Journal of Clinical and Translational Hepatology **2023**
DOI: 10.14218/JCTH.2022.00039

Original Article

Histopathological Features Predicting Long-term Clinical Outcomes in Patients with Vanishing Bile Duct Syndrome

Tingting Lv[1], Haitian Yu[1], Xiao Han[1], Aileen Wee[2], Jimin Liu[3], Min Li[4], Jinghang Xu[5], Xiaoli Hu[6], Jia Li[7], Weijia Duan[1], Tailing Wang[8], Jidong Jia[1#*] and Xinyan Zhao[1#*]

[1]Liver Research Center, Beijing Friendship Hospital, Capital Medical University; National Clinical Research Center for Digestive Diseases, Beijing, China; [2]Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, National University Hospital, Singapore; [3]Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada; [4]Clinical Epidemiology and Evidence-Based Medicine Unit, National Clinical Research Center for Digestive Diseases, Beijing Friendship Hospital, Capital Medical University, Beijing, China; [5]Department of infectious disease, Peking University First Hospital, Beijing, China; [6]Department of infectious disease, Heilongjiang Province Hospital, Heilongjiang, China; [7]Department of Hepatology, Tianjin Second People's Hospital, Tianjin, China; [8]Department of Pathology, China-Japan Friendship Hospital, Beijing, China

Received: 27 September 2022 | **Revised:** 19 December 2022 | **Accepted:** 1 February 2023 | **Published online:** 4 April 2023

Abstract

Background and Aims: The clinicopathological features and long-term outcomes of patients with vanishing bile duct syndrome (VBDS) have yet to be elucidated. The study aims to investigate these features and identify factors associated with poor prognosis. ***Methods:*** This multicenter retrospective study recruited patients with liver biopsy-proven VBDS who were followed up at five hospitals in northern China from January 2003 to April 2022. Clinical and pathological data at time of biopsy were reviewed. Clinical outcomes including cirrhosis, decompensation events, liver transplantation (LT), and liver-related death were recorded. Cox regression analysis was used to identify the risk factors associated with poor outcomes. ***Results:*** A total of 183 patients were included. The median age was 47 years, with 77.6% being women. During a median follow-up of 4.8 years, 88 patients developed compensated or decompensated cirrhosis, 27 died, and 15 received LT. Multivariate Cox regression analysis showed that hepatocellular cholestasis (HR 2.953, 95% CI: 1.437–6.069), foam cells (HR 2.349, 95% CI: 1.092–5.053), and advanced fibrosis (HR 2.524, 95% CI: 1.313–4.851) were independent predictors of LT or liver-related deaths. A nomogram formulated with the above factors showed good consistency with a concordance index of 0.746 (95% CI: 0.706–0.785). ***Conclusions:*** Nearly half of VBDS patients studied progressed to end-stage liver disease and 23% of them had LT or liver-related death within two years of diagnosis. Hepatocellular cholestasis, foam cells and advanced fibrosis rather than the degree of bile duct loss or underlying etiologies were independently associated with poor prognosis in VBDS patients.

Citation of this article: Lv T, Yu H, Han X, Wee A, Liu J, Li M, *et al*. Histopathological Features Predicting Long-term Clinical Outcomes in Patients with Vanishing Bile Duct Syndrome. J Clin Transl Hepatol 2023. doi: 10.14218/JCTH.2022.00039.

Keywords: Bile duct loss; Prognosis; Cholestasis; Foam cells; Advanced fibrosis.

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBIL, direct bilirubin; DILI, drug-induced liver injury; GGT, gamma-glutamyl transpeptidase; IBIL, indirect bilirubin; LT, liver transplantation; PBC, primary biliary cholangitis; TBIL, total bilirubin.

[#]Contributed equally to this work.

[*]**Correspondence to:** Xinyan Zhao and Jidong Jia, Liver Research Center, Beijing Friendship Hospital, Capital Medical University, No. 95 Yong-an Road, Beijing 100050, China. ORCID: https://orcid.org/0000-0002-8016-4368 (XZ) and https://orcid.org/0000-0002-4673-8890 (JJ). Tel: +86-10-63139816, Fax: +86-10-63139246, E-mail: zhao_xinyan@ccmu.edu.cn (XZ) and jia_jd@ccmu.edu.cn (JJ)

Introduction

Vanishing bile duct syndrome (VBDS), also known as ductopenia, is a pathological diagnosis defined by loss of interlobular bile ducts in 50% or more portal tracts in a liver biopsy containing at least 11 portal tracts.[1] It can be caused by many cholestatic disorders including autoimmune, drug/toxin, genetic, infection, or ischemia.[2] Primary biliary cholangitis (PBC) and primary sclerosing cholangitis are two well recognized disorders which can eventually develop VBDS in their later stages.[3,4] Limited studies, including case reports or small case series of neoplastic diseases (particularly lymphoma) or drug-induced liver injury (DILI) has also associated these etiologies with VBDS.[1,5,6] In 2017, the DILI Network published a 10-year prospective study on drug or supplement induced bile duct loss and at least 12 cases of VBDS were identified in the 363 patients studied.[7] However, the study was only based on DILI data, which would not include many other potential VBDS etiologies. Therefore, insufficient information exists on VBDS and its associated histology, clinical course, and long-term prognosis.

The degree of bile duct paucity has been shown to be a good predictor for poor prognosis in patients with either DILI or PBC.[7–9] VBDS, as a higher extent of bile duct loss (≥50%), has been associated with advanced disease and

poor prognosis.[10] However, VBDS has also been observed in early stages of the disease, and disease severity and clinical prognosis can be variable.[11] Some patients regain normal liver function, but others may experience persistent, severe, with unremitting jaundice and high levels of cholestatic enzymes, finally leading to liver transplantation (LT) or death.

Risks factors associated with VBDS prognosis remain unclear, but are in part determined by the balance of bile duct destruction and regeneration.[12] Considering the heterogeneity of VBDS, the specific precipitating cause may influence a patient's overall outcome. In the present study, the hypothesis was that VBDS etiology and/or baseline histological features could help predict long-term outcomes. Therefore, this retrospective study investigated various etiologies, clinical and histological features, and long-term outcomes in patients with VBDS and identified several potential risk factors for predicting poor prognosis.

Methods

Study population

In this multicenter retrospective cohort study, medical records and liver biopsy slides were reviewed for patients with VBDS who underwent biopsy either at Beijing Friendship Hospital, China-Japan Friendship Hospital, Peking University First Hospital, Heilongjiang Province Hospital or Tianjin Second People's Hospital between January 2003 and April 2022. The study was conducted in accordance with the Declaration of Helsinki and all research was approved by the ethical standards for clinical studies by the ethics committee. All patients gave verbal consent to participate in the study.

All included patients were diagnosed based on histological evidence that more than 50% of portal tracts lacked a bile duct in liver biopsies with 11 or more portal tracts.[2,13] Partial portal areas were not counted. Patients were excluded if they had ever experienced chronic rejection or graft versus host reaction after LT or bone marrow transplantation, or if they were lost to follow-up.

Baseline data collection

Clinical characteristics at the time of liver biopsy, including age, sex, biochemical indices [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL)], treatment strategies and etiology of VBDS were collected. The starting date was defined as the date of VBDS diagnosis. The duration of follow-up was defined as the interval between the date of diagnosis and the date of either the last visit, or LT, or death.

Enrolled patients were divided into three groups by their etiology, DILI-VBDS, PBC-VBDS, or mixed liver-related diseases (miscellaneous-VBDS). PBC was diagnosed when patients met at two or more of the following criteria: (1) Biochemical evidence of cholestasis, such as the elevation of ALP and GGT; (2) Presence of antimitochondrial antibodies or PBC-specific antinuclear antibodies, like anti-sp100 or anti-gp210; (3) Histologic evidence of nonsuppurative destructive cholangitis.[14] DILI was diagnosed when (1) ALT level ≥5 × the upper limit of normal (ULN) or ALP level ≥2 × ULN or ALT level ≥3 × ULN and TB >2 × ULN; (2) Roussel Uclaf Causality Assessment Method score ≥3. A final diagnosis of DILI was confirmed by three hepatologists (XYZ, TTL, and HTY).

Histological assessment of liver biopsy

Formalin-fixed, paraffin-embedded liver samples were routinely sectioned and stained with hematoxylin and eosin, Masson trichrome, reticulin, and cytokeratin 7, and 19. They received the same structured histological evaluation by a pathologist (TLW) and a clinical hepatologist and liver pathologist (XYZ), who were blinded to all clinical information. Discrepancies in interpretation were discussed until consensus was achieved. Histological features included interlobular bile duct injury or loss, cholestasis, cholate stasis, foam cells, portal inflammation, interface hepatitis, and stage of fibrosis. All these features were scored according to the published standard descriptions for DILI[15,16] and PBC[17,18] (Supplementary Table 1).

Information on clinical outcomes

Follow-up data and clinical outcomes (decompensated cirrhosis, LT, or death) were obtained through interviews and review of electronic medical records. Cirrhosis was diagnosed histologically or clinically based on modified diagnostic criteria proposed by the Chinese Society of Hepatology.[19] Decompensated cirrhosis was defined as the occurrence of either ascites, variceal bleeding, or hepatic encephalopathy.[19]

Statistical analysis

Descriptive statistics were used to detail the baseline characteristics. Continuous data were presented as median and interquartile range. The Mann-Whitney *U* test was used to compare age, liver biochemistries, follow-up time, and the period from onset to biopsy. The chi-square test was used to compare the categorical variables of etiology, sex, and pathological features. A multivariate Cox proportional hazards model was used to identify prognostic factors associated with long-term survival. Forward stepwise procedures were applied for the final model selection. Additionally, nomograms were developed based on the optimized Cox regression model to facilitate point-of-care risk assessment and calculate the predicted survival probability.[20,21] The statistical analysis was performed with SPSS version 20 (IBM Corp., Chicago, IL, USA) and R software version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org/). For all analyses, *p*-values <0.05 were considered significant.

Results

Demographic characteristics of VBDS patients

Over a 20 year period, 242 subjects with biopsy-proven VBDS from routine consultant cases (to minimize selection bias) were retrospectively reviewed. A total of 59 patients were excluded from the final analysis, three due to chronic allograft rejection after LT and 56 due to loss of follow-up (Fig. 1). A final total of 183 patients were included, the demographics and laboratory data of whom are shown in Table 1. Eighty-two patients were from China-Japan Friendship Hospital, 68 from Beijing Friendship Hospital, 22 from Peking University First Hospital, seven from Tianjin Second People's Hospital, and four from Heilongjiang Province Hospital. Supplementary Table 2 shows that similar clinical characteristics were observed among the included and excluded VBDS patients.

The median age at diagnosis was 47 (38, 55) years of age, 142 (77.6%) patients were women, 167 had been treated with ursodeoxycholic acid, and usage proportion was similar in patients with or without death/LT. The median time from onset to biopsy was one year and no significant difference was observed in the onset-to-biopsy duration among groups.

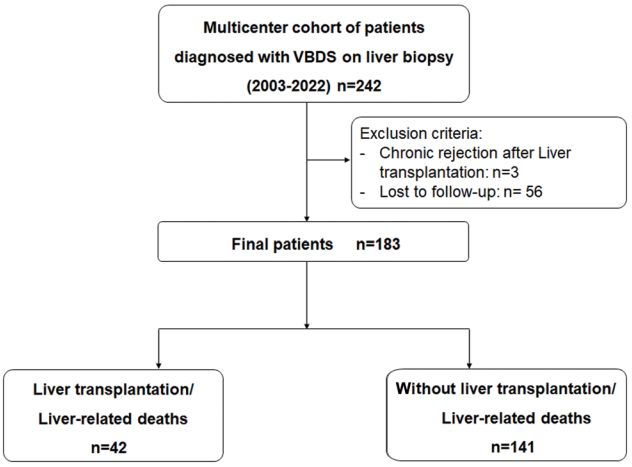

Fig. 1. Flowchart of the retrospective multicenter cohort of patients diagnosed with VBDS on liver biopsy. A final total of 183 patients were included, of which 42 succumbed to liver-related deaths or required liver transplantation. VBDS, vanishing bile duct syndrome.

Clinical and histological features of VBDS patients

Biochemical indices at time of liver biopsy are shown in Table 1. Median ALP was 340.5 (218.3, 538.5) U/L and GGT was 366 (209.0, 632.0) U/L. Median ALT was 93.5 (48.0, 148.0) U/ L, AST was 87.3 (57.9, 127.0) U/L, and TBIL was 28.8 (17.1,75.3) μmol/L. Histopathological changes are summarized in Table 2. The mean length and number of portal tracts in each liver biopsy was 1.4 (1.2, 1.8) centimeters and 13 (11,15), respectively. Overall, 43% of patients had ≥75% bile duct loss and 51.3% had bile duct loss with ductular reaction, and 25% had hepatocellular cholestasis. Other cholestatic features included canalicular cholestasis (22.4%), cholestasis within Kupffer cells (18.0%), cholate stasis (34.4%), and foam cells (19.7%). Advanced fibrosis (≥F3, Metavir stage) was detected in only 30.6% of patients. Additionally, two distinctive forms of VBDS were observed: 35.5% of patients lacked obvious inflammation and fibrosis and the other 64.5% showed obvious portal inflammation (Ludwig score ≥2) or advanced fibrosis (Supplementary Fig. 1). The correlations between onset-to-biopsy time and histological features are shown in Supplementary Table 3. Time to liver biopsy was not associated with either fibrosis stage or duct loss. However, cholate stasis, a histopathological feature of chronic cholestasis, was positively associated with this time period. Lobular inflammation was negatively associated with the clinical course of VBDS.

Comparison of VBDS patients with different etiologies

Among all VBDS cases, 118 were caused by PBC, 29 were associated with DILI, and the remaining 36 had miscellaneous etiologies: idiopathic adult VBDS (*n*=20), primary sclerosing cholangitis (*n*=9), Alagille syndrome (*n*=4), and progressive familial intrahepatic cholestasis (*n*=3). For biochemical measures, ALP, and GGT levels were similar among groups, whereas ALT and bilirubin levels were significantly higher in

Table 1. Demographic and laboratory features of patients with vanishing bile duct syndrome

Characteristics	DILI-VBDS (*n*=29)	PBC-VBDS (*n*=118)	Miscellaneous-VBDS (*n*=36)	*p*-value
Age in years, median (IQR)	47.5 (38.2–56.0)	49.0 (39.0–54.0)	46.5 (38.0–57.8)	0.940
Sex, *n* (%)				*0.002*
Male	10 (34.5%)	17 (14.4%)	14 (38.9%)	
Female	19 (65.5%)	101 (85.6%)	22 (61.1%)	
ALT in U/L	141 (99.8–179.5)	76.8 (45–132.5)	81.55 (48–134.5)	*0.011*
AST in U/L	97 (75–137)	84.65 (63–116.8)	86 (51.25–127.8)	0.548
ALP in U/L	354.1 (265.8–481.3)	341 (201–546)	330 (226.5–488)	0.917
GGT in U/L	456 (209–645)	322 (212–625)	415 (199–579)	0.720
ALB in g/L	40.35 (33.3–43.0)	38.2 (34.6–41.8)	37.1 (33.5–40.4)	0.666
TBIL in μmol/L	54.4 (19.5–163.6)	26.76 (16.1–64.9)	30.85 (18.8–99.7)	0.084
DBIL in μmol/L	32.3 (8.3–107.8)	10.33 (5.42–33.7)	16 (8–82.3)	*0.042*
Clinical outcomes, *n* (%)				*0.004*
No cirrhosis	22 (75.9%)	59 (50%)	14 (38.9%)	
Compensated cirrhosis	2 (6.9%)	22 (18.6%)	3 (8.3%)	
Decompensated cirrhosis	0 (0%)	16 (13.6%)	3 (8.3%)	
Liver transplantation	3 (10.3%)	6 (5.1%)	6 (16.7%)	
Liver-related death	2 (6.9%)	15 (12.7%)	10 (27.8%)	
UDCA administration	21 (72.4%)	115 (97.5%)	31 (86.1%)	0.001
Period from onset to biopsy in months	4.5 (1–14.25)	13 (1–42.5)	12 (3–60)	0.093
Follow-up duration in years	4.93 (2.79–8.30)	5.01 (2.38–7.15)	3.16 (1.54–6.06)	0.139

Italic font indicates statistical significance. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBIL, direct bilirubin; GGT, gamma-glutamyl transpeptidase; LT, liver transplantation; TBIL, total bilirubin; VBDS, vanishing bile duct syndrome.

Table 2. Comparison of histological characteristics of patients with vanishing bile duct syndrome

Histological features	All patients (*n*=183)	DILI-VBDS (*n*=29)	PBC-VBDS (*n*=118)	Miscellaneous-VBDS (*n*=36)	*p*-value
Bile duct loss					0.173
≥1/2 but <3/4, *n* (%)	105	12 (41.4%)	40 (37.7%)	20 (55.6%)	
≥3/4, *n* (%)	78	17 (58.6%)	66 (62.3%)	16 (44.4%)	
Periportal CK7+ staining*					0.208
<1/3, *n* (%)	104	20 (83.3%)	58 (69%)	23 (82.1%)	
≥1/3, *n* (%)	37	4 (16.7%)	26 (31%)	5 (17.9%)	
Ductular reaction					
Absent	89	20 (69%)	56 (47.5%)	13 (36.1%)	*0.047*
Periportal, small	66	7 (24.1%)	46 (39%)	13 (36.1%)	
Periportal, branched	28	2 (6.9%)	16 (13.6%)	10 (27.8%)	
Hepatocellular cholestasis					
Yes	46	12 (41.4%)	22 (18.6%)	12 (33.3%)	*0.018*
None	137	17 (58.6%)	96 (81.4%)	24 (66.7%)	
Canalicular cholestasis					*0.007*
Yes	41	11 (37.9%)	18 (15.3%)	12 (33.3%)	
None	142	18 (62.1%)	100 (84.7%)	24 (66.7%)	
Cholestasis in Kupffer cells					0.195
Yes	33	8 (27.6%)	17 (14.4%)	8 (22.2%)	
None	150	21 (72.4%)	101 (85.6%)	28 (77.8%)	
Cholate stasis					*<0.001*
Yes	63	1 (3.4%)	44 (37.6%)	18 (50%)	
None	120	28 (96.6%)	73 (62.4%)	18 (50%)	
Foam cells					0.800
Yes	36	7 (24.1%)	22 (18.6%)	7 (19.4%)	
None	147	22 (75.9%)	96 (81.4%)	29 (80.6%)	
Lobular inflammation					0.439
None	3	0 (0%)	2 (1.7%)	1 (2.8%)	
Mild or moderate	118	18 (62.1%)	73 (61.9%)	27 (75%)	
Severe	62	11 (37.9%)	43 (36.4%)	8 (22.2%)	
Portal inflammation					*0.001*
Yes	171	7 (24.1%)	4 (3.4%)	1 (2.8%)	
None	12	22 (75.9%)	114 (96.6%)	35 (97.2%)	
Interface hepatitis					*<0.001*
Yes	140	14 (48.3%)	18 (15.3%)	11 (30.6%)	
None	43	15 (51.7%)	100 (84.7%)	25 (69.4%)	
Fibrosis Stage					*<0.001*
None	19	3 (10.3%)	15 (12.7%)	1 (2.8%)	
Stage I–II	108	25 (86.2%)	65 (55.1%)	18 (50%)	
Stage III–IV	56	1 (3.4%)	38 (32.2%)	17 (47.2%)	
Different form of VBDS					*<0.001*
With portal inflammation or advance fibrosis	118	28 (96.6%)	72 (61%)	18 (50%)	
Without portal inflammation and advance fibrosis	65	1 (3.4%)	46 (39%)	18 (50%)	

Italic font indicates statistical significance. *CK-7 staining was not performed in all patients. VBDS, vanishing bile duct syndrome.

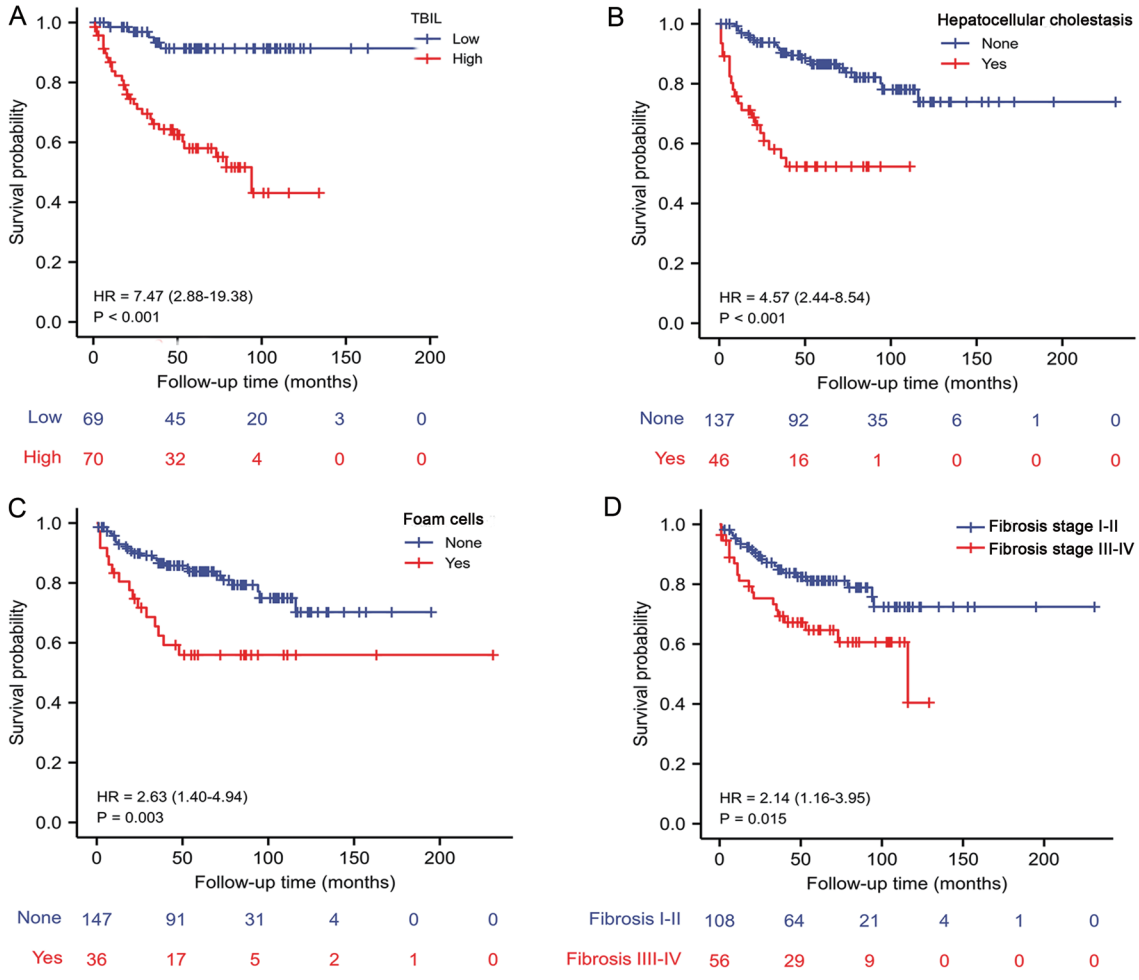

Fig. 2. Kaplan-Meier plots of transplant-free survival in VBDS patients. Stratification by (A) total bilirubin (TBIL) levels, (B) hepatocellular cholestasis, (C) foam cells and (D) fibrosis stage. Significant differences were observed in all the parameters analyzed (p<0.05). VBDS, vanishing bile duct syndrome.

DILI-VBDS patients (ALT: 141.0 vs. 76.8 vs. 81.5 U/L; DBIL: 32.3 vs. 10.3 vs. 16 μmol/L, DILI vs. PBC vs. miscellaneous, respectively; p<0.05, Table 1).

Histological features are shown in Table 2. DILI-VBDS patients had a significantly higher degree of histological cholestasis, followed by miscellaneous etiologies, than PBC-VBDS patients: hepatocellular cholestasis 41.4% vs. 33.3% vs. 18.6%, cholestasis in Kupffer cells 27.6% vs. 22.2% vs. 14.4%, canalicular cholestasis 37.9% vs. 33.3% vs. 15.3% (DILI vs. miscellaneous vs. PBC, respectively, all p<0.05). Miscellaneous etiologies and PBC-VBDS patients exhibited a significantly higher degree of cholate stasis than DILI-VBDS patients: miscellaneous 50%, PBC 37.6%, DILI 3.4% (p<0.05). Portal inflammation and interface hepatitis were significantly higher in DILI-VBDS patients than in the other two groups: portal inflammation 24.1% vs. 3.4% vs. 2.8%, interface hepatitis 48.3% vs. 15.3% vs. 30.6% (DILI vs. PBC vs. miscellaneous, respectively; all p<0.05). Advanced fibrosis was significantly more common in patients with miscellaneous etiologies and PBC-VBDS than in those with DILI-VBDS: 47.2% vs. 32.2% vs. 3.4%, respectively (p<0.05). Other histological features, such as degree of bile duct loss, lobular necroinflammation, foam cells, and periportal CK7+ staining, were similar across groups.

Follow-up and clinical outcomes of VBDS patients

The median follow-up period was 4.8 (2.0, 7.3) years and nearly half the patients (48.1%) developed compensated or decompensated cirrhosis during that time. Forty-two patients (23.0%) succumbed to an end-point event, 15 to LT and 27 to liver-related death (Table 1). There was a higher proportion of males in the LT/death group than in the no-endpoint group (36% vs. 18%, respectively; p<0.05). The overall 1-, 3-, 5-, and 10-year transplant-free survival rates were 91.0%, 83.0%, 78.0% and 67.2%, respectively.

Risk factors of poor VBDS prognosis

Univariate Cox regression analysis demonstrated that lower serum ALB and higher serum bilirubin at biopsy, but not underlying etiology were associated with higher risk of LT or liver-related death (Supplementary Table 4). After adjusting the multivariate Cox regression, only TBIL (HR 1.017, 95% CI: 1.010–1.023, p<0.05) was significantly associated with LT or death of VBDS patients (Fig. 2A).

Histologically, cholestasis within hepatocytes, canaliculi and Kupffer cells, cholate stasis, foam cells and advanced fibrosis rather than underlying etiology were associated with poor outcome (Table 3). Multivariate analysis showed that

Table 3. Univariate and multivariate Cox regression analysis of histological features associated with liver transplantation or liver-related deaths in patients with vanishing bile duct syndrome

Histological features	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Degree of VBDS	1.155 (0.623–2.139)	0.824		
Periportal CK7+ staining	0.939 (0.405–2.178)	0.964		
Ductular reaction	1.427 (0.764–2.662)	0.264		
Hepatocellular cholestasis	4.570 (2.445–8.542)	*<0.001*	2.953 (1.437–6.069)	*0.003*
Canalicular cholestasis	4.434 (2.367–8.303)	*<0.001*		
Cholestasis in Kupffer cells cells	4.348 (2.281–8.286)	*<0.001*		
Cholate stasis	2.514 (1.366–4.627)	*0.003*		
Portal inflammation	1.298 (0.314–5.375)	0.719		
Interface hepatitis	1.216 (0.563–2.627)	0.619		
Lobular inflammation	20.742 (0.001–Inf)	0.539		
Fibrosis degree	2.137 (1.157–3.945)	*0.015*	2.524 (1.313–4.851)	*0.005*
Foam cells	2.626 (1.396–4.942)	*0.003*	2.349 (1.092–5.053)	*0.029*

Italic font indicates statistical significance. VBDS, vanishing bile duct syndrome.

hepatocellular cholestasis (HR 2.953, 95% CI: 1.437–6.069; Fig. 3A–C), foam cells (HR 2.349, 95% CI: 1.092–5.053; Fig. 3D–F), and advanced fibrosis (HR 2.524, 95% CI: 1.313–4.851) were independent predictors of poor prognosis. Kaplan-Meier plots of these features are shown in Figure 2B–D.

Correlations were calculated among the key histological features to avoid multivariable interference (Supplementary Table 5). Fibrosis was positively correlated with portal inflammation (r=0.38, p<0.01), interface hepatitis (r=0.36, p<0.01), cholate stasis (r=0.49, p<0.01), and ductular reaction (r=0.32, p<0.01), and negatively correlated with foam cells (r=−0.15, p<0.05). Hepatocellular cholestasis corre-

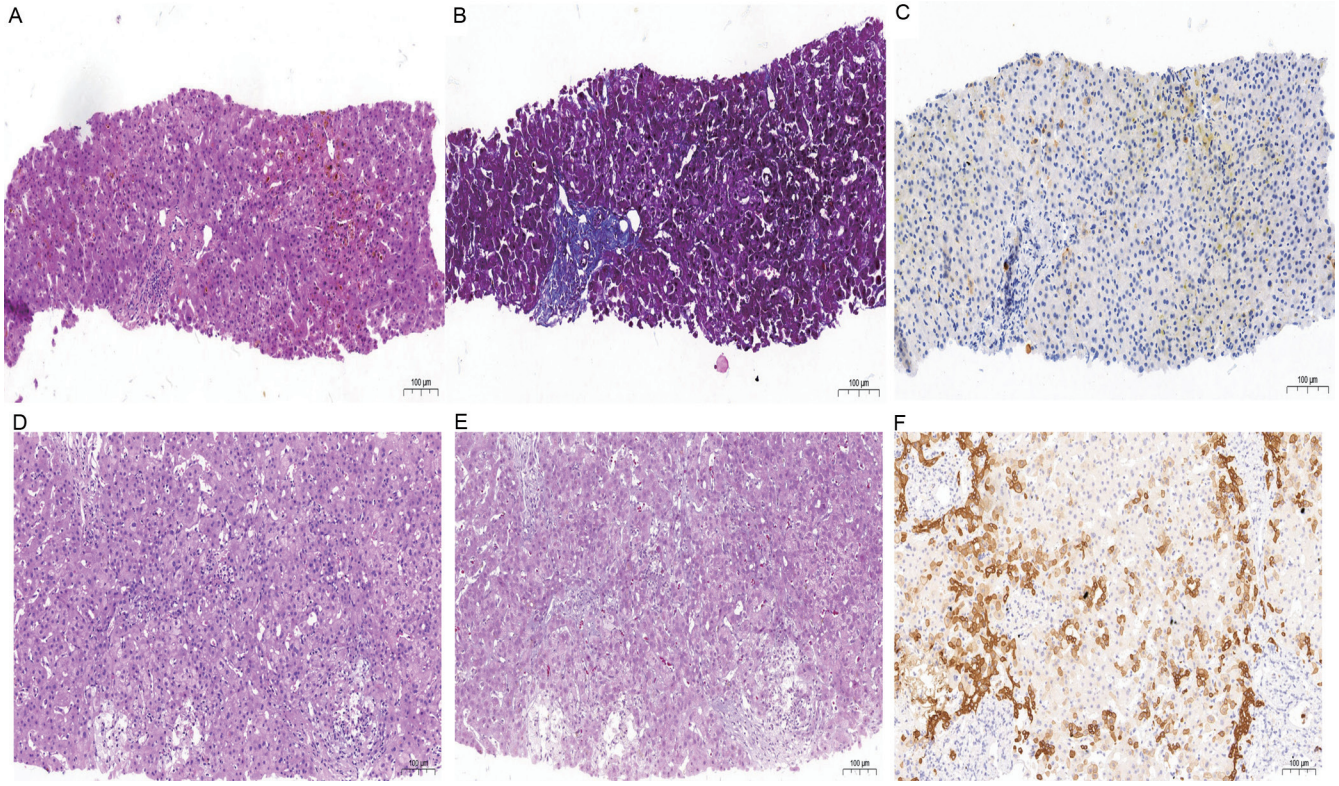

Fig. 3. Prominent histopathological findings in the liver biopsies of VBDS patients with clinical outcomes of liver transplantation or death. (A–C) VBDS accompanied by cholestasis but without obvious inflammation or significant fibrosis. (D–F) VBDS accompanied by clusters of foam cells but without obvious inflammation or fibrosis. CK7+ intermediate hepatocytes are prominent. (A, D: hematoxylin and eosin; B, E: Masson trichrome; C, F: CK7). VBDS, vanishing bile duct syndrome.

Table 4. Estimated survival up to 10 years according to risk factor

Hepatocellular cholestasis	Foam cells	Fibrosis degree	3-year survival, %	5-year survival, %	10-year survival, %
–	+	I–II	83.97	80.04	65.25
–	–	I–II	91.33	89.09	80.13
–	+	III–IV	71.41	65.12	43.93
–	–	III–IV	83.97	80.04	65.25
+	+	I–II	52.26	43.75	20.49
+	–	I–II	71.41	65.12	43.93
+	+	III–IV	28.63	20.03	4.71
+	–	III–IV	52.26	43.75	20.49

lated negatively with portal inflammation (r=−0.26, p<0.01) and positively with foam cells (r=0.40, p<0.01). The degree of VBDS correlated positively with hepatocellular cholestasis (r=0.24, p<0.01) and negatively with fibrosis stage (r=−0.15, p<0.05). Fibrosis stage correlated weakly with cholestasis, and collinearity diagnostics demonstrated that there was no collinearity among the three key histological risk factors (all variance inflation factors, p<3).

Nomogram construction and validation

Based on the three independent histological prognostic factors, a nomogram was developed for the prediction of transplant-free survival in VBDS patients (Supplementary Fig. 2A). Hepatocellular cholestasis was the factor that contributed most to prognosis, followed by advanced fibrosis and foam cells. The 3-, 5-, and 10-year transplant-free survival for VBDS patient was predicted by the cumulative score (Table 4). The predictive performance of the nomogram was measured by concordance index and calibrated with 1,000 bootstrap samples to decrease the overfit bias. The nomogram demonstrated good accuracy in estimating the risk of death or LT, with an unadjusted concordance index of 0.746 (95% CI: 0.706–0.785; Supplementary Fig. 2B). Furthermore, calibration plots showed good agreement of the above-mentioned risk factors.

Discussion

VBDS does not often accompany with poorer outcomes and seeking for the potential risk factors associated with death or LT is interested. Of the 183 patients studied, half recovered or maintained their present disease course and the other half progressed to cirrhosis or cirrhosis-related complications; Moreover, 23% died of end-stage liver disease or required LT. Etiology was not associated with outcome. Clinically, serum bilirubin level was most associated with transplant-free survival. Histologically, hepatocellular cholestasis, foam cells, and advanced fibrosis were predictive of poor prognosis.

In this study, high TBIL was associated with poor VBDS outcomes. This result was compatible with two recent studies of VBDS associated with DILI.[7,22] Hyperbilirubinemia has also been identified as an independent risk factor for poor prognosis in PBC.[14] This phenomenon was also in accordance with the histological presence of hepatocellular cholestasis as an independent predictor of the survival of VBDS patients, suggesting that cholestasis is a hallmark of decompensated bile drainage. Although more than 50% portal tracts lacked a bile duct, only one-third of VBDS patients had clinical jaundice or histological hepatocellular cholestasis. Collective compensatory drainage by a reserve interconnected biliary network can partially explain the extended period of jaundice-sparing despite extensive duct loss.[2,23] Furthermore, whether alteration of hepatobiliary transporter expression was associated with disease progression needs to be studied further.[24] Finally, it was not intermediate hepatocytes (CK7-positive), an early histological sign of cholate stasis, but foam cells, a typical feature of late-stage cholate stasis, that were associated with poor outcomes. Thus, chronic cholestatic features (hepatocellular cholestasis and foam cells) were associated with adverse outcomes in VBDS patients.

Advanced fibrosis rather than portal inflammation was independently associated with poor prognosis in VBDS. The present cohort suggested that there were two distinctive forms of VBDS: one associated with obvious portal inflammation and advanced fibrosis, and the other being "bland" duct loss without obvious portal inflammation or fibrosis (Supplementary Fig. 1). Inflammation seemed to be less predictive of prognosis in patients with bile duct loss. Previous research reported that the necroinflammatory index was not associated with clinical outcomes in patients with DILI-related bile duct loss.[7] Advanced fibrosis has been well recognized as a hallmark of poor prognosis in a variety of liver diseases,[25] such as chronic hepatitis B and C[26] and nonalcoholic fatty liver disease.[27] Portal and periportal fibrosis have been shown to be correlated with poor prognosis in PBC.[28,29] Fibrosis stage has also been associated with poor outcome in children with bile duct paucity.[25] The present data suggested that advanced fibrosis together with bile duct loss was associated with an even worse clinical outcome in patients with VBDS regardless of the underlying etiology; therefore, timely evaluation for LT may be warranted. VBDS can occur even in early stages of disease, and not all patients with advanced stages experience VBDS. In this study, 69.3% of VBDS patients were in the early stage of fibrosis (DILI 96.5%, PBC 67.8% and miscellaneous 52.8%).

Bile duct loss, especially at the 50% cut off, has been regarded as a histological hallmark of poor prognosis.[7,30,31] Our previous data showed that a loss of only 25% of bile ducts was independently associated with liver disease chronicity.[32] In the current study, a loss of 75% or more did not seem to be associated with a worse outcome when compared to patients with 50% or more duct loss; thus, implying that a greater degree of bile duct loss may not have an additive effect on the prognosis of VBDS patients. However, this hypothesis requires evidence from further studies and the underlying mechanism must be probed. Homeostasis of bile ducts is maintained by a balance between cell death and cell renewal or regeneration,[33] and ductular proliferation often

coexists with interlobular bile duct loss.[23] Previous studies have demonstrated that the regenerative ability of interlobular bile ducts was an important factor determining the prognosis of DILI-VBDS.[34] In this study, no significant difference in ductular reaction (or ductular proliferation, 48.2% vs. 61.9%) or extent of CK7-positive hepatocytes (a kind of bipotent hepatic progenitor cell, 27.5% vs. 21.9%), was observed between the two groups. Although etiology was not independently related with VBDS prognosis in this study, the clinical outcome was influenced by the cause of the initiating insult and the degree of injury.[33] DILI-VBDS generally had a better prognosis than those with PBC-VBDS.[35] VBDS associated with COVID-19 is usually transient and resolve with disease resolution, but some COVID-19 related ductopenia is severe.[36]

The study has limitations. First, a minority of patients had an undetermined etiology, and the sample sizes of DILI- and miscellaneous-VBDS were small. That might have been PBC patients have a higher incidence of VBDS than other etiologies. Second, although the study was retrospective in nature, to our knowledge, this is the largest cohort from different providences of China with long-term follow-up outcome data worldwide. The identified risk factors need to be further verified in a prospective cohort study.

Conclusions

In conclusion, of the 183 VBDS patients studied, half progressed to cirrhosis and 23% experienced LT or liver-related death within 2 years of diagnosis. Histological factors such as hepatocellular cholestasis, foam cells, and advanced fibrosis rather than the underlying etiologies of VBDS were independently associated with poor prognosis. The present study provides new evidence to support early liver histological evaluation, as a critical modality for better stratifying high-risk patients with VBDS.

Funding

This work was supported by National Natural Science Foundation of China (No. 82100633 to LTT).

Conflict of interest

JJ has been an executive associate editor of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Study concept and design, (XZ, JJ), acquisition, analysis, or interpretation of data (TL, XH, HY), drafting the manuscript (TL), critical revision of the manuscript for important intellectual content (AW, JL, TW), statistical analysis (TL, ML), study supervision (JX, XlH, JL, WD).

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki and all research was approved by the ethical standards for clinical studies by the Ethics Committee.

Data sharing statement

The data used to support the findings of this study are available from the corresponding author at zhao_xinyan@ccmu.edu.cn upon request.

References

[1] Bessone F, Hernandez N, Tanno M, Roma MG. Drug-Induced Vanishing Bile Duct Syndrome: From Pathogenesis to Diagnosis and Therapeutics. Semin Liver Dis 2021;41(3):331–348. doi:10.1055/s-0041-1729972, PMID:34130334.
[2] Nakanuma Y, Tsuneyama K, Harada K. Pathology and pathogenesis of intrahepatic bile duct loss. J Hepatobiliary Pancreat Surg 2001;8(4):303–315. doi:10.1007/s005340170002, PMID:11521175.
[3] Burt AD, Ferrell LD, Hubscher SG. MacSween's Pathology of the Liver, 7th ed. Philadelphia, PA: Elsevier; 2018.
[4] Pusl T, Beuers U. Ursodeoxycholic acid treatment of vanishing bile duct syndromes. World J Gastroenterol 2006;12(22):3487–3495. doi:10.3748/wjg.v12.i22.3487, PMID:16773706.
[5] Zafar M, Farooq M, Butler-Manuel W, Khattak MF, Rana UI, Muhammad T, *et al*. Vanishing Bile Duct Syndrome Associated With Non-Hodgkin's Lymphoma and Hepatitis E Virus Infection. Cureus 2022;14(1):e21328. doi:10.7759/cureus.21328, PMID:35070583.
[6] Faragalla K, Lau H, Wang HL, Liu J. Cloxacillin-induced acute vanishing bile duct syndrome: A case study and literature review. Br J Clin Pharmacol 2022;88(10):4633–4638. doi:10.1111/bcp.15445, PMID:35730139.
[7] Bonkovsky HL, Kleiner DE, Gu J, Odin JA, Russo MW, Navarro VM, *et al*. Clinical presentations and outcomes of bile duct loss caused by drugs and herbal and dietary supplements. Hepatology 2017;65(4):1267–1277. doi:10.1002/hep.28967, PMID:27981596.
[8] Nakanuma Y, Zen Y, Harada K, Sasaki M, Nonomura A, Uehara T, *et al*. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. Pathol Int 2010;60(3):167–174. doi:10.1111/j.1440-1827.2009.02500.x, PMID:20403042.
[9] Fujinaga Y, Namisaki T, Moriya K, Kitade M, Kawaratani H, Shimozato N, *et al*. Identification of clinical risk factors for histological progression of primary biliary cholangitis. Hepatol Res 2019;49(9):1015–1025. doi:10.1111/hepr.13355, PMID:31021038.
[10] Kumagi T, Guindi M, Fischer SE, Arenovich T, Abdalian R, Coltescu C, *et al*. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol 2010;105(10):2186–2194. doi:10.1038/ajg.2010.216, PMID:20502446.
[11] LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012–. Available from: https://pubmed.ncbi.nlm.nih.gov/31643176/.
[12] Ye LH, Wang CK, Zhang HC, Liu ZQ, Zheng HW. [Clinicopathologic features of drug-induced vanishing bile duct syndrome]. Zhonghua Gan Zang Bing Za Zhi 2017;25(4):317–320. doi:10.3760/cma.j.issn.1007-3418.2017.04.019, PMID:28494557.
[13] Bakhit M, McCarty TR, Park S, Njei B, Cho M, Karagozian R, *et al*. Vanishing bile duct syndrome in Hodgkin's lymphoma: A case report and literature review. World J Gastroenterol 2017;23(2):366–372. doi:10.3748/wjg.v23.i2.366, PMID:28127210.
[14] You H, Ma X, Efe C, Wang G, Jeong SH, Abe K, *et al*. APASL clinical practice guidance: the diagnosis and management of patients with primary biliary cholangitis. Hepatol Int 2022;16(1):1–23. doi:10.1007/s12072-021-10276-6, PMID:35119627.
[15] Kleiner DE, Chalasani NP, Lee WM, Fontana RJ, Bonkovsky HL, Watkins PB, *et al*. Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations. Hepatology 2014;59(2):661–670. doi:10.1002/hep.26709, PMID:24037963.
[16] Wang T, Zhao X, Shao C, Ye L, Guo J, Peng N, *et al*. A proposed pathologic sub-classification of drug-induced liver injury. Hepatol Int 2019;13(3):339–351. doi:10.1007/s12072-019-09940-9, PMID:30977034.
[17] Wendum D, Boëlle PY, Bedossa P, Zafrani ES, Charlotte F, Saint-Paul MC, *et al*. Primary biliary cirrhosis: proposal for a new simple histological scoring system. Liver Int 2015;35(2):652–659. doi:10.1111/liv.12620, PMID:24939754.
[18] Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379(2):103–112. doi:10.1007/BF00432479, PMID:150690.
[19] Xu XY, Ding HG, Li WG, Xu JH, Han Y, Jia JD, *et al*. Chinese guidelines on the management of liver cirrhosis (abbreviated version). World J Gastroenterol 2020;26(45):7088–7103. doi:10.3748/wjg.v26.i45.7088, PMID:33362370.
[20] Steyerberg EW, Vergouwe Y. Towards better clinical prediction models: seven steps for development and an ABCD for validation. Eur Heart J 2014;35(29):1925–1931. doi:10.1093/eurheartj/ehu207, PMID:24898551.
[21] D'Agostino RB, Grundy S, Sullivan LM, Wilson P, CHD Risk Prediction Group. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA 2001;286(2):180–187. doi:10.1001/jama.286.2.180, PMID:11448281.
[22] Wasuwanich P, Choudry H, So JM, Lowry S, Karnsakul W. Vanishing bile duct syndrome after drug-induced liver injury. Clin Res Hepatol Gastroenterol 2022;46(9):102015. doi:10.1016/j.clinre.2022.102015, PMID:36067952.
[23] Strazzabosco M, Fabris L. Development of the bile ducts: essentials for the clinical hepatologist. J Hepatol 2012;56(5):1159–1170. doi:10.1016/j.jhep.2011.09.022, PMID:22245898.
[24] Zollner G, Fickert P, Zenz R, Fuchsbichler A, Stumptner C, Kenner L, *et al*. Hepatobiliary transporter expression in percutaneous liver biopsies of patients with cholestatic liver diseases. Hepatology 2001;33(3):633–646. doi:10.1053/jhep.2001.22646, PMID:11230744.
[25] Meena BL, Khanna R, Bihari C, Rastogi A, Rawat D, Alam S. Bile duct

paucity in childhood-spectrum, profile, and outcome. Eur J Pediatr 2018;177(8):1261–1269. doi:10.1007/s00431-018-3181-3, PMID:29868931.
[26] de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C, Baveno VIIF. Baveno VII - Renewing consensus in portal hypertension. J Hepatol 2022;76(4):959–974. doi:10.1016/j.jhep.2021.12.022, PMID:35120736.
[27] Cotter TG, Rinella M. Nonalcoholic Fatty Liver Disease 2020: The State of the Disease. Gastroenterology 2020;158(7):1851–1864. doi:10.1053/j.gastro.2020.01.052, PMID:32061595.
[28] Warnes T, Roberts S, Smith A, Haboubi N, McMahon RF. Liver biopsy in primary biliary cholangitis: is sinusoidal fibrosis the missing key? J Clin Pathol 2019;72(10):669–676. doi:10.1136/jclinpath-2019-205958, PMID:31371396.
[29] Chen S, Duan W, Li M, Li S, Lv T, Tian Q, *et al*. Prognosis of 732 ursodeoxycholic acid-treated patients with primary biliary cholangitis: A single center follow-up study from China. J Gastroenterol Hepatol 2019;34(7):1236–1241. doi:10.1111/jgh.14521, PMID:30365184.
[30] Chalasani N, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalkar J, *et al*. Features and Outcomes of 899 Patients With Drug-Induced Liver Injury: The DILIN Prospective Study. Gastroenterology 2015;148(7):1340–1352.e1347. doi:10.1053/j.gastro.2015.03.006, PMID:25754159.
[31] Bjornsson ES, Jonasson JG. Idiosyncratic drug-induced liver injury associated with bile duct loss and vanishing bile duct syndrome: Rare but has severe consequences. Hepatology 2017;65(4):1091–1093. doi:10.1002/hep.29040, PMID:28073177.
[32] Tian QJ, Zhao XY, Wang Y, Wee A, Soon GST, Gouw ASH, *et al*. Histologic pattern is better correlated with clinical outcomes than biochemical classification in patients with drug-induced liver injury. Mod Pathol 2019;32(12):1795–1805. doi:10.1038/s41379-019-0314-9, PMID:31300804.
[33] Reau NS, Jensen DM. Vanishing bile duct syndrome. Clin Liver Dis 2008;12(1):203–217. doi:10.1016/j.cld.2007.11.007, PMID:18242505.
[34] Hubscher SG, Buckels JA, Elias E, McMaster P, Neuberger JM. Reversible vanishing bile duct syndrome after liver transplantation: report of 6 cases. Transplant Proc 1991;23(1 Pt 2):1415–1416. PMID:1989250.
[35] Degott C, Feldmann G, Larrey D, Durand-Schneider AM, Grange D, Machayekhi JP, *et al*. Drug-induced prolonged cholestasis in adults: a histological semiquantitative study demonstrating progressive ductopenia. Hepatology 1992;15(2):244–251. doi:10.1002/hep.1840150212, PMID:1735527.
[36] Kulkarni AV, Khelgi A, Sekaran A, Reddy R, Sharma M, Tirumalle S, *et al*. Post-COVID-19 Cholestasis: A Case Series and Review of Literature. J Clin Exp Hepatol 2022;12(6):1580–1590. doi:10.1016/j.jceh.2022.06.004, PMID:35719861.