Source:* [4]

Number needed to harm (NNH): The number of individuals needed to be treated for some specified period of time in order that one person out of those treated would have one harmful event (during some specified time period). NNH is the inverse of the absolute risk difference between a treated and a control group. For example, if the rate of a hepatic event is 5% in the treated group as opposed to 1% in a control group over one year of treatment, the difference is 4%. Thus, on average, 25 people would need to be treated for one year for one person to experience a harmful event (1 in 25 people = 4%). *Source: CIOMS VI, Adapted to include calculation (given in CIOMS VI under "Number needed to treat")*

Odds Ratio (OR): The odds of an event (such as death) in one group compared to the odds in a reference group. Odds are used in betting but have useful mathematical properties in analysis of binary data. For example, if there are 10 individuals studied and 2 experience an event, the probability is 2/10 = 0.2. The odds are 2:8 (2 have the event compared with 8 who do not). Therefore, the odds = 0.25. If these odds are compared with another group in whom the odds are

different, say 0.125, then the odds ratio is 2 (0.25/0.125). With rare events the OR approximates the relative risk. *Source: CIOMS VI*

Passive surveillance (of spontaneous reports): A surveillance method that relies on healthcare providers (and consumers in some countries) to take the initiative in communicating suspicions of adverse drug reactions that may have occurred in individual patients to a spontaneous reporting system. *Source: CIOMS VIII*

Pharmacovigilance: The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems.

Source: [1]

Positive predictive value (PPV): The proportion of those who tested positive who actually have a disease or condition. *Source:* [4]

Post-marketing: The stage when a drug is approved and generally available on the market.

Source: [→16]

Pre-marketing: The developmental stage before a drug is approved and available for prescription or sale to the public. *Source:* [16]

Prevalence: Number of existing cases of an outcome in a defined population at a given point in time. Note. Prevalence is calculated as a proportion (cases divided by total in population), often expressed as a percentage. *Source:* [16]

Product information (PI): Documents proposed by marketing authorisation holders / applicants, amended if required and agreed by regulatory authorities, which provide information to prescribers / healthcare professionals or patients on the appropriate and safe use of a medicinal product. As such the product information constitutes the main tool used for routine risk minimisation. For examples regarding terminology used in different regulatory jurisdictions see Fig. 1.1 in Chapter I of the CIOMS IX report *[reproduced below]*. The EU labelling on the immediate or outer packaging is a part of product information. *Source: CIOMS IX*

Figure 1.1 from CIOMS IX report: Examples of nomenclature for components of product information

PRODUCT INFORMATION (PI)

Product information for HCPs	**Product information for patients**
Summary of product characteristics (SmPC, also sometimes SPC)	Package leaflet
Data sheet	Patient information leaflet
Drug data sheet	Patient product information
Safety data sheet	Patient information
Package insert	Consumer medicines information
Product information	Patient instructions for use
	Patient package insert

Labelling on inner and outer packaging

*HCPs=health care professionals

Proteomics: The study of the structure and function of proteins, including the way they work and interact with each other inside cells. *Source:* [11]

Qualification: A conclusion, based on a formal regulatory process, that within the stated context of use, a medical product development tool can be relied upon to have a specific interpretation and application in medical product development and regulatory review. *Source:* [4]

Real-world data (RWD): Data relating to patient health status and/or the delivery of health care that are routinely collected from a variety of sources. Examples of real-world data include the following: Data derived from electronic health records; medical claims and billing data; data from product and disease registries; patient-generated data, including in-home use and/or other decentralized settings; data gathered from other sources that can inform on health status, such as mobile devices. *Source:* [→17]

Receiver-operating characteristic (ROC) curve: A figure depicting the power of a diagnostic test. The receiver operating characteristic (ROC) curve presents the test's true-positive rate (*i.e.*, sensitivity) on the horizontal axis and the false-positive rate (*i.e.*, 1 – specificity) on the vertical axis for different cut points dividing a positive from a negative test result. An ROC curve for a perfect test has an area under the curve of 1.0, whereas a test that performs no better than chance has an area under the curve of only 0.5. *Source:* [3]

Registry: (Europe) An organised system that uses observational methods to collect uniform data on specified outcomes in a population defined by a particular disease, condition or exposure. *Source:* [15]

(United States) A patient registry is an organized system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition, or exposure, and that serves one or more predetermined scientific, clinical, or policy purposes. *Source:* [→18]

Risk Evaluation and Mitigation Strategy (REMS): A drug safety programme that the U.S. FDA can require for certain medications with serious safety concerns to help ensure the benefits of the medication outweigh its risks. REMS are designed to reinforce medication use behaviors and actions that support the safe use of that medication. While all medications have labeling that informs health care stakeholders about medication risks, only a few medications require a REMS. *Source:* [→19]

Risk factor: Characteristics associated with an increased probability of occurrence of an event or disease.[→20] *Source: CIOMS IX*

Risk management plan (RMP): (In the European Community) A detailed description of the risk management system [DIR 2001/83/EC Art 1(28c)].

The risk management plan established by the marketing authorisation holder shall contain the following elements: (a) an identification or characterisation of the safety profile of the medicinal product(s) concerned; (b) an indication of how to characterise further the safety profile of the

medicinal product(s) concerned; (c) a documentation of measures to prevent or minimise the risks associated with the medicinal product, including an assessment of the effectiveness of those interventions; (d) a documentation of post-authorisation obligations that have been imposed as a condition of the marketing authorisation [IR 520/2012 Art 30(1)].[15]

Source: CIOMS XI

(Note: The CIOMS XI report reflects the definition given in Revision 3 of the EU GVP document; whereas the above entry reflects that in EU GVP Revision 4.)

Risk minimization: In a broader sense the term risk minimisation is used as an umbrella term for prevention or reduction of the frequency of occurrence of an undesirable outcome (see risk prevention) and reduction of its severity should it occur (see risk mitigation). *Source: CIOMS IX*

Risk mitigation: Reduction of the severity of an undesirable outcome should it occur.

Source: CIOMS IX

Risk prevention: Reduction of the frequency of occurrence of an undesirable outcome in a population, population subset or an individual patient. *Source: CIOMS IX*

Sensitivity: The proportion of people with a positive test result among those with the target condition.

Source: [3]

Signal: Information that arises from one or multiple sources (including observations and experiments), which suggests a new potentially causal association or a new aspect of a known association, between an intervention and an event or set of related events, either adverse or beneficial. [Adapted from:→21] *Source: CIOMS VIII*

Specificity: The proportion of people who are truly free of a designated disorder who are so identified by the test. The test may consist of, or include, clinical observations. *Source:* [3]

Sponsor: An individual, company, institution or organisation, which takes responsibility for the initiation, management and/or financing of a clinical trial.[→22] *Source: CIOMS IX*

Structural alerts: In order to identify compounds with potential toxicity problems, particular attention is paid to structural alerts, which are high chemical reactivity molecular fragments or fragments that can be transformed via bioactivation by human enzymes into fragments with high chemical reactivity. The concept has been introduced in order to reduce the likelihood that future candidate substances as pharmaceuticals will have undesirable toxic effects. *Source: [→23]*

Tumour-agnostic therapy: A type of therapy that uses drugs or other substances to treat cancer based on the cancer's genetic and molecular features without regard to the cancer type or where the cancer started in the body. Tumour-agnostic therapy uses the same drug to treat all cancer types that have the genetic mutation (change) or biomarker that is targeted by the drug. It is a type of targeted therapy. Also called tissue-agnostic therapy. *Source:* [11]

Validation: A process to establish that the performance of a test, tool, or instrument is acceptable for its intended purpose *Source:* [4]

References Appendix 1

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APPENDIX 2.

CASE REPORT FORM FOR HEPATIC EVENT

Liver-related signs and symptoms

1. Were liver-related signs and symptoms assessed?	☐ Yes	☐ No
If Yes, please give assessment date, and answer questions 2-23 below:		
a) Assessment date (dd/mm/yyyy)	_____	
Does the patient have:		
2. Fever?	☐ Yes	☐ No
3. Nausea?	☐ Yes	☐ No
4. Vomiting?	☐ Yes	☐ No
5. Abdominal pain?	☐ Yes	☐ No
6. Abdominal tenderness?	☐ Yes	☐ No
7. Joint pain/ arthralgia?	☐ Yes	☐ No
8. Joint swelling?	☐ Yes	☐ No
9. Rash?	☐ Yes	☐ No
10. Urticaria?	☐ Yes	☐ No
11. Mucosal inflammation or ulceration?	☐ Yes	☐ No
12. Asterixis?	☐ Yes	☐ No
13. Confusion/ disorientation?	☐ Yes	☐ No
14. Coma?	☐ Yes	☐ No
15. Jaundice?	☐ Yes	☐ No
16. Ascites?	☐ Yes	☐ No
17. Peripheral oedema?	☐ Yes	☐ No
18. Palmar erythema?	☐ Yes	☐ No
19. Fatigue?	☐ Yes	☐ No
20. Lymphadenopathy?	☐ Yes	☐ No
21. Dark urine?	☐ Yes	☐ No
22. Other liver-related signs or symptoms?	☐ Yes	☐ No
23. If Yes, please specify: _____		

Medical history: Liver-related diseases

(a) Does the subject have a history of:	If Yes, please give start date (dd/mm/yyyy)	(b) If Yes in column (a): Is the condition/ event still ongoing? If No, please give end date (dd/mm/yyyy)
24. Hepatitis A?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
25. Hepatitis B?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
26. Hepatitis C?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
27. Hepatitis D?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
28. Hepatitis E?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
29. Autoimmune hepatitis?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
30. Haemochromatosis?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
31. Nonalcoholic fatty liver disease (NAFLD)?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
32. Nonalcoholic steatohepatitis (NASH)?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
33. Gallbladder disease? Examples: gallbladder stones, cholecystitis, bile-duct stones	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
34. Alcohol-related liver disease? Examples: alcohol related cirrhosis, alcohol related hepatitis, steatosis	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
35. Drug-induced liver injury (DILI)?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
36. Jaundice or hyper-bilirubinaemia?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
37. HIV infection?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
38. Tuberculosis?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
39. Congestive heart failure?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
40. Right heart failure?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
41. Hepatic metastasis?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
42. Diabetes?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___ If ongoing, does the subject require: ☐ Insulin? ☐ Other oral or parenteral agents? ☐ Dietary therapy alone?

(a) Does the subject have a history of:	If Yes, please give start date (dd/mm/yyyy)	**(b) If Yes in column (a):** Is the condition/ event still ongoing? If No, please give end date (dd/mm/yyyy)
43. Inflammatory bowel disease (Crohn's disease or ulcerative colitis)?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
44. Hypotension?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
45. Systemic infection or sepsis?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
46. Seizures?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
47. Recent drop in blood pressure or shock?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
48. Herpes infection?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___
49. Uncontrolled diabetes mellitus?	☐ Yes, started: ___/___/___ ☐ No	☐ Yes ☐ No, ended: ___/___/___

Risk factors for conditions associated with liver disease

Assessment Date: _____

Has any of the following occurred within <u>one week</u> before the hepatic event?	
50. Did the subject engage in vigorous physical exercise?	☐ Yes
51. Has the subject taken acetaminophen (paracetamol)?	☐ Yes
If Yes:	
a) What was the start date of the acetaminophen (paracetamol)?	___ / ___ / ___ (dd/mm/yy)
b) What was the total daily dose of the acetaminophen (paracetamol)?	_____
c) How many days was the subject taking the acetaminophen (paracetamol)?	_____
d) Is the subject still taking the acetaminophen (paracetamol)?	☐ Yes
52. Did the subject eat wild mushrooms?	☐ Yes
If Yes:	
a) On what date did the subject eat wild mushrooms?	___ / ___ / ___ (dd/mm/yy)

Has any of the following occurred within 3 months before the hepatic event?	
53. Assessment date	___ / ___ / ___ (dd/mm/yy)
54. Has the subject gained or lost more than 5 lbs (2 kg) in weight? If Yes: b) Amount of weight gained or lost:	☐ Yes _____ lbs *or* _____kg
55. Has the subject consumed alcohol? If Yes: a) What was the first date of alcohol consumption? c) What was the end date of alcohol consumption? d) What was the amount of beer consumed? e) What was the amount of wine consumed? f) What was the amount of spirits consumed?	☐ Yes ___ / ___ / ___ (dd/mm/yy) ___ / ___ / ___ (dd/mm/yy) ___ g per beverage ____ beverages per day on ___ days per week ___ g per beverage ____ beverages per day on ___ days per week ___ g per beverage ____ beverages per day on ___ days per week
56. Has the subject changed the amount of alcohol consumption recently? If Yes: a) Amount of change in alcohol consumption (+% or –%) b) Date of change	 _____ ___ / ___ / ___ (dd/mm/yy)
57. Has the subject been exposed to anyone with jaundice or hepatitis?	☐ Yes
If Yes, check **ALL** that apply: a) Hepatitis A	☐
b) Hepatitis B	☐
c) Hepatitis C	☐
d) Hepatitis E	☐
e) Jaundice	☐
58. Has the subject obtained a tattoo(s), acupuncture, or piercing?	☐ Yes
59. Has the subject been exposed to an environmental or industrial toxin or a chemical agent?	☐ Yes
If Yes: a) Exposure date:	___ / ___ / ___ (dd/mm/yy)
Check **ALL** that apply: b) Industrial solvent	☐
c) Insecticide	☐
d) Aflatoxin	☐
e) Other	☐ Specify:_____
60. Has the subject travelled to areas at risk for viral hepatitis (A,B,C,D and E)? (Examples include: Hepatitis A–Mediterranean or South America; Hepatitis B–South-East Asia; Hepatitis E–India, Mexico) If Yes: a) Please specify area(s)	☐ Yes _____ _____

Has any of the following occurred within 3 months before the hepatic event?	
61. Has the subject received parenteral nutrition? If Yes: a) What was the parenteral nutrition start date? b) What was the parenteral nutrition end date?	☐ Yes ___ / ___ / ___ (dd/mm/yy) ___ / ___ / ___ (dd/mm/yy)
62. Has the subject had significant weight loss?	☐ Yes
63. Has the subject used recreational drugs or injection drugs? If Yes, check ALL that apply a) Methamphetamines b) Cocaine c) Ecstasy d) Ketamine e) Narcotics f) Other, specify	☐ Yes ☐ ☐ ☐ ☐ ☐ ☐ _____
64. Has the subject had a blood transfusion? If Yes: a) Blood Transfusion Date	☐ Yes ___ / ___ / ___ (dd/mm/yy)
65. List any other risk factors the subject has that were not listed above.	_____ _____ _____ _____ _____

Hepatic imaging studies

66. Was an imaging study performed? If Yes, please answer questions 67-71 below.	☐ Yes
67. Was magnetic resonance imaging (MRI)/ magnetic resonance cholangiopancreatography (MRCP) performed? If Yes:	☐ Yes
a) Date	___ / ___ / ___ (dd/mm/yy)
What were the results? Check ALL that apply:	
b) Hepatomegaly	☐
c) Steatosis	☐ Fat content: _____ %
d) Nodular liver	☐
e) Dilated bile ducts	☐ Result: _____ mm
f) Bile duct stricture	☐
g) Gallstones in gallbladder	☐
h) Gallstones in bile duct	☐
i) Biliary sludge	☐
j) Focal lesion/tumour	☐
k) Portal vein thrombosis	☐
l) Hepatic artery thrombosis	☐
m) Hepatic vein thrombosis	☐
n) Dilated portal vein	☐
o) Ascites	☐
p) Other	☐ Specify: _____
68. Was an abdominal ultrasound performed? If Yes:	☐ Yes
a) Date	___ / ___ / ___ (dd/mm/yy)
What were the results? Check ALL that apply:	
b) Hepatomegaly	☐
c) Steatosis	☐ Fat content: _____ %
d) Nodular liver	☐
e) Dilated bile ducts	☐ Result: _____ mm
f) Bile duct stricture	☐
g) Gallstones in gallbladder	☐
h) Gallstones in bile duct	☐
i) Biliary sludge	☐
j) Focal lesion/tumour	☐
k) Portal vein thrombosis	☐
l) Hepatic artery thrombosis	☐
m) Hepatic vein thrombosis	☐
n) Dilated portal vein	☐
o) Ascites	☐
p) Other	☐ Specify: _____

69. Was a CT scan performed?	☐ Yes
If Yes:	
a) Date	___ / ___ / ___ (dd/mm/yy)
What were the results? Check **ALL** that apply:	
b) Hepatomegaly	☐
c) Steatosis	☐ Fat content: _____ %
d) Nodular liver	☐
e) Dilated bile ducts	☐ Result: _____ mm
f) Bile duct stricture	☐
g) Gallstones in gallbladder	☐
h) Gallstones in bile duct	☐
i) Biliary sludge	☐
j) Focal lesion/tumour	☐
k) Portal vein thrombosis	☐
l) Hepatic artery thrombosis	☐
m) Hepatic vein thrombosis	☐
n) Dilated portal vein	☐
o) Ascites	☐
p) Other	☐ Specify: _____
70. Was ERCP (Endoscopic retrograde cholangiopancreatography) performed?	☐ Yes
If Yes:	
a) Date	___ / ___ / ___ (dd/mm/yy)
What were the results? Check **ALL** that apply:	
b) Dilated bile ducts	☐ Result: _____ mm
c) Bile duct stricture	☐
d) Gallstones in gallbladder	☐
e) Gallstones in bile duct	☐
f) Biliary sludge	☐
g) Focal lesion/tumour	☐
h) Stent placement	☐
i) Ascites	☐
j) Other	☐ Specify: _____
71. Was magnetic resonance spectroscopy (MRS) performed?	☐ Yes
If Yes:	
a) Date	___ / ___ / ___ (dd/mm/yy)
What were the results? Check **ALL** that apply:	
b) Steatosis	☐ Fat content _____ %
c) Other	☐ Specify _____

Liver biopsy

72. Was a liver biopsy performed?	☐ Yes
If Yes:	
a) Date:	___ / ___ / ___ (dd/mm/yy)
Check **ALL** that apply:	
b) Hepatic inflammation	☐
c) Hepatic necrosis	☐
d) Eosinophilic infiltrate	☐
e) Lymphocytic infiltrate	☐
f) Perivenulitis of central veins	☐
g) Central zonal necrosis	☐
h) Hepatic steatosis	☐
i) Steatohepatitis	☐
j) Rosette formation	☐
k) Mallory bodies	☐
l) Emperipolesis	☐
m) Hepatic fibrosis	☐
n) Neoplastic disease	☐
o) Cholestasis	☐
p) Sinusoidal obstruction syndome	☐
q) Vanishing bile duct syndrome	☐
r) Hepatic congestion	☐
s) Granulomas	☐
t) Other	☐ Specify: _____
u) None	☐
v) Unknown	☐

Family history

73. Do any of the subject's first-degree relatives have alpha-1 antitrypsin deficiency?	☐ Yes
74. Do any of the subject's first-degree relatives have autoimmune disease?	☐ Yes
75. Do any of the subject's first-degree relatives have hereditary haemochromatosis?	☐ Yes

Local laboratory tests

Hepatic tests should be monitored during the event until resolution or return to baseline levels, regardless of whether the study drug is continued or not.

76. Were laboratory tests performed?	☐ Yes		
If Yes, please give collection date and answer questions 77-103 below a) Lab specimen collection date:	___ / ___ / ___ (dd/mm/yy)		
Local laboratory test	Result	Units	Normal range
77. Bilirubin, total			
78. Bilirubin, direct			
79. Bilirubin, indirect			
80. Haptoglobin			
81. Aspartate aminotransferase			
82. Alanine aminotransferase			
83. Creatine kinase			
84. Aldolase			
85. Alkaline phosphatase			
86. Gamma glutamyl transferase			
87. Liver-specific alkaline phosphatase			
88. Bone-specific alkaline phosphatase			
89. Prothrombin Time (PT)			
90. PT-INR			
91. Leukocytes (WBC)			
92. Erythrocyte count (RBC)			
93. Haemoglobin			
94. Haematocrit			
95. Platelets			
96. Neutrophils			
97. Lymphocytes			
98. Monocytes			
99. Eosinophils			
100. Basophils			
101. (Other, please specify)			
102. "			
103. "			

Serology tests

104. Were serology tests performed?	☐ Yes			
If Yes, give collection date and answer questions 105-124 below a) Lab specimen collection date:	___ / ___ / ___ (dd/mm/yy)			
Test	Result	Unit	No unit	Not done
105. Urine ethylglucuronide			☐	☐
106. Serum phosphatidylethanol			☐	☐
107. Urine toxicology			☐	☐
108. Antinuclear antibodies			☐	☐
109. Anti-smooth muscle antibody (or anti-actin)			☐	☐
110. Hepatitis B virus surface antigen			☐	☐
111. Hepatitis B virus core antibody			☐	☐
112. Hepatitis B virus DNA			☐	☐
113. Hepatitis B virus surface antibody			☐	☐
114. Hepatitis A virus antibody IgM			☐	☐
115. Hepatitis A virus antibody			☐	☐
116. Hepatitis E virus IgG antibody			☐	☐
117. Hepatitis E virus IgM antibody			☐	☐
118. Hepatitis E virus IgA antibody			☐	☐
119. Hepatitis E virus RNA			☐	☐
120. Hepatitis C virus antibody			☐	☐
121. Hepatitis C virus RNA			☐	☐
122. (Other, please specify)			☐	☐
123. "			☐	☐
124. "			☐	☐

Concomitant medications, or dietary/nutritional supplements

Name of drug	Daily dose	Start date (dd/mm/yy)	End date (dd/mm/yy)	**OR:** Tick if ongoing
125.		___ / ___ / ____	___ / ___ / ____	☐
126.		___ / ___ / ____	___ / ___ / ___	☐
127.		___ / ___ / ____	___ / ___ / ___	☐
128.		___ / ___ / ____	___ / ___ / ___	☐
129.		___ / ___ / ____	___ / ___ / ___	☐
130.		___ / ___ / ____	___ / ___ / ___	☐
131.		___ / ___ / ____	___ / ___ / ___	☐
132.		___ / ___ / ____	___ / ___ / ___	☐
133.		___ / ___ / ____	___ / ___ / ___	☐
134.		___ / ___ / ____	___ / ___ / ___	☐
135.		___ / ___ / ____	___ / ___ / ___	☐
136.		___ / ___ / ____	___ / ___ / ___	☐
137.		___ / ___ / ____	___ / ___ / ___	☐
138.		___ / ___ / ____	___ / ___ / ___	☐

APPENDIX 3.

DILI CASE NARRATIVES

- In this appendix you will find two case reports. The diagnostic work-up described for these cases is performed to exclude other (*i.e.,* non-drug) causes for the liver injury.
- For Case 2 the patient has given a written informed consent that the case may be used for the purpose of this report.
- These cases are not intended to be a template for narratives of DILI but to illustrate the complexity of DILI.
- The presentation of these cases is not intended to endorse any specific recommendation about the treatment of DILI. Rather it is meant as an instructional exercise describing clinical scenarios that are typical for DILI and some associated health care management principles.

Case 1: DILI in a clinical trial participant
This is a hypothetical case based on a composite of data from several real cases [→1–2].

An 80-year-old Caucasian woman was enrolled in a Phase 3 clinical trial of ximelagatran versus warfarin for prevention of stroke in patients with atrial fibrillation. She was randomized to receive ximelagatran 36 mg twice daily. Her past medical history included atrial fibrillation, coronary heart disease, angina, hypertension, hyperlipidaemia, osteoporosis, and hypothyroidism.

Additional medications included atenolol, calcium, digoxin, ergocalciferol, retinol, calcium carbonate, estrogen, furosemide, levothyroxine, sodium phenobarbital, atropine methonitrate, glyceryl trinitrate, theophylline, papaverine, potassium chloride and ramipril. There was no history of alcohol drinking, other substance abuse, or acetaminophen overdose, and the patient denied recent use of herbal or dietary supplement. She reported no prior history of liver disease or drug allergies.

Her baseline liver tests included ALT=15 U/L (ULN: 45), AST=8 U/L (ULN: 40), ALP=74 U/L (ULN: 120) and TBL=8 µmol/L (ULN: 21).

On **Day 61** of the trial she was asymptomatic, but routine blood test showed a serum ALT of 180 U/L (4 × ULN), and AST 128 U/L (3.2 × ULN) while TBL and ALP were within normal limits. She had no fever, rash, or abdominal pain on examination and her complete blood count revealed no eosinophilia. Evaluation included viral serological tests for hepatitis A (HAV total antibody, HAV IgM antibody), hepatitis B (HBV surface antigen, HBV core antibody), hepatitis C (HCV antibody), and autoimmune serological tests (anti-nuclear antibody, anti-smooth muscle antibody, quantitative immunoglobulins), which were all negative.

A follow-up bloodwork on **Day 68** showed an increased ALT of 447 U/L (9.9 × ULN) and AST of 270 U/L (6.8 × ULN). Serum TBL and ALP remained within the normal limits. The study drug was permanently discontinued, but all other concomitant medications were continued. The patient was started on open label warfarin for stroke prophylaxis. Additional evaluation which included serological tests for cytomegalovirus (CMV) antibody and Epstein-Barr Virus (EBV) antibody was negative. The patient remained asymptomatic.

On **Day 78**, 10 days after permanent discontinuation of the study drug, ALT and AST continued to increase. ALT was 1240 U/L (28 × ULN) and AST 590 U/L (15 × ULN). ALP was minimally elevated at 131 (ULN: 125) and TBL was within normal limits at 14 µmol/L (ULN: 21) although moderately elevated compared to baseline value. An abdominal ultrasound was performed and was found to be normal.

On **Day 85**, 17 days after discontinuation of the study drug, the patient was complaining of fatigue, right upper quadrant abdominal discomfort, and dark urine. There was no fever or skin rash. ALT and AST were further increased at 1419 U/L (32 × ULN) and 729 (18 × ULN). TBL was 48 µmol/L (2.3 × ULN) and ALP was 141 U/L (1.2 × ULN). Direct bilirubin (DBL) was 39 µmol/L (10 × ULN), INR was 1.1.

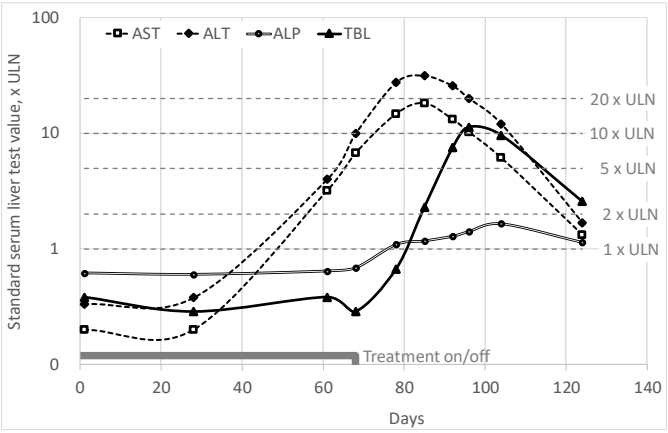
On **Day 92,** 24 days after discontinuation of the study drug, the patient was still complaining of fatigue and dark urine. Physical examination showed jaundice, scleral icterus, and moderate tenderness in the right upper abdominal quadrant. ALT and AST were mildly lower than previous results, 1158 U/L (26 × ULN) and 527 U/L (13 × ULN) respectively, however, TBL was markedly increased to 158 µmol/L (7.5 × ULN). ALP was mildly elevated 155 U/L (1.3 × ULN). INR remained normal.

On **Day 96**, 28 days after discontinuation of the study drug, the patient was still complaining of fatigue and was visibly jaundiced. Abdominal examination revealed mild tenderness in the right upper quadrant. ALT and AST showed further improvement (903 U/L and 410 U/L respectively), but TBL continued to increase to 238 µmol/L (11 × ULN). ALP was mildly elevated at 170 U/L (1.4 × ULN). INR remained within the normal limits.

On **Day 104**, 36 days after drug discontinuation, the patient was feeling better. ALT and AST showed marked improvement to 544 U/L and 245 U/L respectively, and TBL showed a mild decrease compared to the previous result, 202 µmol/L (9.6 × ULN). On subsequent days the patient continued to improve clinically, and liver tests showed gradual improvement. On **Day 124**, two months after the discontinuation of the study drug, ALT and AST were 76 U/L and 53 U/L (1.7 × ULN and 1.3 × ULN, respectively), and TBL decreased to 54 µmol/L (2.6 × ULN).

The patient's liver tests returned to normal range eight weeks after discontinuation of the study drug (**Figure 1)**. The severity of liver injury was graded as a Level 3 using the DILIN categorical scoring system (see Table 3 on page 10). Due to the typical time to onset and the positive dechallenge, in the absence of any alternative cause, the liver injury was adjudicated by expert opinion as 'highly likely' in its causal association with ximelagatran. RUCAM scoring was not performed because this algorithmic method currently has several limitations in the clinical trial setting.

Appendix 3, Figure 1.
Time course of serum liver safety biomarker test results of Patient 1
Hypothetical case based on data from several real cases [1]



ALP=alkaline phosphatase, **ALT**=alanine aminotransferase, **AST**=aspartate aminotransferase, **TBL**=Total bilirubin, **ULN**=upper limit of normal

Genomic testing was performed using genome-wide scanning complemented by other methods. The patient was found to be positive for *HLA-DRB1*07:01* and *HLA-DQA1*02:10* allelic markers, both associated with an increased risk for ximelagatran–related liver injury.[2]

Discussion

This patient presented with a clinical picture of acute hepatocellular liver injury, which has been shown to be the typical signature of ximelagatran-related DILI. Time to onset was 61 days, which is consistent with the typical 2–12 weeks window of susceptibility observed with most drugs causing acute idiosyncratic hepatocellular DILI. Concomitant medications were not found to be a likely cause and extensive evaluation, which included viral serology tests for hepatitis A, B, C, EBV, and CMV, autoimmune serology and abdominal ultrasound, did not reveal alternative causes of liver injury. Ximelagatran treatment was discontinued when ALT was 9.9 × ULN and TBL was within normal range; however aminotransferases (ATs) continued to increase over the following two weeks. This increase in serum ALT and AST was associated with an increase in serum TBL and direct bilirubin. Although discontinuation of the causing drug is typically followed by a relatively rapid decrease in liver enzymes (*i.e.*, positive dechallenge), a temporary worsening is not uncommon, and occasionally may be prolonged. TBL elevation was accompanied by mild hepatic symptoms (*i.e.*, fatigue and dark urine). However, hypersensitivity symptoms (*e.g.*, fever, skin rash) were notably absent, which is typical for ximelagatran-related liver injury.

The increase in serum TBL is a worrisome sign, as it may reflect a worsening liver injury and imminent liver failure. In such cases the patient should be hospitalized, and if elevated TBL persists, an urgent transfer to a liver transplant centre should be considered. In this case, the decrease in serum ATs was eventually followed by a slow decrease in TBL. The delayed decrease in serum TBL despite a substantial improvement in ATs may reflect delayed bilirubin clearance rather than worsening of the liver injury.

Case 2: DILI in the post-marketing setting

This case concerns an 85-year-old woman with atrial fibrillation and a minor stroke in the medical history but otherwise healthy for her age. As regular medication she was treated with warfarin and antihypertensive agents (atenolol 50 mg, bendroflumethiazide 12.5 mg and enalapril 10 mg) with no other concomitant medications.

A few days after a cat bite, the patient developed a wound infection and was referred to the infectious disease department where she was treated with two doses of piperacillin/tazobactam and thereafter oral treatment with amoxicillin-clavulanate 500 mg three times daily for 10 days.

Approximately two to three weeks after the end of treatment with amoxicillin-clavulanate, when the infection was resolved, the patient developed itching, and after an additional week she consulted her General Practitioner (GP) because of itching, jaundice and discoloured faeces but not associated with abdominal pain or other symptoms. The GP ordered some blood tests and found high levels of total bilirubin (8 × ULN), ALP (2.7 × ULN) and ALT (2 × ULN).

The GP suspected a malignancy and referred the patient to the hospital, where a CT scan of the abdomen was performed. This investigation was normal, and ultrasonography of the liver was normal as well. There was no calculus or any signs of malignancy or bile duct obstruction. The laboratory results on admission are shown in **Table 1**.

Appendix 3, Table 1. Laboratory results of Patient 2 on admission to hospital

Laboratory investigation	Local laboratory result		Fold elevation	Reference range
	Conventional units	SI units	× ULN	SI units
Total bilirubin	16 mg/dl	275 µmol/L	11	5–25 µmol/L
ALP	282 U/L	4.7 µkat/L	2.6	0.6–1.8 µkat/L
ALT	69 U/L	1.15 µkat/L	1.05	0.15–1.10 µkat/L
PK-INR*	1.0	1.0	(no elevation)	0.9–1.2
Albumin	310 g/dL	31 g/L	(no elevation)	36–48 g/L

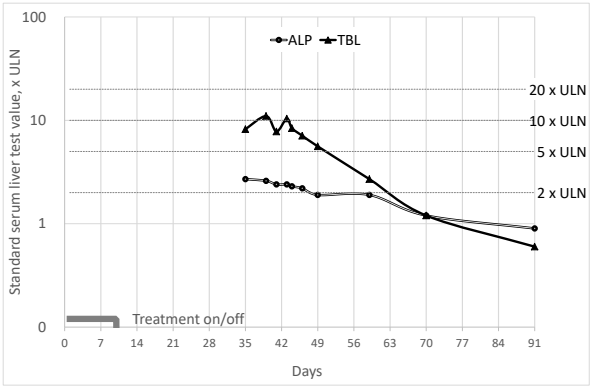
*Warfarin stopped

The patient was routinely investigated with blood samples for viral hepatitis and chronic liver disorders. All these tests—hepatitis A, hepatitis B, hepatitis C, anti-smooth muscle autoantibody (ASMA), anti-mitochondrial antibody (AMA) and antinuclear antibody (ANA)—were negative. Protein electrophoresis was normal except for a slightly increased alpha-1-antitrypsin as a marker of inflammation. There was only a slight elevation of ALT throughout the clinical course.

Clinical course

After admission to the hepatology department the bilirubin level decreased rapidly the first week after admission. The patient was discharged to home after 10 days of hospital care and the bilirubin levels returned to normal during the following months. After two months the liver values were all normal and the patient had completely recovered (**Figure 2**). The patient continued with her antihypertensive agents during this time, and warfarin was reintroduced when she was discharged.

Appendix 3, Figure 2. Time course of ALP and total bilirubin of Patient 2



ALP=alkaline phosphatase, **TBL**=total bilirubin, **ULN**=upper limit of normal

Days 1-10: treatment with amoxicillin-clavulanate, Day 35: initial blood tests, Day 39: hospital admission, Day 45: discharge from hospital

Discussion and clinical diagnosis

DILI caused by amoxicillin-clavulanate was strongly suspected since no other reasonable explanation for the jaundice was found. The most important differential diagnoses in this case would be a malignancy or a calculus involving the bile ducts.

The patient recovered spontaneously. No liver biopsy was performed because of the rapid improvement and considering the potential risks with a liver biopsy especially in elderly patients. The liver injury associated with amoxicillin-clavulanate appears to be caused mainly by clavulanate rather than amoxicillin. This is described on the LiverTox® webpage on amoxicillin-clavulanate,[→3] which includes a case report where re-exposure to amoxicillin alone was not associated with recurrence of liver injury.[→4]

R value calculation *(see also Section 1.1.2)*

R = (ALT value ÷ ALT ULN) ÷ (ALP value ÷ ALP ULN)
= (1.15 ÷ 1.1) ÷ (4.7 ÷ 1.8)
= 1 ÷ 2.6
= **0.38**

Definitions for enzyme pattern: R>5: hepatocellular; R<2: cholestatic; 2 < R > 5: mixed.
The laboratory picture showed a cholestatic pattern (R value=0.4)

RUCAM score (cholestatic liver injury)

1)	Time to onset: (from cessation of the drug ≤30 days)	+1
2)	Course: decrease ALP and bilirubin ≥50% within 180 days	+2
3)	Risk factors. Age ≥ 55	+1
4)	Concomitant drugs	0
5)	Exclusion of other causes of liver injury	+2
6)	Previous information on hepatotoxicity of the drug (label)	+2
7)	Rechallenge (not performed)	0

In this case the RUCAM score was 8. This indicates that it was "probable" that amoxicillin-clavulanate was responsible for the liver injury (see Section 2.4.2 for more information on the RUCAM scale).

The clinical picture with a cholestatic pattern after about two weeks of treatment with amoxicillin-clavulanate is typical for this agent, which is known as one of the most common causes of DILI. It belongs to Category A according to a published categorization system [→5] which is used on the LiverTox® website to classify drugs into five categories based on the number of published cases of DILI [→6][21]. With >100 published DILI cases amoxicillin-clavulanate belongs to Category A and can be considered as a well-established cause of clinically apparent liver injury.

In conclusion, this case was assessed as a DILI secondary to treatment with amoxicillin-clavulanate. The patient improved rapidly, did not develop any complications and could return to her home in good health.

References Appendix 3

→1 Avigan MI, Muñoz MA. Perspectives on the Regulatory and Clinical Science of Drug-Induced Liver Injury (DILI). In: Chen M, Will Y, eds. Drug-Induced Liver Toxicity, Methods in Pharmacology and Toxicology, New York, Humana Press, 2018, 367-93. (Abstract)

→2 Kindmark A, Jawaid A, Harbron CG, *et al.* Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. Pharmacogenomics J. 2008;8(3):186–195. (Journal full text)

→3 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury (Internet). Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-2020. Amoxicillin-Clavulanate. Updated 30 Mar 2016. (Webpage)

→4 Peroux JL, Peroux E, Jais F, Philit F, Chichmanian RM. [Augmentin hepatotoxicity: responsibility of clavulanic acid? Apropos of a case]. [Article in French]. Gastroenterologie clinique et biologique. 1992;16(1):102-3. (PubMed)

→5 Björnsson ES, Hoofnagle JH. Categorization of drugs implicated in causing liver injury: Critical assessment based on published case reports. Hepatology (Baltimore, Md). 2016;63(2):590-603. (AASLD free access)

→6 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury (Internet). Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-20. Categorization Of The Likelihood Of Drug Induced Liver Injury. Updated 2019 May 4. (Webpage)

[21] Category A: more than 50 case reports – **well known**, well described and well reported to cause liver injury; Category B: 12–50 case reports, **known** or **highly likely** to cause idiosyncratic liver injury; Category C: 4–11 case reports, **probably** linked to idiosyncratic liver injury; Category D: 1–3 case reports, **possible** hepatotoxin and only a rare cause of liver injury, Category E: no published case reports, **not believed** or **unlikely** to cause liver injury.
Two additional categories are: E*: no convincing cases in the medical literature, but acute liver injury cases reported to regulatory agencies or mentioned in large clinical studies, but no specifics or details supportive of causality assessment available, **unproven, but suspected** to cause liver injury; and X: for medications recently introduced into or rarely used in clinical medicine, inadequate information on the risk of liver injury, **unknown**.
Agents cause liver injury but only when given in high doses, as might occur with a drug overdose, are categorized using [HD] after the category of A, B, C or D.

APPENDIX 4.

MEMBERSHIP AND MEETINGS OF THE CIOMS DILI WORKING GROUP

CIOMS Working Group on Drug-Induced Liver Injury (DILI)

Name	Organization
Guruprasad P AITHAL	University of Nottingham, United Kingdom
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Amel BENKRITLY*	Sanofi
Einar S BJÖRNSSON	National University Hospital of Iceland
Michele BORTOLINI	Roche
Robert J FONTANA	University of Michigan Medical Centre, U.S.
Stewart GEARY	Eisai
Alexandre KIAZAND*	Astra Zeneca
Gerd A KULLAK-UBLICK	Novartis
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M Isabel LUCENA	International Union of Basic and Clinical Pharmacology (IUPHAR) / University of Málaga, Biomedical Research Institute of Málaga (IBIMA), Spain
Yimin MAO*	RenJi Hospital, Shanghai Jiao Tong University School of Medicine, China
John MARCINAK	Takeda (until September 2019, then: AbbVie)
Michael MERZ	University of Zurich, Switzerland
Manfred OSTER	Sanofi
Shanthi PAL*	World Health Organization (WHO)
Xingmin QIU*	Pfizer
Lembit RÄGO	CIOMS
Arie REGEV	Eli Lilly
John Michael SAUER*	Critical Path Institute (C-Path)
Elmer SCHABEL*	Federal Institute for Drugs and Medical Devices (BfArM), Germany
Monica SOARES	Brazilian Health Regulatory Agency (Anvisa)

Name	Organization
Haibo SONG*	China Food and Drug Administration (CFDA)
James SOUTHERN	South African Health Products Regulatory Authority
Walter STRAUS	Merck
Hajime TAKIKAWA	Teikyo University, Japan
Daisuke TANAKA*	World Health Organization (WHO)
Mari THÖRN	Swedish Medical Products Agency (SMPA)
Shinobu UZU	Pharmaceuticals and Medical Devices Agency (PMDA), Japan
Jean-Marc VIDAL*	European Medicines Agency (EMA)
Javier WAKSMAN	FibroGen
Jia-bo WANG*	Fifth Medical Center of Chinese PLA General Hospital, China
Hui-Talia ZHANG	Bayer

*=Participated in fewer than three meetings.

List of Working Group meetings

Date	Location	Host
April 2017	Geneva, Switzerland	CIOMS
November 2017	Málaga, Spain	University of Málaga, Biomedical Research Institute of Málaga (IBIMA)
May 2018	Reykjavík, Iceland	National University Hospital of Iceland
November 2018	Aix-en-Provence, France	CIOMS
May 2019	Tallinn, Estonia	CIOMS

Drug-induced liver injury (DILI) is a growing challenge because of the ever-increasing number of drugs used in medical care. DILI is rare but can be serious and is largely unpredictable. It is an important cause of mortality and liver transplantation, and a leading cause of attrition in drug development. Progress is under way in identifying genetic risk factors, exploring new mechanistic concepts of the complex underlying interactions, and developing new biomarkers that can predict or diagnose DILI. The pharmaceutical industry has a key role in advancing these initiatives, and prospective DILI registries must adopt standard procedures for biological sample collection and storing. There is a strong need for standard guidelines to support these efforts.

The consensus report of the CIOMS DILI Working Group aims to provide a critical framework and essential set of tools to detect, diagnose and manage DILI during drug development and in the post-marketing setting. The report is intended for clinical and basic pharmaceutical industry investigators who capture, analyze and communicate liver safety data in drug development. It is also intended for regulatory scientists and expert consultants who comprehensively evaluate new products and emerging biomarkers for their association with DILI risk, and for health care professionals who monitor and manage patients treated with potentially hepatotoxic drugs in clinical practice.

Drug-Induced Liver Injury (DILI). Current status and future directions for drug development and the post-market setting. A consensus by a CIOMS Working Group. Geneva: Council for International Organizations of Medical Sciences (CIOMS), 2020.

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