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Histopathological characteristics of liver biopsy performed at different time points in drug-induced liver injury

Yu Wang[1], Zikun Ma[1], Tiantian Guo[1], Jimin Liu[2], Min Li[3] and Xinyan Zhao[1]

[1]Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing, China, [2]Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada and [3]Clinical Epidemiology and Evidence-Based Medicine Unit, National Clinical Research Center for Digestive Diseases, Beijing Friendship Hospital, Capital Medical University, Beijing, China

Summary. Background and Aims. Liver biopsy can provide critical information in patients with drug-induced liver injury (DILI). Our study aimed to compare the histopathological features of DILI at different time points from the onset to liver biopsy.

Methods: We conducted a single-centre retrospective observational study. The clinical and follow-up data were extracted, and the pathological slides were reviewed.

Results. 129 patients were included. The median age was 52 and 75% were women. They were divided into <1 month, 1-3 months, and >3 months groups according to the durations from onset of the disorder to liver biopsy. The aminotransferase, alkaline phosphatase, and bilirubin levels showed no significant differences at onset but significantly decreased with time among the three groups (all $p<0.05$) at the time of liver biopsy. Histological injury patterns were significantly different among the three groups ($p<0.01$). Hepatocellular, canalicular, and cholestasis of Kupffer cells were significantly less frequent in the >3 months group ($p<0.01$). For patients taking herbs, bridging necrosis and cholestatic injury were significantly more frequent in the <1 month group ($p<0.01$). Furthermore, ductopenia, cholate stasis, and foam-like cells were equally distributed in the three groups but were significantly associated with poor prognosis.

Conclusions. Biopsy time significantly affects liver pathology: the earlier, the more acute cholestatic-hepatitic pattern, the later, the more chronic injury patterns. The prognostic features (ductopenia, cholate stasis, and foam-like cells) occurred equally in all three groups. Our study provides valuable information for liver pathologists aiding in their better interpretation of the liver biopsy from patients with DILI.

Key words: Pathology, Histopathology, Timing, Outcome

Introduction

The incidence of drug-induced liver injury (DILI) has been increasing globally and is becoming one of the major aetiologies of liver dysfunction in hepatology (Sgro et al., 2002; Bjornsson et al., 2013; Shen et al., 2019). Many drugs can cause liver injury, which is the main reason for the premature termination of drug development and postmarketing withdrawal according to the Food and Drug Administration (FDA) (Downing et al., 2017; Garcia-Cortes et al., 2020). However, DILI lacks specific biomarkers for diagnosis at present, and it is a diagnosis of exclusion (Suzuki et al., 2011; Church et al., 2019). Similarly, liver histopathology of DILI has no pathognomonic findings; however, it can help confirm or exclude a diagnosis (Kleiner, 2017).

There have been several studies on the histopathological characteristics of DILI. 18 histological injury patterns were summarized from a drug-induced liver injury network (DILIN) prospective cohort, 5 of

Corresponding Author: Xinyan Zhao, M.D., Ph.D:, Liver Research Center, Beijing Friendship Hospital, National Clinical Research Center for Digestive Disease, Capital Medical University, Beijing, China. e-mail: zhao_xinyan@ccmu.edu.cn

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Abbreviations. ALB, albumin; ALF, acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DB, direct bilirubin; DILI, drug-induced liver injury; DILIN, Drug-Induced Liver Injury Network; FDA, Food and Drug Administration; GGT, gamma glutamyltransferase; HDS, herbal and dietary supplements; IAIHG, International Autoimmune Hepatitis Group; INR, international normalized ratio; LT, liver transplantation; RUCAM, Roussel Uclaf Causality Assessment Method; TB, total bilirubin; ULN, upper limit of normal.

which (acute hepatic, chronic hepatic, acute cholestatic, chronic cholestatic, and cholestatic-hepatitic) accounted for up to 83% of the patients (Kleiner et al., 2014). In addition, histopathological subclassification based on the injury targets and severity of injury was proposed (Wang et al., 2019a) . Moreover, histopathological classification of DILI has significantly greater value in predicting clinical outcomes than biochemical R classification (Tian et al., 2019). Furthermore, many studies have described histological features and their associated clinical manifestations of different drugs, such as azithromycin, azathioprine, He Shou Wu, and Bu Gu Zhi (Martinez et al., 2015; Siramolpiwat and Sakonhaya, 2017; Wang et al., 2019b, 2020). However, liver histopathology of DILI changes rapidly and significantly with time, in parallel to the changes in liver biochemical parameters. Liver histological characteristics and their dynamic changes at different time points of liver biopsy along the natural evolution of DILI have not been well established and warrant further study.

Ideally, examinations of sequential biopsies would be a reliable approach for the assessment of the microscopic evolution of DILI; however, it is not ethical to obtain the subsequent liver biopsy in a majority of resolved DILI patients. Therefore, we attempted to clarify the histopathological evolutional features of DILI at the different time points of liver biopsy with reference to the onset of DILI (<1 month, 1-3 months, and >3 months). We further correlated the histological findings with the corresponding clinical information and prognosis. We hypothesized that the histological injury patterns and degrees of severity correlate with clinical findings at different time points. Therefore, the study contributes to a better understanding of DILI liver histopathology from longitudinal and disease evolutional perspectives.

Materials and methods

Study design

This was a single-centre retrospective observational study. Hospitalized patients with suspicion of DILI from January 2009 to December 2020 were screened. The electronic medical records of these cases and liver biopsy slides were retrospectively reviewed. All of the data were analyzed. Ethical issues: This study was approved by the Institutional Ethical Review Board (2022-P2-063-01).

Inclusion criteria: (1) the liver biochemistry fulfilled one of the following criteria: ALT level ≥5× ULN or ALP level ≥2×ULN or ALT level ≥3×ULN and TB >2×ULN; (2) Roussel Uclaf Causality Assessment Method (RUCAM) score ≥6 or if the RUCAM score 3-6 (Danan and Benichou, 1993), and if two out of three hepatologists (YW, XYZ, and ZKM) agreed the case(s) were probable DILI; and (3) at least one liver biopsy was available.

Exclusion criteria: (1) viral hepatitis A, B, C, and E, and the presence of nonhepatotropic viral infections (cytomegalovirus and Epstein-Barr virus); (2) alcohol liver disease (excessive alcohol consumption >40 g/d for men and >20 g/d for women, lasting for 5 years); (3) autoimmune hepatitis (International Autoimmune Hepatitis Group [IAIHG] simplified score ≥6) (Alvarez et al., 1999); (4) toxin-induced liver injury; (5) Antimitochondrial antibody(AMA) positive with nonsuppurative destructive cholangitis (primary biliary cholangitis, PBC); and (6) nonalcoholic steatohepatitis (NASH).

Indications for liver biopsy: (1) patients who cannot be distinguished between DILI and other liver disorders according to clinical and laboratory tests; (2) patients who had persistent biochemical abnormalities (Chalasani et al., 2021); and (3) patients who required a determination of the severity of DILI.

Definition of time points from DILI onset to liver biopsy: (1) <1 month group: patients who underwent liver biopsy within 30 days of onset; (2) 1-3 months group: patients who underwent liver biopsy 30-90 days from onset; and (3) >3 months group: patients who underwent liver biopsy over 90 days from onset.

Definition of prognosis of DILI patients: (1) recovery was defined as biochemical normalization (ALT and AST for hepatocellular, ALP for mixed and cholestatic patients, and TB <1.5×ULN); (2) chronic DILI was defined as liver biochemistry abnormalities persisting for more than 6 months; and (3) end-point events were defined as patients who underwent liver transplantation or who died.

Data collection

The demographic, clinical, and laboratory data were extracted from the electronic medical record system. The patterns of injury were defined as hepatocellular (R≥5), mixed (2<R<5), and cholestatic (R≤2) according to the onset R value (ratio of serum alanine aminotransferase [ALT]/upper limit of normal [ULN] to serum alkaline phosphatase [ALP]/ULN) (Aithal et al., 2011). Case severity was graded as mild, moderate, severe, and acute liver failure (ALF) to fatal (death or received liver transplantation attributed to DILI) (Watkins et al., 2008). Follow-up information was obtained by an electronic medical tracing system, which was divided into two groups according to two types of outcomes (recovered vs. unrecovered groups or having end-point events vs. non-end-point events groups).

All of the pathological slides stained with haematoxylin-eosin (H&E), Masson's trichrome, reticulin, periodic acid-Schiff (PAS), periodic acid-Schiff diastase (PASD), Prussian blue (Iron), CK7, and CK19 for the enrolled patients were reviewed by an experienced expert of clinical liver pathology (XYZ) by using the scoring proposed by Kleiner et al. (2014). Clinical, laboratory, and follow-up data of patients were

blinded to XYZ during the slide review process.

Statistical analysis

Statistical analysis was performed by using SPSS software (version 26.0; IBM, Armonk, NY). Demographic characteristics and histopathological features were described as percentages for the categorical variables and means with standard errors (SEs) or quartiles for the continuous variables, as appropriate. Differences between groups were evaluated by using the Chi-square test for the categorical variables, the t test analysis for normally distributed variables, and the Mann-Whitney U test for non-normally distributed continuous variables.

Results

Description of the clinical characteristics of the DILI patients

From January 2009 to December 2020, 129 out of 741 clinically suspected DILI patients underwent liver biopsy and were included in this study. The process of patient selection is shown in Figure 1.

The median age was 52 years (range: 14-77 years), and 97 (75%) patients were female (Table 1). The median time from DILI onset to liver biopsy was 42 days (23, 96), the median time from hospitalization to liver biopsy was 4 days (2, 9), and the median latency was 30 days (14, 88). The most common culprit drugs were herbal products (53%), followed by a combination

Table 1. Demographic and clinical characteristics of DILI patients in the study.

Age, year, median (range)	52 (14, 77)
Female, n (%)	97 (75%)
Period of onset to biopsy, days, median	42 (23, 96)
Period of hospitalized to biopsy, days, median	4 (2, 9)
Latency, days, median	30 (14, 88)
Follow-up time, months	42 (23, 76)
Culprit drugs, n (%)	
Herbal products	69 (53%)
Biochemical drugs	24 (19%)
Combination	27 (21%)
HDS	9 (7%)
Biochemical injury pattern, n (%)	
Hepatocellular (R≥5)	91 (71%)
Mixed (2<R<5)	21 (16%)
Cholestatic (R≤2)	17 (13%)
RUCAM score, n (%)	
Highly probable (≥9)	22 (17%)
Probable (6~8)	79 (61%)
Possible (3~5)	28 (22%)
Severity, n (%)	
Mild	45 (35%)
Moderate	8 (6%)
Severe	67 (52%)
More than severe (ALF to fatal)	9 (7%)

HDS, Herbal and dietary supplement; ALF, Acute liver failure; Combination, History of taking both herbal products and biochemical drugs together.

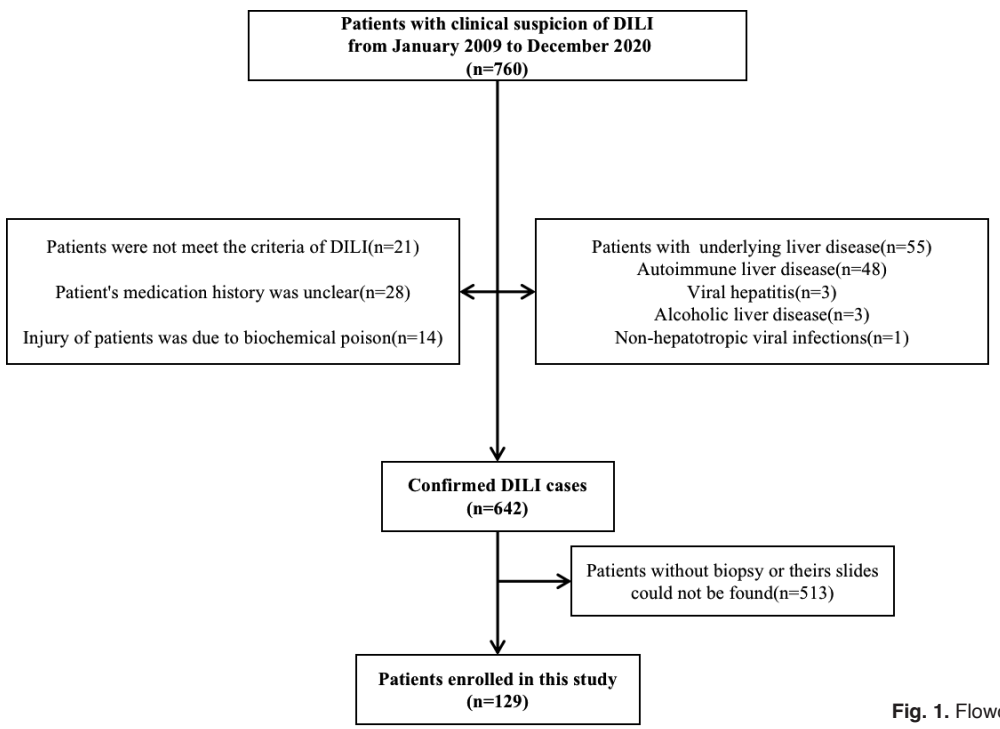

Fig. 1. Flowchart of patients included in this study.

of herbal and biochemical drugs (21%). Of these 129 patients, the main clinical injury pattern was hepatocellular (71%), and mixed and cholestatic injury patterns were 16% and 13%, respectively. Severe cases accounted for 52% (67/129). RUCAM scores ≥9 comprised 17% (28/129), scores of 6-8 comprised 61% (79/129), and scores of 3-5 comprised 22% (22/129) of the patients.

DILI patients with liver biopsy (n=129) had comparable biochemical classification, ALP, GGT, TB, DB, and prognosis parameters compared to those patients without liver biopsy (n=493), except for significantly lower ALT and AST serum levels at DILI onset.

Comparison of liver biochemical tests at DILI onset among the three groups (<1 month, 1-3 months, and >3 months)

Clinical and laboratory data based on the above

Table 2. Comparison of the onset clinical characteristics at different time points of liver biopsy.

	Overall n=129	<1 month n=46	1-3 months n=49	>3 months n=34	P value
Age, year, median	52 (14, 77)	48 (15, 73)	48 (14, 77)	49 (26, 75)	0.955
Female, n (%)	97 (75%)	37 (80%)	32 (65%)	28 (82%)	0.126
Body mass index, mean ± standard deviation	22.8±3.0	22.9±2.7	22.4±2.9	23.0±3.7	0.612
ALT (IU/L), median	455 (188, 857.5)	514.0 (285.8, 885.0)	410.0 (171.5, 657.5)	571.5 (148.5,1025.3)	0.359
AST (IU/L), median	275.0 (127.0, 658.0)	411 (144.4, 758.5)	225.0 (122.3, 583.0)	202.0 (106.5, 821.5)	0.251
ALP (IU/L), median	159.0 (100.5, 236.0)	170.1 (116.0, 239.5)	159.0 (105.5, 278.5)	121.0 (86.3, 207)	0.089
GGT (IU/L), median	169.0 (76.5, 256.5)	184.0 (106.9, 307.8)	164.0 (75.0, 252.2)	172.5 (59.5, 225.8)	0.356
TB (umol/L), median	69.1 (22.0, 130.7)	92.1 (27.5, 181.2)	70.4 (19.0, 132.1)	36.4 (17.5, 105.6)	0.086
DB (umol/L), median	40.4 (7.1, 93.2)	61.3 (7.6, 128.8)	38.1 (4.9, 96.5)	15.8 (7.0, 75.1)	0.128
INR, median	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	1.0 (1.0, 1.2)	1.1 (1.0, 1.2)	0.875
Severity score, n (%)					0.334
Mild	45 (35%)	12 (26%)	17 (35%)	16 (47%)	
Moderate	8 (6%)	1 (2%)	4 (8%)	3 (9%)	
Severe	67 (52%)	32 (70%)	25 (51%)	10 (29%)	
ALF/Fatal/Liver transplantation	9 (7%)	1 (2%)	3 (6%)	5 (15%)	
RUCAM score, n (%)					0.566
Possible (3~5)	28 (22%)	9 (20%)	11 (22%)	8 (24%)	
Probable (6~8)	79 (61%)	29 (63%)	27 (55%)	23 (68%)	
Highly probable (≥9)	22 (17%)	8 (17%)	11 (22%)	3 (9%)	
Recovery, n (%)					<0.001
Yes	103 (80%)	42 (91%)	43 (88%)	18 (53%)	
No	26 (20%)	4 (9%)	6 (12%)	16 (47%)	
End-point events, n (%)					0.015
Yes	10 (8%)	0 (0)	4 (8%)	6 (18%)	
No	119 (92%)	46 (100%)	45 (92%)	28 (82%)	

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, Glutamyl transpeptidase; TB, Total bilirubin; DB, Direct bilirubin; INR, International normalized ratio.

Table 3. Comparison of clinical characteristics before liver biopsy at different time points of liver biopsy.

	Overall n=129	<1 month n=46	1-3 months n=49	>3 months n=34	P value
Age, year, median	52 (14, 77)	48 (15, 73)	48 (14, 77)	49 (26, 75)	0.955
Female, n (%)	97 (75%)	37 (80%)	32 (65%)	28 (82%)	0.126
ALT (IU/L), median	177.0 (85.5, 312.5)	231.5 (136.5, 439.0)	181.0 (83.5, 286.0)	132.5 (69.3, 248.0)	0.011
AST (IU/L), median	119.5 (68.6, 268.9)	178.5 (75.4, 315.3)	118.0 (71.5, 247.5)	86.5 (58.6, 218.3)	0.111
ALP (IU/L), median	140 (97.5, 189.5)	158.0 (116.5, 237.8)	145.0 (90.5, 184,0)	111.5 (77.8, 148.5)	0.002
GGT (IU/L), median	122.0 (69.0, 215.4)	157.0 (82.0, 254.8)	123.0 (71.0, 206.9)	96.0 (49.0, 200.8)	0.093
TB (umol/L), median	40.1 (17.3, 180.5)	103.8 (26.2, 236.1)	42.1 (16.4, 182.7)	19.2 (13.3, 38.8)	0.001
DB (umol/L), median	22.8 (4.9, 109.7)	58.0 (10.3, 113.8)	22.8 (4.6, 113.6)	5.7 (3.5, 21.8)	0.001
ALB (g/L), median	35.8 (33.2, 38.7)	36.2 (32.4, 39.7)	34.5 (32.0, 38.6)	36.8 (33.9, 38.8)	0.263
INR, median	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	1.1 (1.0, 1.1)	1.0 (1.0, 1.2)	0.757

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, Glutamyl transpeptidase; TB, Total bilirubin; DB, Direct bilirubin; ALB, Albumin; INR, International normalized ratio.

groups are shown in Table 2. There were 34 cases in the >3 months group, of whom the indication for biopsy was in 23 (67.6%) cases for differentials with AIH, 5 (14.7%) cases for chronicity, 3 (8.8%) cases for liver biochemistry abnormalities persisting beyond 180 days, and 3 (8.8%) cases for aggravation of liver disease (Chalasami et al., 2021). There were no significant differences in age, sex, or BMI among the three groups. The onset serum levels of ALT, AST, ALP, GGT, TB, and DB, as well as the severity score, were not significantly different among the three groups. However, serum ALT (p=0.011), ALP (p=0.002), TB (p<0.001), and DB (p<0.001) levels were significantly decreased with time among the three groups (Table 3). The RUCAM score remained similar among the three groups. In addition, the degree of injury (severe cases, 70%) tended to be higher in patients who underwent biopsy <1 month from onset. Those patients undergoing liver biopsy >3 months tended to have more ALF/fatal/liver transplantations events (15%). With the extension of time points from liver biopsy, the proportion of unrecovered cases significantly increased in a stepwise manner (p<0.001). None of the patients in the <1 month group had end-point events, and 8% of the patients in the 1-3 months group and 18% of patients in the >3 months group died or underwent LT (p=0.015).

Comparison of histological findings among the three groups (<1 month, 1-3 months, and >3 months)

The distribution of histological findings is shown in Table 4. Overall, The most common histological injury pattern was acute hepatitic (61/129, 47%), followed by cholestatic-hepatitic (46/129, 36%, Fig. 2). Spotty (100/129, 77%) and confluent necrosis (94/129, 72%) were commonly observed (Fig. 3), which were mainly located in zone 3 (71%). Portal (65/129, 50%) and periportal (84/129, 65%) inflammatory activities were mild or mild to moderate in the majority of cases. More than half of the cases had sinusoidal lymphocytic infiltration (73%) and portal tract eosinophilic infiltration (62%). Additionally, approximately half of the cases had some degree of cholestasis, including hepatocellular (48%), canalicular (44%), and Kupffer cell cholestasis (40%).

The major injury patterns were significantly different among the three groups (p=0.003) (Fig. 4). The prominent injury patterns were acute hepatitic and cholestatic-hepatitic. Acute cholestatic injury was higher in <1 month group compared to the other two groups. Chronic cholestatic injury trended up from <1 month group to >3 months group. However, the degrees of portal inflammation, necrosis, infiltrated inflammatory

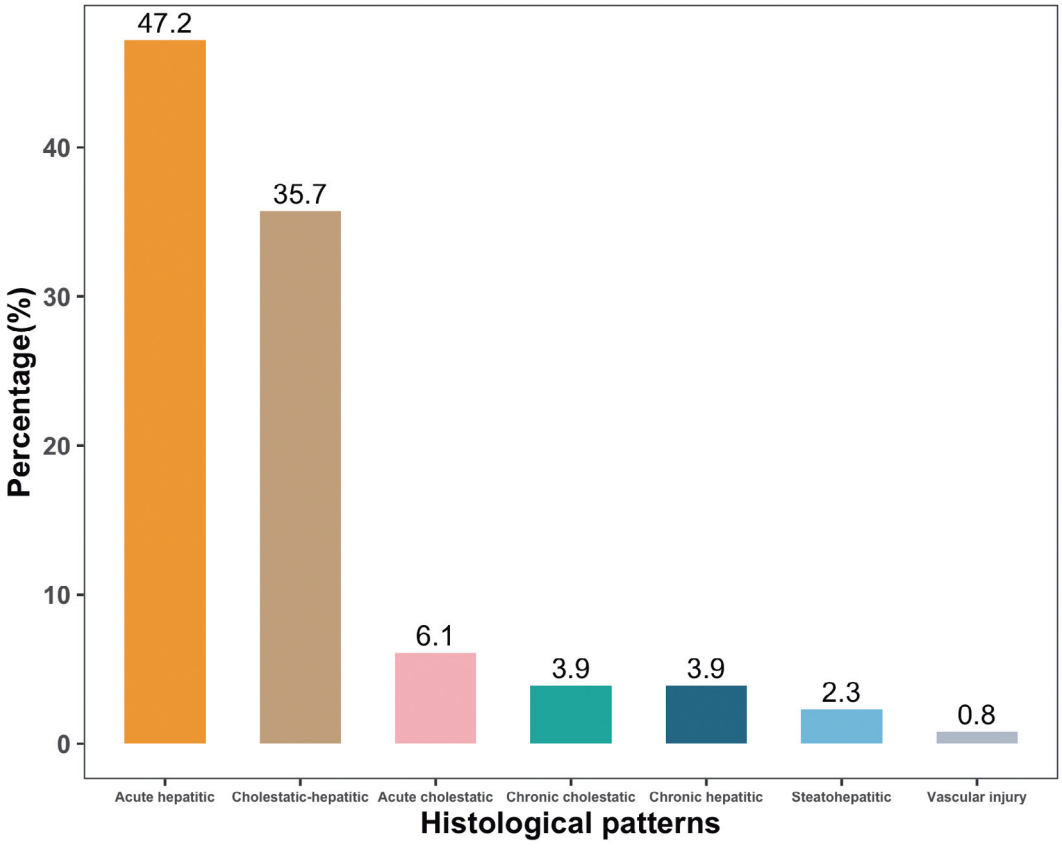

Fig. 2. Distribution of liver histopathological injury patterns of patients in this study.

Table 4. Comparison of histological findings at different time points of liver biopsy.

Feature	Overall n=129	<1 month n=46	1-3 months n=49	>3 months n=34	P value
Necrosis					
Spotty necrosis, median	2 (1,2)	1 (1,2)	2 (1,2)	2 (1,2.25)	0.865
Confluent necrosis, median	2 (0,4)	2.5 (0,4)	3 (0,3)	1.5 (0,4)	0.943
Bridging/multiacinar necrosis, median	0 (0,1)	0 (1,2)	0 (0,0)	0 (0,1)	0.591
Apoptosis, n (%)					0.249
None	27 (21%)	10 (22%)	8 (16%)	9 (26%)	
1 per 40x hpf	79 (61%)	32 (70%)	29 (59%)	18 (53%)	
≥2 per 40x hpf	23 (18%)	4 (9%)	12 (25%)	7 (21%)	
Degree of necrosis, median	2 (1,2)	2 (1,2)	2 (1,2)	1.5 (1,2)	0.826
Location of necrosis, n (%)					0.552
None	4 (3%)	3 (6%)	0 (0)	1 (3%)	
Zone 1	0 (0)	0 (0)	0 (0)	0 (0)	
Zone 3	92 (71%)	33 (72%)	36 (74%)	23 (68%)	
Panacinar	1 (1%)	1 (2%)	0 (0)	0 (0)	
Azonal	32 (25%)	9 (20%)	13 (27%)	10 (29%)	
Inflammation					
Portal inflammation, median	1 (1,2)	1 (1,2)	1 (0,1)	1 (1,2)	0.686
Periportal inflammation, median	1 (0.5,2)	1 (0.75,2)	2 (1,2)	1 (1,2)	0.942
Inflammatory cell infiltration					
Sinusoidal lymphocytic infiltration, n (%)					0.828
None	34 (26%)	11 (24%)	13 (27%)	10 (29%)	
Scattered	74 (57%)	31 (67%)	26 (53%)	17 (50%)	
Diffused	21 (16%)	4 (9%)	10 (20%)	7 (21%)	
Eosinophils infiltration, n (%)					0.65
None	48 (37%)	19 (41%)	16 (33%)	13 (38%)	
<5/each small portal area	56 (43%)	18 (39%)	22 (45%)	16 (47%)	
>5/each small portal area	25 (19%)	9 (20%)	11 (22%)	5 (15%)	
Cholestasis					
Grade of cholestasis, median	0 (0,2)	1 (0,2)	1 (0,1)	0 (0,0.25)	0.004[a,b]
Canalicular cholestasis, median	0 (0,1.5)	1 (0,2)	0 (0,1)	0 (0,0.25)	0.018[a,b]
Hepatocellular cholestasis, median	0 (0,1)	1 (0,2)	1 (0,1)	0 (0,0.25)	0.006[a,b]
Kupffer cells cholestasis, n (%)					0.033[a]
None	77 (60%)	22 (48%)	29 (60%)	26 (76%)	
Scattered	31 (24%)	13 (28%)	13 (26%)	5 (15%)	
Numerous	21 (16%)	11 (24%)	7 (14%)	3 (9%)	
Fibrosis					
Fibrosis, median	0 (0,2)	0 (0,1)	0 (0,1)	0 (0,2)	0.559
Sinusoidal fibrosis, median	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0.616
Steatosis					
Grade of steatosis, median	0 (0,1)	0 (0,0.25)	0 (0,1)	0 (0,1)	0.546
Other Findings					
PAS- (+) macrophages, n (%)					0.096
None	18 (14%)	5 (11%)	5 (10%)	8 (24%)	
Scattered	70 (54%)	23 (50%)	28 (57%)	19 (56%)	
Clusters	41 (32%)	18 (39%)	16 (33%)	7 (21%)	
Acidophil bodies, n (%)					0.951
None	51 (40%)	16 (35%)	20 (41%)	15 (44%)	
≤3/20x	72 (56%)	30 (65%)	26 (53%)	16 (47%)	
>3/20x	6 (5%)	0 (0)	3 (6%)	3 (9%)	
Sinusoidal dilation, n (%)					0.678
Absent/Mild	106 (82%)	36 (78%)	41 (84%)	29 (85%)	
Moderate to marked	23 (18%)	10 (22%)	8 (16%)	5 (15%)	
Ductopenia, n (%)					0.892
None	120 (93%)	43 (94%)	45 (92%)	32 (94%)	
≤50%	5 (4%)	3 (6%)	1 (2%)	1 (3%)	
>50%	4 (3%)	0 (0)	3 (6%)	1 (3%)	
Foam-like cells, n (%)					0.646
None	117 (91%)	41 (89%)	46 (94%)	30 (88%)	
Few	11 (9%)	5 (11%)	2 (4%)	4 (12%)	
Cluster	1 (1%)	0 (0)	1 (2%)	0 (0)	
Lipid-laden stellate cells, n (%)					
Not predominant	121 (94%)	44 (96%)	47 (96%)	30 (88%)	0.295
Predominant	8 (6%)	2 (4%)	2 (4%)	4 (12%)	

[a], >3 months group vs. <1 month (P<0.05); [b], >3 months vs. 1-3 months (P<0.05); Hpf, High power filed.

Table 5. Comparison of characteristics of culprit drugs at different time points of liver biopsy.

Herbal	Overall n=67	<1 month n=27	1-3 months n=22	>3 months n=18	P value
Bridging/Multiacinar necrosis, n (%)					0.024
None	47 (70%)	15 (56%)	20 (91%)	12 (67%)	
Bridging	19 (28%)	11 (41%)	2 (9%)	6 (33%)	
Multiacinar	1 (1%)	1 (4%)	0	0	
Grade of cholestatic, median	0 (0, 2)	1 (0, 2)	0 (0, 1)	0 (0, 0)	0.032
Hepatocytic regeneration, n (%)					0.046
Absent	2 (3%)	0	1 (5%)	1 (6%)	
Focal	50 (75%)	20 (74%)	20 (91%)	10 (56%)	
Diffused	15 (22%)	7 (26%)	1 (5%)	7 (39%)	
Combination (herbal+chemical Drugs)	Overall n=29	<1 month n=10	1-3 months n=12	>3 months n=7	P value
Bridging/Multiacinar necrosis, n (%)					0.033
None	21 (72%)	10 (100%)	8 (67%)	3 (43%)	
Bridging	8 (28%)	0	4 (33%)	4 (57%)	
Multiacinar	0	0	0	0	
Lobular disarray, n (%)					0.029
Absent	22 (76%)	10 (100%)	9 (75%)	3 (43%)	
Present	7 (24%)	0	3 (25%)	4 (57%)	

Combination: History of taking both herbal products and biochemical drugs together.

Table 6. Comparison of prognosis among three groups at different time points of liver biopsy.

	Overall n=129	Recovery		P value	End-point events		P value
		Yes n=103	No n=26		Yes n=10	No n=119	
Histological injury patterns, n%				0.006			0.008
Acute hepatitic	61 (47%)	51 (50%)	10 (38%)		2 (20%)	59 (50%)	
Chronic hepatitic	5 (4%)	2 (2%)	3 (12%)		1 (10%)	4 (3%)	
Acute cholestatic	8 (6%)	7 (7%)	1 (4%)		0 (0)	8 (7%)	
Chronic cholestatic	5 (4%)	1 (1%)	4 (15%)		3 (30%)	2 (2%)	
Cholestatic-Hepatitic	46 (36%)	39 (38%)	7 (27%)		4 (40%)	42 (35%)	
Sinusoidal lymphocytic infiltration, n%				0.028			0.128
Absent	34 (26%)	30 (29%)	4 (15%)		1 (10%)	33 (28%)	
Scattered	74 (57%)	60 (58%)	14 (54%)		6 (60%)	68 (57%)	
Diffused	21 (16%)	13 (13%)	8 (31%)		3 (30%)	18 (15%)	
Eosinophil cell infiltration, n%				0.007			0.168
None	48 (37%)	34 (33%)	14 (54%)		5 (50%)	43 (36%)	
<5/each small portal area	56 (43%)	44 (43%)	12 (46%)		5 (50%)	51 (43%)	
>5/each small portal area	25 (19%)	25 (24%)	0 (0)		0 (0)	25 (21%)	
Grade of Cholestasis, median	0 (0,2)	0 (0,2)	0.5 (0,2)	0.701	2 (0.75,2.25)	0 (0,2)	0.027
Canalicular cholestasis, median	0 (0,1)	0 (0,1)	0 (0,2)	0.571	1.5 (0.75,2.25)	0 (0,1)	0.015
Ductopenia, n%				0.051			0.002
None	120 (93%)	98 (95%)	22 (85%)		7 (70%)	113 (95%)	
<50% of each small portal area	5 (4%)	4 (4%)	1 (4%)		0 (0)	5 (4%)	
>50% of each small portal area	4 (3%)	1 (1%)	3 (11%)		3 (30%)	1 (1%)	
Acute Cholangitis, n%				0.364			0.150
Not Present	127 (98%)	102 (99%)	25 (96%)		9 (90%)	118 (99%)	
Present	2 (2%)	1 (1%)	1 (4%)		1 (10%)	1 (1%)	
Cholate stasis, n%				0.005			<0.001
None	127 (98%)	103 (100%)	24 (92%)		8 (80%)	119 (100%)	
Minimal	1 (1%)	0 (0)	1 (4%)		1 (10%)	0 (0)	
Majority	1 (1%)	0 (0)	1 (4%)		1 (10%)	0 (0)	
Foam-like cells, n%				0.047			0.016
None	117 (91%)	96 (93%)	21 (81%)		7 (70%)	110 (92%)	
Minimal	11 (8%)	7 (7%)	4 (15%)		2 (20%)	9 (8%)	
Cluster	1 (1%)	0 (0)	1 (4%)		1 (10%)	0 (0)	
Lipid-laden stellate cells, n%				0.086			0.016
Not predominant	121 (94%)	99 (96%)	22 (85%)		7 (70%)	114 (96%)	
Predominant	8 (6%)	4 (4%)	4 (15%)		3 (30%)	5 (4%)	

cell types, fibrosis, and steatosis did not show any significant differences among the three groups. Similarly, other findings, such as acidophil bodies, sinusoidal dilation, ductopenia, and foam-like cells, did not show any significant differences. However, the degrees of overall cholestasis was significantly lower in the >3 months group than in the <1 month group (p=0.003 for cholestasis, p=0.009 for canalicular, p=0.017 for Kupffer cell cholestasis) or the 1-3 months group (p=0.018 for cholestasis, p=0.046 for canalicular, p=0.173 for Kupffer cell cholestasis) (Fig. 5). These histological findings of cholestatic changes were consistent with their liver biochemical changes.

Comparison of culprit drugs among the three groups (<1 month, 1-3 months, and >3 months)

In general, there were no significant differences in the classification of culprit drugs among the three groups. For patients taking herbs, bridging necrosis (p=0.024) and cholestasis (p=0.032) were significantly more frequent at <1 month than at 1-3 months and >3 months. For patients taking combined herbal and drugs, bridging necrosis and lobular disarray were significantly more frequent at 1-3 months (p=0.033) and >3 months

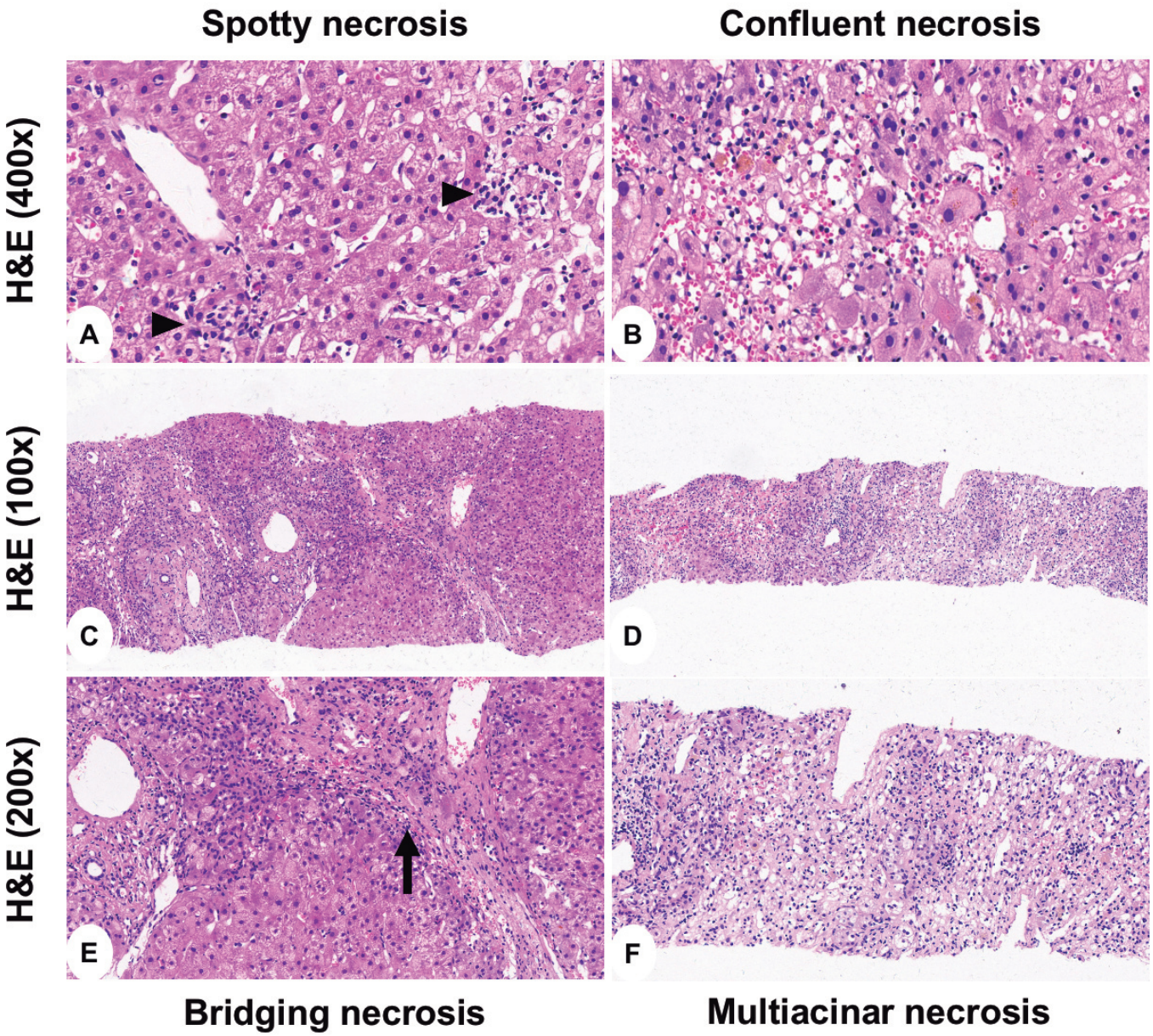

Fig. 3. Examples of histopathological features of different degrees of necrosis in patients. **A.** Spotty necrosis (arrowhead). **B.** Confluent necrosis. **C, E.** Bridging necrosis (arrow). **D, F.** Multiacinar necrosis. H&E. A, B, x 400; C, D, x 100; E, F, x 200.

(p=0.029) than at <1 month (Table 5).

Comparison of histopathological features between patients with different outcomes

The median follow-up time was 42 (23, 76) months. Features associated with the prognosis of DILI patients are shown in Table 6. The major injury patterns were significantly different between the recovered and unrecovered groups (p<0.001). The majority of acute hepatic and cholestatic-hepatitic injury patterns were identified in the recovery group, whereas chronic hepatic

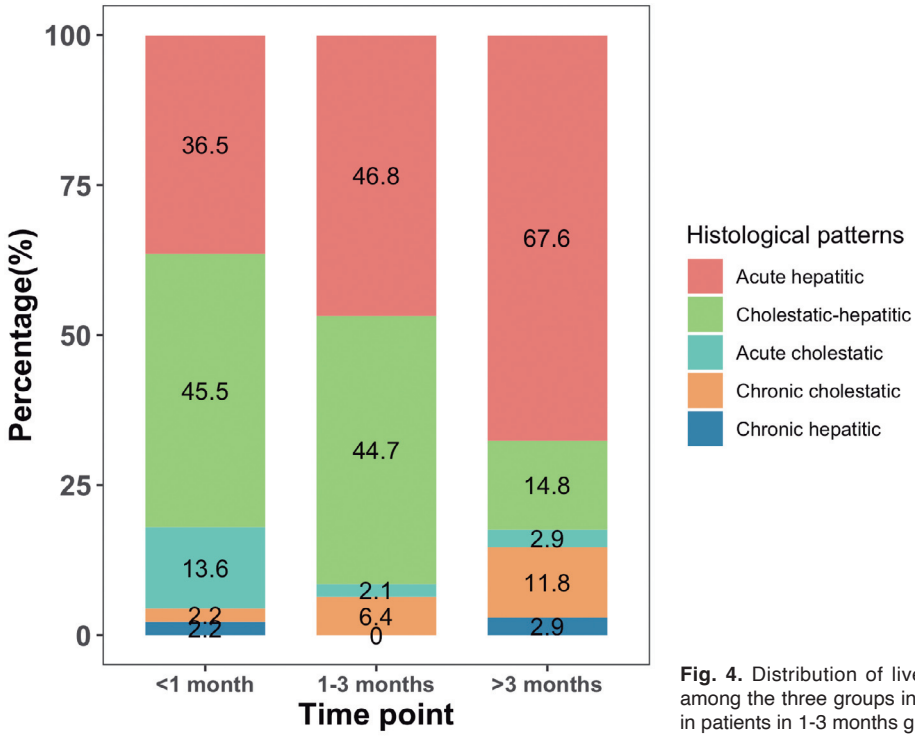

Fig. 4. Distribution of liver histopathological injury patterns of patients among the three groups in this study ("0": No chronic hepatic injury pattern in patients in 1-3 months group.).

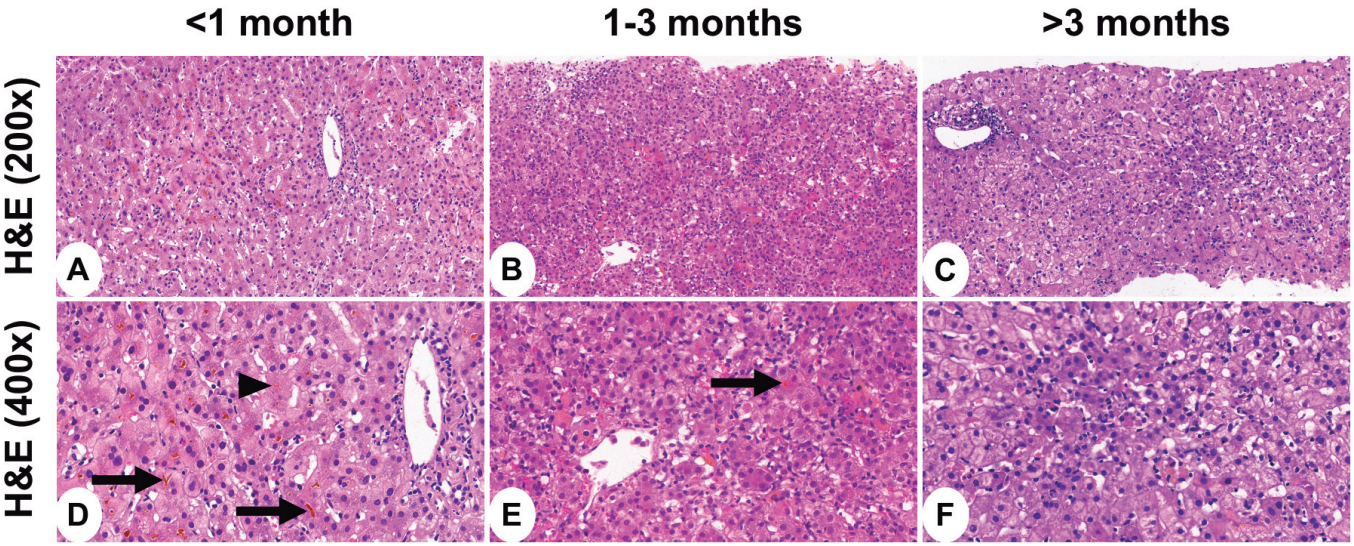

Fig. 5. Examples of histopathology of different degrees of cholestasis in patients at different time points of liver biopsy. **A, D.** In <1 month group, more severe cholestasis, including canalicular cholestasis (arrow), hepatocellular cholestasis (arrowhead) and Kupffer cells cholestasis. **B, E.** In 1-3 months group, mild to moderate cholestasis (arrow for canalicular cholestasis). **C, F.** In >3 months group, no cholestasis. H&E. A-C, x 200; D-F, x 400.

and chronic cholestatic injuries were significantly more frequent in the unrecovered group. When regarding the individual pathological characteristics, diffusely sinusoidal lymphocytic infiltration (p=0.028), cholate stasis (p=0.005), and foam cells (p=0.047) were significantly more frequent in the unrecovered group, whereas eosinophilic infiltration in the portal tract was significantly more frequent in the recovered group than in the unrecovered group (p=0.007), although these features were equally distributed in the three groups.

The major histopathological injury patterns were significantly different between the end-point event group and the non-end-point event group (p=0.015). Overall cholestasis (p=0.027), canalicular cholestasis (p=0.015), ductopenia (p=0.002) , cholate stasis (p<0.001), foam-like cells (p=0.016), and lipid-laden stellate cells

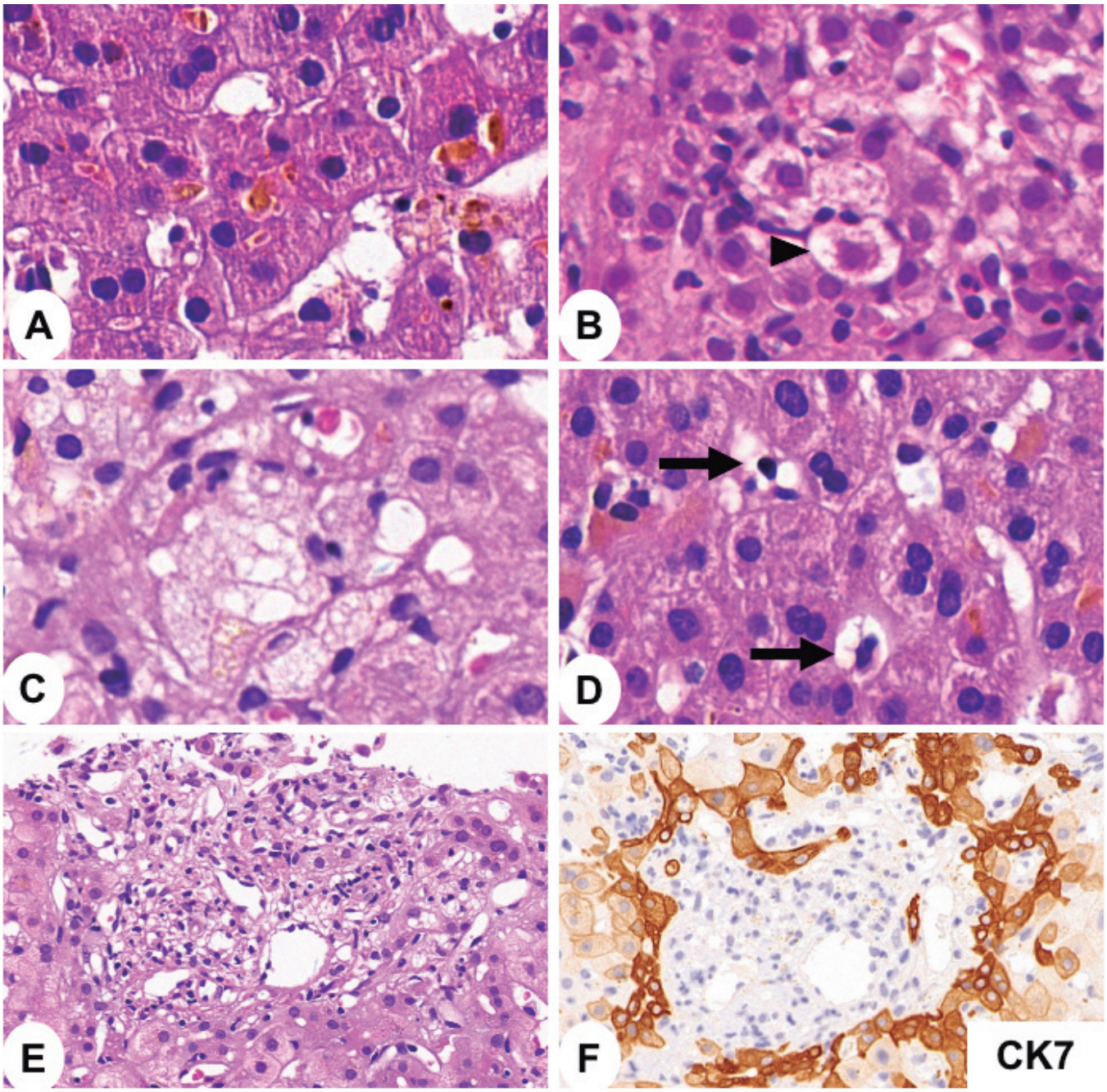

Fig. 6. Examples of histopathology of liver in this study. **A-D.** Cholestasis **(A)**, cholate stasis (**B**, arrowhead), foam-like cells **(C)** and lipid-laden stellate cells (**D**, arrow), respectively. **E, F.** Bile duct loss. CK7: cell keratin 7. H&E. A-D, x 400; E, F, x 200.

(p=0.016) (Figure 6) were more significant in the end-point event group than in the non-end-point event group, although these features were equally distributed among the <1 month, 1-3 months and >3 months groups.

Discussion

Liver biopsy can provide valuable information on injury patterns and severity in an intuitive way, which constitutes the basis of clinical manifestations and laboratory alterations (Kleiner, 2018). To some extent, liver pathology can help in the differential diagnosis of DILI. In this study, we found that acute and chronic hepatic patterns, acute and chronic cholestatic patterns, and cholestatic-hepatitic patterns were the major histological injury patterns, which is similar to previous reports from either China or the USA (Kleiner et al., 2014; Tian et al., 2019). Moreover, the histological injury patterns changed significantly regarding the time of biopsy; specifically, an earlier DILI onset corresponded to more acute injury patterns (acute hepatic, cholestatic, and cholestatic-hepatitic), and later DILI onset corresponded to more chronic injury patterns (chronic hepatic and cholestatic). Interestingly, we found that acute cholestatic histological changes, such as hepatocellular, canalicular, and Kupffer cell cholestasis, significantly subsided with time. In fact, these histological changes were in accordance with the changes in serum bilirubin levels.

In this study, we retrieved liver biochemical parameters both at DILI onset and just before the time of liver biopsy. Interestingly, we found that there were no significant differences in any liver biochemical indices at DILI onset when referenced to the time of liver biopsy. However, some biochemical parameters (ALT, ALP, and TB/DB) significantly decreased before liver biopsy. In addition, serum bilirubin levels decreased more rapidly than ALP levels, whereas the decrease in the ALT level was comparable among the three groups. Therefore, it is worth referencing the biochemical parameters both at disease onset and before liver biopsy, with the latter providing a more precise relationship to the findings of liver biopsy.

In this study, we also found that some key histological features, such as sinusoidal lymphocytic infiltration, cholate stasis, foam-like cells, lipid-laden stellate cells, and ductopenia, were equally distributed among the three groups without significant differences. However, these features are important prognostic indicators, which have previously been described to be associated with either chronicity or end-point events, such as liver-related death or LT (Björnsson et al., 2017). These results were similar to the findings of DILIN and to the results of our previous study (Tian et al., 2019). Moreover, portal tract eosinophilic infiltration was equally identified in the three groups, but its presence was associated with an earlier DILI recovery. This finding was in accordance with the study by Björnsson et al. (2007).

For liver injury induced either by herbal or a combination of herbal and drug treatments, there were significantly more frequencies of bridging/multiacinar necrosis, whereas lobular disarray was more frequent in the group of combined herbal and drug insults. This finding was in accordance with the reports by Spanish DILI Registry (Medina-Caliz et al., 2018), thus suggesting that herbal-induced liver injury was associated with a higher degree of liver necrosis, warranting awareness by liver pathologists and hepatologists.

The strength of this study was that we used a well-accepted scaling system to evaluate histological findings (Kleiner et al., 2014). All of the pathological slides were carefully reviewed by an experienced clinical liver pathologist who was blinded to the patients' information. The pathological findings were carefully and thoroughly correlated with liver biochemical markers at different time points and long-term prognosis. However, there were some limitations of this study. First, this was a single-centre retrospective study with a relatively small number of patients, which may result in a selection bias. Therefore, our results need to be validated by other cohorts or hepatologists. Second, patients with liver biopsy seem to have milder severity (lower ALT and AST levels at onset) than patients without liver biopsy. However, there were no significant differences in peak ALT/AST levels, severity scores, or outcomes between patients with or without liver biopsy. Third, all of the patients who were included in the study only had a single liver biopsy at different time points, which may result in unavoidable sample errors.

In conclusion, the time points from onset to liver biopsy significantly affect the histopathological findings; specifically, the earlier that a liver biopsy is taken, the more acute cholestatic hepatic injury patterns that are observed, with later performances of biopsy corresponding to more chronic pathological injury patterns. Some key histological features, such as ductopenia, cholate stasis, and foam-like cells, can occur equally at different time points after DILI onset but have strong prognostic values. Therefore, the early recognition of these prognostic factors through liver histopathological examinations plays a critical role in DILI management.

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