POINT OF VIEW

Hepatotoxicity in 2011 - advancing resolutely

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INTRODUCTION

Liver toxicity from drugs, and also from alternative medicine products such as herbalist's remedies and dietary supplements, is currently an increasingly relevant health issue. A great majority of hepatic adverse reactions seen in clinical practice are unpredictable (unrelated to a drug's pharmacological characteristics) and basically result from an interaction of three circumstances: a drug with potential to generate hepatotoxic radicals in a genetically susceptible individual under certain environmental factors. This type of reaction, which occurs only rarely, goes undetected during a drug's development process, and hence typically manifests when dozens of thousands of patients are exposed to it post-marketing; this still represents the first cause for a drug's market withdrawal. On the other hand, the absence of objective diagnostic tests and variable clinical presentation commonly entail a delayed diagnosis of liver toxicity. In the present article, we review recent advances in this area and new consensus as result of investigators from different countries.

EPIDEMIOLOGY

The actual incidence of liver toxicity in clinical practice is little known. Only one prospective population study in France established the crude annual incidence of hepatic drug reactions to be 14 cases per 100,000 population (16 times higher than reported to regulatory agencies via the yellow card system) (1). In practice the risk for idiosyncratic hepatotoxicity associated with the use of most drug agents is thought to oscillate between 1/10,000 and 1/100,000 exposed individuals. The diagnosis of drug-related hepatotoxicity is substantially less common than that of other causes of liver disease. Toxicity is believed to represent 4-10% of jaundice cases admitted to general hospitals (2). Among inpatients the incidence of idiosyncratic hepatotoxicity has been estimated between 0.7% and 1.4% (3). Antibacterials, nonsteroidal anti-inflammatory drugs, and anticonvulsants rank at the top of the list of drug classes involved in hepatotoxicity (4,5), and amoxicillin/clavulanic acid is in absolute figures the most commonly involved molecule (4,5). Geographical variability is high regarding agents responsible for liver damage (Table I). Most cases in western countries are associated with antibiotics, anticonvulsants, and psychotropic drugs; less than 10% correspond to herbal remedies and dietary supplements, but this proportion has been increasing in the last few years (1,4-8). In Asia herbal remedies are a relevant cause of hepatotoxicity (9). A recent study showed that idiosyncratic hepatotoxicity and fulminant liver failure occur more commonly with drugs administered at a daily dose ≥ 50 mg/day, as compared to those administered at lower doses (10).

RISK FACTORS

Literature references on hepatotoxicity risk factors such as age, sex, alcohol ingestion, smoking, concomitant drugs, underlying liver disease, and genetic factors are abundant. However, the fact that liver toxicity is no isolated disorder

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Table I. A summary of hepatotoxicity registry studies performed in various countries

Country	Switzerland	Spain	USA	Korea	Japan	Singapore
Years	1975-2005	1994-2008	2004-07	2005-07	1997-2006	
n	784	603	300	371	1,676	31
Type of study	State registry	45 sites	Prospective, 5 sites	Prospective, 17 sites	Retrospective, multicenter	Prospective, population study
HC/Mixed/Cholestatic %	52/21/29	55/21/25	56/20/24	ND	59/20/21	74/6/19
Mean age/range (years)	58 (42-74)	54 (13-88)	48 ± 18	49 ± 14.5	55	51
Women %	57	49	60	63.3	57	45
Inpatients %		54	54	100		
Deaths or transplants %	9.2	5.4	10.1	1.3	3.7	9.6
Chronic outcome %		16.9	13.6		8.4	
Most common agents (%)	Antibiotics (27) Painkillers (5) Disulfiram (3.4) Carbamazepine (2.2) Lipid-low. drugs (1)	Antibiotics (39) CNS agents (15) Painkillers (11) Lipid-low. drugs (5)	Antimicrobials (45.5) CNS agents (15) Herbs (9) Immunomod. (5.5) Painkillers (5) Antihypertensives (5) Endocrine agents (4) Lipid-lowering drugs (3.4)	Herbs (27.5) Drugs (20.8) Diet. supplm. (13.7) Medicinal plants (9.4) Folk remedies (8.6) OTC drugs (6.5)	Antibiotics (14) CNS agents (10) Diet. supplm. (10) Painkillers (9.9) Chinese h. (7.1)	Tradition. CM (54) Drugs (26) Malaysian h. (16)

CM: complementary medicine; HC: hepatocellular; NA: not available; OTC: over the counter; CNS: central nervous system. Lipid-low. drugs: lipid lowering drugs; Immunomod.: immunomodulators; Diet. supplm.: dietary supplements; Tradition. CM: traditional CM; Malaysian h.: Malaysian herbs; Chinese h.: Chinese herbs. Adapted from reference 23.

but includes a wide variety of liver responses to numerous chemicals should be considered, hence risk factors do not usually apply to many causal agents. Advanced age represents an important risk factor for isoniazide-related hepatotoxicity (11), while valproate-induced liver damage (12) and Reye's syndrome from aspirin ingestion are more common in children (13). It has been suggested that women might be more susceptible to liver toxicity (9), perhaps because they use a greater number of drugs. However, extensive population studies could not find a higher frequency of hepatotoxicity among females (1,4,5). The black race is more susceptible to anticonvulsant hypersensitivity syndrome (14), whereas Caucasian whites seem to have an increased risk for liver toxicity in association with flucloxacillin (15).

Genetic factors play a significant role in drug-induced liver damage. Recently, candidate gene studies and genome-wide analyses have identified several genetic markers associated with increased hepatotoxicity risk. Thus, flucloxacillin-induced hepatotoxicity is closely linked to *HLA5701* (15), the same allele responsible for susceptibility reactions to abacavir, which is present in at least 4% of Caucasian Europeans (16). Estrogen-induced cholestasis is associated with mutations in gene *ABC B11*, which codes for the bile salt export pump (17), and valproate-related liver toxicity is linked to mutations in the gene encoding mitochondrial DNA polymerase gamma, *POL G1* (18). On the other hand, a double null genotype for cytosolic glutathione transferase (*GST M1 T1*) –a key enzyme in the defense against oxidative stress– entails an increased risk

for liver toxicity with various drugs, particularly non-steroidal anti-inflammatory drugs and antibacterials (19). No single risk factor to date has proven sufficiently predictive of hepatotoxicity in a given individual.

CASE DEFINITION, PHENOTYPES, AND PROGNOSTIC DETERMINANTS

Liver toxicity may present with clinical and pathological manifestations that are virtually reminiscent of every known liver condition, and whose severity may oscillate from asymptomatic liver enzyme elevations to fulminant liver failure. The most common presentation form is a clinical picture that mimics acute viral hepatitis, but other forms may occur, including chronic hepatitis, liver cirrhosis, primary biliary cirrhosis-like disease, veno-occlusive disease, and even neoplasms (20).

However, patients very often manifest only mild changes in liver tests and no histological data are available. Hence, in searching for homogeneity in the definition of hepatotoxicity an international consensus group established that liver toxic damage would be present when in the presence of drug exposure an elevation in serum alanine-aminotransferase (ALT), conjugated bilirubin, or combined bilirubin, ALT and alkaline phosphatase (AP) levels > 2 times the upper limit of normal (ULN) is identified (21). Recently a new group of experts redefined the case concept according to said biochemical criteria because of their perception that, given the increasing number of patients with high ALT sec-

Table II. A classification of liver damage type according to the "Council for International Organizations of Medical Sciences" (21), recently updated by the "Phenotype Standardization Project" from the "International Serious Adverse Event Consortium" (22)

Hepatocellular: Increase $> 5 \times \text{ULN}$ ALT or $R \geq 5$

Cholestatic: Increase $> 2 \times \text{ULN}$ AP or $R \leq 2$ $R = \text{ALT} \times \text{N} / \text{AP} \times \text{N}$

Mixed: Increase $> 2 \times \text{ULN}$ ALT & AP or $2 < R < 5$

ULN: upper limit of normal; ALT: alanine-aminotransferase; AP: alkaline phosphatase.

ondary to non-alcoholic fatty liver, ALT threshold levels should be increased to > 5 times the ULN to minimize false negative results, whereas AP could keep its previously established threshold ($> 2 \times \text{ULN}$). A new case category would be the combination of increased ALT $> 3 \times \text{ULN}$ and total bilirubin $> 2 \times \text{ULN}$ (22) (Table II).

In line with this method, toxic liver injury is characterized, based on serum ALT and AP levels (when separately elevated), and on the relationship (R) between both (when concurrently elevated), as hepatocellular ($R \geq 5$), cholestatic ($R \leq 2$) and mixed ($R = 2-5$) (21). R must be estimated from an initial blood sample since pattern may change (from hepatocellular to cholestatic or mixed) in later stages because of the varying resolution times of different liver enzymes (faster for ALT *versus* AP) (23), and its value is critical for the CIOMS/RUCAM scale. In situations where ALT or AP are elevated before drug administration, baseline values will be used as ULN. The categorization of liver lesions according to this criterion exhibits a good overall correlation with underlying injuries and is excellent for prognostic purposes (4,24). Age and sex influence the hepatocellular and cholestatic expression of hepatotoxicity. The former is more common in women younger than 60 years, and the latter is more common in males above 60. In contrast, mixed expression has no relation to these variables (25).

Liver toxicity symptoms are nonspecific, similar to those in any acute liver condition, and include asthenia, anorexia, nausea, abdominal pain, fever, jaundice, choloria, and pruritus. Early pruritus is typical of cholestatic forms and only develops late –if ever– in hepatocellular patterns. Hypersensitivity symptoms may help in the diagnosis of hepatotoxicity. Symptoms such as rash, fever, and facial edema, associated with eosinophilia and atypical lymphocytosis are helpful for the diagnosis, and are characteristic of liver toxicity from selected drugs, including aromatic anticonvulsants, sulfamides, and allopurinol (23).

Acute hepatocellular expression is the most common presentation of toxic liver disease, and may be caused by a wide variety of drugs. Acute toxic hepatocellular damage with jaundice entails death or a need for liver transplantation in 10% of patients on average (26), a fact known as "Hy's

rule". An analysis of *Registro Español de Hepatotoxicidad* and the Swedish SADRAC database has validated this, and using a multivariate analysis also found that other variables such as advanced age, female gender, and AST levels were independently associated with poor prognosis (4,24). In contrast, an analysis of the prospective cohort in the collaborative DILIN group in the USA failed to validate Hy's rule but identified diabetes as a risk factor for serious disease progression (5).

While in most cases hepatotoxicity resolution is complete with no apparent sequels, a subgroup oscillating between 5.7% in the Spanish Registry (4) and 14% in the US Registry show biochemical evidence of chronicity. In the Spanish Registry study acute cholestatic/mixed patterns had a greater tendency toward chronicity when compared to the hepatocellular type (9 vs. 4%, respectively; $p < 0.031$) (27), although residual lesion severity was greater in the latter (30% of cirrhosis and 20% of chronic hepatitis). Cardiovascular and CNS drug classes are more prone to chronic hepatotoxicity induction (27). Very recent analyses suggest that, regardless of the lesion's hepatocellular, cholestatic, or mixed pattern, most liver profile normalizations occur within one year, hence this cutoff could be most suitable to tell prolonged resolution from true chronic progression (28).

CAUSALITY ASSESSMENT

The availability of molecular markers for liver toxicity that could be used in clinical practice still seems far removed. Therefore, the diagnosis of hepatotoxicity remains a challenge for clinicians. Only seldom may a conclusive diagnosis with hepatotoxicity be reached. This includes the measuring of plasma levels for some intrinsic liver toxins such as acetaminophen or aspirin.

Under usual clinical conditions the causality attribution process is based on the suspicion of liver toxicity together with an appropriate exclusion of specific causes (Table III). A key element to maximize diagnostic yield is a high degree of suspicion that any liver disease may be related to drug exposure, followed by an attentive research of toxic exposure, a consistent time sequence, an analysis of the hepatotoxic potential of identified agents, and a cautious exclusion of specific causes of liver disease (29) (Fig. 1).

Liver damage latency is defined as the time from the first day with drug exposure to symptom development. This period is variable since liver toxicity may develop even following the discontinuation of its causal agent (typical in hepatotoxicity from amoxicillin-clavulanate) (30). In 80% cases, symptoms occur in the first three months from the beginning of treatment (9). Causality attribution resides in the availability of detailed information on drug exposure in advance of liver damage, as well as an appropriate exclusion of alternative causes (31). An exclusion of other liver disease causes is necessary, including acute hepatitis A, B or C; autoimmune hepatitis; Wilson's disease; primary scler-

Table III. Main causes of liver disease to be investigated before liver toxicity is diagnosed

<i>Test/Clinical setting</i>	<i>Disease</i>	<i>Comment</i>
Viral serology Anti-HAV IgM Anti-HBc IgM Anti-HCV, HCV-RNA IgM anti-HEV, HEV-RNA CMV IgM EBV IgM	Viral hepatitis	More common in younger women Investigate risk factors Similar outcome after solving liver toxicity
Bacterial serology (if persistent fever, diarrhea) <i>Salmonella, Campylobacter, Listeria, Coxiella</i>	Bacterial hepatitis	Systemic symptomatology
Syphilis serology	Secondary syphilis	Multiple sex partners. AP levels disproportionately high
Autoantibodies (ANA, ANCA, AMA, ASMA, anti-LKM-1)	Autoimmune hepatitis Primary biliary cirrhosis	Female gender. Uncertain course after drug discontinuation. Associated autoimmune diseases
Ceruloplasmin, urinary copper (patients < 40 years)	Wilson's disease	Always rule out in younger patients
Alfa-1 antitrypsin	Alfa-1 antitrypsin deficiency	Associated lung disease
Transferrin saturation (anicteric hepatocellular injury)	Hemochromatosis	Anicteric hepatocellular damage. Older women, men at midlife
Hypotension, shock, heart failure Vascular disease, elderly	Ischemic hepatitis	Disproportionately high AST levels. Hypotension, shock, surgery, recent heart failure
Imaging techniques: radiographic/endoscopic (abdominal US, CAT, cholangioresonance, ERCP)	Biliary obstruction	Colicky abdominal pain. Mixed or cholestatic pattern

Anti-HAV: hepatitis-A antibody; Anti-HBc: hepatitis-B core antibody; Anti-HCV: hepatitis-C antibody; Anti-HEV: hepatitis-E antibody; Anti-LKM-1: type-1 liver-kidney microsomal antibody; AMA: antimitochondrial antibody; ANA: antinuclear antibody; ANCA: anti-neutrophil cytoplasmic antibody; ASMA: anti-smooth muscle antibody; CMV: cytomegalovirus; EBV: Epstein-Barr virus; ERCP: endoscopic retrograde cholangiography; CAT: computerized axial tomography.

rosing cholangitis; hepatitis E; graft-*versus*-host disease; and a pancreato-biliary origin. Ruling out hepatitis E is presently vital because of its increasing incidence in the last few years, particularly in eastern European countries. Detailed history taking is important with a focus on alcohol abuse, heart failure, hypotension, hyperthermia, and hypoxia before symptom onset, as these may cause liver ischemia. Sepsis and parenteral nutrition must be ruled out in inpatients, as these may result in cholestatic liver damage. Criteria favoring a diagnosis with hepatotoxicity include identifying hypersensitivity manifestations and rapid clinical and biological improvement following drug discontinuation (dechallenge). The gold standard for the diagnosis of hepatotoxicity is a recrudescence of clinical and biological changes after rechallenge. However, because of ethical reasons this is unjustifiable except under exceptional circumstances. Most re-exposures occur unintentionally (6% in the Spanish hepatotoxicity registry) and may entail an increased risk of undesirable outcomes (32). Notwithstanding, careful questioning may on occasion unveil subtle evidence of accidental re-exposure, which will significantly help during diagnosis. In such cases the first episode following drug exposure surely lacked jaundice and symptoms were nonspecific (abdominal discomfort, fever, asthenia), which made its identification challenging to a doctor not familiar with liver toxicity (33).

The role of liver biopsy in the diagnosis of hepatotoxicity is uncertain (5,10). While the presence of eosinophils, granulomata, necrosis, and cholestasis adds to the suspicion of liver toxicity, no specific features may confirm the diagnosis (34-36). Figure 1 shows a diagnostic algorithm for hepatotoxicity.

Special situations in causality assessment

Hepatotoxicity causality attribution exhibits relevant differences in children as compared to adults. Drug prescription is lower in children *versus* adults, and the former seem to be less susceptible to hepatotoxicity. Exceptions exist, including Reye's syndrome and hepatotoxicity from aspirin and valproate. These age-related differences in susceptibility may result from cytochrome P-450's genetic expression (37).

Individuals with chronic viral (B or C) liver disease have an increased risk for idiosyncratic liver toxicity, at least with some drugs, owing to pharmacokinetic changes, dysregulated cytokine expression, and altered drug metabolism pathways (38-43). Non-alcoholic steatohepatitis and obesity have shown no greater risk for liver toxicity but in cases induced by methotrexate and tamoxifen (43-46). Multiple studies have shown an increased risk of elevated ALT in patients coinfecting with HIV and HBV or HCV as compared to HIV

Fig. 1. Diagnostic algorithm for hepatotoxicity.

infection alone during antiretroviral therapy, but telling hepatotoxicity from an underlying liver disease exacerbation is sometimes challenging (41-43). The role of alcohol as susceptibility factor and its potential influence in the course of toxic liver disease is controversial. In the CIOMS/RUCAM scale (see below) for the assessment of suspected hepatotoxicity, alcohol consumption is worth one point, and thus increases diagnostic probabilities, but no scientific evidence supports this. On the other hand, in the multivariate analysis of factors with a potential impact on serious hepatotoxicity outcomes in DILIN, alcohol use during the previous 12 months behaved as a protective factor (OR = 0.33) (5). Autoimmune hepatitis represents a particularly difficult issue when it comes to differentiate it from idiosyncratic hepatotoxicity, since both conditions lack specific markers and some drugs induce autoimmune hepatitis-like syndromes (minocycline, nitrofurantoin, and methyl dopa) (47-59). Other drugs such as interferon alfa or anti-TNF-alfa antibodies may unmask latent autoimmune hepatitis. In fact, the development of hepatitis with autoimmunity phenomena during drug therapy poses the dilemma of whether it is a true autoimmune hepatitis, and onset coincided with the taking of an unrelated drug, or the drug gave rise, either directly or through low-grade liver injury, to autoimmune hepatitis in a genetically

predisposed patients (50). Selected drugs, even in the absence of liver damage, may favor the development of antibodies.

Diagnostic scales

Causality attribution scales for hepatotoxicity attempt to semi-quantitatively estimate the likeliness that a pharmacological agent may be responsible for liver injury. These tools have both strengths and weaknesses (51), but their main drawback is maybe that they cannot be compared to an objective diagnostic parameter. Two diagnostic scales or algorithms are currently used to assess causality in hepatotoxicity: the CIOMS/RUCAM scale (52), and the María & Victorino scale (53), also known as Clinical Diagnostic Scale (CDS). Both scales provide a scoring system for 6 items in the decision-making strategy. Responses correspond to weighted values that add up to provide a total score. These ratings are translated into suspicion categories. The former scale showed in a study a higher consistency with clinical judgment (54), but its reproducibility among experts has also been questioned by a recent study (55). The primary disadvantage of the CIOMS/RUCAM scale is its complexity, which renders its use in daily practice difficult. The María & Victorino scale

adds an assessment of immuno-allergic phenomena such as fever, rash, or cytopenias, and excludes factors such as pregnancy and alcohol ingestion. This scale obtained an excellent kappa when 50 hepatotoxicity cases were compared to expert opinions (56). Despite this, in a wide independent series the María & Victorino scale showed inferior results as compared to the RUCAM scale (54). Other diagnostic tools are less applicable. Thus, Naranjo's Adverse Reaction Probability Scale provides a scoring system to attribute drug-related adverse reactions using simple questions, and is user-friendly; however, it is of little use for hepatotoxicity (57). Japanese researchers added the results of lymphocyte stimulation tests and eosinophilia tests to the RUCAM scale (58). No comparison of the Japanese RUCAM scale to expert opinions has been carried out to date.

TREATMENT

The primary therapeutic measure is immediate discontinuation of any non-essential drugs since the ongoing presence of a drug responsible for hepatotoxicity may result in higher probabilities of fulminant or chronic outcome (26,27). Drug discontinuation must lead to clinical improvement, albeit a worsening of liver function may be on occasion detected for several days or even weeks. The way to restored liver function is variable. Clinical improvement is slower for the cholestatic *versus* the hepatocellular pattern. N-acetylcysteine has shown some effectiveness in reducing the need for transplantation in acute liver failure unrelated to acetaminophen intoxication (including idiosyncratic toxic hepatitis) provided it is administered early during encephalopathy (59). N-acetylcysteine may probably be also effective to prevent hepatotoxicity progression toward fulminant forms. Corticoids have not been effective for serious cases (60) but may be used for some patients with hypersensitivity manifestations. In non-controlled studies ursodeoxycholic acid seems effective for prolonged ductopenia and toxic cholestasis (61). Novel therapy targets may include cytoprotective Keap1-Nrf2 signaling, which is started in liver inflammation by Nrf2 translocation into the nucleus, where it activates the transcription of a number of antioxidant genes such as NQO1, HO-1, and yGCS. Nrf2-knockout mice exhibit greater liver toxicity in experimental studies. Thus, in a post-injury liver regeneration model serum total bilirubin levels increased 5-fold in these mice ($p < 0.001$), which proves a reduced liver functional capacity in the absence of Nrf2 activity (62). Nrf2 activators might thus have liver protective properties in cases of toxic liver injury. Another potential target would be nuclear receptors, which are involved in multiple physiological liver functions. Of late, the farnesoid X receptor agonist obeticholic acid, which showed choleretic and antifibrotic properties in experimental models, has been successfully tried to improve cytotoxicity and cholestasis indices in patients with primary biliary cirrhosis and deficient response to ursodeoxycholic acid (63), and might theoretically be also effective in patients with prolonged toxic cholestasis.

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