Optimizing diagnostic approach to drug-induced liver injury

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ABSTRACT

Drug-induced liver injury (DILI) is often a trial even to expert clinicians, because sometimes diagnosis is not easy to be made. Guidelines of the American College of Gastroenterology (ACG) yielded in 2014, help to better understand the problem. The diagnosis of DILI is made through a detailed evaluation of clinical, serological, radiological and histological aspects. Biochemical data include liver function tests that allow to assess the pattern of damage, such as hepatocellular, cholestatic and mixed liver injury; serological data include testing for major and possibly minor hepatotropic viruses, non-organ specific autoantibodies. Clinical scenario might include jaundice, nausea, vomiting and extra-hepatic manifestations such as fever, pruritus, rash and eosinophilia. Investigation of the potential culprit drugs should involve firstly the temporal relationship between intake of the medication and onset of symptoms, thus the improvement after drug withdrawal. Overall, to complete the diagnostic evaluation, an abdominal ultrasound can be performed, as well as measurement of liver stiffness by transient elastography, and finally liver biopsy, which still represents the most accurate method to definitely assess liver damage. Sometimes, in such cases, computed tomography scan and magnetic resonance could help in the diagnosis of cases presenting with focal lesions of the liver, with cholestatic-like disease or vascular alterations, such as veno-occlusive disease. DILI diagnostic criteria help clinicians thinking of liver injury induced by drug, excluding other causes of liver disease. According to severity of liver damage and type of drug, it is possible to carefully predict the patient's outcome.

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Burden of drug-induced liver injury

Drug-induced liver injury (DILI) is an uncommon condition, which sometimes is responsible for acute liver failure (ALF) and consequently urgent liver transplant.[1] Incidence of DILI was between 1 in 10,000 and 1 in 100,000 according to previously published data, however, more recent studies reported that incidence appears higher.[1] Recently, the American College of Gastroenterology (AGA) yielded guidelines on *Diagnosis and Management of Idiosyncratic Drug-Induced Liver Injury*,[2] providing indications regarding clinical presentation, diagnosis and management. European Countries still did not formulate appropriate rules, but there are several registries both in Western[3-7] and in Asian[8] countries, which have provided useful information as regards the etiology, pathogenesis as well as the clinical presentation, diagnosis and management of DILI. Further, another help comes from a really useful website, *The Liver Tox* (available at http://livertox.nlm.nih.gov), which provides information on documented hepatotoxicity of certain drugs, herbal remedies and dietary supplements (HSD). In Europe many projects are ongoing with the final proposal of formulating appropriate guidelines to help clinicians in the management of this still debated issue. For this proposal, the Pro Euro DILI Registry has been created and is actually working on recruiting DILI cases

(available at http://nddcbru.org.uk/study/pro-euro-dili-registry).

Prognosis of DILI is generally good, even if only 10% have ALF with coagulopathy and encephalopathy. In these cases, outcome results poor, with a need for liver transplantation in 40% of cases or death of the patient.[2] In these cases, according to Hy's law, mortality risk is 1 in 10, if those three criteria are present: i) aminotransferases more than three times to normal; ii) total bilirubin more than two times to normal, without initial signs of cholestasis; iii) exclusion of other causes of liver disease such as hepatitis viruses A, B and C (HAV, HBV, HCV) hepatitis or other preexisting acute liver diseases.[2]

Definition of drug-induced liver injury cases

The drug-induced liver injury is defined as liver damage caused by drugs, dietary supplements and herbal products, at usual dose.[1] We can distinguish two different types of DILI: intrinsic DILI, with a predictable and dose dependent action (*e.g.* paracetamol, methotrexate, chemotherapeutic agents); idiosyncratic DILI, unpredictable and not dose dependent. It is less frequent, in susceptible subjects, with variable latency, presentation and course.[2,9]

Predisposition to idiosyncratic DILI is linked to different variables: associated with the patient (age, gender, race, comorbidity, genetic susceptibility, previous episodes of DILI), associated with environment (alcohol, tobacco, diet, obesity), associated with drug (dosage, chemical structure, administration rout, interaction with other drugs).[2,9] Female patients have a greater risk of developing an adverse reaction, because of a slower metabolizing capacity and hormonal interference.[10] Potentially all drugs can be involved, but liver damage is commonly caused by antibiotics, non-steroidal anti-inflammatory drugs, statins, anti-platelets, immunosuppressant and HSD.[1,11-14]

DILI definition criteria changed in the last thirty years. Initially, through the Council of International Organization of Medical Scientists (CIOMS), DILI was defined as an increase of aminotransferases (ALT/AST), alkaline phosphatase (AP), and bilirubin of at least 2 times normal value. After this, RUCAM scores introduced the concepts of latency, as the temporal relationship between drug intake and clinical presentation; the dechallenge, as the clinical course after drug discontinuation, and finally the rechallenge, as the reiteration after drug administration.[15,16] RUCAM scores also include assessment of literature reports for the suspected drug. Other scores, as Maria and Victorino,[17] added parameters of exclusion of alternative causes such as common viral, alcoholic and autoimmune liver disease, and the presence of coexistent extrahepatic and immune-allergic manifestations such as pruritus, rash, fever, eosinophilia, arthralgia.[17] According to the ACG Guidelines,[2] the diagnosis is made by evaluating the alteration of liver enzymes, clinical history and physical examination of the patient. Pattern of liver injury is assessed by the ratio R=(ALT/UNL)/(ALP/UNL) and it allows us to define if the DILI has a hepatocellular (R>5), a cholestatic (R<2) or a mixed pattern (2<R<5).[2]

Predictable drug-induced liver injury cases

Intrinsic or predictable cases of DILI are dose dependent, being due to an overdose of assuming drug. Thus, intrinsic DILI can be usually due to paracetamol, chemotherapeutic agents or methotrexate.

DILI due to paracetamol (acetaminophen, APAP) happens, whenever this drug is taken in excessive doses, for example with suicidal purpose. It can cause acute hepatitis or acute liver failure with a need for urgent liver transplant. Paracetamol causes lobular central necrosis, which manifests with high blood levels of aminotransferases. N-acetyl-cysteine used as antidote, 8 to 24 h after ingestion of high amounts of paracetamol (200 mg/kg or 10 g are toxic dose), can reduce the oxidative effect of free radicals, which are basic to liver damage. Despite the initial severity of acute hepatitis, prognosis is favorable, related to the rapid regeneration of hepatocytes.[9,18]

The metabolism of altered chemotherapeutic agents can result in systemic toxicity with high or persistent raised drug blood levels. Sometimes, the adverse reaction to chemotherapeutic agents manifests with hepatocellular injury, inflammation and cholestasis, or causes endothelial damage and thrombosis leading to vascular complications such as veno-occlusive disease, also called hepatic sinusoidal obstruction syndrome (SOS). SOS frequently occurs in patients undergoing hematopoietic cell transplantation or receiving combination of fluorouracil with oxaliplatin/irinotecan as neoadjuvant therapy. The National Cancer Institute (NCI) and the World Health Organization (WHO) have developed standardized criteria to quantify the severity of treatment-related abnormalities in liver function tests of patients undergoing chemotherapy.[19,20]

Herbal and dietary supplements

HDS are commonly responsible for herb-induced liver injury (HILI), because they are not *real drugs*, so they do not follow the pre-marketing phases of clinical trials, designed to evaluate efficacy and safety of drugs. Further, being a mixture of substances (such as seeds, leaves and roots) herbal drugs can also increase the risk of toxicity.[11] Clinical manifestations

can be acute, as fulminant hepatitis and acute liver failure, or chronic, as cholestatic hepatitis, veno-occlusive forms and cirrhosis. People using these products are generally highly educated women, which prefer the use of these products believing they are *natural*, thus *safe*. For these reasons, pregnant women, children and elder people, use HDS as well. However, different substances may interact with other drugs, as commonly happens in the elderly, when people are on polytherapy (assuming more than 10-12 tablets a day), thus adding an herbal product can be very hazardous.[11] It is noteworthy that HDS are usually purchased rapidly through the Internet, without any medical prescription, and are frequently used as slimming aids or muscle building. In a certain percentage of the cases, hormonal products used as body building can cause jaundice with a favorable prognosis as compared to other substances (like green tea extracts) which instead determines an unfavorable outcome finally leading to liver transplantation.[21]

Diagnosis of drug-induced liver injury

It is made through a detailed clinical history, supported by serological markers, clinical imaging and histological features. There is a lack of consensus on which laboratory threshold criteria should be used to confidently identify DILI cases. In fact, DILI cannot be merely defined by the rise in serum aminotransferases, it requires causality assessment as well.[22] Basically, it remains a diagnosis of exclusion (Figure 1).

In this review, we aim to describe the multistep process for making a diagnosis of DILI/HILI after that clinical, serological and histological assessments have been performed. Thus, radiological data stemmed by abdominal ultrasound (US), transient elastography (TE), computed tomography (CT) scan and magnetic resonance imaging (MRI) of the liver, will further help clinician to confirm or exclude the clinical scenario of DILI.

Clinical and serological evaluation

Clinicians should carefully evaluate medications history of patients. Firstly, they should collect demographical data about age, sex, race, morbidity, use of alcohol or other drugs. Secondly, the investigation of a possible drug involved, as first or second exposition, time of drug exposure, temporal relationship between intake and onset of symptoms and possible improvement after drug discontinuation.[1] More and more frequently, the culprit drug has been substituted by HDS. In fact, they are usually marketed without prescription and patients do not disclose to physician their use. DILI manifestations can be evident even months after the start of involved drug, the time to onset of DILI after drug administration varying widely, from few days to even more than one year, making it difficult to adequately find the implicated drug, especially in patients assuming multiple drugs.[21]

Clinical course is changing from asymptomatic disease (diagnosis made occasionally, as a result of examinations routinely performed) to acute clinical presentation with jaundice, dark urine, malaise, anorexia, nausea, skin rash and in the most severe cases signs of hepatic encephalopathy. Extra-hepatic manifestations such as pruritus, rash, fever and eosinophilia in case of hypersensitivity could be investigated, as well. Biochemical assessment of the patient with DILI include liver function tests (LFTs) such as AST/ALT and AP, allowing to define the type of pattern of liver injury. Albumin level decreasing with an increase in total bilirubin and international normalized ratio are usually associated with severe liver dysfunction. When total bilirubin and ALP are persistently elevated, as 30-60 days after the diagnosis, a chronic outcome can be possible.[21] A complete blood count is performed to evaluate eosinophilia, typical in case of hypersensitivity syndrome in DILI.

The first condition to be excluded is acute viral hepatitis, thus through anti-HAV, anti-HCV, HBsAg, anti-HBc major hepatotropic viruses should be rule out; when negative, even minor hepatotropic viruses (cytomegalovirus, Epstein-Barr virus, *Herpes simplex* virus) should be serologically tested.[8]

Then serum IgG and non-organ specific autoantibodies (ANA, AMA, ASMA, LKM) are useful to exclude autoimmune liver damage. It is noteworthy, that in some cases, a slightly positivity for ANA (1:80 or 1:160) with a speckled pattern can be present. This finding does not mean that the patient is suffering from an autoimmune hepatitis needing corticosteroids or immunosuppressant therapy. In such cases, drugs can unmask an underlying autoimmune disease, but the diagnosis is histological. AMA and p-ANCA can be investigated when there is a cholestatic pattern, to completely exclude primary biliary cholangitis or primary sclerosing cholangitis (Figure 1).[23,24]

Alfa-1 antitrypsin, ceruloplasmin, and iron markers are evaluated to exclude genetic disease as cystic fibrosis, Wilson disease and hemochromatosis, and all of them can be evaluated at a later time, also because of the usual character of chronicity of these diseases.

Emerging biomarkers

Transaminases, ALT and AST, AP and total bilirubin are currently the only DILI biomarkers approved in clinical practice. Although playing an important role in the diagnosis of all liver diseases, they are not specific for hepatotoxicity, as the increase

in these values is common in all pathological liver conditions. The usefulness of having biomarkers with high specificity and sensitivity would allow identifying earlier potentially hepatotoxic drugs in the drug development process and subsequently to reduce the number of post-marketing drugs withdrawn. However, these biomarkers could be useful in assessing DILI, not really available in clinical practice. Further, the ability to validate new DILI biomarker candidates is restricted due to the current lack of fully functional animal models for idiosyncratic DILI. The Pro-Euro-DILI Registry is collecting serial biological samples from DILI onset to normalization of enrolled patients, for future DILI biomarker studies and validations.[25,26]

Among the DILI candidate new biomarkers, there are the mechanistic-based biomarkers such as glutamate dehydrogenase (GLDH), the high mobility group box 1 protein (HMGB1) and keratin-18 (K18), which can also provide information on the mechanism of necrosis of the different causal agents.

GLDH is a mitochondrial enzyme that is found mainly in the liver as compared with kidneys and muscles. An increase in the level of circulating GLDH indicates mitochondrial dysfunction, which usually occurs during hepatocellular necrosis. GLDH correlates well with the increase of ALT in patients with various forms of liver injury, including overdose of APAP, although GLDH does not precede ALT elevation resulting from APAP hepatotoxicity. HMGB1 is a chromatin-binding protein with pro-inflammatory activity;[27] K18, full length and caspase cleaved-K18, indicates the degree of cellular necrosis and apoptosis that occurs in the subject. It has also been found that these biomarkers are more sensitive than ALT, with early increases compared to ALT in patients at the first presentation after APAP overdose.[28]

MicroRNAs (miRNAs) are small non-coding RNAs (20-24 nucleotides) involved in post-transcriptional regulation of gene expression. Recently they received a lot of attention as potential non-invasive DILI biomarkers candidates.[29] MiR-122 and

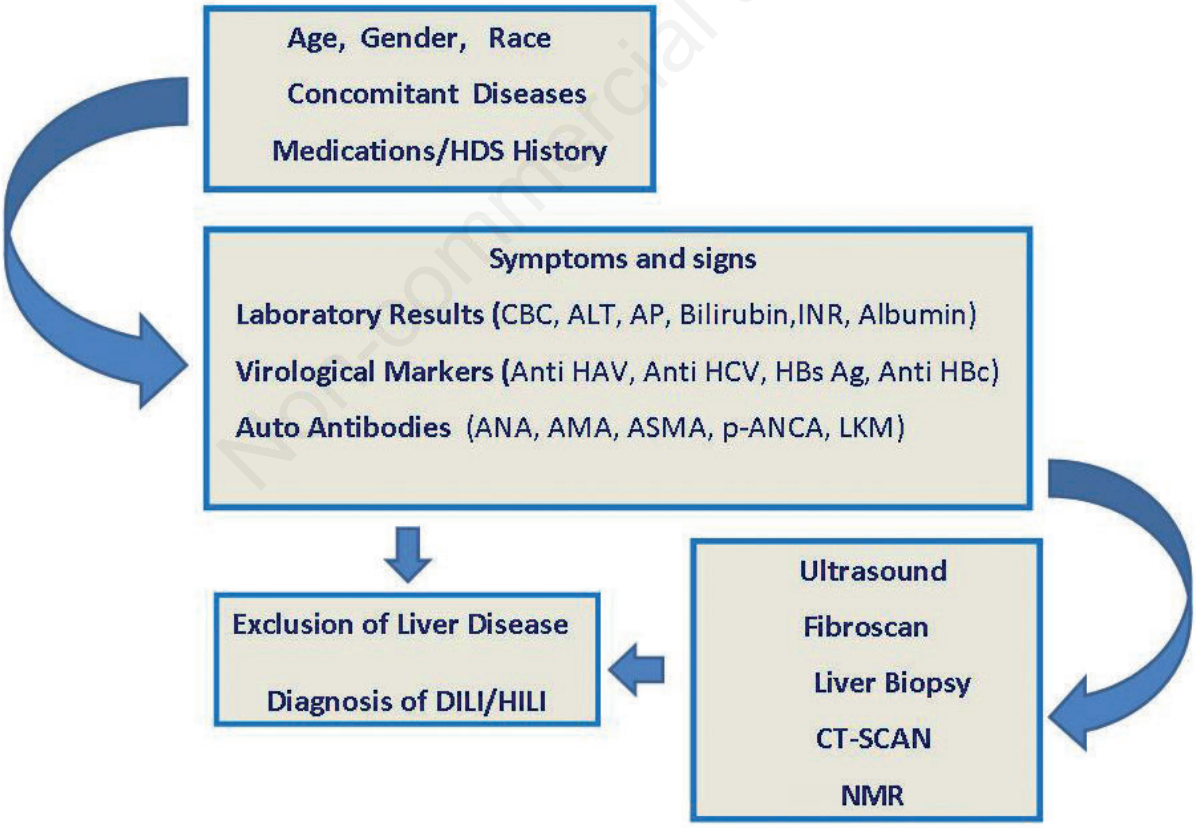

Figure 1. Algorithm to diagnose drug-induced liver injury. HDS, herbal and dietary supplements; CBC, complete blood count; ALT, alanine aminotransferase; AP, alkaline phosphatase; INR, international normalized ratio; Anti HAV, hepatitis A virus antibodies; Anti HCV, hepatitis C virus antibodies; HBs Ag, hepatitis B surface antigen; Anti HBc, hepatitis B core antibodies; ANA, antinuclear antibodies; AMA, antimitochondrial antibodies; ASMA, antismooth muscle antibodies; p-ANCA, anti-neutrophil cytoplasmic antibodies; LKM, antimicrosomial liver kidney antibodies; DILI, drug-induced liver injury; HILI, herbal-induced liver injury; CT-scan, computed tomographic scan; NMR, nuclear magnetic resonance.

miR-192 were the first circulating miRNAs that were shown to increase after doses of toxic APAP in mice and soon thereafter confirmed that they behaved similarly in humans, even before hepatocellular damage occurred.[28] Emerging data also indicate that miRNA-122 may have a prognostic value, with higher early serum levels reported in patients with APAP overdose who met the King's College criteria for liver transplantation.[30]

Th1 [interleukin (IL)12, interferon γ, IL2, IL15] and Th2 (IL4, IL5, IL13) cytokines are usually activated by an acute stimulus. If inflammation does not resolve, it can evolve into a chronic response of immunological system (Th17/Th19) with a severe prognosis.[31]

Biomarkers derived from *Omics*, as metabolomics and proteomics, are a large number of molecules (metabolites, proteins, DNA) that allow the identification of the *toxicity signature*; these could be used to improve preclinical safety assessment and DILI disease diagnostics. An exploratory comparison of global serum proteomes in DILI has reported promising results with apolipoprotein E expression demonstrating the greater power to differentiate DILI from controls (Table 1).[9,32] Unfortunately, these biomarkers are not yet available in clinical practice, but only experimentally.

Histological pattern

Even if liver biopsy is often not required, it still represents the golden standard to assess liver damage by drugs, confirming a doubtful clinical suspicion and ruling out underlying chronic liver disease (viral, alcoholic, metabolic).[33] Characteristics of a liver biopsy in DILI are fibrosis, steatosis, necro-inflammation, granulomas, lipofuscin, cholestasis. DILI could be associated with autoimmune hepatitis (DIAIH), which occurs mainly in women, with positivity of auto-antibodies, high levels of transaminases and gamma-globulin, features of liver histology such interface hepatitis, lymphoplasmacytic infiltrate, rosettes and cholestasis, and with a better prognosis[34] as compared with autoimmune hepatitis properly defined. Recent data, suggest an association with certain HLA gene variants, with alteration of specific cytokines[35] and inhibition of some hepatobiliary transporters.[36] Drug-Induced Liver Injury Network (DILIN) prospective study showed that most cases of DILI attributed to nitrofurantoin or minocycline and about half of cases due to methyldopa and hydralazine had a phenotype of autoimmunity similar to AIH. These features decrease with recovery of the injury and are not associated with the typical HLA alleles found in patients with idiopathic AIH.[37] The clinical characteristics of DILI, which showed histological findings similar to AIH, were revealed. In such patients, a liver biopsy is recommended in order to determine the appropriate treatment strategy. In DILI with histology-like AIH patients, long-term follow-up is needed to perceive the relapse.[38] LFT results positively correlated with

Table 1. Useful biomarkers in clinical practice associated with drug-induced liver injury.

Biomarker	Drug-induced liver injury
ALT, AST, AP, Bilirubin	Acute and chronic
Sorbitol dehydrogenase	Acute
Glutathione S-transferase	Liver and kidney
Serum cytokine profiles Th1 Th2 Th17/Th19	Acute and chronic
miRNAs miR-122 miR-192	Acute and chronic APAP overdose
Glutamate dehydrogenase	Marker of necrosis APAP overdose
HMGB-1	Marker of necrosis APAP overdose prior ALT
K18 (full-length and ccK18)	Marker of apoptosis APAP overdose prior ALT
Apolipoprotein E	Acute

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; Th1, Th2, Th 17/Th19, T helper lymphocytes; miRNAs, micro RNA; APAP, acetaminophen; HMGB-1, high mobility group box-1 protein; K18, keratin 18. *Modified from Licata, 2016.*[9]

histological findings. DILI can manifest as predominantly hepatitis, bile duct injury or combination. Histological pattern recognition in the liver biopsy may help to identify specific hepatotoxic agents causing DILI.[39] Liver biopsies from subjects with DILI were characterized by low counts of mature B cells and natural killers (NK) cells in portal tracts in contrast with viral hepatitis. NK cells were found only in viral cases, whereas both (AIH and viral hepatitis) showed higher counts of B cells than DILI. Plasma cells were most strongly associated with AIH.[40] Chemotherapy-induced liver injury involves oxidative stress with pathological findings such as sinusoidal dilatation, peliosis, perisinusoidal fibrosis, regenerative nodular hyperplasia. In other cases, there are deposition of lipid vesicles, lobular inflammation causing steatosis and steatohepatitis. These conditions can mimic liver metastasis, as well.[41]

Radiological assessment

Abdominal ultrasound

The abdominal US in the DILI is one of the first diagnostic tools that has been performed, because, in the presence of liver disease, this method, inexpensive, non-invasive, readily available and acceptable to patients, gives a series of very useful information. However, US in DILI does not show specific signs but it is mainly used to exclude other liver diseases and to address diagnosis. For example, in cholestatic liver damage, US will exclude obstructive jaundice because it will not detect dilated bile ducts and will make us thinking of a medical rather than surgical cholestasis.

One of the most frequent signs in DILI, is the steatosis, seen at ultrasound as the *bright liver echo pattern* which could be determined by various drugs.[42,43] This echo pattern shows markedly increased the *liver/kidney contrast*, defined as the relative brightness of the liver to the renal parenchyma when compared in longitudinal scanning involving both these organs (Figure 2).[44,45] However, steatosis is common in obese patients and ultrasound did not show specific signs allowing us to differentiate between steatosis by drugs or steatosis by steatohepatitis, so even in this case liver US relieves only a small piece in the complex mosaic that usually is the diagnosis of DILI.[46-48]

Sinusoidal obstruction syndrome (SOS), previously named veno-occlusive disease, is a disease resulting from the use of chemotherapeutic drugs and it affects prevalently patients undergoing regimes in the setting of haematopoietic stem cell transplantation.[49] There are no typical ultrasound signs but in the clinical context the evidence of one of them must make SOS suspect. The most frequent signs are: signs of portal hypertension showed by Doppler ultrasound, liver and spleen enlargement, recanalization of para-umbilical

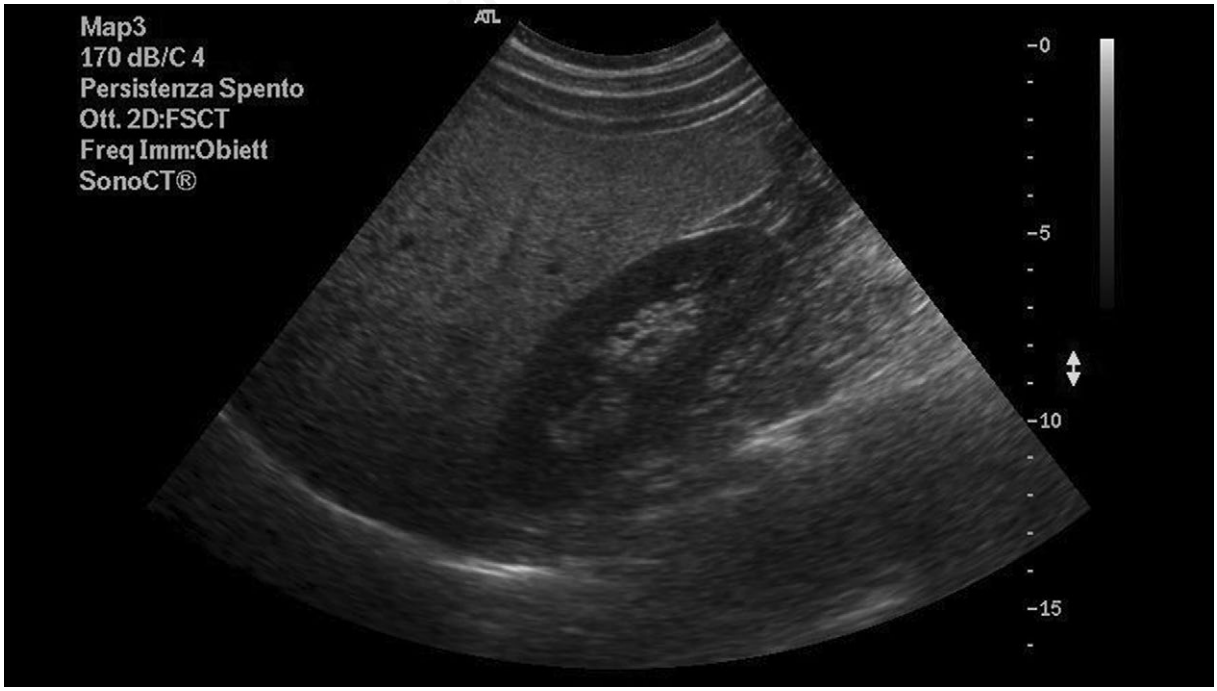

Figure 2. The bright liver. The relative brightness of the liver to the renal parenchyma when compared in longitudinal scanning involving both these organs.

vein, ascites, gallbladder wall thickening, and portal vein thrombosis. Doppler evaluation of portal flow shows either maximal velocity <10 cm/s, biphasic flow, or complete flow reversal in severe cases (Figure 3).[49,50]

Portal vein thrombosis is a rare complication of DILI and ultrasound identifies echoes inside the vessel (thrombi) obstructing partially or completely.[51]

In chronic DILI ultrasound can also show irregular surfaces of the liver, variable echo patterns, signs of portal hypertension and spleen enlargement, as in all chronic liver diseases.

Transient elastography

New diagnostic tools have been developed which, when supported by ultrasound, permit the estimation of fibrosis.[51-53]

Transient elastography is a useful non-invasive technique for monitoring liver fibrosis in patient taking methotrexate (MTX)[54] because suffering from psoriasis, with abnormal results (7-11 Kpa). Presence of overweight or obesity, duration of MTX therapy and cumulative dose were correlated with abnormally elevated TE results.[55] It is also a good tool for evaluating hepatic injury after FOLFOX treatment.[56] In fact, a clear change in liver stiffness was observed after chemotherapy within 48 h, and it became normal in most of cases after 2 weeks. Some patients can show aberrant elevation of TE values after a FOLFOX treatment, showing pathologically liver injury.[56] Liver stiffness increase, in association with raised liver enzymes, have been linked to acute inflammation or acute intrahepatic cholestasis.[57] TE could be used to differentiate acute and acute on chronic liver failure (ACLF), in fact patients with acute liver damage have significantly lower fibrosis (thus lower liver stiffness estimation) than patients with ACLF.[58]

The hepatic toxicity showed by altered hepatic biochemical tests and by symptoms of liver disease needs information obtained through liver biopsy or by assessing the hepatic elasticity or stiffness through elastography. Thus, elastography is useful in assessing the chemotherapy related hepatotoxicity with cancer, also in children.[59]

Computed tomography and magnetic resonance imaging

With the increasing use of CT and MRI, most DILI can be initially suspected at imaging instead of clinical examination. Imaging findings, however, are often non-specific and a clinical-biochemical correlation is required to achieve the correct diagnosis.

A severe and life-threatening DILI is acute hepatitis. CT and MRI findings are non-specific, and include hepatomegaly with decreased parenchymal enhancement, periportal edema, gallbladder wall thickening and ascites (Figure 4).[60] Hepatic SOS is the result of blockage of small hepatic veins with consequent hepatic venous stasis. CT and MRI cannot directly demonstrate vascular damage, but certain imaging findings due to hepatic venous stasis can

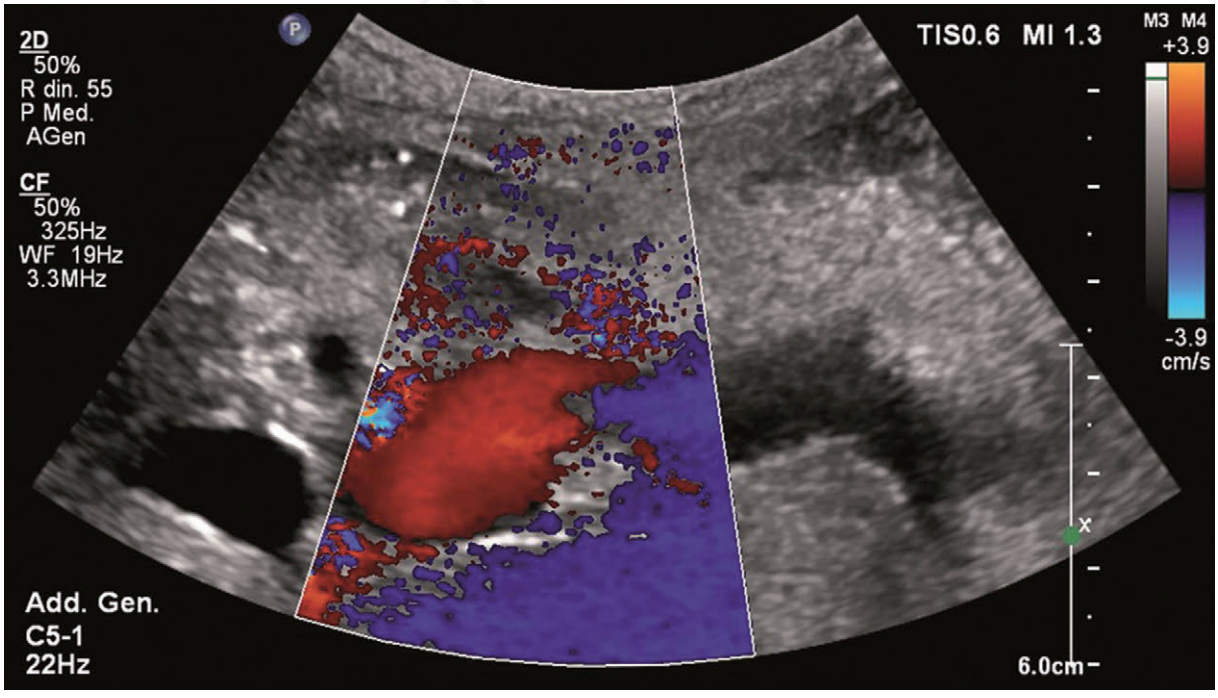

Figure 3. Patient with sinusoidal obstruction syndrome, complete flow reversal in portal vein.

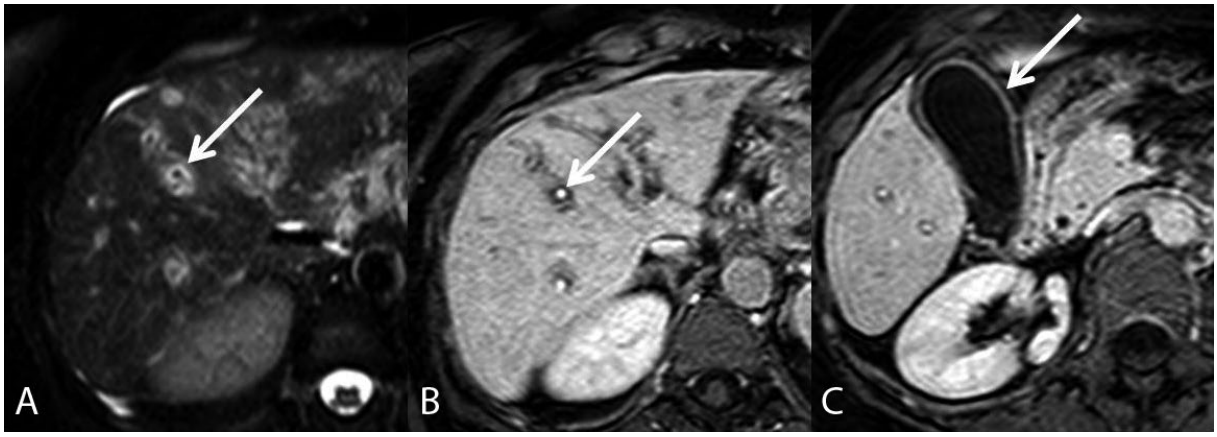

Figure 4. Magnetic resonance images in a 64-year-old woman with acute hepatitis: A and B) Fat-suppressed T2-weigthed and portal-venous phase images show periportal edema (arrows). C) Portal-venous phase image shows gallbladder wall thickening (arrow).

suggest a correct diagnosis in an appropriate clinical setting. These include hepatomegaly with patchy parenchymal enhancement, periportal edema, gallbladder wall thickening and narrowing of main hepatic veins. MR can also show multiple hyperintense areas on T2-weighted images, and reticular hypointensity of the liver on hepatobiliary phase with Gd-EOB-DTPA.[61,62] Signs of portal hypertension such as splenomegaly, esophageal varices and ascites can also be seen, and indicate a more advanced stage of SOS.[62] SOS can also manifest as a focal lesion, and radiologically mimic a metastasis. Non-spherical shape, ill-defined margin, intermingled hypo- or hyperintensity on hepatobiliary phase MR images, and isointensity on high-b-value diffusion-weighted MR images favor a diagnosis of metastasis over focal SOS.

Drug-induced sclerosing cholangitis is usually difficult to diagnose at imaging. CT and MR can show a similar appearance to primary sclerosing cholangitis, with narrowing of bile ducts.[63,64] Magnetic resonance cholangiopancreatography helps differentiate these two entities by demonstrating primary involvement of bile ducts at the hepatic portal in drug-induced sclerosing cholangitis.[63] Contrast-enhanced CT or MRI can also show periductal edema, enhanced and thickened bile duct wall, and heterogeneous liver enhancement.[64]

Conclusions

In this review we wanted to underline that, DILI, although epidemiologically affecting a lower percentage of cases compared to other liver diseases, sometimes can be severe as life threatening unless a transplant is performed. Despite the numerous and increasing evidence in the literature concerning the attempt to identify damage patterns related to a specific drug, as well as biomarkers useful not only in diagnosing but also in predicting the outcome, even today the diagnosis of DILI is still a *diagnosis of exclusion*. An ever-increasing support comes from both radiological and ultrasonographic imaging techniques. In fact, they do not allow to make the diagnosis, but to exclude other pathologies, whose resolution is not spontaneous, and to show clinical pictures (steatosis or VOS), which are typical of patients with DILI. CT and MRI are second level techniques of imaging and should be used for a differential diagnosis (to exclude gallstones, pancreatic disease, sclerosing cholangitis) or when we want to provide particular information of some aspect of DILI (*e.g.* SOS). The presence of networks dedicated to this pathology both in the USA (DILIN Network) and in Europe (ProEuroDili Network) will surely allow in the near future knowing with greater certainty the pathogenic mechanisms that underlie DILI and therefore the possibility to predict it.

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