REVIEW

Diagnostic and prognostic assessment of suspected drug-induced liver injury in clinical practice

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Abstract

Idiosyncratic drug-induced liver injury (DILI) is a challenging liver disorder because it can present with a range of phenotypes, mimicking almost every other hepatic disease, and lacks specific biomarkers for its diagnosis. Hence, a confident DILI diagnosis is seldom possible as it relies on the precise establishment of a temporal sequence between the exposure to a given prescription drug or sometimes hidden herbal product/over the counter medication as well as the exclusion of other aetiologies of liver disease. However, an accurate diagnosis is of most importance, as prompt withdrawal of the causative agent is essential in DILI management. Indeed, DILI can be severe and even fatal or in a fraction of cases evolve to chronic damage, but specific biomarkers for predicting mortality/liver transplantation or a chronic outcome in the very early phases of DILI are not yet available. In this article, we discuss the best diagnostic and prognostic approach of a DILI suspicion by judiciously choosing and interpreting the standard tests currently used in clinical practice.

KEYWORDS
biomarkers, chronic DILI, diagnostic tools, drug-induced liver injury, phenotype, prognostic models

1 | INTRODUCTION

Idiosyncratic drug-induced liver injury (DILI), as opposed to intrinsic DILI, is an unexpected adverse drug reaction that occurs rarely owing to interactions between drug properties and host factors (genetics, alcohol intake, diet, coexisting diseases, associated medications and microbiome among others). The variety of interactions accounts for individual susceptibility, DILI phenotypic expression and outcome.[1] Genetic variations in genes involved in drug metabolism phases 1 (bioactivation), phase 2 (conjugation) and phase 3 (cellular excretion)

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are believed to affect the accumulation of reactive metabolites to a critical threshold leading to cellular stress that potentially initiates cell damage and active immune responses.[2] Drug chemical properties are probably of most importance in the initiation of mild injury, but once injury begins the responses to injury insult (ie immune response, inflammation, tissue injury and repair) are mainly driven by host factors.[1]

The complexity of the mechanism underlying idiosyncratic DILI and the variability from one subject to another might also explain the particular signature of this adverse hepatic reaction, namely the ability to present with a wide range of phenotypes and severity. Indeed, idiosyncratic DILI is one of the most challenging clinical scenarios in hepatology. This is because of the impressive number of drugs used in clinical practice but also herbs and dietary supplements that have shown hepatotoxic potential, its low frequency compared with other acute or chronic liver disease, the variety of clinical and histological phenotypic presentation and, most importantly, the current absence of specific biomarkers able to distinguish DILI form other liver disorders. All these factors jeopardize the correct assessment of DILI, whose diagnosis still relies on a high degree of suspicion in addition to careful exclusion of alternative aetiologies of liver damage. Idiosyncratic DILI is also the most frequent cause of acute liver failure (ALF) in the USA and Europe. Notably, drug-induced ALF carries a particularly poor prognosis so an early prediction is of paramount importance.[3]

Recent concerted efforts on biomarker discovery and validation in the Innovative Medicines Initiative Safer and Faster Evidence-based Translation (IMI SAFE-T) Consortium[4] have brought hope to the area of new serum biomarkers to improve the diagnostic performance of currently used aminotransferases in patients with DILI. However, despite the identification of promising soluble markers for predicting outcome, the specificity of these new analytes in terms of distinguishing DILI from other hepatic injuries is yet limited.[5] Hence, correct assessment of DILI suspicions requires nowadays the optimization of the current laboratory and imaging tests available for a better diagnostic approach and prognostic prediction. In this article, we aim to discuss the best approach to diagnostic and prognostic DILI assessment in a post-market setting.

2 | CLINICAL SPECTRUM OF DILI

2.1 | Clinical presentation

Idiosyncratic DILI can mimic any other hepatic disease in presentation and hence requires a high degree of awareness and suspicion from the clinicians. It can occur either in subjects without pre-existing liver disease or in patients with known or undiagnosed liver disorders, including patients in which hepatotoxicity may further decompensate underlying cirrhosis leading to acute-on chronic liver failure[6] (Figure 1). The most frequent form of presentation is an acute episode mimicking viral hepatitis or acute cholestatic syndrome. Clinical features can include low-grade fever, asthenia,

Key points
- Drug-induced liver injury can present with a wide range of phenotypes, but a hepatitis or cholestasis "like" syndrome are the most common types in clinical practice.
- A diagnosis of DILI is mainly based on excluding other aetiologies of liver injury, but the pharmacological history and monitoring of liver tests upon drug discontinuation are also crucial for the diagnosis.
- Prospective cohort studies have identified demographic and laboratory variables that are predictive of short-term severe or chronic outcome.
- Genetic factors identified in genome-wide association studies, while not useful for pre-treatment risk minimization, can help to distinguish DILI from other liver diseases.

abdominal discomfort, anorexia, jaundice, encephalopathy and ascites, all of them unspecific for DILI. Jaundice is the most frequent manifestation present in 69%-71% of the cases.[7,8] ALF is described from 4% to 14% of cases of DILI.[9-12] Nonetheless, clinical symptoms, albeit not specific for hepatotoxicity, can be useful to identify some typical drug signatures, establish alternative causes and predict outcome. Hepatotoxicity usually resolves spontaneously after drug cessation except for a minority of instances that progress to ALF and chronic DILI, showing persistent increases in aminotransferases and or/alkaline phosphatase (ALP) and/or radiological or histopathological evidence of liver damage despite drug withdrawal.[13]

Many prescription and over the counter drugs, herbs and dietary supplements have been associated with liver injury, which complicates the adjudication process particularly when the subject is receiving several agents in combination. Resources such as the LiverTox webpage[14] provide updated information on the potential for hepatotoxicity of many medications in common use. Careful inquiry, retrieving information on treatment start and stop dates with regard to the initiation of symptoms and the course of the clinical syndrome upon drug discontinuation is needed to establish a compatible temporal relationship with the suspected causative agent. Time to onset can range from a few days to several months but the majority of subjects develop DILI within the first 3 months of therapy, although in some instances (eg amoxicillin-clavulanate-related DILI) the hepatic reaction can occur with a considerable delay after treatment interruption.[15]

3 | PHENOTYPES

The first step to correctly appraise a suspicion of DILI is to characterize the phenotype (Figure 1). Acute DILI is usually identified and classified using biochemical criteria, which by consensus include one of the following thresholds: (a) alanine amino transferase (ALT) ≥5 × upper limit of normal (ULN), (b) ALP ≥2 × ULN after
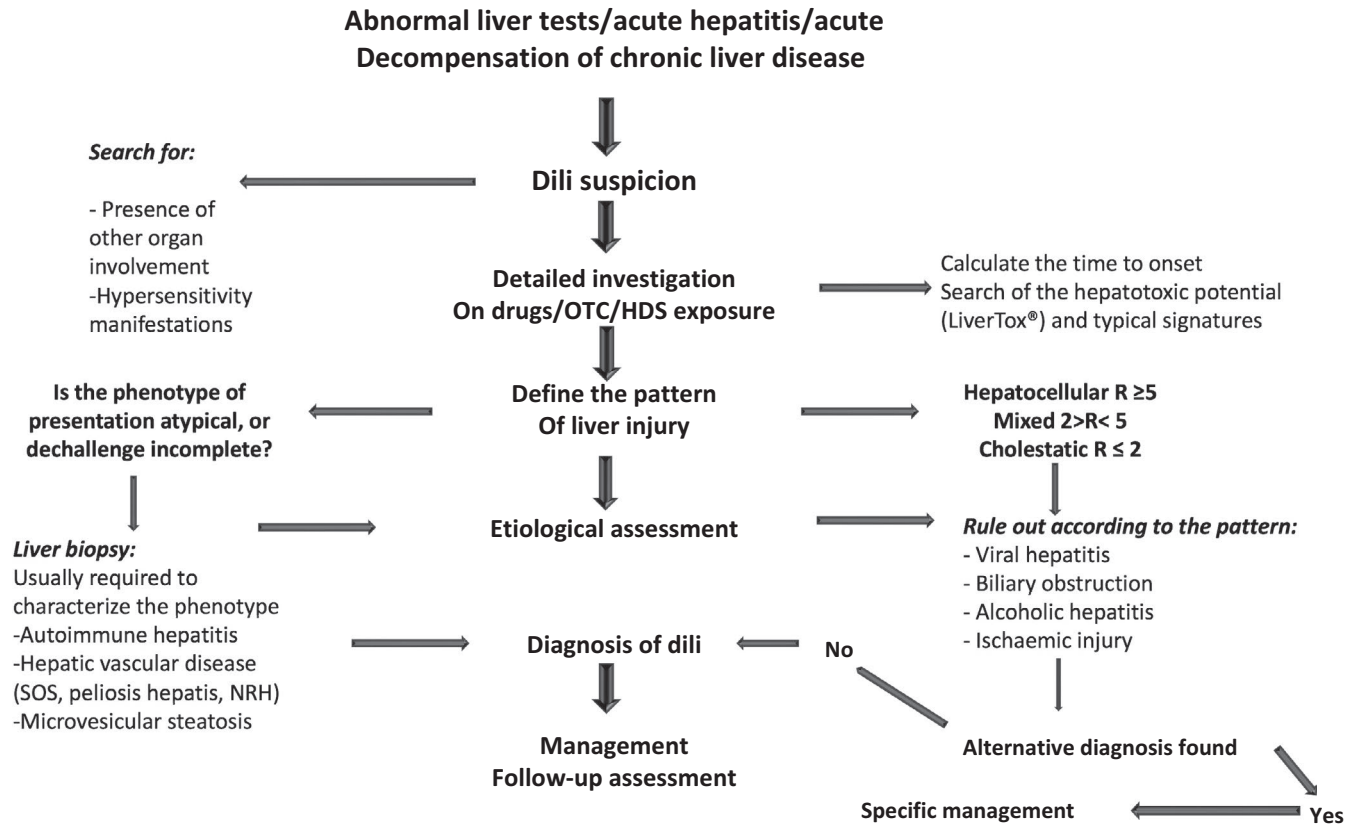
FIGURE 1 Algorithm for approaching drug-induced liver injury diagnosis

ruling out bone pathology or (c) ALT ≥3 × ULN plus total bilirubin (TBL) >2 × ULN. Thus, pattern of liver injury is classified according to the first available liver profile values rather than being based on histology as liver biopsy is not commonly performed. Hepatocellular pattern of liver injury is defined as the ratio (R) ALT (expressed in ULN) divided by ALP (expressed in ULN) being 5 or higher. Cholestatic pattern is defined by R ≤2. When R is <5 and >2, the pattern is considered as mixed.[16] The progress of liver enzyme elevations over time tend to diminish the R value and make the pattern more cholestatic.[17]

Although most of the drugs have the potential to induce all the patterns of liver injury (hepatocellular, cholestatic and mixed), some drugs have characteristic signatures that can help clinicians to differentiate the causal agent when many drugs have been taken at the same time period. Thus, drugs with typical DILI signatures include anabolic steroids, which characteristically induced mild or moderate increases in transaminases and ALP but very high elevations in TBL[18] and herbs that are typically associated with a hepatocellular pattern and high levels of transaminases.[19] DILI caused by isoniazid,[20] flutamide[21] and diclofenac[22] show in almost all instances a hepatocellular pattern of injury, while liver injury owing to amoxicillin-clavulanate,[23] azathioprine[24] and oestrogens are predominantly mixed or cholestatic.[25] Nevertheless, host factors such as age have been shown to influence the pattern of injury regardless of the drug, with increasing age being associated with cholestatic pattern of liver injury.[26]

However, DILI can present with a myriad of other acute and chronic syndromes (Table 1) that would be inaccurately classified using the biochemical criteria explained before. The majority of these phenotypes are indistinguishable from those not related to medications with regard to clinical, imaging and histopathological features and DILI should therefore be suspected in the setting of an exposure to drugs known to be associated with these particular presentations of DILI. In some instances (eg nodular regenerative hyperplasia, indolent fibrosis or granulomatous hepatitis), the link between specific drugs (eg oxaliplatin, methotrexate and allopurinol, respectively) and the phenotype is so evident that DILI arises as the first diagnostic choice.

4 | DIAGNOSTIC TOOLS FOR DRUG-INDUCED LIVER INJURY ASSESSMENT

4.1 | Liver tests for liver injury assessment

The absence of diagnostic DILI biomarkers has led to that serum ALT/aspartate aminotransferase (AST), ALP and TBL levels still remain the pillars for DILI case detection and qualification.[27] Minor and reversible increases in ALT/AST that occurs with some drugs such as statins or that may indicate pre-existing liver disease (ie fatty liver) should not be classified as DILI. Moreover, ALT lacks specificity as a rise in serum values can also be related to other organ damages, most often muscle injury, which can be drug induced (ie rhabdomyolysis)

TABLE 1 Phenotypes in DILI

Phenotypes	Case characteristics	Associated drugs	Elements in assessment and management
Hepatocellular pattern of liver injury	ALT (or AST) raised ≥5 fold above ULN or ratio (R) ALT/ALP ≥5	Isoniazid,[20] flutamide,[21] diclofenac[22] and herbs[19]	
Mixed or cholestatic pattern of liver injury	2<R>5 (mixed) ALP ≥2 ULN or R ≤2 (cholestatic)	Amoxicillin-clavulanate,[23] azathioprine[24] and oestrogens[25]	
Hypersensitivity syndrome	DRESS[78] syndrome involving several organs including the liver in 60%-100% of cases and 10% of mortality. SJS/TEN[79] with mortality in the presence of DILI is even higher (36% to 46%)[80,81]	Carbamazepine, allopurinol, lamotrigine, sulfasalazine, phenobarbital, nevirapine, phenytoin, abacavir, mexiletine, dapsone and vancomycin,[78] minocycline[82]	Important HLA associations include HLA-B*15:02 and SJS/TEN associated with carbamazepine, HL A-B*13:01and DRESS associated with dapsone, HLA-B*35:02 and minocycline, HL A-B*58:01 and SJS/TEN and DRESS associated with allopurinol[82]
Drug-induced autoimmune hepatitis	Acute or chronic damage with serological and/or histological features of AIH	Nitrofurantoin, minocycline, statins, diclofenac and anti-TNFα agents[83]	Often needs immunosuppression with corticosteroid. Stop of immunosuppression after remission is typically not followed by relapse unlike in idiopathic AIH[54]
Fatty liver disease	Non-alcoholic fatty liver disease related to specific drugs	Amiodarone	Steatohepatitis, Mallory bodies, ballooning, fibrosis and cirrhosis[84]
		Methotrexate	Long-term exposure has been associated with fatty infiltration, fibrosis with potential progression to cirrhosis[85]
		Tamoxifen	Doubles the risk of presenting fatty liver disease[86]
		Irinotecan	Steatosis and steatohepatitis[87]
Nodular regenerative hyperplasia (NRH)	Characterized by a widespread benign transformation of hepatic parenchyma into small regenerative nodules. NRH may lead to the development of portal hypertension	Azathioprine,[88] HAART,[89] oxaliplatin, 6-thioguanine, bleomycin, busulphan, cyclophosphamide, cytosine arabinoside, chlorambucil, doxorubicin and carmustine	Oxiplatin is the drug more frequently associated with NRH[90]
Liver tumours	Adenoma or hepatocellular carcinoma detected by biopsy/imaging	Oral contraceptives[91]	Increase the incidence of liver cell adenoma from 3 per million per year in the general population, to 3-4 per 100 000
		Androgens[92] xymetholone and methyltestosterone and danazol	Hepatic adenomas, hepatocellular carcinomas, cholangiocarcinoma and angiosarcoma
Secondary sclerosing cholangitis	Acute damage with imaging and/or histological features mimicking primary sclerosing cholangitis	Amoxicillin-clavulanate, amiodarone, atorvastatin, infliximab, 6-mercaptopurine, and venlafaxin sevoflurane[44,93]	May evolve to chronic DILI
Granulomatous hepatitis	Central accumulation of mononuclear cells, primarily macrophages, with a surrounding rim consisting of lymphocytes and fibroblasts	Allopurinol, carbamazepine, phenytoin, quinidine, methyldopa and sulphonamides[94]	
Acute fatty liver	Acute onset of microvesicular steatosis	Sodium valproate, nucleoside analogue reverse transcriptase inhibitors, amiodarone and salicylate	Salicylate capable of inducing the 'Reye's syndrome' in children, a rare form of hepatotoxicity[95-97]

TABLE 1 (Continued)

Phenotypes	Case characteristics	Associated drugs	Elements in assessment and management
Liver injury related to immune check point inhibitors	Immune-related adverse events, including hepatotoxicity[98,99]	Ipilimumab, pembrolizumab and nivolumab, atezolizumab, avelumab and durvalumab	ICIs-related hepatitis is typically 'seronegative', not presenting ANA, ASMA or other AIH-associated autoantibodies and with no recurrence after immunosupressant withdrawal[100,101]
Vanishing bile duct syndrome[102]	Unresolving cholestasis leading to progressive loss of intrahepatic bile ducts	Azathioprine, amoxicillin-clavulanate, carbamazepine, chlorpromazine, erythromycin, flucloxacillin, phenytoin, terbinafine and co-trimoxazole	
Peliosis hepatis	Proliferation of sinusoidal hepatic capillaries that results in cystic blood-filled cavities distributed randomly throughout the liver[103]	Anabolic steroids,[104,105] tamoxifen, and azathioprine	

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibodies; ASMA, antismooth muscle antibodies; DILI, drug-induced liver injury; DRESS, Drug rash with eosinophilia and systemic symptoms; HAART, highly active antiretroviral therapy; HLA, human leukocyte antigens; ICI, immune check point inhibitors; NRH, Nodular regenerative hyperplasia; R, ratio; SJS/TEN, Stevens-Johnson syndrome/ Toxic epidermal necrolysis; TNF, tumour necrosis factor; ULN, upper limit of normal.

and is generally accompanied by a disproportionate increase in AST with regard to ALT. Testing for creatine phosphokinase (CPK) can assist in distinguishing between liver- and muscle-driven ALT elevations. An isolated elevation in TBL usually related to its unconjugated fraction does not qualify as DILI as it generally indicates haemolysis or Gilbert syndrome.[16] Nonetheless, a rise in serum ALT is highly sensitive for hepatocyte injury, and when accompanied by an elevation in TBL becomes a reliable biomarker of liver injury in DILI and liver dysfunction (the so-called Hy's law, see the next section).[28] Cholestatic damage is characterized by markedly elevated serum ALP in association with raised gamma glutamyl transferase (GGT). However, an isolated elevation of GGT is insufficient to qualify as DILI as it does not indicate liver damage.[16] The performance of AST and GGT in replacing ALT and ALP, respectively, when the latter are unavailable at DILI recognition was analysed in a study of 588 patients included in the Spanish DILI Registry. Whereas AST values can reliably substitute ALT in calculating the pattern of injury, the utility of GGT in replacing ALP is limited.[29]

Importantly, liver biochemical analyses should be performed when DILI is first recognized as these values more accurately reflect the actual liver injury. However, abnormal liver tests when first found do not represent the true onset time of liver cell injury, which may already be advanced, subsiding or past.[30] To clarify this issue, serial aminotransferase measurements are necessary. Liver biochemistry should also be tested in DILI patients until complete normalization for diagnostic reassurance. Steady decline of aminotransferases supports the diagnosis of DILI, whereas flare-ups and/or incomplete resolution of biochemical abnormalities suggest competing aetiologies. In addition, persistently elevated aminotransferases may indicate a chronic outcome. Importantly, elevation of liver enzymes upon re-exposure to the suspected agent provides strong evidence for causality although the required threshold for this elevation is still controversial.[31]

Clinicians should also bear in mind that elevated serum aminotransferases inaccurately reflect the extent to which the liver is damaged in insidious or atypical varieties of DILI such as indolent fibrosis (methotrexate), sinusoidal obstruction syndrome, cirrhosis or microvesicular steatosis secondary to mitochondrial toxicity. In such instances, the threshold values defined for case qualification may not be reached and DILI must be suspected and diagnosed, according to compatible histological/imaging findings in the context of exposure to specific drugs/toxicants.[31]

Laboratory assessment of a DILI suspicion should also include coagulation parameters and serum albumin to further scrutinize potential severity of the liver damage. Elevated international normalized ratio (INR) values (>1.5), which indicates impending liver failure, should prompt referral to a liver transplant unit.

4.2 | Serology and other laboratory tests for excluding alternative causes

Because of the current absence of specific biomarkers, the diagnosis of DILI still relies on the exclusion of alternative causes of liver injury. Classification of injury pattern can assist in the initial diagnostic approach guiding the necessary work-up to exclude the most common causes of hepatitis and cholestasis (Figure 1). Patient age and a detailed medical history to exclude alcohol abuse, comorbidities (such as sepsis, congestive heart failure, recent episodes of syncope or hypotension, which would indicate ischaemic hepatitis), should be retrieved. In addition, ascertainment of risk factors for viral hepatitis and the local burden of infectious diseases potentially affecting the liver are paramount to correctly assess the case.

As a first step, serology tests for viral hepatitis A, B, C and E should be performed, particularly in patients with hepatocellular and

mixed type of liver damage. Potentially challenging cases include, for example, patients who are hepatitis B surface antigen (HBsAg) carriers, in whom hepatitis B virus (HBV) reactivation as the cause of liver injury should be excluded by testing HBV-deoxyribonucleic acid (HBV-DNA). As there is no specific biomarker for acute hepatitis C (HCV), this variety of viral hepatitis can also be misdiagnosed as DILI. Indeed, in 1.3% of adjudicated DILI cases in the Drug-Induced Liver Injury Network (DILIN) prospective cohort, HCV-ribonucleic acid (HCV-RNA) tested positive making the diagnosis a challenge.[8] Besides, hepatitis E (HEV) is a common cause of viral hepatitis in Eastern countries but it is also an emerging cause in Western countries and can subsequently be a DILI confounder.[32,33] Anti-HEV IgM-positive cases ranged from 3% in the DILIN database[33] to 7% in the Spanish DILI Registry.[34] Drugs initially thought to be responsible for the anti-HEV IgM positive DILI cases in Spain actually had low hepatotoxicity potential, showed less compatible temporal sequences and/or presented with higher aminotransferase levels compared with anti-HEV IgM-negative cases.[34] However, anti-HEV serology has not yet reached consensus worldwide as a diagnostic test for active HEV infection.[35] Despite this limitation, a search for HEV infection as an alternative diagnosis is advisable in patients being assessed for DILI, particularly in cases in which the time to onset is less compatible with the drug signature of the suspected medication and in those with transaminase levels in the range of viral hepatitis.

When suspected DILI presents with a hepatocellular pattern, autoimmune hepatitis (AIH) is a potential alternative diagnosis that should be evaluated with autoantibodies (antinuclear antibodies (ANA); anti-smooth muscle antibodies (ASMA)) and serum IgG. However, DILI associated with drugs such as nitrofurantoin, minocycline, anti-tumour necrosis factor (TNF)-α and statins among many others[36-38] can exhibit an AIH phenotype indistinguishable from idiopathic AIH, making the differential diagnosis a challenge. In such instances, the history of exposure to the medication and a resolution of biochemical abnormalities with no relapse either spontaneously or upon corticosteroids tapering and withdrawal support the diagnosis of *drug-induced* AIH. Likewise, when suspected hepatotoxicity presents with a cholestatic pattern, primary biliary cholangitis needs to be excluded by anti-mitochondrial antibody (AMA) testing.[15] Alcoholic hepatitis rarely masquerades as DILI; a history of alcohol abuse with a predominance of AST over ALT elevation with ALT values usually below 300 IU/L and other biochemical features of chronic alcoholism such as high values of GGT and erythrocyte mean corpuscular volume make the diagnosis evident.

In younger patients with acute or chronic hepatitis, Wilson's disease should be ruled out by measuring ceruloplasmin levels. When ceruloplasmin—an acute phase reactant—is diminished or only slightly decreased, which may occur in Wilson's disease presenting as acute hepatitis, a 24-hour urine cooper excretion, ophthalmologic examination for Kayser-Fleischer rings and genetic testing of the *ABCB7* gene are required.[39] Ischaemic hepatitis needs to be excluded in older patients and those with pre-existing cardiac disease, although prior hypotension or syncope was documented in only 53% of the cases in a systematic review of ischaemic hepatitis.[40] Very high

aminotransferases values with a predominance of AST over ALT typically followed by a fast resolution is the biochemical hallmark of liver ischaemia.

As a second step, testing for cytomegalovirus, Epstein-Barr virus and herpes virus infection is usually performed. However, this should only be mandatory when liver damage is seen in association with extrahepatic manifestations such as rash, lymphadenopathy and atypical lymphocytes.

4.3 | Imaging

Liver imaging in DILI is used to exclude alternative aetiologies. An abdominal ultrasound is advisable in all DILI suspicions regardless of the biochemical pattern of damage to evaluate the biliary tract and to exclude parenchymal focal lesions. Additional imaging techniques would be justified in the clinical context of accompanying prominent abdominal pain and/or mixed or cholestatic injury. Thus, computerized tomography and magnetic resonance cholangiography (MRCP) are sometimes required to exclude gallstone disease and other competing aetiologies.[31] In rare instances, toxic damage to the biliary tract presenting as sclerosing cholangitis has been described with chemotherapeutic agents such as 5-fluorodeoxyuridine after hepatic intra-arterial infusions for treatment of hepatic metastases; these are consequences of ischaemic injury to the biliary tract rather than toxicity.[41,42] Likewise, dilatation of bile ducts has been attributed to ketamine abuse in some case reports. Secondary sclerosing cholangitis has also been reported in association with methimazole and docetaxel.[43] Recently, it was reported that a small proportion of unselected acute cholestatic/mixed DILI cases undergoing MRCP or endoscopic retrograde cholangiopancreatography (ERCP) had secondary sclerosing cholangitis-like changes. The implicated agents varied and included amoxicillin-clavulanate, amiodarone, atorvastatin, gabapentin, infliximab, 6-mercaptopurine, sevoflurane and venlafaxine. All the 10 patients were females and 70% presented with jaundice and had a longer time to resolution.[44] Thus, in a patient being assessed for suspected DILI, the identification of sclerosing cholangitis-like changes does not necessarily mean that the subject has primary sclerosing cholangitis as an alternative diagnosis.[31]

4.4 | Liver biopsy

Histological assessment in acute and chronic liver diseases is currently less frequently indicated than non-invasive tests, which are considered reliable particularly for staging fibrosis. Moreover, in acute liver injury, the degree of inflammation may lead to increased values of transient elastography, overestimating fibrosis.[45] However, as DILI lacks specific serum biomarkers, liver biopsy has long been considered a complementary diagnostic tool that can assist and reinforce the diagnostic process.[46] In a review of liver biopsies from 249 patients with DILI from a prospective observational cohort, the authors tried to establish correlations between pre-defined histological patterns and biochemical phenotypes. Although the hepatocellular and cholestatic biochemical patterns did not match perfectly with their histological

counterparts, more severe inflammation and cell death were associated with hepatocellular pattern compared to higher frequency of bile plugs and ductal paucity in those with cholestasis.[47]

However, liver biopsy is not routinely performed in suspected DILI cases because it does not provide definite diagnostic information in most instances. Notable exceptions are when the de-challenge is incomplete or negative after drug discontinuation, which makes an alternative diagnosis more likely or the presentation suggest one of the phenotypes listed in Table 1, which require histology for a full characterization. One example that requires a liver biopsy is the appraisal of ductopenia caused by some agents that can lead to vanishing bile duct syndrome.[48] Also, in the case of AIH, whose diagnosis is established in patients with hepatocellular injury on the grounds of the detection of typical serum autoantibodies and elevated IgG, a compatible liver histology strongly reinforces the diagnosis.[31] Moreover, DILI can be indistinguishable from AIH even after detailed investigations, as described for 9% of instances.[49] Indeed, serum criteria used for the diagnosis of AIH are largely unspecific as a high prevalence of ANA (15%-24%), ASMA (up to 43%), anti-liver-kidney-microsomal antibody (anti-LKM, 1%) and raised immunoglobulin G levels (5%) can be found among asymptomatic individuals.[50] Hence, histological findings, albeit not pathognomonic, are included in the simplified diagnostic scale currently used for the diagnosis of AIH[51] making liver biopsy a necessary tool to properly assess AIH including cases suspected to be drug induced. Histological findings of AIH (n = 28) and DILI (n = 35) were blindly assessed by three expert pathologists in a study; hepatocellular cholestasis and portal neutrophils was indicative of DILI, while presence of fibrosis suggested the diagnosis of AIH.[52] Using dual immunohistochemistry staining of liver biopsies to characterize portal inflammatory infiltrates in another study, it was shown that inflammatory cells in DILI (that included cases of drug-induced AIH) were predominantly cytotoxic (CD8$^+$) T cells, whereas mature B cells (CD20$^+$) were more prominent in AIH.[53] Nevertheless, long-term follow-up of patients after drug discontinuation would be required to differentiate idiopathic from drug-induced AIH as the latter does not usually recur after withdrawal of the drug and resolution of liver damage.[54]

4.5 | Genetic testing

Recent genome-wide association studies have identified a number of human leucocyte antigens (HLA) genotypes and haplotypes associated with DILI related to a selected group of drugs. Nowadays, HLA genotyping is widely accessible, affordable and can assist diagnosis in selected clinical contexts.[55] As with most polygenic disorders, the pre-treatment value of genetic testing (to prevent DILI in carriers of a specific allele) is very low, but the high negative predictive values (>95%) of some of these alleles can be used to exclude DILI when the subject is not a carrier and the clinical picture could be ascribed to an alternative aetiology. Besides this, when the subject is receiving a combination of medications, genetic testing may help to clarify the role of a particular drug if the patient carries a specific HLA allele associated with hepatotoxicity for one

of the agents. An additional value of HLA typing could be in the differential diagnosis of DILI vs AIH as carriage of an HLA risk allele associated with a specific drug[56] (eg *HLA-A* 33:01* in a subject taking terbinafine that experience acute liver injury with autoimmunity features) would favour the diagnosis of DILI, whereas its absence and the presence of the typical HLA allele associated with AIH, such as *HLA-DRB1*03:01 and DRB1*04:01* would support the diagnosis of AIH. Although HLA typing is applicable to a very limited group of drugs, genome-wide association studies (GWAS) have also recently identified non-HLA genetic variants associated with DILI in general, which could also be useful for clarifying ambiguous cases.[57]

4.6 | Scales used in DILI causality assessment

The lack of specific tests or biomarkers to confirm a DILI diagnosis makes it very important to include a systematic evaluation to confidently attribute a liver injury episode to a drug. Several causality assessment methods specific for DILI have been developed over the past decades, which provide a framework for a more objective evaluation in suspected cases of DILI. Of the several diagnostic scales in place, the Council for International Organizations of Medical Sciences (CIOMS) scale, also called Roussel Uclaf Causality Assessment Method (RUCAM), is still considered the most reliable and reproducible method that correlates better with expert review.[58] RUCAM gives points to seven distinct domains: (a) temporal relationship between exposure to a particular drug and liver injury (both its onset and course), (b) exclusion of alternative non-drug-related aetiologies, (c) exposure to other medications that could explain DILI, (d) risk factors for the adverse hepatic reaction, (e) evidence in the literature regarding DILI from the drug in question and (f) response to re-exposure to the medication. The total score ranges from −9 to +10 and classifies the event as highly probable (>8), probable (6-8), possible (3-5), unlikely (1-2) or excluded (≤0) according to its likelihood of being DILI.[59] However, the RUCAM scale has some limitations: (a) when there is missing information (frequently when reviewing retrospective cases) or (b) no data on de-challenge (cases of acute liver failure), (c) when the patient takes multiple drugs during the same time period or (d) when a drug typically produces delayed DILI (eg amoxicillin-clavulanate). In addition, (e) the questions posed by the scale require some degree of subjectivity by the user to interpret as well as answer. Also, (f) the value added by the risk factors is controversial. Despite its limitations, the RUCAM is the most commonly used diagnostic tool for DILI, and its use increases consistently and objectivity in causality assessment.[60]

5 | DIFFERENT PROGNOSTIC SCORES APPLIED TO INDIVIDUAL CASES OF SUSPECTED DILI

5.1 | Predicting serious DILI outcome

Prediction of severe outcome at DILI recognition remains a challenge in clinical practice; however, it is crucial for improving

patient management. Early recognition of cases with potential to develop ALF can help the clinician to identify those patients who need closer observation, hospitalization or transferal to a liver transplant centre. ALF is a sudden deterioration in liver function in which the patient develops encephalopathy, jaundice and co-agulopathy in the absence of underlying chronic liver disease.[61] These features are delayed, severe and irreversible in most pa-tients,[16] with a high mortality rate (from 60% to 90%) without liver transplantation (LT). DILI is responsible of over 50% of ALF in the USA,[62] UK[63] and Sweden[64] and is the main indication for liver transplantation in ALF cases.[65]

Liver biopsy findings can help to predict a worse prognosis. In a review of biopsies performed on DILI patients in the Swedish Adverse Drug Reactions Advisory Committee (SADRAC) retrospec-tive database, presence of necrosis was predictive of lower rate of survival, while eosinophilia was associated with more favourable DILI outcomes.[66] Likewise, liver failure and death were associated with higher degrees of necrosis, fibrosis stage, microvesicular ste-atosis and ductular reaction, whereas eosinophils and granulomas predominated in those with milder DILI outcome in the DILIN cohort prospective study including 249 liver biopsies.[47] Similarly, evidence of bile duct loss in patients with acute DILI (generally presenting

TABLE 2 Prognostic scores applied to individual cases of suspected DILI

Score's name	Characteristics	Comments
Hy's Law[67]	TBL >2 ULN ALT >3 ULN	High sensitivity, low specificity
New Hy's Law (nHy's law)[9]	TBL >2 ULN nR ≥5* *nR = ALT or AST, whichever the highest/ULN ÷ alkaline phosphatase/ULN value	Similar sensitivity but higher speci-ficity AUROC than traditional Hy's Law. Developed by Spanish DILI Registry and vali-dated by US DILIN
Prognostic algo-rithm by Robles et al[9]	Patients with AST >17.3 × ULN and TBL >6.6 × ULN were found to have the highest risk of ALF/LT; out of patients with AST ≤17.3 × ULN, those with AST/ALT ratio >1.5 have increased risk of ALF/LT in this group	Improvement in specificity and AUROC compared with traditional Hy's Law and nHy's Law but with lower sensitivity
DrILTox ALF Score[70]	DrILTox ALF Score = −0.00691292*platelet count [per 109/L] + 0.19091500*TBL [per 1.0 mg/dL]	Study of a retro-spective cohort of 15 353 DILI patients
Model for End-stage Liver Disease (MELD) score[71,106]	MELD score=10 * [(0.957 * ln(Creatinine)) + (0.378 * ln(Bilirubin)) + (1.12 * ln(INR))] + 6.43	Developed from cirrhotic patients that undergoing elective TIPS and later for reduction in mortality on the wait list for LT
Acute liver failure study group (ALFSG) model[73]	ALFSG based on encephalopathy grade, vaso-pressor requirement, aetiology, TB and INR index	Model is used to predict 21-day survival without LT in patients with ALF
King's college criteria (KCC)[11]	KCC: in APAP-ALF: arterial pH <7.3 or INR >6.5, sCr >300 mmols/L + encephalopathy grade III or IV. In non-APAP-ALF: INR >6.5; or, 3 of the following criteria: age of <11 or >40, TBL >300 mmols/L, time from jaundice to coma >7 d, INR> 3.5 or, drug toxicity	The criteria differ regarding whether ALF is caused by APAP overdose or any other aetiology

Abbreviations: ALF, acute liver failure; ALFSG, Acute liver failure study group; ALT, alanine aminotransferase; APAP, acetaminophen; AST, aspartate aminotransferase; DrILTox ALF Score, drug-induced liver toxicity acute liver failure score; INR, international normalized ratio; KCC, King's college criteria; LT, liver transplant; MELD, Model for End-stage Liver Disease; nHy's law, new Hy's law; sCr, serum creatinine; TB, total bilirubin; TBL, total bilirubin level; TIPS, transjugular intrahe-patic portosystemic shunt; ULN, upper limit of normal.

with cholestatic pattern) indicates the development of vanishing bile duct syndrome with progressive cholestasis leading to liver failure requiring LT or death.[48]

The Food and Drug Administration endorsed many years the denominated 'Hy's law' for detecting serious liver signals during drug development. The Hy's law (Table 2) is based on the observation of Hyman Zimmerman[67] that hepatocellular DILI with jaundice, ruling out other potential aetiologies, denotes a severe reaction with a 10%-50% mortality rate from liver failure (before LT were performed).[68]

The Hy's law has been further validated in the post-marketing setting in three large DILI populations from the Spanish DILI Registry,[7] SADRAC retrospective database[69] and DILIN[8] showing 11.7%, 9.2% and 15% mortality/liver transplantation, respectively, in DILI patients with hepatocellular pattern of liver damage and jaundice.

A number of international consortia that prospectively recruit bona fide DILI cases have evaluated the performance of laboratory and clinical variables for early ALF prediction in DILI patients. The Spanish DILI Registry analysing a cohort of 771 patients proposed a new Hy's Law (Table 2). It demonstrated more accuracy compared with traditional Hy's law (similar sensitivity (90% vs 93%) and higher specificity (63% vs 43%) and also higher area under the receiver operating characteristic (AUROC) (0.77 vs 0.67)). In this study, an independent new prognostic algorithm for ALF in DILI was also developed (Table 2), which demonstrated a good balance between sensitivity and specificity with validation in an independent cohort (specificity, 82%; sensitivity, 80%; AUROC 0.80).[9]

Using a retrospective cohort of 15,353 DILI patients from the Kaiser Permanente database in California, Lo Re et al[70] developed a model including platelet count and total bilirubin (Table 2). The model had the highest discrimination (C statistic, 0.87) with decreasing platelet count and increasing total bilirubin as strong predictors of ALF. This model had a sensitivity of 0.91 and specificity of 0.76 for ALF. In an external validation of the model, high sensitivity was maintained for ALF (0.89). In the initial cohort, traditional Hy's Law criteria showed high specificity (0.92), but low sensitivity (0.68), while a Model for End-stage Liver Disease (MELD) (Table 2) Score of ≥10 showed higher sensitivity (0.84) for ALF.[70]

The performance of MELD score for early prediction of mortality in DILI patients has also been explored in several studies. Thus, in a retrospective study of DILI patients who visited emergency departments in Seoul, Korea from 2010 to 2012, a logistic regression identified MELD [Odds ratio (OR)] 1.21 and haemoglobin (OR 0.77) as independent predictors of poor outcomes.[71] Rathi et al evaluated MELD score in a prospectively collected Indian DILI cohort of 82 patients with 8 liver-related deaths. In a multivariate logistic regression analysis, jaundice, encephalopathy, MELD score and alkaline phosphatase at 1 week independently predicted mortality.[12]

The Drug-Induced Liver Injury Network evaluated MELD score as well as Hy's law and nHy's law as ALF predictor in 1089 DILI patients, including 107 death/LT, in 68 of which DILI had a primary role. In this multivariate analysis, Hy's law was significantly associated

with poor outcome owing to DILI (hazard ratio (HR), 2.2), nR Hy's law showed a stronger association (HR, 6.2), and the predictive capacity of MELD was still stronger (1.2 per MELD point). The C statistic for a MELD cut-off of 19 was 0.83 compared to 0.73 for nR Hy's law and 0.60 for Hy's law. In this study, leukocytosis, coagulopathy, higher bilirubin and thrombocytopenia were independently associated with DILI mortality.[10] It is important to keep in mind that mortality in DILI patients can have other causes than ALF. In 22 of the 107 patients who died up to 2 years from the onset of liver damage in this study, DILI did not play a role in mortality.

Finally, in an Indian cohort of 905 DILI patients of whom 128 (14%) developed ALF, only total protein and INR were independent predictors of mortality.[72] Performance of MELD score, King's college criteria (KCC)[11] score and Acute Liver Failure Study Group (ALFSG) index[73] (described in Table 2) with regard to mortality was compared with receiver operating characteristic (ROC) curve. ROC curve for MELD and ALFSG index was 0.76, but only 0.51 for KCC. The sensitivity and specificity for MELD scores were 72% and 74%, respectively, and 41% and 51%, respectively, for KCC. The best cut-off value for MELD score was ≥28.5.[72]

The comparison of all these studies is difficult because of differences in populations included, definition of DILI, follow-up and the statistical methods used. In addition, the ROC and other quantitative measures determined in each of the studies have been ascertained in a variety of different ways such that cross-study comparisons of their performance characteristics are highly limited. It is worth noting that some of these scores highlight the presence of findings with metrics that signify advancing liver injury while this is not the case for Hy's law. Hence, the reliability of these scores is different and they cannot be strictly compared. Furthermore, Hy's law, although primarily useful for predicting liability of drugs in clinical drug development, is also being used in the post-marketing setting (Registry studies) for prediction of severe outcome. To overcome some of these limitations, the scores discussed in this review should be prospectively validated in similar cohorts of DILI patients with common diagnostic criteria to obtain comparable and more reliable results.

5.2 | Predicting chronicity

The ability to predict those DILI patients in whom the injury will peb rpetuate and become chronic is an important task for clinicians. The first difficulty, however, is to reach a consensus on what chronic DILI is. A former international consensus meeting defined chronic DILI as perpetuating liver damage after 3 months of drug withdrawal,[74] whereas the DILIN group advocated for 6 months,[8,75] and a consensus of DILI experts in 2011 recommended persistence of liver test abnormalities more than 3 and 6 months for hepatocellular and cholestatic/mixed liver damage, respectively.[16] Because these definitions were not evidence based, the Spanish DILI Registry undertook a prospective follow-up study with rigorous exclusion criteria to avoid confounding factors in 298 DILI patients. This study demonstrated that the best cut-off point for defining chronicity was the persistence of liver

tests alterations 1 year after drug discontinuation independent of the type of liver injury (hepatocellular, cholestatic or mixed).[13] Reported risk factors for chronic DILI have been older age,[13] females,[13,17,75] diabetes,[13,75,76] dyslipidaemia, hypertension and use of statins.[13] Because the elderly have a higher prevalence of metabolic syndrome, it is possible that this can account for an increased risk of chronicity rather than the older age itself. Similarly, the use of statins as a risk factor for chronic DILI could be a reflection of the presence of dyslipidaemia in this group of patients or the result of an immune response triggered by these drugs as they have been associated with drug-induced AIH.[77]

Other risk factors for chronic DILI are jaundice at presentation[13] and need for hospitalization.[13,75] These findings probably reflect that more severe liver damage need longer time to resolve. Increased ALP values at DILI onset has also been identified as a risk factor for chronicity.[13,76] Furthermore, Medina-Cáliz et al developed a prognostic model for chronicity based on values of ALP and TBL, showing that persistently elevated TBL ($>2.8 \times$ ULN) and ALP ($>1.1 \times$ ULN) in the second month from DILI onset predict increased risk of chronic DILI.[13]

6 | THE FUTURE

New specific biomarkers are urgently needed not only to confidently diagnose DILI but also to accurately predict it during the early phases of drug development. This would enable the development of safer drugs and a better characterization of liver safety profiles of marketed medications. In addition, this would reduce the uncertainty for physicians and patients when prescribing additional drugs within the same drug class as the causative agent. In the meantime, maintenance of existing prospective DILI cohort studies and establishing new collaborative initiatives will allow a better understanding of DILI signatures that can assist clinicians when assessing a potential case of hepatotoxicity.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

None.

REFERENCES

1. Chen M, Suzuki A, Borlak J, Andrade RJ, Lucena MI. Drug-induced liver injury: interactions between drug properties and host factors. *J Hepatol.* 2015;63:503-514.
2. Yuan L, Kaplowitz N. Mechanisms of drug-induced liver injury. *Clin Liver Dis.* 2013;17:507-518.
3. Hillman L, Gottfried M, Whitsett M, et al. Clinical features and outcomes of complementary and alternative medicine induced acute liver failure and injury. *Am J Gastroenterol.* 2016;111:958-965.
4. SAFE-T consortium The Drug induced liver injury work package of Innovative Medicines Initiative SAFE-T Consortium and The Hepatotoxicity Working Group of Critical Path Institutes PSTC. 2016. http://www.imi-safe-t.eu/files/files-inline/DILIBMSummaryDataPackage-20170105_final_updated.pdf
5. Church RJ, Kullak-Ublick GA, Aubrecht J, et al. Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: an international collaborative effort. *Hepatology.* 2019;69:760-763.
6. Arroyo V, Moreau R, Kamath PS, et al. Acute-on-chronic liver failure in cirrhosis. *Nat Rev Dis Prim.* 2016;2:16041.
7. Andrade R, Lucena M, Fernandez M, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology.* 2005;129:512-521.
8. Chalasani N, Fontana RJ, Bonkovsky HL, et al. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology.* 2008;135(1924–1934):e4.
9. Robles–Diaz M, Lucena MI, Kaplowitz N, et al. Use of Hy's law and a new composite algorithm to predict acute liver failure in patients with drug-induced liver injury. *Gastroenterology.* 2014;147:109-118.
10. Hayashi PH, Rockey DC, Fontana RJ, et al. Death and liver transplantation within 2 years of onset of drug-induced liver injury. *Hepatology.* 2017;66:1275-1285.
11. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology.* 1989;97:439-445.
12. Rathi C, Pipaliya N, Patel R, Ingle M, Phadke A, Sawant P. Drug induced liver injury at a tertiary hospital in India: etiology, clinical features and predictors of mortality. *Ann Hepatol.* 2017;16(3):442–450.
13. Medina-Caliz I, Robles-Diaz M, Garcia-Muñoz B, et al. Definition and risk factors for chronicity following acute idiosyncratic drug-induced liver injury. *J Hepatol.* 2016;65:532-542.
14. LiverTox, clinical and research information on drug-induced liver injury. 2017. https://livertox.nih.gov
15. Kullak-Ublick GA, Andrade RJ, Merz M, et al. Drug-induced liver injury: recent advances in diagnosis and risk assessment. *Gut.* 2017;66:1154-1164.
16. Aithal GP, Watkins PB, Andrade RJ, et al. Case definition and phenotype standardization in drug-induced liver injury. *Clin Pharmacol Ther.* 2011;89:806-815.
17. Andrade RJ, Lucena MI, Kaplowitz N, et al. Outcome of acute idiosyncratic drug-induced liver injury: long-term follow-up in a hepatotoxicity registry. *Hepatology.* 2006;44:1581-1588.
18. Robles-Diaz M, Gonzalez-Jimenez A, Medina-Caliz I, et al. Distinct phenotype of hepatotoxicity associated with illicit use of anabolic androgenic steroids. *Aliment Pharmacol Ther.* 2015;41:116-125.
19. Medina-Caliz I, Garcia-Cortes M, Gonzalez-Jimenez A, et al. Herbal and dietary supplement-induced liver injuries in the Spanish DILI registry. *Clin Gastroenterol Hepatol.* 2018;16:1495-1502.
20. Black M, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. *Gastroenterology.* 1975;69:289-302.
21. García Cortés M, Andrade RJ, Lucena MI, et al. Flutamide-induced hepatotoxicity: report of a case series. *Rev Esp enfermedades Dig.* 2001;93:423-432.
22. Schmeltzer PA, Kosinski AS, Kleiner DE, et al. Liver injury from nonsteroidal anti-inflammatory drugs in the United States. *Liver Int.* 2016;36:603-609.
23. Lucena MI, Andrade RJ, Fernández MC, et al. Determinants of the clinical expression of amoxicillin-clavulanate hepatotoxicity: a prospective series from Spain. *Hepatology.* 2006;44:850-856.

24. Björnsson ES, Gu J, Kleiner DE, Chalasani N, Hayashi PH, Hoofnagle JH. Azathioprine and 6-Mercaptopurine-induced Liver Injury: Clinical Features and Outcomes. *J Clin Gastroenterol*. 2017;51:63-69.

25. Chitturi S, Farrell GC. Adverse effects of hormones and hormone antagonists on the liver. In: Kaplowitz N, DeLeve LD, eds. *Drug-Induced Liver Disease*. San Diego, CA:Academic Press; 2013:605-619.

26. Lucena MI, Andrade RJ, Kaplowitz N, et al. Phenotypic characterization of idiosyncratic drug-induced liver injury: the influence of age and sex. *Hepatology*. 2009;49:2001-2009.

27. Ortega-Alonso A, Stephens C, Lucena MI, Andrade RJ. Case characterization, clinical features and risk factors in drug-induced liver injury. *Int J Mol Sci*. 2016;17:e714.

28. Senior JR. Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury–past, present, and future. *Clin Pharmacol Ther*. 2012;92:332-339.

29. Robles-Diaz M, Garcia-Cortes M, Medina-Caliz I, et al. The value of serum aspartate aminotransferase and gamma-glutamyl transpeptidase as biomarkers in hepatotoxicity. *Liver Int*. 2015;35:2474-2482.

30. Senior JR. Can rechallenge be done safely after mild or moderate drug-induced liver injury? *Hepatology*. 2016;63:691-693.

31. EASL. Clinical practice guidelines on drug-induced liver injury. *J Hepatol*. 2019;70(6):1222-1261.

32. Dalton HR, Fellows HJ, Stableforth W, et al. The role of hepatitis E virus testing in drug-induced liver injury. *Aliment Pharmacol Ther*. 2007;26:1429-1435.

33. Davern TJ, Chalasani N, Fontana RJ, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology*. 2011;141:1665-1672.

34. Sanjuan-Jimenez R, Robles-Díaz1 M, Sanabria J, et al. Prevalence of hepatitis E markers in Spanish patients with suspected drug-induced liver injury [abstract]. *Hepatology*. 2017;66:423A.

35. Hoofnagle JH, Nelson KE, Purcell RH. Hepatitis E. *N Engl J Med*. 2012;367:1237-1244.

36. de Boer YS, Kosinski AS, Urban TJ, et al. Features of autoimmune hepatitis in patients with drug-induced liver injury. *Clin Gastroenterol Hepatol*. 2017;15:103-112.

37. Perdices EV, Medina-Cáliz I, Hernando S, et al. Hepatotoxicity associated with statin use: analysis of the cases included in the Spanish Hepatotoxicity Registry. *Rev Esp enfermedades Dig*. 2014;106:246-254.

38. Björnsson ES, Gunnarsson BI, Gröndal G, et al. Risk of drug-induced liver injury from tumor necrosis factor antagonists. *Clin Gastroenterol Hepatol*. 2015;13:602-608.

39. Clinical E. Practice guidelines: Wilson's disease. *J Hepatol*. 2012;56:671-685.

40. Tapper EB, Sengupta N, Bonder A. The incidence and outcomes of ischemic hepatitis: a systematic review with meta-analysis. *Am J Med*. 2015;128:1314-1321.

41. Ludwig J, Kim CH, Wiesner RH, Krom RA. Floxuridine-induced sclerosing cholangitis: an ischemic cholangiopathy? *Hepatology*. 1989;9:215-218.

42. Aldrighetti L, Arru M, Ronzoni M, Salvioni M, Villa E, Ferla G. Extrahepatic biliary stenoses after hepatic arterial infusion (HAI) of floxuridine (FUdR) for liver metastases from colorectal cancer. *Hepatogastroenterology*. 2001;48:1302-1307.

43. Horsley-Silva JL, Dow EN, Menias CO, et al. Docetaxel induced sclerosing cholangitis. *Dig Dis Sci*. 2015;60:3814-3816.

44. Gudnason HO, Björnsson HK, Gardarsdottir M, et al. Secondary sclerosing cholangitis in patients with drug-induced liver injury. *Dig Liver Dis*. 2015;47:502-507.

45. Sagir A, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology*. 2007;47:592-595.

46. Kleiner DE. Histopathological challenges in suspected drug-induced liver injury. *Liver Int*. 2018;38:198-209.

47. Kleiner DE, Chalasani NP, Lee WM, et al. Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations. *Hepatology*. 2014;59:661-670.

48. Bonkovsky HL, Kleiner DE, Gu J, et al. Clinical presentations and outcomes of bile duct loss caused by drugs and herbal and dietary supplements. *Hepatology*. 2017;65:1267-1277.

49. Licata A, Maida M, Cabibi D, et al. Clinical features and outcomes of patients with drug-induced autoimmune hepatitis: a retrospective cohort study. *Dig Liver Dis*. 2014;46:1116-1120.

50. Craig WY, Ledue TB, Johnson AM, Ritchie RF. The distribution of antinuclear antibody titers in «normal» children and adults. *J Rheumatol*. 1999;26:914-919.

51. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology*. 2008;48:169-176.

52. Suzuki A, Brunt EM, Kleiner DE, et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. *Hepatology*. 2011;54:931-939.

53. Foureau DM, Walling TL, Maddukuri V, et al. Comparative analysis of portal hepatic infiltrating leucocytes in acute drug-induced liver injury, idiopathic autoimmune and viral hepatitis. *Clin Exp Immunol*. 2015;180:40-51.

54. Björnsson E, Talwalkar J, Treeprasertsuk S, et al. Drug-induced autoimmune hepatitis: clinical characteristics and prognosis. *Hepatology*. 2010;51:2040-2048.

55. Aithal GP. Pharmacogenetic testing in idiosyncratic drug-induced liver injury: current role in clinical practice. *Liver Int*. 2015;35:1801-1808.

56. Nicoletti P, Aithal GP, Bjornsson ES, et al. Association of liver injury from specific drugs, or groups of drugs, with polymorphisms in HLA and other genes in a genome-wide association study. *Gastroenterology*. 2017;152:1078-1089.

57. Cirulli ET, Nicoletti P, Abramson K, et al. A missense variant in PTPN22 is a risk factor for drug-induced liver injury. *Gastroenterology*. 2019156:1707-1716.e2.

58. Rockey DC, Seeff LB, Rochon J, et al. Causality assessment in drug-induced liver injury using a structured expert opinion process: comparison to the Roussel-Uclaf causality assessment method. *Hepatology*. 2010;51:2117-2126.

59. Danan G, Benichou C. Causality assessment of adverse reactions to drugs—I. A novel method based on the conclusions of international consensus meetings: Application to drug-induced liver injuries. *J Clin Epidemiol*. 1993;46:1323-1330.

60. García-Cortés M, Stephens C, Lucena MI, Fernández-Castañer A, Andrade RJ. Causality assessment methods in drug induced liver injury: strengths and weaknesses. *J Hepatol*. 2011;55:683-691.

61. Bernal W, Wendon J. Acute liver failure. *N Engl J Med*. 2013;369:2525-2534.

62. Reuben A, Tillman H, Fontana RJ, et al. Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. *Ann Intern Med*. 2016;164:724-732.

63. Bernal W, Hyyrylainen A, Gera A, et al. Lessons from look-back in acute liver failure? A single centre experience of 3300 patients. *J Hepatol*. 2013;59:74-80.

64. Wei G, Bergquist A, Broomé U, et al. Acute liver failure in Sweden: etiology and outcome. *J Intern Med*. 2007;262:393-401.

65. Reuben A, Koch DG, Lee WM. Acute liver failure study group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology*. 2010;52:2065-2076.

66. Björnsson E, Kalaitzakis E, Olsson R. The impact of eosinophilia and hepatic necrosis on prognosis in patients with drug-induced liver injury. *Aliment Pharmacol Ther*. 2007;25:1411-1421.

67. Zimmerman HJ. The spectrum of hepatotoxicity. *Perspect Biol Med*. 1968;12:135-161.

68. U.S. Department of Health and Human Services FDA, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for industry. Drug-induced liver injury: premarketing clinical. *Evaluation*. 2009. https://www.fda.gov/downloads/Guidances/UCM174090.pdf. Accessed December 20, 2018.

69. Björnsson E, Olsson R. Outcome and prognostic markers in severe drug-induced liver disease. *Hepatology*. 2005;42:481-489.

70. Lo Re V, Haynes K, Forde KA, et al. Risk of acute liver failure in patients with drug-induced liver injury: evaluation of Hy's law and a new prognostic model. *Clin Gastroenterol Hepatol*. 2015;13:2360-2368.

71. Jeong R, Lee Y-S, Sohn C, Jeon J, Ahn S, Lim KS. Model for end-stage liver disease score as a predictor of short-term outcome in patients with drug-induced liver injury. *Scand J Gastroenterol*. 2015;50:439-446.

72. Devarbhavi H, Patil M, Reddy VV, Singh R, Joseph T, Ganga D. Drug-induced acute liver failure in children and adults: results of a single-centre study of 128 patients. *Liver Int*. 2017;38:1322-1329.

73. Bernal W, Donnelly MC, Durkalski-Mauldin VL, Simpson K, Wang Y, Wendon J. External validation of the USALFSG acute liver failure outcome prediction criteria. *J Hepatol*. 2017;66:S343-S344.

74. Bénichou C. Criteria of drug-induced liver disorders report of an international consensus meeting. *J Hepatol*. 1990;11:272-276.

75. Fontana RJ, Hayashi PH, Gu J, et al. Idiosyncratic drug-induced liver injury is associated with substantial morbidity and mortality within 6 months from onset. *Gastroenterology*. 2014;147:96-108.

76. Fontana RJ, Hayashi PH, Barnhart H, et al. Persistent liver biochemistry abnormalities are more common in older patients and those with cholestatic drug induced liver injury. *Am J Gastroenterol*. 2015;110:1450-1459.

77. Lucena MI, Kaplowitz N, Hallal H, et al. Recurrent drug-induced liver injury (DILI) with different drugs in the Spanish Registry: the dilemma of the relationship to autoimmune hepatitis. *J Hepatol*. 2011;55:820-827.

78. Cacoub P, Musette P, Descamps V, et al. The DRESS syndrome: a literature review. *Am J Med*. 2011;124:588-597.

79. Kardaun SH, Sidoroff A, Valeyrie-Allanore L, et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br J Dermatol*. 2007;156:609-611.

80. Chalasani N, Bonkovsky HL, Fontana R, et al. Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study. *Gastroenterology*. 2015;148:1340-1352.

81. Devarbhavi H, Dierkhising R, Kremers WK, Sandeep MS, Karanth D, Adarsh CK. Single-center experience with drug-induced liver injury from India: causes, outcome, prognosis and predictors of mortality. *Am J Gastroenterol*. 2010;105:2396-2404.

82. Devarbhavi H, Raj S. Drug-induced liver injury with skin reactions: Drugs and host risk factors, clinical phenotypes and prognosis. *Liver Int*. 2018;39:802-811.

83. Björnsson ES, Aithal GP. *Immune-mediated drug-induced liver injury. En: liver immunology*. Cham: Springer International Publishing; 2014:401-412.

84. Raja K, Thung S, Fiel MI, Chang C. Drug-induced steatohepatitis leading to cirrhosis: Long-term toxicity of amiodarone use. *Semin Liver Dis*. 2009;29:423-428.

85. Aithal GP. Hepatotoxicity related to methotrexate. In: Kaplowitz N, DeLeve LD, eds. *Drug-induced liver disease*. San Diego, CA: Elsevier; 2013:593-604.

86. Bruno S, Maisonneuve P, Castellana P, et al. Incidence and risk factors for non-alcoholic steatohepatitis: prospective study of 5408 women enrolled in Italian tamoxifen chemoprevention trial. *BMJ*. 2005;330:932.

87. Vauthey J-N, Pawlik TM, Ribero D, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol*. 2006;24:2065-2072.

88. Vernier-Massouille G, Cosnes J, Lemann M, et al. Nodular regenerative hyperplasia in patients with inflammatory bowel disease treated with azathioprine. *Gut*. 2007;56:1404-1409.

89. Mallet V, Blanchard P, Verkarre V, et al. Nodular regenerative hyperplasia is a new cause of chronic liver disease in HIV-infected patients. *AIDS*. 2007;21:187-192.

90. Hartleb M, Gutkowski K, Milkiewicz P. Nodular regenerative hyperplasia: evolving concepts on underdiagnosed cause of portal hypertension. *World J Gastroenterol*. 2011;17:1400-1409.

91. Rooks JB, Ory HW, Ishak KG, et al. Epidemiology of hepatocellular adenoma. The role of oral contraceptive use. *JAMA*. 1979;242:644-648.

92. Velazquez I, Alter BP. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. *Am J Hematol*. 2004;77:257-267.

93. Gudnason HO, Björnsson ES. Secondary sclerosing cholangitis in critically ill patients: current perspectives. *Clin Exp Gastroenterol*. 2017;10:105-111.

94. Drebber U, Kasper H-U, Ratering J, et al. Hepatic granulomas: histological and molecular pathological approach to differential diagnosis - a study of 442 cases. *Liver Int*. 2008;28:828-834.

95. Stewart JD, Horvath R, Baruffini E, et al. Polymerase γ gene POLG determines the risk of sodium valproate-induced liver toxicity. *Hepatology*. 2010;52:1791-1796.

96. Margolis AM, Heverling H, Pham PA, Stolbach A. A review of the toxicity of HIV medications. *J Med Toxicol*. 2014;10:26-39.

97. Jones DB, Mullick FG, Hoofnagle JH, Baranski B. Reye's syndrome-like illness in a patient receiving amiodarone. *Am J Gastroenterol*. 1988;83:967-969.

98. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. 2016;54:139-148.

99. Haanen J, Carbonnel F, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2017;28:iv119-iv142.

100. Suzman DL, Pelosof L, Rosenberg A, Avigan MI. Hepatotoxicity of immune checkpoint inhibitors: an evolving picture of risk associated with a vital class of immunotherapy agents. *Liver Int*. 2018;38:976-987.

101. De Martin E, Michot J-M, Papouin B, et al. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. *J Hepatol*. 2018;68:1181-1190.

102. Desmet VJ. Vanishing bile duct syndrome in drug-induced liver disease. *J Hepatol*. 1997;26(Suppl 1):31-35.

103. Elsayes KM, Shaaban AM, Rothan SM, et al. A comprehensive approach to hepatic vascular disease. *Radiographics*. 2017;37:813-836.

104. Zafrani ES, von Pinaudeau Y, Dhumeaux D. Drug-induced vascular lesions of the liver. *Arch Intern Med*. 1983;143:495-502.

105. Bagheri SA, Boyer JL. Peliosis hepatis associated with androgenic-anabolic steroid therapy. A severe form of hepatic injury. *Ann Intern Med*. 1974;81:610-618.

106. Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg P. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology*. 2000;31:864-871.