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DRUG-INDUCED LIVER INJURY: MECHANISMS, BIOMARKERS AND CAUSALITY ASSESSMENT

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Abstract

Drug-induced liver injury (DILI) is a leading reason for drug withdrawal, regulatory warnings and acute liver failure, yet it is still diagnosed largely by exclusion. This narrative review summarises published work on how DILI is classified, the mechanisms proposed for intrinsic and idiosyncratic injury, the status of newer circulating biomarkers beyond aminotransferases, and the tools used to judge whether a drug caused a given liver event. Mitochondrial stress, bile-salt transport inhibition and HLA-linked immune responses are the best-supported mechanisms. Causality scores remain imperfect and biomarkers are not yet in routine use. The article is a review and reports no new data.

1. INTRODUCTION

The liver meets almost every drug that is swallowed, and it is therefore no surprise that it is also where many drugs cause trouble. Drug-induced liver injury (DILI) covers a range of events, from a transient rise in serum aminotransferases that settles on its own to fulminant failure that ends in transplantation or death. It has halted promising compounds in late clinical development and prompted withdrawal or restriction of marketed ones, and it remains the most frequent cause of acute liver failure in several Western countries once paracetamol overdose is included [1, 2].

For clinicians the difficulty lies in the diagnosis. There is no test that confirms a drug as the cause. Doctors must first rule out viral hepatitis, biliary obstruction, autoimmune disease, alcohol and ischaemia, and only then accept that a drug is the likeliest explanation, often among several candidate drugs [3, 4]. For researchers the

challenge is different, since the commonest form of the disease is rare in any single exposed person and almost impossible to predict from standard preclinical toxicology.

This review covers three linked topics suggested by the title. The first is how DILI is defined, classified and counted. The second is what is known about mechanisms, including genetic susceptibility. The third is the practical toolkit for deciding whether a given drug caused a given liver event, comprising clinical scoring systems, expert opinion processes and a growing list of experimental biomarkers. The article draws on published guidelines, registries and mechanistic studies up to 2022 and reports no new data.

2. CLASSIFICATION AND EPIDEMIOLOGY

The traditional division is between intrinsic and idiosyncratic injury. Intrinsic DILI is predictable and dose-dependent, appears in most people exposed to a sufficient dose, and can be reproduced in animals. Paracetamol is the classic example, with injury following when the glutathione-dependent detoxification of its reactive metabolite is overwhelmed [5, 6]. Idiosyncratic DILI is uncommon, not clearly dose-dependent in the textbook sense, variable in latency and presentation, and depends on host susceptibility. Most drugs implicated in clinical practice, other than paracetamol, fall into this second group [6, 7].

Operational definitions are needed for research and regulation. An international consensus defined DILI biochemically as an alanine aminotransferase (ALT) level at least five times the upper limit of normal (ULN), an alkaline phosphatase (ALP) at least twice the ULN, or ALT at least three times the ULN with bilirubin more than twice the ULN [5, 8]. The pattern is then classed by the R ratio, which divides the ALT multiple of ULN by the ALP multiple. A value of five or more indicates hepatocellular injury, two or less cholestatic, and values between the two a mixed picture [4, 5].

Population figures depend on how cases are found. A prospective study in Iceland that followed a whole population estimated the annual incidence at about nineteen cases per 100,000 inhabitants, with antibiotics the most frequent group of causes [9]. Registries from Spain and the United States, which collect cases referred to specialist centres, show a similar profile, with amoxicillin-clavulanate leading among single agents and herbal and dietary supplements making up a growing share [10, 11]. These centre-based series over-represent severe disease, and the true burden of mild, self-limited injury is certainly higher.

Clinical phenotypes add another layer. Latency from first dose to onset is usually between a few days and a few months, but with some drugs it is longer, and reactions to amoxicillin-clavulanate may appear after the course has finished. Some patients show immunoallergic features such as fever, rash and eosinophilia. Others develop an autoimmune-like hepatitis with autoantibodies and a histological picture that resembles the primary disease, a pattern described with nitrofurantoin and minocycline. Prolonged cholestasis and loss of small bile ducts can follow severe cholestatic injury [4, 10]. Recognising the phenotype guides both the work-up and the choice of treatment.

Injury is also an outcome of host factors. Age, sex and ethnicity influence which drugs are implicated and how the liver is affected. Cholestatic and mixed injury become more common in older adults, whereas hepatocellular patterns are relatively frequent in younger patients. Pre-existing liver disease increases the consequence of injury more than the likelihood, according to most registry analyses, and the effect of alcohol is uncertain outside specific drugs such as paracetamol [10, 12]. Higher daily doses and lipophilic drugs have been linked with more idiosyncratic reactions, which offers a partial bridge between the two categories.

Under-recognition limits all of these figures. Mild injury is frequently missed unless liver tests are routinely repeated, spontaneous reports capture only a fraction of events, and many cases are labelled as viral or alcoholic liver disease without a medication history being taken properly. Comparisons between countries are further confused by differences in prescribing, in herbal medicine use and in

thresholds for referral. The result is that incidence estimates for individual drugs, often expressed per 10,000 or 100,000 prescriptions, vary widely and should be read with caution [11, 13].

Outcome data matter for deciding who needs attention. Follow-up of the US Drug-Induced Liver Injury Network cohort found that a minority of patients with jaundice died or required transplantation within six months, and that a larger proportion had persistent liver test abnormalities after half a year [10, 14]. Hepatocellular injury with jaundice carries the worst short-term prognosis, which is the observation behind Hy's law, discussed below [15, 16].

3. MECHANISMS OF INJURY

For intrinsic injury the mechanistic story is reasonably clear. Cytochrome P450 enzymes convert paracetamol to a reactive quinone-imine, normally neutralised by glutathione. When glutathione is depleted the metabolite binds covalently to mitochondrial and cytosolic proteins, oxidative stress follows, and hepatocytes undergo necrosis. This sequence explains the success of acetylcysteine, which replenishes glutathione, and illustrates the main principle that liver injury often starts with metabolic activation rather than with the parent compound [5, 6].

Idiosyncratic injury is more complex, and several interacting hypotheses are favoured. Mitochondrial dysfunction is one. Drugs that inhibit respiratory chain complexes, fatty acid oxidation or mitochondrial DNA replication can create a silent burden that later tips cells into death, particularly where underlying genetic variants or other drugs add to the load [17]. Amiodarone, valproate and some nucleoside analogues are cited examples. The idea explains delayed onset and variable susceptibility, but it is hard to prove in the individual patient because mitochondrial damage is rarely measured in routine care.

A second mechanism involves bile acid handling. The bile salt export pump (BSEP) moves bile acids from hepatocytes into the canaliculus, and inhibition of this transporter leads to intracellular accumulation of toxic bile acids. In vitro studies found that inhibition of BSEP correlates with the risk of cholestatic or mixed injury in humans, and screening for this property is now common in drug development [18, 19]. The picture is not simple, since many BSEP inhibitors have not caused clinical injury, perhaps because compensatory transporters protect most people.

The immune system provides the third strand, and genetic evidence for it is the strongest available. Carriage of HLA-B*57:01 raises the risk of flucloxacillin-induced liver injury by an enormous factor, although only a very small share of carriers are affected [20]. Amoxicillin-clavulanate injury is associated with HLA class II alleles, especially DRB1*15:01, together with class I variants [21]. Minocycline hepatotoxicity has been linked to HLA-B*35:02 [22]. These associations suggest drug-specific adaptive immune responses, probably triggered by drug-modified peptides or by direct binding to HLA, though the effector cells and antigens remain mostly uncharacterised.

Most researchers now see these ideas as layers rather than rivals. A drug may produce reactive metabolites, stress mitochondria, and impair bile acid transport to different degrees, and whether this leads to clinical injury depends on the immune context, which determines whether the early damage resolves or escalates. The danger signal concept suggests that stressed hepatocytes release molecules that activate innate immune cells, which in turn present the drug-altered antigens to T cells [7, 12]. Since the sequence is individual, a single in vitro assay will probably never predict human DILI satisfactorily.

Drug properties influence risk in ways that are useful to prescribers and chemists. Review of idiosyncratic cases suggests that daily doses above 50 to 100 mg, high lipophilicity and extensive hepatic metabolism are over-represented among implicated drugs, so that even a rare reaction becomes more probable when the liver is exposed to a large amount of compound or its reactive products [12]. These are statistical tendencies, not rules, and many exceptions exist. Even so, they have been used to screen candidates early in development, along with in vitro assays for reactive metabolite

formation, mitochondrial toxicity and BSEP inhibition [12, 18, 19].

One practical barrier is that standard preclinical species rarely reproduce idiosyncratic injury. Rodents given a candidate for weeks may show nothing, and the same compound can later injure a handful of people per thousand. Liver cell lines have limited metabolic capacity, and primary human hepatocytes lose function quickly in culture. More complex cell models, with several liver cell types and longer culture, improve sensitivity, but none has been shown to predict idiosyncratic outcomes reliably. For now, the predictive value of preclinical packages for rare human reactions stays low [12, 17].

4. BIOMARKERS

ALT has been the workhorse of liver safety for decades, and its limits are well known. It rises with muscle injury and strenuous exercise, it is not specific for the cause, and it tells little about prognosis. Its sensitivity is generally good, but a rise can lag behind the initial injury. In the setting of paracetamol overdose, ALT may still be normal at presentation when the liver has already been exposed to a lethal dose, which is why clinicians rely on timed paracetamol concentrations and not enzyme levels [2, 23].

Newer candidate markers fall into several groups. MicroRNA-122 is abundant in hepatocytes and appears in the circulation earlier and in larger relative amounts than ALT after paracetamol injury [23, 24]. Keratin-18 occurs in full-length and caspase-cleaved forms, which can help to distinguish necrosis from apoptosis. Glutamate dehydrogenase is a mitochondrial enzyme that is liver-specific and not raised by muscle damage. High mobility group box 1 protein, a danger signal, has also been studied and appears to correlate with injury severity. In healthy volunteers, keratin-18 and microRNA-122 behaved as complementary markers to ALT [23, 25].

An international collaborative effort examined candidate biomarkers for both diagnosis and prognosis and concluded that several showed promise, but that none had been validated sufficiently for clinical decision-making [26]. The obstacles are familiar. Samples were often taken at different times after onset, patient numbers were small, causes varied, and comparator groups with other liver disease were insufficient. Qualification for regulatory use needs consistent performance in large, well-characterised populations, and that has only started to be assembled.

Prognostic markers would be especially useful. At present, prognosis rests on bilirubin, international normalised ratio and the presence of encephalopathy, all of which indicate that serious damage has already occurred. Hy's law was proposed from clinical observation that drug-induced hepatocellular jaundice, without another explanation, carries a mortality of around ten per cent or more [15]. Regulators adopted it as a signal in trials [27], and subsequent work has tested composite algorithms that combine ALT, ALP and bilirubin and perform better at predicting acute liver failure than the original rule [16].

Regulatory practice has built on these observations. The US guidance on premarketing evaluation describes the combination of ALT or aspartate aminotransferase above three times ULN with bilirubin above twice ULN, without initial cholestasis, as a warning signal for a drug with serious hepatotoxic potential, and recommends stopping rules for trial participants whose liver tests rise [15, 27]. A drug that produces a hepatocellular signal with jaundice in a few trial participants is likely to cause a larger number of serious cases once marketed to thousands, and development has been halted on this basis more than once.

5. CAUSALITY AND MANAGEMENT

Causality assessment is the most common practical task. The Roussel Uclaf Causality Assessment Method (RUCAM) scores time to onset from drug start and from drug stop, the course of enzymes after withdrawal, risk factors such as age over 55 years and alcohol use, concomitant drugs, exclusion of other causes, previous knowledge of hepatotoxicity for the drug, and response to rechallenge, if any [28, 29]. Totals translate into categories

from excluded through unlikely, possible and probable to highly probable. An updated version was published in 2016 to clarify definitions and separate hepatocellular from cholestatic patterns [29].

RUCAM has strengths, in being structured and widely used, but it also has problems. Studies of reproducibility have shown that experienced assessors given the same case often differ in their scores. The method gives limited weight to alternative causes that cannot be excluded in practice, and it penalises older drugs for which hepatotoxicity is poorly documented. A comparison with a structured expert opinion process, used in the DILIN study, showed only moderate agreement between the two approaches, and the expert process was judged more accurate in some analyses [10, 30]. No method is a gold standard because the true cause is rarely known.

Rechallenge, in which the drug is restarted after recovery, offers the most direct evidence but carries obvious risk. A review of published cases found that a recurrence of injury after rechallenge was common for some drugs and uncommon for others, with the risk higher for drugs that caused severe initial injury or immunoallergic features [31]. Accidental rechallenge is by far the most frequent source of such information. Deliberate rechallenge may be justified when the drug is essential and there is no alternative, as with some antituberculosis regimens, and should be done under close monitoring.

Management begins with stopping the suspected drug and, in patients with several candidates, those with the best supporting evidence. Paracetamol overdose has a specific antidote, acetylcysteine, which is most effective early. Intravenous acetylcysteine improved transplant-free survival in early-stage non-paracetamol acute liver failure in a randomised trial, although benefit was confined to patients with low-grade encephalopathy [32]. Corticosteroids are sometimes used when features of immunoallergic injury are present, and ursodeoxycholic acid in cholestatic disease, but both have limited trial support. Referral to a transplant centre is needed for acute liver failure [3, 33].

Many patients recover completely within weeks of stopping the culprit, but some follow a prolonged course. Persistent enzyme abnormalities beyond six months are classed as chronic DILI, and a proportion of these patients have underlying vanishing bile duct syndrome or fibrosis. Follow-up should therefore continue until liver tests have normalised, and referral to hepatology is appropriate when abnormalities persist. Long-term monitoring data are limited, though the US network reported that chronic injury was more frequent after cholestatic episodes and in older patients [4, 14, 33].

6. LIMITATIONS AND FUTURE DIRECTIONS

The principal evidence is brought together in table 1, which lists the main mechanisms, genetic associations, biomarkers and assessment tools reviewed above, and the distribution of its entries by theme is shown in fig. 1. The table makes one point visually: the evidence is rich for mechanisms and genetics but thinner for diagnostic tools that are actually in routine use. Most entries also derive from observational work or in vitro experiments, and few have been tested prospectively in clinical decision-making.

Table 1: Selected mechanisms, genetic associations, biomarkers and assessment tools in DILI

Topic	Reported finding	Ref.
Paracetamol reactive metabolite	Glutathione depletion and covalent protein binding cause predictable, dose-dependent necrosis	[5, 6]
Mitochondrial injury respiratory chain	Impaired respiration or fatty acid oxidation proposed as a cause of delayed, idiosyncratic injury	[17]
BSEP inhibition bile acid transport	In vitro inhibition correlates with risk of cholestatic or mixed injury	[18, 19]
HLA-B*57:01 flucloxacillin	Very strong association, with only a small minority of carriers affected	[20]
HLA-DRB1*15:01 amoxicillin-clavulanate	Class II and class I alleles influence susceptibility	[21]

Topic	Reported finding	Ref.
HLA-B*35:02 minocycline	Identified as a risk factor for minocycline hepatotoxicity	[22]
MicroRNA-122 and keratin-18 circulating	Appear early after paracetamol injury and complement ALT in healthy volunteers	[23, 24, 25]
Candidate panels multi-centre	Promising but not validated for clinical decisions	[26]
Hy's law jaundice with hepatocellular injury	Predicts serious outcomes; composite algorithms may improve on it	[15, 16, 27]
RUCAM scoring	Structured scoring of timing, course, risk factors and exclusion of alternatives	[28, 29]
Expert opinion DILIN process	Moderate agreement with RUCAM, judged more accurate in some comparisons	[30]

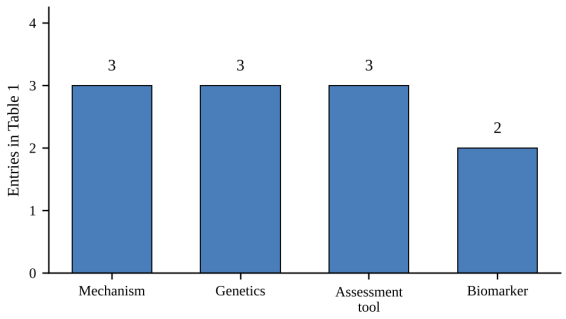


Fig. 1: Number of entries in table 1 by theme

Collaboration between academia, regulators and industry has been central to progress, and it will remain so. A multi-stakeholder conference convened in the mid-2000s set the research agenda that later produced the prospective networks, the standard case definitions and much of the biomarker work discussed here [34]. Sharing of clinical samples and harmonised phenotyping, rather than isolated single-centre efforts, has proved to be the only workable route to adequate numbers for rare outcomes. Continued data sharing between pharmacovigilance centres, trial sponsors and hepatology networks will be needed if pre-treatment genetic tests or validated biomarkers are ever to reach the clinic.

Several limitations apply to the literature as a whole. Cases are often detected late and incompletely documented. Registry cohorts select the sicker patients, and exposure to herbal products and supplements is hard to confirm. Genetic studies have small numbers of cases, and their findings seldom transfer across ancestry groups. Biomarker studies rely heavily on paracetamol, which is atypical because it is intrinsic and its timing is known. Findings from it may not apply to idiosyncratic injury, which is what clinicians usually face [12, 23, 26].

Priorities for the next decade are clear enough. Genome-wide studies in larger multi-ethnic cohorts should identify further risk alleles, and in turn may support pre-treatment testing where the positive predictive value is high enough. Biomarker panels need to be tested against clinical endpoints in prospective cohorts. Better models of the human liver, including co-cultures with immune cells and organoids, could help bridge the preclinical gap. Finally, a causality method combining structured scoring with expert review and quantitative biomarker data would reduce the variability that now limits clinical and regulatory decisions [26, 30].

7. CONCLUSION

Drug-induced liver injury is best seen as a family of conditions with partly shared mechanisms. Intrinsic injury is well understood and, for paracetamol, effectively treatable. Idiosyncratic injury arises from an interplay of metabolic activation, mitochondrial and bile-acid stress, and immune responses shaped by HLA genotype, which explains both its rarity and its unpredictability. Registries have clarified which drugs are usual suspects, but their data come from referral centres [9, 10, 11, 20].

Diagnosis still depends on exclusion and structured judgement. RUCAM and expert opinion are useful but imperfect, and circulating biomarkers such as microRNA-122 and keratin-18 need validation before they can alter care [26, 29, 30]. For now the most dependable measures are simple: prompt withdrawal of the suspected drug, careful history including supplements, early specialist referral when jaundice or coagulopathy develops, and thorough reporting. Larger genetic studies and clinical biomarker trials are the likeliest routes to better prediction [16, 32, 33].

8. ABBREVIATIONS

DILI-Drug-induced liver injury, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase, ULN-Upper limit of normal, BSEP-Bile salt export pump, HLA-Human leukocyte antigen, RUCAM-Roussel Uclaf Causality Assessment Method, DILIN-Drug-Induced Liver Injury Network

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

1. Andrade RJ, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, *et al.* Drug-induced liver injury. Nat Rev Dis Primers 2019;5:58.
2. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, *et al.* Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 2005;42:1364–72.
3. Chalasani NP, Hayashi PH, Bonkovsky HL, Navarro VJ, Lee WM, Fontana RJ. ACG clinical guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. Am J Gastroenterol 2014;109:950–66.
4. Hoofnagle JH, Björnsson ES. Drug-induced liver injury: types and phenotypes. N Engl J Med 2019;381:264–73.
5. Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. J Hepatol 1990;11:272–6.
6. Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov 2005;4:489–99.
7. Uetrecht J, Naisbitt DJ. Idiosyncratic adverse drug reactions: current concepts. Pharmacol Rev 2013;65:779–808.
8. Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, *et al.* Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther 2011;89:806–15.
9. Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. Gastroenterology 2013;144:1419–25.
10. Chalasani N, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalkar J, *et al.* Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study. Gastroenterology 2015;148:1340–52.
11. Andrade RJ, Lucena MI, Fernández MC, Pelaez G, Pachkoria K, García-Ruiz E, *et al.* Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology 2005;129:512–21.
12. Chen M, Suzuki A, Borlak J, Andrade RJ, Lucena MI. Drug-induced liver injury: interactions between drug properties and host factors. J Hepatol 2015;63:503–14.
13. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis 2009;29:337–47.
14. Fontana RJ, Hayashi PH, Gu J, Reddy KR, Barnhart H, Watkins PB, *et al.* Idiosyncratic drug-induced liver injury is associated with substantial morbidity and mortality within 6 months from onset. Gastroenterology 2014;147:96–108.
15. Zimmerman HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1999.
16. Robles-Diaz M, Lucena MI, Kaplowitz N, Stephens C, Medina-Cáliz I, González-Jimenez A, *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;147:109–18.
17. Boelsterli UA, Lim PL. Mitochondrial abnormalities: a link to idiosyncratic drug hepatotoxicity? Toxicol Appl Pharmacol 2007;220:92–107.
18. Morgan RE, Trauner M, van Staden CJ, Lee PH, Ramachandran B, Eschenberg M, *et al.* Interference with bile salt export pump function is a susceptibility factor for human liver injury in drug development. Toxicol Sci 2010;118:485–500.
19. Dawson S, Stahl S, Paul N, Barber J, Kenna JG. In vitro inhibition of the bile salt export pump correlates with risk of cholestatic drug-induced liver injury in humans. Drug Metab Dispos 2012;40:130–8.

20. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, *et al.* HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet 2009;41:816–9.
21. Lucena MI, Molokhia M, Shen Y, Urban TJ, Aithal GP, Andrade RJ, *et al.* Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. Gastroenterology 2011;141:338–47.
22. Urban TJ, Nicoletti P, Chalasani N, Serrano J, Stolz A, Daly AK, *et al.* Minocycline hepatotoxicity: clinical characterization and identification of HLA-B*35:02 as a risk factor. J Hepatol 2017;67:137–44.
23. Antoine DJ, Dear JW, Lewis PS, Platt V, Coyle J, Masson M, *et al.* Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. Hepatology 2013;58:777–87.
24. Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DG, Antoine DJ, *et al.* Circulating microRNAs as potential markers of human drug-induced liver injury. Hepatology 2011;54:1767–76.
25. Thulin P, Nordahl G, Gry M, Yimer G, Aklillu E, Makonnen E, *et al.* Keratin-18 and microRNA-122 complement alanine aminotransferase as liver safety biomarkers in healthy volunteers. Toxicol Appl Pharmacol 2014;281:105–13.
26. Church RJ, Kullak-Ublick GA, Aubrecht J, Bonkovsky HL, Chalasani N, Fontana RJ, *et al.* Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: an international collaborative effort. Hepatology 2019;69:760–73.
27. US Food and Drug Administration. Guidance for industry: drug-induced liver injury: premarketing clinical evaluation. Rockville: FDA; 2009.
28. Danan G, Benichou C. Causality assessment of adverse reactions to drugs-I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. J Clin Epidemiol 1993;46:1323–30.
29. Danan G, Teschke R. RUCAM in drug and herb induced liver injury: the update. Int J Mol Sci 2016;17:14.
30. Rockey DC, Seeff LB, Rochon J, Freston J, Chalasani N, Bonacini M, *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;51:2117–26.
31. Hunt CM, Papay JI, Stanulovic V, Regev A. Drug rechallenge following drug-induced liver injury. Hepatology 2016;63:584–97.
32. Lee WM, Hynan LS, Rossaro L, Fontana RJ, Stravitz RT, Larson AM, *et al.* Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. Gastroenterology 2009;137:856–64.
33. European Association for the Study of the Liver. EASL clinical practice guidelines: drug-induced liver injury. J Hepatol 2019;70:1222–61.
34. Watkins PB, Seeff LB. Drug-induced liver injury: summary of a single topic clinical research conference. Hepatology 2006;43:618–31.