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ORIGINAL ARTICLE

A proposed pathologic sub-classification of drug-induced liver injury

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Abstract

Background The aim of this study was to establish a new pathologic sub-classification of drug-induced liver injury (DILI) in combination with serum chemistry parameters and clinical observations.

Methods From 777 DILI cases diagnosed in China–Japan Friendship Hospital from 2003 to 2014, 590 cases without other concomitant liver diseases were selected for the study. Pathological classification was established. Pathology and serum biochemical correlation analyses in 208 acute cases with complete biochemical data and prognostic information were conducted.

Results We established a pathological classification of DILI according to the target cells of the liver (hepatocytes, bile duct epithelial cells, liver vascular and sinusoidal endothelial cells). In the 590 cases of DILI analyzed, hepatocyte injury accounted for 67.0%, bile duct epithelial injury (including cholestasis and mixed type of injury) 23.9%, and vascular injury 8.8%; about half of them were caused by the administration of traditional Chinese herbal medicines. Acute hepatocyte injury (lobular hepatitis) is further divided into mild, moderate and severe subtypes, while the mixed type of injury is categorized as cholestatic hepatitis and mixed hepatitis. The dynamic liver enzyme curves were established between lobular hepatitis and mixed-type hepatitis based on the combined consideration of histopathology and serum chemistry data. We proved that R value > 5 with cholestasis is a special feature of mixed hepatitis, which clarified the suspicion of the previous clinical classification of R value. Greater attention should be paid to drug-induced bile duct vanishing syndrome and drug-induced vascular injury.

Conclusion The pathological classification is simple to adopt and practically useful, which demonstrates the consistency between clinical features and liver pathology. The correlation between pathology and clinical biochemistry is an important way to acquire further understanding of DILI.

Keywords Drug-induced liver injury · Liver histology · Herb-induced liver injury · Pathological classification · Histological injury pattern

Introduction

The incidence of DILI has been increasing every year [1, 2]. DILI is currently a common liver disease in China due to the extensive use of herbal medicines without strict regulations or evidence of safety assessment. Herbal medicines are also used in home remedies or as alternative medicines [3]. According to recent data from national annual reports, drug-induced adverse events increased from 4700 cases in 2000, to 1,317,000 cases in 2013 [4], and jumped up to 1,430,000 cases in 2016. More remarkably, in 2016 alone, cases of new and severe adverse events were 423,000, an increase of 7.4% compared to 2015 [5]. Since DILI may be one of the more serious types of adverse events, it is imperative that DILI is further investigated [5].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12072-019-09940-9) contains supplementary material, which is available to authorized users.

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It is well known that the majority of drugs are lipid soluble and must be converted into water-soluble substances within the liver and excreted through bile or urine. During this conversion process, some of the metabolites have toxic effects and may elicit an immune reaction which can damage not only hepatocytes, but also the bile duct epithelium and sinusoidal endothelium (the three major targets of drug-induced injury) [1]. In China, it has long been thought that numerous drugs can cause a wide variety of hepatic injury with a wide spectrum of histopathological changes and, therefore, DILI lacks common pathological features. Pathological lesions with relatively distinctive characteristics, such as the infiltration of eosinophils and microvesicular fatty changes, are sometimes still used as standard criteria to diagnose DILI [6]. Up to now, there has been no well-established pathological criterion of DILI diagnosis domestically within China. As such, pathological study of DILI has resulted in inconsistent and unreproducible findings between pathological and clinical research.

This article therefore aims to: (1) follow the progression of international research on DILI and set up a simple and useful pathological sub-classification, which fits well with the clinical classification; (2) try to clarify and uniformly name the histopathological features of injury patterns of DILI and establish a set of pathological criteria for classification and assessing the severity of liver injury patterns; and (3) through this correlation, study the pathological injury patterns and serum biochemical tests in detail and to fundamentally promote DILI research in China and worldwide.

Methods

Sample selection

The study included a total of 777 cases of DILI confirmed by an expert consensus including two pathologists (Tai ling wang and Lihong Ye) and one hepatologist (Xinyan Zhao). Medical history, laboratory tests, and liver pathology were carefully reviewed, and competitive causes were excluded before being recruited in this retrospective study.

Our preliminary unpublished data showed that intra- and inter- reproducibility of RUCAM for herbal compounds was significantly lower than that for conventional drugs, which is in accordance with recent publications. Therefore, we did not adopt RUCAM because more than half of liver injuries are induced by herbal compounds, and RUCAM does not work very well in the assessment of causality of herbal compounds-induced liver injury [7, 8]. These samples were selected from the China–Japan Friendship Hospital between 2003 and 2014. During re-examination, each specimen was stained with hematoxylin and eosin (H&E), periodic acid–Schiff diastase (PAS-D), reticulin and Masson, cytokeratin 7 (CK7), and cytokeratin 19 (CK19).

(a) All liver tissue samples were classified according to injury target histologically (hepatocytes, biliary epithelial cells, liver vascular and sinusoidal endothelial cells) into different pathological categories and degrees of severity.
(b) Results of liver biochemistry tests (including ALT, AST, ALP, GGT, TBIL, and DBIL) within 1–3 months of DILI onset were comprehensively collected and organized from medical records of the patients. Medication history, corresponding data of liver biochemistry tests, and outcome information obtained by telephone follow-ups were included in the data sets. Hepatotropic viral hepatitis (hepatitis A, B, C, and E viruses) was excluded by serum markers (we routinely test all viral markers during daily practice). EBV and CMV infections were excluded by testing CMV IgM or EBV IgM and or CMV-DNA, EBV-DNA markers. Autoimmune liver diseases (patients with autoimmune simplified score higher than six points suspected as autoimmune hepatitis were excluded), alcoholic and nonalcoholic fatty liver diseases, biliary obstruction, metabolic liver diseases, and ischemic hepatitis were all excluded. 208 out of 590 patients had been followed up until normalization of liver chemistries (ALT or aspartate aminotransferase (AST) $< 1\times$ULN, ALP $< 1\times$ULN and total bilirubin (TBIL) $< 1.5\times$ULN).
(c) The correlation between the statistical analysis of biochemical results and pathological classification was analyzed.

Inclusion criteria

(a) Diagnosis criteria of DILI: (1) chronological relationship between drug or herb(s) exposure and liver injury. (2) Initial liver biochemical test ALT ≥ 5 ULN or ALP ≥ 2 ULN. (3) Extensive exclusion of other liver diseases (see above). (4) Histology compatible with DILI without other characteristic histological manifestations suggesting other liver diseases.
(b) A causal relationship between medication and symptoms of abnormal liver tests.
(c) Qualified liver biopsy samples collected, including at least ten portal areas for each tissue slide.

Exclusion criteria

(a) All patients were tested for serum markers of viral hepatitis (A–E). 118 samples were excluded due to concurrent infection with hepatotropic or non-hepatotropic viral hepatitis, bile duct diseases, steatohepatitis, liver

transplantation, and systemic diseases in which the liver had been involved.

(b) 69 other samples were excluded due to uncertain medical history or unqualified liver biopsy samples.

Statistical analysis

Quantification data were presented as a median value. Enumeration data were presented as percentages. Data from each group underwent normality tests and variance analyses. In this study, the quantification data did not coincide with normal distribution, so the Kruskal–Wallis test was used to assess nonparametric data for comparison among multiple groups. Comparison between two groups was carried out using the Mann–Whitney test. Multiple comparisons used the Bonferroni correction. Comparison of counted data used the Chi square test. Two-sided p values < 0.05 were considered statistically significant. All data were analyzed using SPSS (version 17.0; SPSS, Inc., Chicago, IL).

Results

Flowchart for patient enrollment (Fig. 1)

A total of 777 patients with satisfactory liver biopsy samples were diagnosed as having DILI. Among them, 118 patients with other concomitant liver disease and 69 patients with incomplete data were excluded. The rest of the 590 patients were included in the histological evaluations. 208 patients with acute DILI had complete biochemical test data and follow-up information. The correlation analysis was performed among these 208 cases (marked in red in Fig. 1).

Demographic characteristics of patients

There were 184 (31.19%) males and 406 (68.81%) females in the total of 590 patients. DILI was more common in females than in males. The ages ranged from 3 months to 80 years. Cases among the 31–50 years age group and 51–70 years age group were the largest. The mean age of onset was 45.1 ± 14.8 years. Detailed baseline demographic data are summarized in Supplement Table 1.

Distribution of medication class used in the 590 patients

Of all cases of DILI, 43.8% were due to traditional Chinese medicines, 0.4% due to natural herbal medicines, and 5.9% due to dietary supplements, accounting for just over half (50.1%). Traditional Chinese medicines were the most common class of agents inducing DILI.

Western medicines accounted for 43.6% of the proportion. Antibiotics, antipyretic analgesics, and cardiovascular drugs were the most common ones, followed by anti-diabetic drugs, statins, hormones, and digestive and nervous system

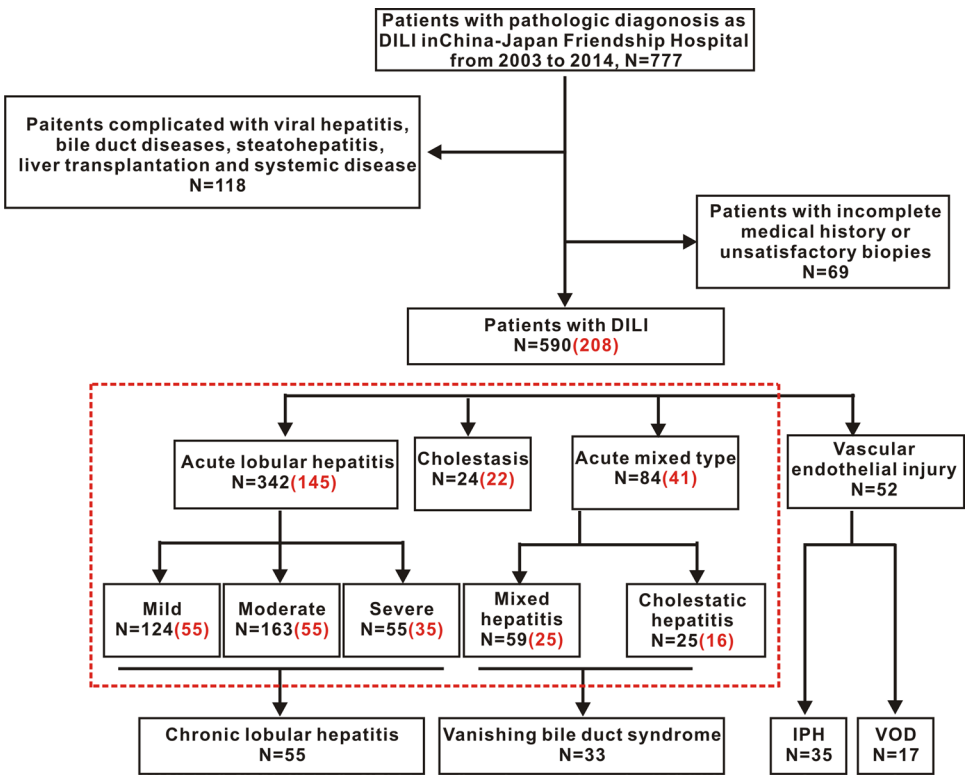

Fig. 1 Flowchart for patient enrollment

drugs in the second place. Furthermore, environmental factors (hair dye, pesticides, and chemical contact) accounted for 6.3% of the proportion of drugs inducing liver injury (Supplement Table 2). We compared the difference between herb(s)- and conventional drug-induced liver injury. The result is listed in Supplement Table 2.

Target-based classification on histopathological evaluations

(a) Hepatocyte injury, majority of which was lobular hepatitis (totally 397 cases). There was no main injury pattern featured as macro/microvesicular steatosis or granulomas in this category.

  (i) Acute lobular hepatitis was the most common type (342 cases). The pathologic feature was lobular inflammatory necrosis which was mostly found in the centrilobular region (Fig. 2a) and showed focal, confluent, bridging, or multilobular necrosis combined with mixed inflammatory cell infiltration, including monocytes, neutrophils, and/or a few eosinophils and microgranulomas (Fig. 2b). Inflammation in the portal area extended outward, giving the portal areas a star-like configuration (Fig. 2d). According to the type (degree) of necrosis, lobular hepatitis can be divided into mild, moderate, and severe, with reference to "Standards for the division of inflammatory activity of chronic hepatitis." [9] Focal necrosis was considered as mild; confluent necrosis (Fig. 2c) with or without a few bridging necrosis was considered moderate; multi-lobular necrosis or substantial bridging necrosis was considered severe (Fig. 2e).
  (ii) In the 55 cases of chronic lobular hepatitis, 43 cases were results from recurrent moderate or severe lobular hepatitis, while the other 12 cases were caused by long-term administration of medications. The morphological characteristics were similar to chronic viral hepatitis (Fig. 2f) and fibrosis was often obvious.

(b) Bile duct epithelial damage, including cholestatic pattern (bile canaliculi and bile duct injury at all levels) and mixed with hepatocellular injury (141 cases).

  (i) Acute cholestasis and mixed hepatocellular-cholestatic (108 cases).

    (i) Simple cholestasis (24 cases): mainly featured with cholestasis in the centrilobular region, without significant hepatocellular injury or inflammation of the portal areas.
    (ii) Hepatocellular–cholestatic mixed type (84 cases): characterized by hepatocanalicular cholestasis (bile plugs in canaliculi) near the central veins; regional hepatocyte enlargement containing bile pigment granules, with enlarged nucleus, binuclear or multinuclear hepatocytes; Kupffer cell hypertrophy in the sinusoids, and phagocytosis of bile plugs were also seen. Mild degree of hepatocellular injury (with spotty necrosis) is named cholestatic hepatitis (25 cases) (Fig. 3a); severe degree hepatocellular injury with confluent necrosis or bridging necrosis is called mixed hepatitis (59 cases) (Fig. 3b). Inflammation at the portal area in these two subtypes was more evident and sometimes showed bile duct injury (Fig. 3c).

  (ii) Chronic cholestasis (33 cases).

This pattern mostly evolved from mixed or cholestatic hepatitis and mainly affected the bile duct. The disease duration was more than 1 year and accompanied by vanishing bile duct syndrome. According to the disease duration, the presence or lack of jaundice was divided into two groups:

(i) The group with no jaundice (15 cases) had elevated levels of alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT). Inflammation and fibrosis in the portal area were mild (Fig. 3d).
(ii) The group with jaundice (18 patients) had obvious portal fibrosis and inflammation. Bile canaliculus plugs, biliary rosettes, and foam cell aggregation were noticed in the hepatic lobule. Cholate stasis presented in periportal hepatocytes. A teenager who had progressed to advanced liver cirrhosis after an illness period of 1 year was treated by liver transplantation (Supplement Fig. 1.).

(c) Vascular injury (51 cases).

  (i) Obliteration of portal venules (idiopathic portal hypertension, 34 cases). The pathological features included interstitial fibrosis of the portal tracts and the disappearance of portal venules. Due to portal hypertension, dilatation and herniation of small branches of the portal vein toward the liver parenchyma could be found, along with smooth muscle proliferation and thickening of medium-sized branch walls of the portal veins.

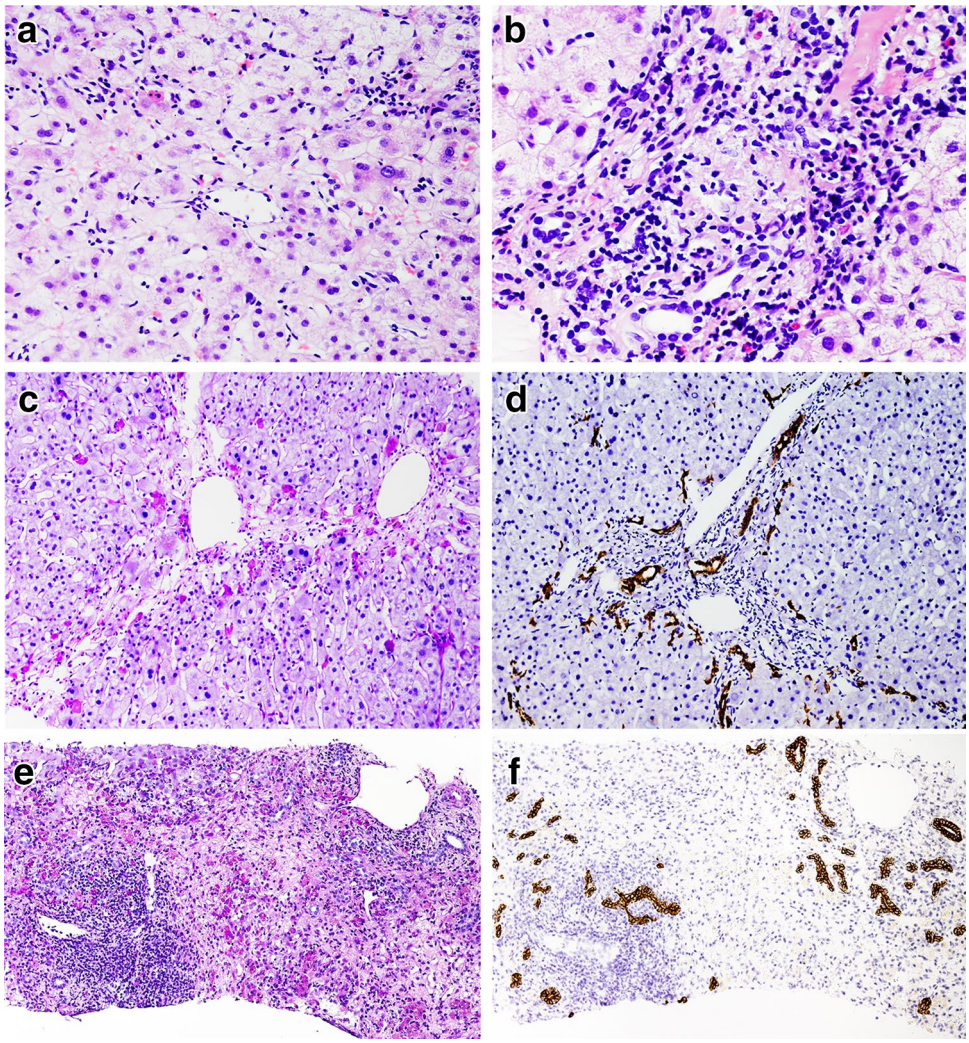

Fig. 2 Mild, moderate, and severe lobular hepatitis. Female, 69 years old. Disease onset after continuous use of "Xuekangning" for one year. Liver biopsy was done 1 month after onset. **a** Multiple spotty necrosis and apoptotic body in the perivenous area, with various inflammatory cellular infiltration (H&E staining). **b** Expanded portal tract, various inflammatory cellular infiltration, and a few eosinophils and microgranuloma. **c** Female, 45 years old. Disease onset 1 week after taking paracetamol orally and metronidazole intravenously with an ALT level of 1747 IU/L. Liver biopsy was done 2 weeks after onset. Liver histology shows confluent necrosis and mild inflammation with a clear broad line in the perivenous area. Several ceroid cells were found in the focal necrotic area as well as in the sinusoids (PAS-D staining). **d** Mild mixed inflammatory cellular infiltration expanded with mild ductular reaction and proliferation (CK7 staining). **e** Female, 27 years old. Disease onset after taking allergy medicine due to a skin disease. Liver biopsy was done 1 month after onset. **e** Multiple lobular necrosis, with extensive inflammatory cells infiltration (H&E staining). **f** Prominent periportal ductular reaction (CK7 staining). *H&E* hematoxylin and eosin stain, *ALT* alanine aminotransferase, PAS-D periodic acid–Shiff-diastase, *CK 7* cytokeratin 7

Nodular regenerative hyperplasia of hepatocytes could be seen in eight cases.

(ii) Veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) (17 cases) was characterized pathologically by endothelial injury of the centrilobular region sinusoids and small veins, subintimal edema, and fibrous tissue proliferation, causing sinusoids and small hepatic vein fibrotic obliteration. As the disease advances, affected sinusoids and hepatic veins may become completely obliterated with fibrosis. Atrophy and necrosis of hepatic plates could be seen with sinusoidal congestion and dilatation. One of the patients showed congestive cirrhosis of the liver.

Histological diagnostic criteria of this sub-classification are listed in Table 1.

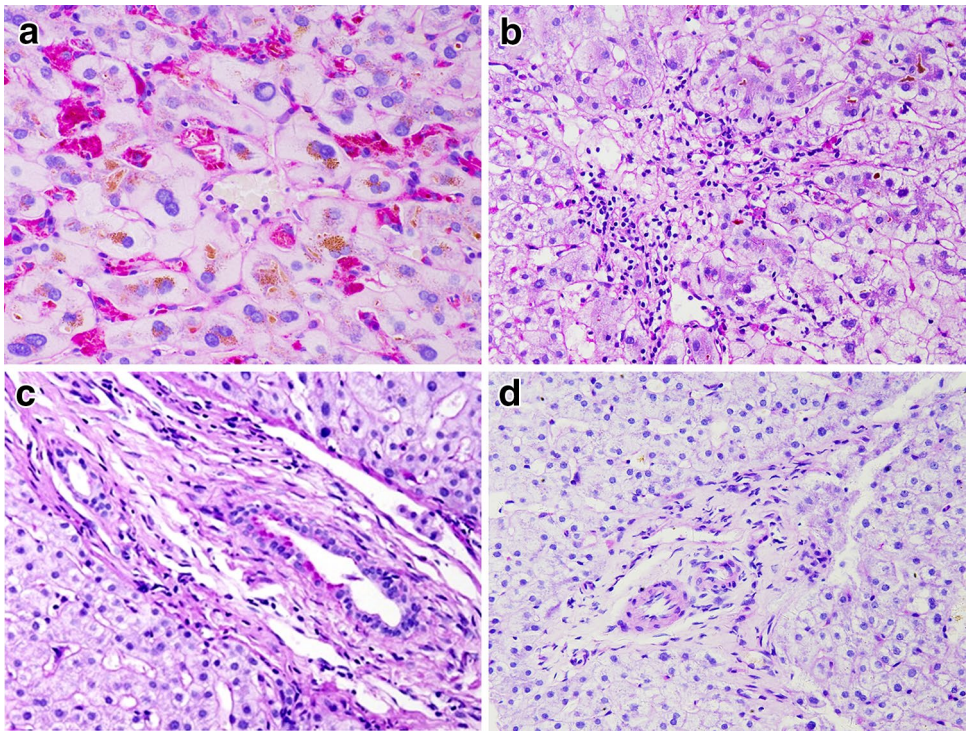

Fig. 3 Cholestatic and mixed hepatitis. **a** Cholestatic hepatitis. Female, 73 years old. Disease onset after 1 week into a 3-week course of herbal decoction for shingles. Liver biopsy was done 15 days after onset. The biopsy showed bile canaliculi plugs around the central vein (brown), hepatocytes swelled with bile pigment granules, and with an enlarged nucleus or dual nuclei. Kupffer cell hypertrophy (PAS-D +, red) could be seen in the sinusoids. Small bile plugs and a few focal necrosis can be seen in the cytoplasm (PAS-D). **b** Mixed hepatitis. Female, 32 years old. Disease onset 2 days after taking antipyretic analgesics, presenting with nausea and yellow urine. Liver biopsy was done 1 month after onset. The biopsy showed the centrilobular zone characterized by confluent necrosis and hepatocyte swelling, and feathery degeneration. The bile plugs were prominent in the bile canaliculi (PAS-D +). **c** Small bile duct damage. Female, 34 years old. Disease onset after receiving a 7-day course of herbal decoction containing cinnabar and other herbs for insomnia, presenting with jaundice and fatigue. The biopsy showed cholestatic hepatitis, done 5 months after onset. Duct epithelium was irregular with mild inflammatory infiltration (PAS-D). **d** Vanishing small bile duct. Female, 27 years old. She had a 5-year history of abnormal liver function tests. ALP levels fluctuated between 278 IU/L and 155 IU/L. TBIL levels fluctuated between 55 μmol/L and 27 μmol/L. Portal fibrosis was mild, and bile ducts could not be seen (PAS-D). *PAS-D* periodic acid–Shiff-diastase, *ALP* alkaline phosphatase, *TBIL* total bilirubin

Analysis of liver biochemical tests and clinical course in 208 patients with acute drug-induced liver injury (Table 2)

Acute lobular hepatitis was observed in 145 of the 208 patients, including mild (55 cases), moderate (55 cases), and severe (35 cases) cases; mixed hepatitis was observed in 25 cases, cholestatic hepatitis was observed in 16 cases, and simple cholestasis in 22 cases.

(i) Comparison of mild, moderate, and severe lobular hepatitis.

Serum ALT and AST levels of the moderate and severe groups were significantly higher than that in the mild group, with $p < 0.001$, $p = 0.031$ (Fig. 4a) and $p = 0.003$, $p < 0.001$ (Fig. 4b), respectively. There was no significant difference between the moderate and severe groups. There was no significant difference in serum ALP levels among all three groups (Fig. 4c). Serum TBIL levels of the moderate group (73 μmol/L) and the severe group (100 μmol/L) were significantly higher than that in the mild group (29 μmol/L), with $p = 0.003$ and $p < 0.001$ (Fig. 4d), respectively. Despite the fact that serum TBIL level of the severe group was higher than that in the moderate group, there was no statistically significant difference between these two groups.

(ii) Comparison of mixed hepatitis, cholestatic hepatitis, and simple cholestasis.

Serum ALT and AST levels of the mixed hepatitis group were significantly higher than that of the cholestatic hepatitis and simple cholestasis groups ($p < 0.001$, Fig. 5a, b). There was no significant difference in ALP (Fig. 5c) and TBIL (Fig. 5d) levels among the three groups ($p > 0.05$).

Table 1 Histological definition of each sub-classification category of DILI

	Definition
Acute lobular hepatitic	Lobular predominant inflammation and apoptosis with lobular disarray in more severe cases
Mild	Spotty necrosis mainly around the perivenular areas
Moderate	Predominant lobular activity manifested as confluent necrosis or occasional bridging necrosis, portal inflammation usually mild
Severe	Multiple bridging necrosis or multiple lobular necrosis is present with a higher degree of inflammatory cell infiltration, mixed in nature
Chronic hepatitic	Portal predominant inflammation with generally mild to moderate lobular inflammation. Fibrosis not required
Cholestatic hepatitic	Combination pattern with visible hepatocellular or canalicular bile with different severities of necro inflammation
Cholestatic hepatitic	Hepatocanalicular cholestasis with spotty necrosis
Mixed injury	Hepatocanalicular cholestasis with confluent or bridging necrosis
Acute cholestasis	Hepatocellular or canalicular bile with little inflammation
Chronic cholestasis	Definite cholate stasis or copper accumulation associated with a chronic hepatitic pattern of inflammation and bile duct injury or loss
VBDS	More than 50% of portal tracts (≥ 11) has no visible bile ducts with signs of cholate stasis
Chronic cholestatic hepatitis	Less than 50% percent of portal tracts (≥ 11) has no visible bile ducts with signs of cholate stasis
Vascular injury	Sinusoidal dilatation or sinusoidal endothelium injury with/without peliosis. Efferent or afferent abnormal vascular injuries
IPH	Hepatoportal sclerosis with portal-based fibrosis or abnormal portal/periportal vessels or herniated vessels
SOS	Sinusoidal obstruction syndrome with or without occlusion of central venules

Table 2 Liver biochemical tests and recovery time in different pathological types of DILI

Cases ($N=$)	Lobular hepatitis			Mixed hepatitis	Cholestatic hepatitis	Simple cholestasis
	Mild	Moderate	Severe			
	55	55	35	25	16	22
ALT (U/L)	661 (300, 1063)	957 (690, 1601)	900 (574, 1325)	1059 (866, 1738)	426 (279, 527)	74 (36, 266)
AST (U/L)	468 (181, 753)	714 (413, 927)	808 (488, 1121)	1030 (733, 1398)	210 (152, 592)	63 (36, 103)
ALP (U/L)	127 (90, 167)	149 (111, 219)	159 (112, 240)	187 (150, 236)	150 (120, 233)	213 (158, 240)
GGT (U/L)	136 (61, 240)	215 (120, 305)	150 (68, 225)	179 (92, 270)	186 (105, 463)	88 (48, 224)
TBIL (μmol/L)	29 (15, 66)	73 (34, 142)	101 (36, 163)	217 (60, 579)	215 (103, 627)	207 (68, 799)
Recovery time (days)	31 (20–41)	48 (31–69)	159 (81–230)	97 (45–102)	113 (30–268)	92 (21–161)

(iii) The peak of TBIL in mixed hepatitis, cholestatic hepatitis, and simple cholestasis groups were all significantly higher than those in mild, moderate, and severe lobular hepatitis groups ($p<0.001$).

(iv) In mixed hepatitis, the pathological changes were characterized by both hepatocyte and canaliculi injuries. The process of canalicular injury caused by DILI resulted from the toxic drug metabolites excreted by the bile impair transporters (e.g., water channels, BSEP, MRP2, MDR3) on canalicular membranes, resulting in intraluminal cholestasis and bile plugs in canaliculi. Tight junctions of canaliculi were damaged, followed by bile leakage into the sinusoids and blood. Serum TBIL gradually increased and reached a peak after 11–20 days. Once injury ceases, bile plugs drop into sinusoids and the structure of the canaliculi begins repair. Canalicular functional recovery is slower than that of hepatocytes. Usually, serum TBIL levels may return to normal within 3–4 months. Recovery times of these three groups with canalicular injuries are roughly the same, regardless of the severity of hepatocyte injury (Table 2). Therefore, after the onset of mixed hepatitis, hepatocyte damage occurs first with a higher level of ALT. During ALT decline, TBIL will gradually increase to a peak, forming a characteristic bimodal biochemical curve (Fig. 6b).

(v) What is worth highlighting is the R value of mixed hepatitis. After analyzing a total of 208 DILI cases (results not attached to this text), we found "R value ≥ 5 accompanied with cholestasis" was exactly characteristic of mixed hepatitis. It is only necessary

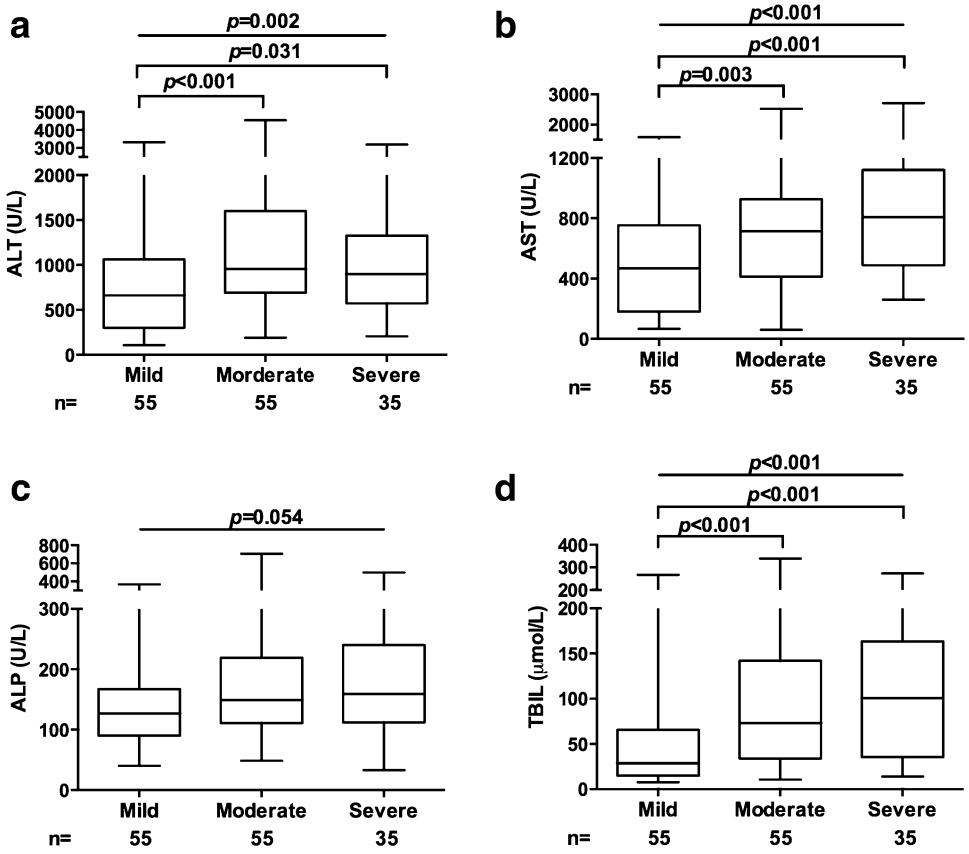

Fig. 4 Comparison of liver biochemistry of lobular hepatitis with different severities. Serum ALT and AST levels of the moderate and severe groups were significantly higher than that in the mild group, with $p < 0.001$, $p = 0.031$ (**a**) and $p = 0.003$, $p < 0.001$ (**b**), respectively. There was no significant difference between the moderate and severe groups. There was no significant difference in serum ALP levels among all three groups (**c**). Serum TBIL levels of the moderate group (73 μmol/L) and the severe group (100 μmol/L) were significantly higher than that in the mild group (29 μmol/L), with $p < 0.001$ and $p < 0.001$ (**d**), respectively. *ALT* alanine aminotransferase; *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *TBIL* total bilirubin

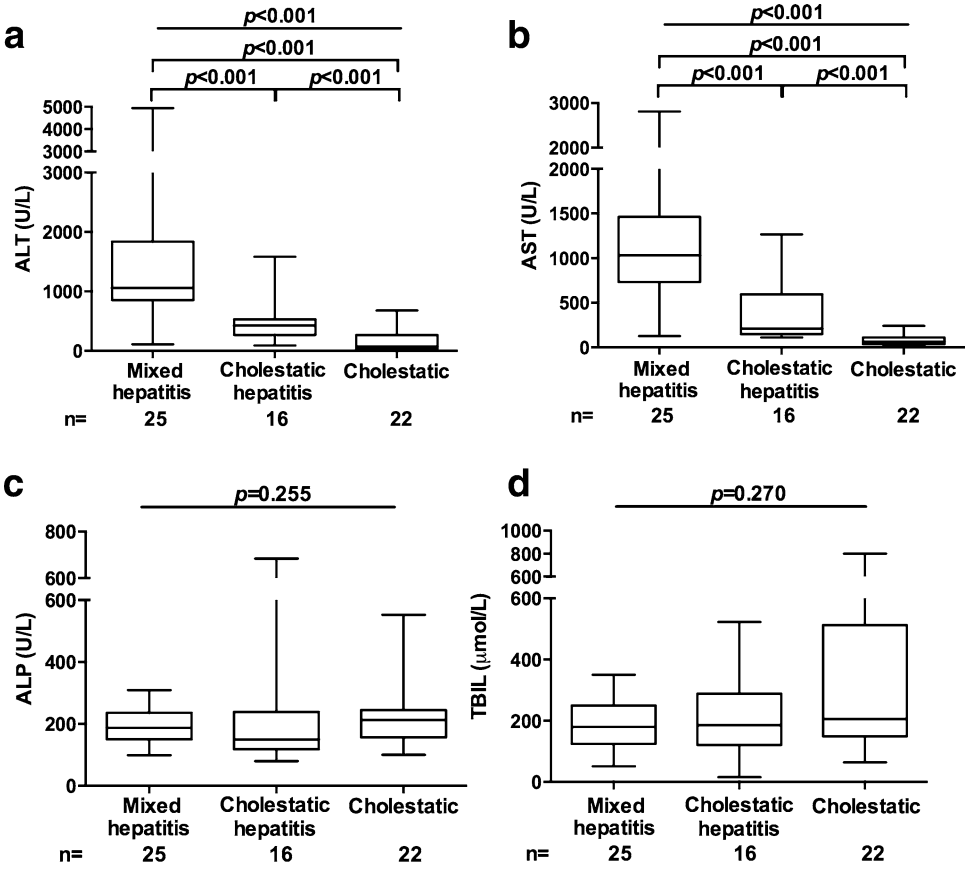

Fig. 5 Comparison of liver biochemistry of simple cholestasis, cholestatic hepatitis, and mixed hepatitis. Serum ALT and AST levels of the mixed hepatitis group were significantly higher than that of the cholestatic hepatitis and simple cholestasis groups ($p < 0.001$, **a**, **b**). There was no significant difference in ALP (**c**) and TBIL (**d**) levels among the three groups ($p > 0.05$). *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *TBIL* total bilirubin

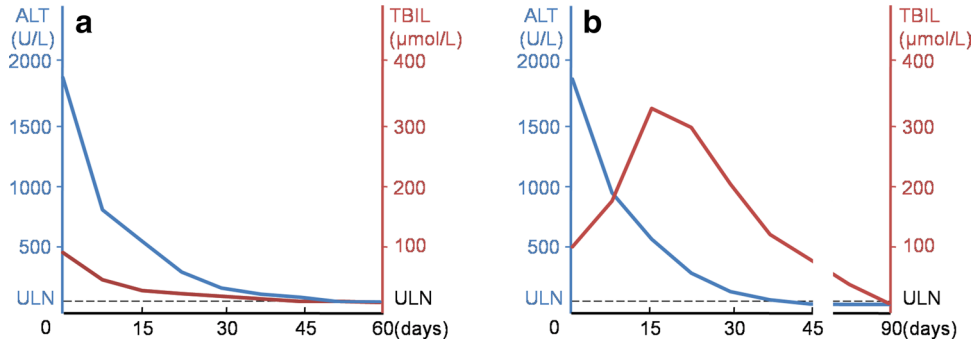

Fig. 6 Comparison of patterns of biochemistry changes (ALT and TB) after dechallenge in lobular hepatitis and mixed hepatitis. In lobular hepatitis, serum ALT reaches a peak in a short time. Along with ALT degradation, ALT levels can be reduced by 50% or more from the peak within 1 week. Meanwhile, slightly elevated TBIL decrease simultaneously, resulting in a rapidly declining biochemical curve with a single overlap peak (**a**). **b** After the onset of mixed hepatitis, hepatocyte damage occurs first with a higher level of ALT. During ALT decline, TBIL will gradually increase to a peak, forming a characteristic bimodal biochemical curve. *ALT* alanine aminotransferase, *TBIL* total bilirubin

to supplement the feature of mixed hepatitis to it, which is why we subdivided clinically mixed DILI into mixed hepatitis (R value ≥ 5 accompanied with cholestasis) and cholestatic hepatitis ($2 < R < 5$).

(vi) Comparison of recovery times in liver biochemical tests in different pathological types of DILI. The time to recovery in patients with severe lobular hepatitis (159 days) was significantly longer than that in the moderate group (48 days) and mild group (31 days) ($p < 0.001$), and the time to recovery in the moderate group was significantly longer than that of the mild group ($p < 0.001$). The time to recovery for patients in the mixed hepatitis, cholestatic hepatitis, and simple cholestasis groups ranged from 92 to 113 days, with no significant difference between them.

Discussion

Accumulation of liver biopsies strengthened our recognition of DILI. Along with the increasing usage of super active drugs, more and more DILI cases have emerged, especially among those with jaundice in the 1950s to 1960s. Due to the unclear pathogenesis and difficulties in classification, the clinical diagnosis was extremely problematic. Popper et al. proposed classification of DILI in 1965 into six categories [10], based on his microstructure and histological characteristic observations of 155 pathological specimens (including liver biopsy, surgery resection, and 56 cases of autopsies): (1) toxic injury; (2) simple cholestasis; (3) nonspecific drug-induced hepatitis with or without cholestasis; (4) viral hepatitis-like reactions; (5) drug-induced steatohepatitis (only one case); and (6) reactive hepatitis accompanied by drug-induced injury of other organs. This classification has been commended by Kleiner as a milestone for pathological classification [11]. In 1990, CIOMS clinically classified DILI into hepatocellular, mixed, and cholestatic patterns [12]. In the same period, Zimmerman comprehensively described the pathological changes in drug/chemical-induced liver injury based on around 1000 DILI biopsies and relevant references [13]. They histologically classified DILI into acute and chronic types, giving detailed descriptions of hepatocellular, cholestatic, and vascular injury as well as liver tumors, listing poisoning liver injury separately. After that, multiple hepatopathologists expounded upon the DILI classification for pathological changes in drug- and chemical-induced liver injury. In recent years, Kleiner has recommended a classification of DILI according to the injury patterns and suggested that the exact nomenclature and injury severity should be reflected by the pathologic classification. In light of the above ideas, we proposed the sub-classification of DILI according to the injury targets of the drugs in this study.

It was proven that the injury of each of the targets of the liver had certain specific characteristics through observing histopathological changes of the 590 DILI cases. Classification became easier and practically useful according to the injury target. We classified the pathological patterns before dividing them into subtypes based on the injury severity. Various pathological injury patterns of DILI were sorted out in a simple and appropriate way. The most common pathological patterns of 590 cases were hepatocellular injury (67.3%) and cholestasis with mixed hepatitis (23.9%), which was consistent with the data reported worldwide; and the rest of it was vascular endothelial damage (8.8%). Recently, Kleiner summarized the pathological patterns of liver injury caused by 48 kinds of drugs that had been reported over the years (more than 50 cases of DILI associated with each drug were enrolled) [14]. Most patterns were of acute and chronic hepatitis, acute and chronic cholestasis and cholestatic

hepatitis, and a few were vascular injury or steatosis, as was consistent with the main types among 249 cases according to the DILIN reported in 2014 in the USA as well as our data. It was fully demonstrated that the drug reactions of each liver injury target and the basic injury pattern were relatively constant, regardless of the class of the drugs and patients' racial backgrounds. The classification defined based on injury targets made a harmonization of both pathology and the clinic; it was quite helpful to understand the dynamic changes and response manners of the target cells to a variety of injuries by analyzing the combined morphological and biochemical characteristics of the liver.

Referencing to the "Standards for the division of inflammatory activity of chronic hepatitis", the inflammatory degree of lobular hepatitis was divided into mild, moderate, and severe levels [9]. We found that the biochemical tests were significantly different among mild, moderate, and severe lobular hepatitis in the 208 cases, indicating that this division criteria were fully applicable to DILI. At the same time, we noticed that the clinical course of severe lobular hepatitis was three to four times longer than that of mild and moderate lobular hepatitis. The difference in clinical course depends not only on the extent of hepatocyte necrosis, but also on proliferation and regeneration. It is clear that mild and moderate necrosis are immediately replenished by regeneration of adjacent mature hepatocytes. Therefore, the necrosis would be completely repaired in 1–2 months without scars. The repair of severe multi-lobular necrosis gradually progresses by the activation of progenitor cells around the portal area within the necrotic zone, as ductular reaction, followed by hepatocyte differentiation. It usually takes months, or even up to a year, to recover. Regeneration and repair processes are affected by age, nutrition, concomitant diseases, and many other factors. If the regeneration was poor, it usually develops scars or turns into chronic hepatitis, indicating poorer prognosis. In addition, patients with severe lobular hepatitis often have accompanying elevated immunoglobulin levels and the infiltration of majority of plasma cells in interstitial tissue, which needs to be differentiated with autoimmune hepatitis [1].

New R criterion has been defined as $R > 5$ with jaundice at DILI onset. It has a greater ability to predict acute liver failure [15]. Our sub-classification of DILI has mixed hepatitic phenotype, which is defined as hepatocanalicular cholestasis with higher extent of necrosis (bridging necrosis). Mixed hepatitic phenotype is the histological basis of new R criterion. Cholestasis plus high extent of necrosis can explain why new R criterion has a greater ability for predicting acute liver failure in patients with DILI. [16].

We analyzed the association between pathology and biochemistry in the two most common types of DILI. First, in lobular hepatitis, the pathology feature is characterized by hepatocyte necrosis, followed by ALT releases into the blood. Serum ALT reaches a peak in a short time (in moderate lobular hepatitis, ALT may increase to 1000–2000 IU/L). Along with ALT degradation, ALT levels can be reduced by 50% or more from the peak within 1 week. Meanwhile, slightly elevated TBIL decreases simultaneously, resulting in a rapidly declining biochemical curve with a single peak (Fig. 6a). Biochemical parameters can be returned to normal within 1–2 months due to the rapid proliferation and regeneration of hepatocytes.

What is worth highlighting is the R value of mixed hepatitis. The R value has been used to identify different clinical types of DILI for more than 20 years. A part of R value ≥ 5 accompanied with cholestasis aroused controversy over R value adjustment. After analyzing a total of 208 DILI cases (results not attached to this text), we found "R value ≥ 5 accompanied by cholestasis" was exactly characteristic of mixed hepatitis. Thus, there was no need to revise the R value; it is only necessary to supplement the feature of mixed hepatitis to it, which is why we subdivided clinically mixed DILI into mixed hepatitis (R value ≥ 5 accompanied by cholestasis) and cholestatic hepatitis ($2 < R < 5$) [16].

There are two issues in the DILI classification that warrant greater attention:

1. Acute drug-induced bile duct injury often affects the interlobular bile duct, but because of a lack of specific indications, it is difficult to detect early on in clinical practice. In fact, it is common in liver biopsies of mixed hepatitis or cholestatic hepatitis. Although most of these injured small bile ducts can be recovered, some lesions could persist and even progress to acute vanishing bile duct syndrome [17]. A 16-year-old girl as shown in Supplement Fig. 1 presented with jaundice after taking rifampicin for diarrhea. Liver biopsy within 50 days after onset showed mixed hepatitis, but there was no interlobular bile duct in each portal area. Because of persistent clinical jaundice, she underwent liver transplantation 1 year after onset. Examination of the explanted liver showed biliary cirrhosis, in which the interlobular bile ducts had disappeared from most of the portal areas and dense lymphocyte had infiltrated into the portal area, suggesting that there may be excessive immune response involved. After reviewing the existing literature, we found nine cases of children that presented with acute vanishing bile duct syndrome due to DILI (confirmed by liver biopsy). Except for one case that died on the 19th day [1], two cases developed liver cirrhosis and after 6 months [1] and 8 months after onset of disease [1], respectively, both had liver transplantations. One patient received liver transplantation 13 months after onset [1]. In the remaining five cases, there was one case where liver inflammation ceased after receiving large doses of immunosuppressant due to the diagnosis of small bile

duct reduction confirmed by liver biopsy. The other four cases also improved by effective treatment. These cases suggest that we need to pay greater attention to timely detection and treat the drug-induced disappearance of the small bile ducts.

2. Drug-induced liver vascular injury also warrants great attention. Sinusoidal or vascular injury caused by DILI is not uncommon. Mild cases may have sinusoidal endothelial injury or dilation of local sinusoids and the formation of peliosis. Severe cases may have intrahepatic vein endothelial injury, even leading to idiopathic portal hypertension (IPH) or SOS/VOD [18].

The incidence of IPH in western countries is very low, but relatively high in Japan and India [19], and not uncommon in China [20]. A total of 279 Chinese cases were diagnosed with pathology in our department between 2003 and 2014. A small number of cases were derived from lupus erythematosus; 34 cases had clear medication history, with the remaining having unknown reasons. Among them, one case was of a young girl who was hospitalized with liver cirrhosis and upper gastrointestinal bleeding. Liver biopsy confirmed IPH with 12 years of medication history of bezoar detoxification pills (containing realgar As2S4) due to persistent constipation at her school. Three years later, arsenic poisoning lesions appeared, and blood arsenic concentration increased to 30 times higher than normal. If the girl had not been found to have arsenic poisoning, her IPH would not have been clearly recognized. As Wanless said, clinical symptoms of IPH tend to appear long after the original vascular lesions, after the original lesions may have already "healed," leading to difficulty in clarifying reasons for illness. In reviewing these cases in our department, many cases had unknown causes of IPH, but the pathological pattern is concise and consistent, so it is difficult to say that they were not drug- or chemical-induced vascular injury.

Of the 17 SOS/VOD cases, nine were caused by taking "TuSanQi" (gynura segetum) by boiling the leaves in water as a medication to treat flesh wounds or self-cultivating the plant to make tonic drinks. In 2012, Gao H et al. [21] found that the strain of gynura segetum containing pyrrolidine alkaloid was the pathogenic ingredient rather than the strain of gynura segetum without pyrrolidine alkaloid. Over the years, pyrrolidine alkaloid-induced VOD has not been uncommon; both official and individual action must be taken to avoid VOD.

The pathological classification of DILI is summarized in Fig. 7. DILI is classified by the target cells of the injury (hepatocytes, biliary epithelial cells, liver vascular and sinusoidal endothelial cells). By corresponding to the clinical classification of CIOMS and dividing the degree of acute patients according to the pathology and clinical features, the hepatocyte injury type (lobular hepatitis) was subdivided into mild, moderate, and severe degrees. The mixed type was subdivided into mixed hepatitis and cholestatic hepatitis according to the hepatocellular injury degree. In the cholestatic type, acute bile duct vanishing syndrome is very important. The characteristics of various chronic DILI types are clearly known, and they can progress into fibrosis and cirrhosis, respectively. For the sake of clarity, rare hepatocellular injuries (large, small (micro) patterns like steatosis and granulomatous-type and drug-induced tumors) were not included in Fig. 7.

Bjornsson and Hoofnagle [22] divided 346 drugs into four categories based on incidence from over 60 years of reports on drugs that have caused liver injury: Category

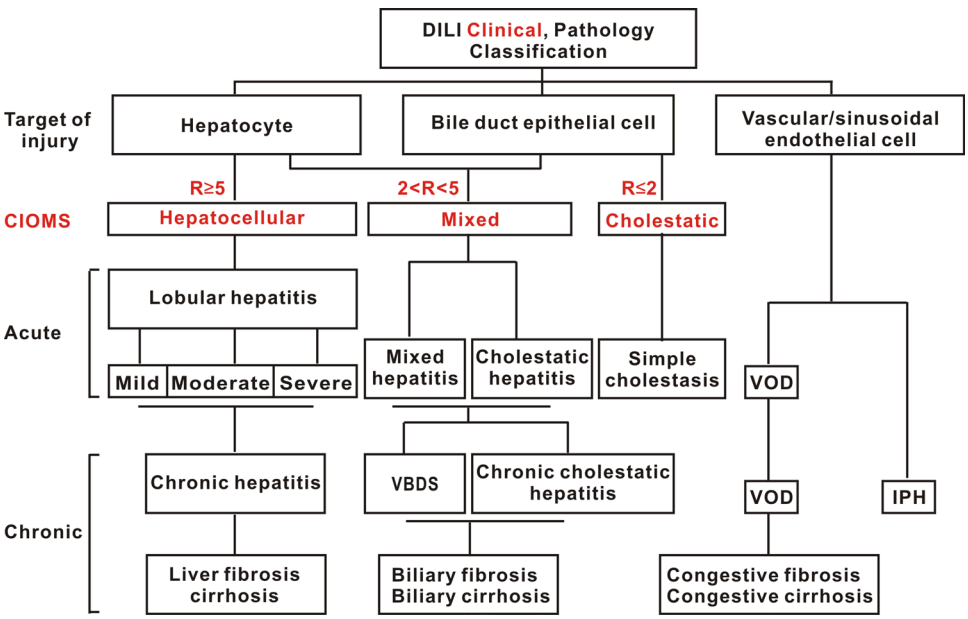

Fig. 7 A proposed pathological sub-classification of DILI in accordance with clinical *R* classification along with sub-classification according to different severity of liver injuries

A, > 50; category B, 12–49; category C, 4–11; category D, < 3. They uploaded the clinical manifestations and histopathological changes on the Internet for enquiry and reference. This method was heralded as a new approach toward drug safety research in 2016 for evaluating hepatotoxicity of drugs. However, Bjornsson did not summarize the toxicity of traditional Chinese medicine due to lack of information. Nowadays, DILI in China has become quite common, especially injury caused by traditional Chinese medicines [23]. It is predicted that over 50% of DILI is caused by traditional Chinese medicine [24]. Thus, we are able to and are responsible for further research in this field.

Clinical use of the study is according to our data, subclassification of DILI into mild, moderate, and severe cases which have significant difference in severity and time of full recovery. Patients with severity in liver histology had higher clinical severity score and longer time of recovery. A majority of DILI cases with chronic hepatitis and chronic cholestatic injury pattern with follow-up information had persistent liver biochemical tests at 6 months after the DILI episode. A majority of patients with acute hepatic or acute cholestatic injury pattern had ALT or TB normalization within 3 months.

Limitation of the study is that for the majority of DILI cases in this study, we did not have information with regard to the incidence of acute liver failure and final outcome. Therefore, we cannot make any conclusion about this sub-classification of DILI and its predictability on drug-induced acute liver failure. Further study is needed.

In conclusion, a pathological classification according to injury targets (hepatocytes, biliary epithelial cells, liver vascular, and sinusoidal endothelial cells) and injury severity is simple and practical and emphasizes the concordance between clinical features and liver pathology. Through liver biopsy in combination with clinical manifestation and medication history, we are able to diagnose DILI with clarity. By clearly defining DILI classification and degree of pathological changes, we can assess the disease and its prognosis. The correlation between pathology and liver biochemistry is essential for acquiring a deeper understanding of the pathogenesis of DILI.

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Compliance with ethical standards

Conflict of interest Tailing Wang, Xinyan Zhao, Chen Shao, Lihong Ye, Jing Guo, Na Peng, Honglei Zhang, Jian Li, Yuanyuan Kong, Hong You, Jidong Jia declare no conflict of interest.

Ethical approval This article does not contain any study with human participants or animals performed by any of the authors.

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