Clinical Presentations and Outcomes of Bile Duct Loss caused by Drugs and Herbal and Dietary Supplements

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Abstract

Bile duct loss during the course of drug induced liver injury is uncommon but can be an indication of vanishing bile duct syndrome. In this work we assess the frequency, causes, clinical features and outcomes of cases of drug induced liver injury with histologically proven bile duct loss. All cases of drug induced liver injury enrolled into a prospective database over a ten year period that had undergone liver biopsies (n=363) were scored for the presence of bile duct loss and assessed for clinical and laboratory features, causes and outcomes. 26 of the 363 patients (7%) with drug, herbal or dietary supplement associated liver injury had bile duct loss on liver biopsy which was moderate to severe (<50% of portal areas with bile ducts) in 14 and mild (50–75%) in 12. The presenting clinical features of the 26 cases varied, but the most common clinical pattern was a severe cholestatic hepatitis. The implicated agents included amoxicillin/clavulanate (n=3), temozolomide (n=3), various herbal products (n=3), azithromycin (n=2) and 15 other medications or dietary supplements. Compared to those without, those with bile duct loss were more likely to develop chronic liver injury (94% vs 47%), which was usually cholestatic and sometimes severe. Five patients died and two others underwent liver transplantation for progressive cholestasis

despite treatment with corticosteroids and ursodiol. The most predictive factor of poor outcome was the degree of bile duct loss on liver biopsy.

Conclusions—Bile duct loss during acute cholestatic hepatitis is an ominous early indicator of possible vanishing bile duct syndrome, for which at present there are no known means of prevention or therapy.

Introduction

Drug induced liver injury represents a broad array of forms of hepatic injury grouped together only because they are all caused by drugs or herbal and dietary supplements (HDS). The clinical patterns vary widely, from an acute hepatitis-like picture, to acute hepatic necrosis, cholestatic injury, fatty liver disease, sinusoidal obstruction syndrome, nodular regenerative hyperplasia and cirrhosis. Some of the variation relates to the mode of cellular injury (necrosis, apoptosis, mitochondrial damage), but some relates to the liver cell type that bears the brunt of injury: whether hepatocytes, cholangiocytes, sinusoidal lining cells or venular endothelial cells. In this regard, the common forms of cholestatic liver injury from medications might reflect injury first and foremost to mature cholangiocytes or biliary epithelium or their progenitor cells. Although liver biopsies taken during acute drug induced liver injury not infrequently show injury to bile ducts, they rarely demonstrate loss of bile ducts despite prominent cholestasis and inflammation. The exception to this generalization is the vanishing bile duct syndrome (VBDS), a rare but serious complication of some cases of cholestatic drug injury to the liver.[1–12]

Vanishing bile duct syndrome is an uncommon but potentially severe form of chronic liver disease. Known causes of VBDS include graft-vs-host disease, primary biliary cirrhosis, sclerosing cholangitis, paraneoplastic syndromes, Alagille syndrome and drugs. Rarely, VBDS arises without a known cause and can be referred to as idiopathic. The full spectrum of VBDS, particularly that due to medications, is not well known. VBDS has been described largely in isolated case reports or small case series that generally represent the most severe and dramatic examples of this injury. The frequency of bile duct loss during drug induced liver injury and its overall course and outcome, particularly whether it invariably leads to VBDS, have not been well characterized. In a large, long-term prospective study of drug induced liver injury in the United States, we have assessed the frequency, causes, clinical patterns and outcomes of cases in which liver biopsies demonstrated appreciable bile duct loss.

Materials and Methods

The Drug Induced Liver Injury Network (DILIN) is a prospective, collaborative study of drug-induced liver injury in the United States, which was initiated in 2003 as a cooperative agreement funded by the National Institutes of Health (NIH)[13,14] Additional details are in Supplementary Material.

After 6 months of follow up, cases were adjudicated for the likelihood that the injury was due to the implicated drugs or HDS by a causality committee.[15] All cases were scored as being definite (1: 95% likelihood), highly likely (2: 75–94%), probable (3: 50–74%),

possible (4: 25–49%) or unlikely (5: <25%). For cases with more than one implicated agent, each drug or HDS was scored separately in a similar manner. For the purposes of this analysis, only cases scored as probable, highly likely or definite were used.[14] All cases were also graded for severity on a scale of 1 to 5 as mild, moderate, moderate and hospitalized, severe or fatal using standardized criteria.[13] For the current analyses, chronicity was scored for both severity and biochemical pattern at 6, 12 and 24 months and at the last visit as none (0: serum ALT, Alk P in reference ranges, total bilirubin 1.2 mg/dL and INR < 1.5 or missing); mild (1: ALT 1 to 3 times and/or Alk P 1 to 2 times ULN and/or bilirubin >1.2 mg/dL but <2.5 mg/dL, and INR < 1.5 or missing); moderate (2: ALT > 3 times or Alk P > 2 times ULN but bilirubin < 2.5 mg/dL and INR < 1.5 or missing); moderately severe (3: ALT or Alk P elevated above ULN, serum bilirubin 2.5 mg/dL and INR <1.5); or severe (4: ALT or Alk P elevated above ULN, bilirubin 2.5 mg/dL with INR 1.5 or other signs of liver failure) (Supplementary Table 2). The pattern of persistent injury was characterized as cholestatic, mixed or hepatocellular based upon R ratio, where R = (ALT value/ALT ULN) divided by (Alk P value/Alk P ULN). By usual convention values of R< 2 are defined as cholestatic, R> 5 as hepatocellular and R=2–5 as 'mixed'.[13]

All deaths and liver transplants recorded in the DILIN Prospective study were assessed by committee in a standardized manner, and the role of the drug- or HDS- induced liver injury was scored as the primary cause, a contributory cause or not related.[17]

A liver biopsy was not required as a part of the DILIN Prospective Protocol, but if performed in the course of routine medical care, a request was made that de-identified, recut, unstained slides be prepared and sent to the Laboratory of Pathology, National Cancer Institute, in the NIH Clinical Center (Bethesda, MD). Biopsies were read by the DILIN hepatic pathologist (D.E.K.) without specific clinical information and scored for multiple findings.[16] In this system, bile duct paucity was scored as 0 (none or normal: > 75% of portal areas had bile ducts), 1+ (mild loss: 50–75% of portal areas had bile ducts) or 2+ (moderate-to-severe loss: <50% of portal areas had bile ducts). Cases were also analyzed for the number of portal areas and the number with identifiable bile ducts, which allowed calculation of the fraction of portal areas with bile ducts.

Results are presented as median values and ranges. Statistical significance among groups was determined by Wilcoxon rank-sum test for continuous variables, Fisher's exact test for binary variables, Chi-Square for categorical variables and log-rank tests for time-to-event variables. The statistical analyses were done using SAS 9.4 (SAS Institute, Cary, NC), and p values of <0.05 were considered significant.

Results

Cohort of patients with bile duct loss

Over a ten year period (September 2004 to September 2014), 1433 subjects with suspected drug induced liver injury were enrolled in the DILIN Prospective Protocol, among whom 1296 completed 6 months of follow up data accrual and underwent central adjudication of causality. Among the adjudicated cases, 1056 (81.5%) were judged to be probable, highly likely or definite drug-induced liver injury, and, among these, 363 (34%) had liver biopsies

that were available and deemed adequate for histopathological interpretation. Among these 363 cases, 26 (7%) had evidence of bile duct loss, which was scored as mild in 12 and moderate-to-severe in 14. The process of development of two cohorts (with and without bile duct loss) is shown in Figure 1.

Clinical features of cohort

The demographic, clinical, laboratory and histologic features of the 26 cases with bile duct loss are summarized in Table 1. The median age was 53 years (range 11 to 87), all except one were adults, and 54% were women. All except one (96%) were jaundiced (serum total bilirubin > 2.5 mg/dL). Other common symptoms included itching (77%), nausea (46%), fatigue (42%) and abdominal pain (42%). The time to onset after starting the implicated medication ranged from 3 to 551 days with a median of 38 days. The laboratory results at onset were typically cholestatic with prominent elevations in Alk P (median and range: 368; 71 to 1261 U/L) and mild to moderate increases in ALT levels (296; 57 to 1268 U/L). The median R ratio was 1.7 but ranged from 0.6 to 8.0; the R ratio being in the low range for hepatocellular injury in 5 cases (19%: 6.3 to 8.0), in the mixed range in 6 (23%: 2.4 to 3.7) and cholestatic range in 15 (58%: <2.0). Rash was reported in 10 patients (39%), fever in 12 (46%) but peripheral eosinophilia in only 4 (15%). Among the 10 patients with rash, half were diagnosed with a severe cutaneous reaction: two with drug reaction with eosinophilia and systemic signs (DRESS syndrome) and one each for Stevens Johnson syndrome, toxic epidermal necrolysis and erythema multiforme.

Comparison of patients with and without bile duct loss

Table 1 also provides a comparison of the 26 cases with and the 337 cases without bile duct loss on liver biopsy. The two groups were similar in age, sex and race, but those with bile duct loss were more likely to have jaundice and a cholestatic pattern of liver enzyme elevations (R <2.0 in 57% vs 23%). Cases with duct loss were also more likely to have rash and fever than the control group. Overall, the peak bilirubin and initial and peak Alk P levels were higher in the bile duct loss group, while initial and peak ALT levels were lower. Importantly, the mortality rate was higher in those with bile duct loss vs those without (27% vs 9%, p = 0.01) as was the rate of chronicity among patients followed for at least 6 months (94% vs 47%, p < 0.001).

We also compared the 26 cases with bile duct loss to all those with R values ≥ 8 who underwent liver biopsies. These control subjects have clinical features and types of liver injury that more closely resemble those of the study cohort. The results are summarized in Suppl Table 3. Differences in those with bile duct loss include a trend for greater frequency of African Americans [6/26 (23%) vs 20/193 (10%), p= 0.097, Fisher's exact test, 2 sided], higher levels of serum Alk P and total bilirubin, significantly higher INR, higher scores for severity at baseline, and much greater risk of chronicity and likelihood of poor outcomes.

Drugs implicated in causing bile duct loss

Adjudication of the causality identified 2 cases as definite, 14 highly likely and 10 probable. However, many patients had taken multiple medications within two months of onset, and the specific agent that caused the liver injury was not always clearly defined. The various agents

that were implicated in the 26 cases of drug-induced liver injury with bile duct loss are listed in Table 2, which also shows the numbers of cases attributed to these agents among all 363 patients who underwent liver biopsy. The most commonly associated agents in the cohort with bile duct loss included amoxicillin-clavulanic acid, HDS products, azithromycin and the fluoroquinolones, but these were also commonly associated agents in the control population of cases. In the biopsy cohort, 3 of the 4 temozolomide cases demonstrated bile duct loss. Similarly, the only cases of liver injury attributed to several other agents in this cohort represented cases in the bile duct loss group, in particular thalidomide and its derivative, lenalidomide. In many cases, however, the implicated agent was considered only "probable" or "possible" and there were other possibly implicated agents. Indeed, for the cohort with bile duct loss, the mean number of other medications being taken within two months of onset of liver injury was 9.6, the median was 7.5, and the range was 1 to 35. Similarly, among the 337 subjects who underwent liver biopsies that did not show bile duct loss, the mean number of concomitant drugs was 6.9, the median was 5, and the range was 1–51. Differences between the two groups were not significant [p=0.09]. Among the other agents taken within two months of onset were several drugs that have been linked to VBDS[12], including cephalosporins (n=8), fluoroquinolones (n=2), azithromycin (n=4), erythromycin (n=1), clindamycin (n=2), amoxicillin (n=1), carbamazepine (n=1), lamotrigine (n=1), ibuprofen (n=2), acetaminophen (n=6), omeprazole (n=10), lansoprazole (n=2), atorvastatin (n=4), fenofibrate (n=1) and metoclopramide (n=1).

Frequently implicated agents among cases that underwent liver biopsy but did not show bile duct loss included drugs associated with purely hepatocellular injury such as nitrofurantoin, isoniazid and minocycline. Important causes of cholestatic liver injury that were not linked to any cases of duct loss included the anabolic steroids and estrogens. Thus, among 16 cases of anabolic steroid associated jaundice who underwent liver biopsies and were enrolled in the DILIN database, none demonstrated significant bile duct loss.

Histopathological findings [Figure 2]

Histopathological changes were diverse. Usually, inflammatory infiltrates were mild, with little or no direct interaction with the remaining ducts. Residual ducts showed reactive epithelial changes consistent with injury or repair. Chronic cholestatic changes were common with periportal pseudoxanthomatous changes of hepatocytes, copper accumulation and, sometimes, marked ductular reaction. Sclerosing duct changes reminiscent of sclerosing cholangitis were seen in a few ducts in four of the 26 cases. Acute large duct obstruction can cause zone 3 cholestasis but would not cause duct loss. Chronic large duct obstruction could be considered in some cases, but would also not cause duct loss and, furthermore, had been excluded by imaging.

Outcomes of cases with bile duct loss

Outcomes of the liver injury among the 26 cases with bile duct loss are shown in Table 3. By the time of the 6 month follow up visit, 5 patients had died and 5 others had been lost to follow up. Of the 16 patients with 6 months of follow up, 15 (94%) had biochemical evidence of persistent injury, which was cholestatic in all 15 adults (median R ratio = 0.8) and mixed in the one adolescent in the cohort (R = 3.4). The persistent injury at 6 months

was scored as severe in 1 (evidence of hepatic failure), moderately severe in 3 (serum total bilirubin >2.5 mg/dL), moderate in 9 (Alk P > twice ULN) and mild in two. One year follow up was available on 13 and two year on 9 of those with persistent injury at 6 months, all except two of whom continued to have biochemical evidence of cholestatic liver injury. With time, median values of Alk P and bilirubin decreased and median chronicity score declined from 2.0 at 6 months to 2.0 at one year and 1.0 at two years. Among the original 26 patients with bile duct loss, 7 died and 2 underwent liver transplantation. Among those who died, the liver injury was scored as the primary cause in 2, a contributory cause in 3 and unrelated in 2 cases.

Early liver biopsies showing bile duct loss

In 19 patients, the liver biopsy demonstrating bile duct loss was done within 3 months of onset; the remaining 7 being done 7 to 22 months later. Indeed, 6 patients with bile duct loss on a late biopsy had had initial biopsies within 3 months of onset that did not show significant duct loss. These 6 patients did not differ in clinical, biochemical features or even in other histologic features from those who had duct loss on early biopsy. However, the early biopsies not showing bile duct injury had fewer numbers of portal areas (median 7, range 4 to 9) than the biopsies that did show bile duct loss (median 14, range 7 to 28) (p = 0.002), suggesting that the early biopsies in these 6 patients may have been sub-optimal for reliable assessment of duct loss (Suppl Table 4).

Among the 19 patients with early liver biopsies showing bile duct loss, 9 were scored as mild, 5 of whom had 6 months of follow up, at which time 4 had evidence of persistent cholestatic liver injury. In further follow up, none of these patients underwent liver transplantation or died of progressive liver disease (two died of brain cancer unrelated to the drug reaction). Among the 10 patients with early liver biopsies showing moderate-to-severe bile duct loss, 6 month follow up was available in 8, of whom 4 died. The liver injury was considered the primary cause of death in 2 and contributory in 2. The remaining 4 patients all had persistent cholestatic liver injury that was scored as moderate or severe at 6 months and was still moderate or severe when they were last seen, one undergoing liver transplantation at 22 months after onset and one dying with liver injury considered a contributory cause.

Predictive factors for poor outcome

Analysis of predictive factors for a poor outcome was done limiting the analysis to the 20 patients with at least 6 months of follow up or death before 6 months. A poor outcome was considered one of the following: (1) death in which the liver injury was considered the primary (n=2) or a contributory (n=3) cause, (2) liver transplantation (n=2), or (3) persistent liver injury, which on final assessment was still moderate or severe (chronicity severity score 2, 3 or 4: n=7). Using these criteria, 13 patients were considered to have poor and 7 benign outcomes. The demographic, clinical, biochemical and histologic features of the two groups are compared in Table 4. As shown, the benign vs poor outcomes groups tended to differ somewhat in median age (63.5 vs 48.2 years, p = 0.08) and race (14% vs 38% African American, p = 0.35) but not in regard to sex, duration of drug use to onset, or treatment with corticosteroids or ursodiol. Laboratory test results at the onset of injury were similar in those

with a benign vs poor outcome, but by the time of liver biopsy, those with a poor outcome had more abnormal laboratory test results. Histologic features of disease activity (HAI scores), fibrosis, copper accumulation, and evidence of bile duct injury were similar in those with benign and poor outcomes. The factor most closely related to poor outcome was the degree of bile duct loss on liver biopsy: those with moderately severe to severe bile duct loss being invariably associated with a poor outcome. All biopsies were re-reviewed by the hepatopathologist and the number of adequately sized portal tracts and number of those with an identifiable bile duct were counted. The average percent of portal areas with bile ducts in those with a benign outcome was 64% compared to only 17% in those with a poor outcome (p = 0.003).

Selected representative case summaries are given in the Supplemental Material, including patients with bile duct loss with subsequent progressive cholestasis resulting in death (Case 1) or liver transplantation (Case 2); severe acute cholestasis with residual injury 2 to 3 years after onset (Cases 3, 4 and 5); and marked acute cholestasis with complete resolution by 6 months (Case 6) or after several years (Case 7).

Discussion

In this cohort, 26 of 363 (7%) cases of drug-induced liver injury undergoing liver biopsy had histologic evidence of bile duct loss. Analysis of the characteristics of those with bile duct loss demonstrated that they typically had a moderate-to-severe acute cholestatic liver injury with immunoallergic features, some patients having severe cutaneous reactions such as DRESS, Stevens Johnson syndrome or toxic epidermal necrolysis. Importantly, the histologic finding of bile duct loss was associated with evolution to chronic liver injury (94%) and a high liver-related morbidity and mortality (26%). The major causes of VBDS in this cohort included many of the common causes of cholestatic hepatitis such as amoxicillin/clavulanate [4–6, 18], azithromycin [8,19] and fluoroquinolones [4,9, 20]. Isolated cases were due to allopurinol, thalidomide, lenalidomide, montelukast and cephalosporins. Single cases were due to agents that are very rare causes of liver injury such as omeprazole, lansoprazole and enalapril. In some it was difficult to confidently attribute the injury to one specific agent. Strikingly, many common causes of drug-induced liver injury were not linked to any of these bile duct loss cases, examples including isoniazid, minocycline, nitrofurantoin, diclofenac or common causes of "bland cholestasis" such as estrogens and anabolic steroids. A special exception to the rarity of bile duct injury was temozolomide, a relatively recently introduced alkylating agent that crosses the blood-brain barrier and is used extensively in the treatment of malignant brain tumors. [21, 22]

In this case series, 2 of the 26 patients with bile duct injury on liver biopsy ultimately died with severe, progressive cholestatic liver injury and 2 others underwent liver transplantation with a similar clinical syndrome suggestive of VBDS. Three other patients died and the cholestatic liver injury was considered contributory. Thus, the overall mortality of acute drug induced liver injury with bile duct loss may be as high as 27%. In one instance of liver transplantation in this cohort, complete absence of bile ducts was documented in the explanted liver. In the other cases VBDS was assumed to be the cause of the progressive injury.

While the mortality rate of liver injury with bile duct loss was high, some patients recovered clinically and a few resolved all biochemical evidence of liver injury or cholestasis. Thus, in follow up, 2 of 26 patients (11%) with bile duct injury and paucity initially (both with mild duct loss on biopsy) had complete resolution with no symptoms and normal liver tests when seen 6 months after onset. Another 8 patients (31%) had mild to moderate alkaline phosphatase abnormalities but had no symptoms or bilirubin elevations, suggesting residual, subclinical bile duct loss that might be considered mild or a form fruste of VBDS. The best predictor of a benign vs poor outcome in this study was the degree of bile duct loss. There was a trend for poor outcomes to be associated with younger age at onset and African-American race. The numbers of cases in this series was not sufficient to perform multivariate analyses of these factors, but certainly the roles of age and race in influencing the course and outcome of drug-induced liver injury are important topics for further investigation. A high proportion of patients were treated with corticosteroids and ursodiol (Table 4), but with little evidence of effect in individual cases or overall.

The pathogenesis of bile duct loss and VBDS is not known, but it is clearly idiosyncratic and likely to be due to immunologically mediated injury to bile ducts. Supportive of this concept is that the major causes of idiosyncratic cholestatic hepatitis are common causes of VBDS, whereas the major causes of acute hepatocellular injury (and acute liver failure) are uncommon causes of VBDS. The association of the most severe cases of VBDS with severe cutaneous reactions such as Stevens Johnson syndrome suggests that VBDS may be due to an aberrant hypersensitivity reaction affecting cholangiocytes in addition to keratinocytes, perhaps because of shared immunogenic proteins or shared ability to present drug-protein-adducts or immunogenic drug metabolites on their cell surface.

Strengths of this study include the number of cases of suspected VBDS, the availability of liver histology from early in the course of injury, the standardized fashion of evaluation, causality assessment, grading and staging and the central "blinded" histologic readings. This series also represents the full spectrum of this form of liver injury, including mild cases that resolve and severe cases that lead to death or liver transplantation. Another strength is that all cases of suspected drug-induced liver injury were enrolled and not just classic and clear cut instances. The complexity of many cases and the multitude of drugs to which they were exposed might appear to be a weakness in this study, but actually represents a more unbiased representative sample of what occurs in clinical practice.

Weaknesses of the study must also be considered. Not all patients enrolled in DILIN undergo liver biopsies, and the decision to perform biopsies is made locally based upon clinical judgment and not as a part of a standardized protocol. In support of the potential for selection bias in the patients undergoing liver biopsies, the overall incidence of chronic liver injury (49%) was substantially higher in this subgroup of patients compared to the 17% rate we previously reported in 899 consecutively enrolled patients. This difference was likely due to the selection of patients with non-resolving laboratory abnormalities to undergo liver biopsies [14]. In addition, many other cases of bile duct loss and vanishing bile duct syndrome may have been enrolled in the DILIN database, but without liver biopsies such cases could not be included in this series. Furthermore, the liver biopsies subjected to central review were recuts of the original specimens, and one reason for some patients not having

identifiable bile duct loss on early biopsies may have been the limited size of the recut sample. Indeed, these data suggest that a minimum of ten portal tracts is needed to reliably exclude significant bile duct loss and possibility of ultimately developing vanishing bile duct syndrome. Another limitation of the study is that, despite assiduous efforts, follow-up of subjects was incomplete.

In summary, the finding of bile duct loss on liver biopsy during an acute liver injury has a poor prognosis, especially if the bile duct loss is moderate or severe (i.e. fewer than 50% of portal areas with an identifiable bile duct). The assessment requires an adequate biopsy specimen and careful enumeration of the number of portal tracts and the number without identifiable bile ducts. Many drugs are capable of causing bile duct loss and vanishing bile duct syndrome, but predominantly those that cause acute cholestatic or mixed hepatitis with immunoallergic features. Although not formally studied in this work, therapies, including corticosteroids and ursodiol do not appear to have major salutary effects on the course and outcome of bile duct injury. Other approaches to diagnosis and management of this potentially severe complication of cholestatic drug-induced liver injury are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Alk P alkaline phosphatase

ALT alanine aminotransferase

ANA serum anti-nuclear antibodies

AST aspartate aminotransferase

CK cytokeratin

DCRI Duke Clinical Research Institute

DILI[N] Drug Induced Liver Injury [Network]

DRESS drug rash with eosinophilia and systemic signs

HAI histology activity index

HDS herbal and dietary supplements

INR international normalized ratio

NCI National Cancer Institute

NIH National Institutes of Health

NIDDK National Institute of Diabetes and Digestive and Kidney Diseases

PA portal area[s]

R	the ratio of serum ALT/ULN for ALT divided by serum Alk P/ULN for Alk P
SMA	serum anti-smooth muscle antibodies
ULN	upper limit of normal
VBDS	vanishing bile duct syndrome

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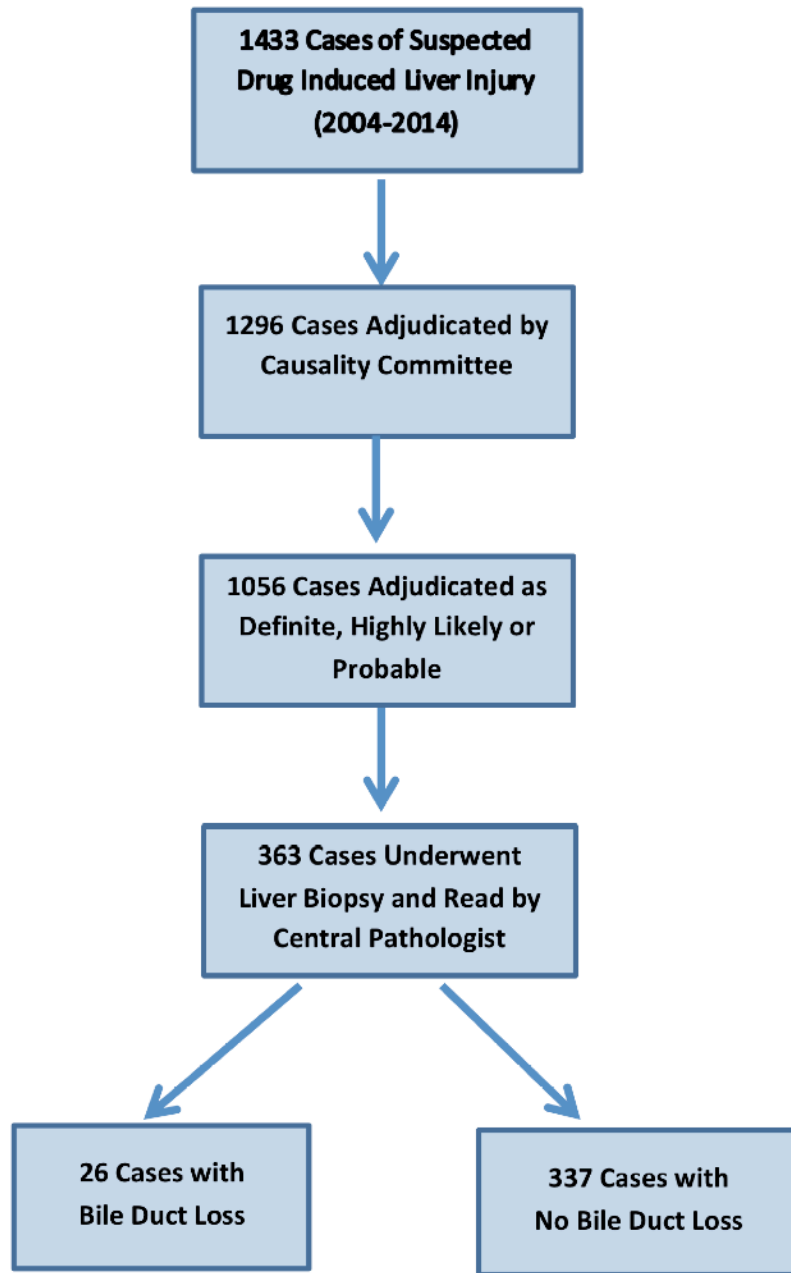
Figure 1. Summary Flow Diagram of how the Analytic Cohort was Developed
Among 1433 patients enrolled in the DILIN Prospective study between September 2004 and September 2014, 1296 underwent full causality assessment by the time of this analysis, of whom 1056 were considered definite, highly likely or probable drug induced liver injury. Among these 363 underwent liver biopsies that were available for analysis, 26 of which showed bile duct loss.
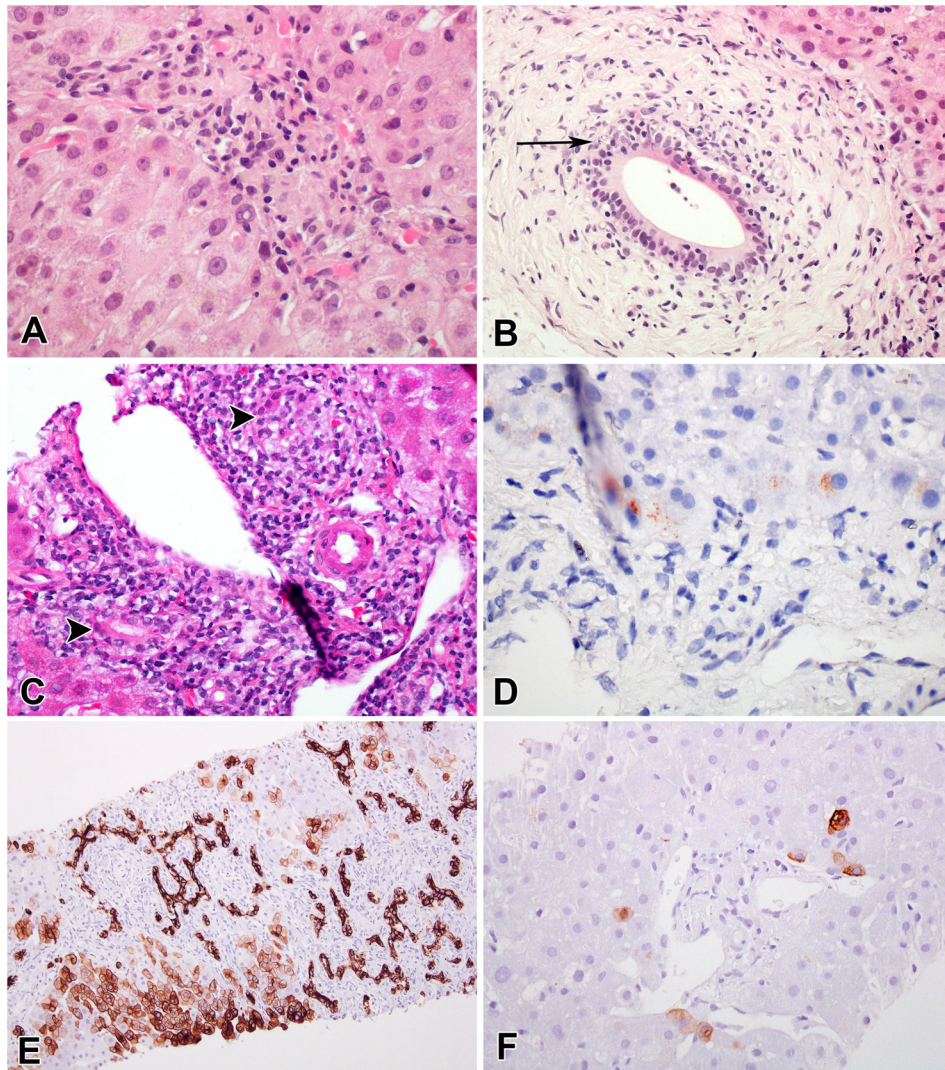
Figure 2. Representative Histopathology
A, B: Loss of bile ducts due to montelukast. A. PA infiltrated by lymphocytes and macrophages without discernible duct (H&E, 600x). B. Infiltrated PA with apoptotic cell (arrow) (H&E, 400x). C, D: Mild bile duct paucity due to traditional Chinese medicine. C. PA with a infiltrate of lymphocytes that often obscured bile ducts (arrow heads) (H&E, 400x). D. Chronic cholestasis confirmed by positive copper stain (red granules) (Copper, 600x). E, F. CK 7 staining showed extensive ductular reaction and hepatocellular CK 7 expression (E) or loss of both bile ducts and canals of Herring (F). (anti-CK 7, 200x and 400x, respectively).

Table 1

Selected Features of Subjects with Bile Duct Loss Compared to Biopsied Subjects without Duct Loss

Feature	Bile Duct Loss (n=26)	No Duct Loss (n=337)	P values
Sex (Female)	14 (54%)	201 (60%)	0.68
Race			0.16
White	20 (77%)	266 (79%)	
Black	6 (23%)	45 (13%)	
Other	0	26 (8%)	
Age* (years)	53 (11–87)	50 (8–86)	0.12
Symptoms (any)	25 (96%)	317 (94%)	1.00
Jaundice	25 (96%)	263 (78%)	0.02
Itching	20 (77%)	198 (59%)	0.10
Fatigue	11 (42%)	179 (53%)	0.31
Abdominal Pain	11 (42%)	154 (46%)	0.84
Rash	10 (39%)	87 (26%)	0.17
Fever	12 (46%)	84 (25%)	0.04
Eosinophils >500/μL	4 (15%)	43/326 (13%)	0.76
ANA--positive	5 (19%)	98/328 (30%)	0.37
SMA--positive	4/25 (16%)	77/319 (24%)	0.47
Latency* (days)	38 (3–551)	58 (1–7046)	0.05
Initial: Bilirubin* (mg/dL)	7.2 (0.2–34.1)	5.9 (0.2–32.5)	0.49
ALT* (U/L)	296 (57–1,268)	543 (6–10,000)	0.01
Alk P* (U/L)	368 (71–1,261)	215 (41–1,952)	<0.001
R ratio*	1.7 (0.6–8.0)	6.4 (0.1–100)	<0.001
Peak: Bilirubin* (mg/dL)	21.5 (0.6–59)	13.9 (0.3–55)	<0.01
ALT* (U/L)	497 (97–3,388)	713 (9–10,000)	0.17
Alk P* (U/L)	804 (357–2,414)	297 (65–2,865)	<0.001
INR*	1.6 (1.0–6.8)	1.2 (0.9–13.1)	0.11
Bilirubin peak to <2.5 mg/dL, median in days	70 (n=23)	34 (n=274)	<0.01
Corticosteroid therapy	20 (77%)	142/328 (43%)	<0.01

Feature	Bile Duct Loss (n=26)	No Duct Loss (n=337)	P values
Ursodiol therapy	16 (62%)	88/328 (27%)	<0.001
Severity Score *	3.5 (1–5)	3.0 (1–5)	0.04
Severity Score:			0.04
1 (mild)	1 (4%)	57 (17%)	
2 or 3 (moderate)	12 (46%)	168 (50%)	
4 or 5 (severe or fatal)	13 (50%)	114 (34%)	
Chronicity at 6 months	15/16 (94%)	98/209 (47%)	<0.001
Liver Transplantation ‡	2 (8%)	24 (7%)	0.71
Death, all causes ‡	7 (27%)	30 (9%)	0.01
Primary	2 (8%)	14 (4%)	
Contributory	3 (12%)	7 (2%)	
Unrelated	2 (8%)	9 (3%)	

*
= Median (range);

‡
= at any time point within 2 years of onset

Table 2

Agents Associated with Bile Duct Loss

Agent	Bile Duct Loss (n = 26)	Total Biopsied (n=363)
Amoxicillin-Clavulanate	3 (11%)	34 (9%)
HDS products [*]	3 (11%)	18 (5%)
Temozolomide	3 (11%)	4 (1%)
Azithromycin	2 (8%)	10 (3%)
Fluoroquinolones	2 (8%)	13 (4%)
Lenalidomide/Thalidomide	2 (8%)	2 (<1%)
Allopurinol	1	4 (1%)
Cefalexin	1	1
Cefazolin	1	11 (3%)
Enalapril	1	1
Infliximab	1	1
Lansoprazole	1	1
Mesalamine	1	1
Metoclopramide	1	1
Montelukast	1	1
Olanzapine	1	1
Omeprazole	1	1

[*] The names of the botanical/herbal agents were as follows: Artemisia annua, 500 mg capsules; Gluco-Ease Plus, Proprietary blend, 525, mg capsules; traditional Chinese medicine, incriminated in the third case due to HDS could not be ascertained.

Agents most frequently implicated in cases without bile duct loss, which are not in the list above, include nitrofurantoin (n=21), anabolic steroids (n=16), minocycline (n=14), isoniazid (n=8) and trimethoprim/sulfamethoxazole (n=8).

Table 3

Liver Test Abnormalities and Chronicity Severity Scores in 26 Patients with Bile Duct Loss

Time after Onset	6 months	1 Year	2 Years
Number still followed	16	13	9
Laboratory values			
Bilirubin *(mg/dL)	1.5 (0.2–35.2)	0.8 (0.3–31.6)	0.8 (0.4–19.3)
ALT *(U/L)	112 (25–483)	91 (35–318)	48 (23–169)
Alk P *(U/L)	395 (94–940)	335 (153–509)	268 (87–1560)
Chronicity score *	2.0 (0–4)	2 (1–4)	1 (1–2)
0 (n)	2	0	0
1 (n)	2	2	5
2 (n)	9	10	4
3 (n)	3	0	0
4 (n)	1	1	0

Of the initial 26 patients, seven died, two underwent liver transplant and eight were lost to follow up within two years of onset; ten before 6 months, three between 6 months and 1 year and another four between 1 and 2 years.

*
Mean and range of laboratory values and chronicity scores at each time point are given as well as the distribution of individual chronicity severe scores (0 to 4).

Table 4

Demographic, Clinical and Laboratory Features by Outcome

Feature	Benign Outcome	Poor Outcome	p values
Number	7	13	
Age [*] (years)	64(42–83)	48 (11–80)	0.08
Sex (Female)	4 (57%)	9 (69%)	0.65
Race:			0.35
White	6 (86%)	8 (62%)	
African American	1 (14%)	5 (38%)	
Symptoms			
Jaundice	6 (86%)	13 (100%)	0.35
Itching	6 (86%)	9 (69%)	0.61
Fatigue	4 (57%)	6 (46%)	1.00
Abdominal Pain	2 (29%)	7 (54%)	0.37
Rash	2 (29%)	7 (54%)	0.37
Fever	1 (14%)	9 (69%)	0.06
Time to onset (days)	39 (11–496)	32 (3–551)	0.53
Initial Laboratory results			
Bilirubin [*] (mg/dL)	11 (0.2–34.)	7.2 (0.4–20.2)	0.61
ALT [*] (U/L)	542 (57–1268)	276 (91–779)	0.55
Alk P [*] (U/L)	482 (281–1261)	366 (71–925)	0.23
R ratio [*]	1.5 (0.6–7.9)	1.8 (1.0–8.0)	0.22
ANA	2 (29%)	1 (8%)	0.27
SMA	1 (14%)	2 (15%)	1.00
Eosinophilia (>500/uL)	1 (14%)	3 (23%)	1.00
Laboratory results at biopsy [*]			
Bilirubin [*] (mg/dL)	7.5 (0.6–14.3)	18.5 (9.0–25.8)	0.04
ALT [*] (U/L)	121.0 (62–350)	297.5 (113–849)	0.11

Feature	Benign Outcome	Poor Outcome	p values
Alk P [*] (U/L)	280.0 (272–828)	746.0 (321–986)	0.04
R ratio [*]	1.0 (0.6–2.6)	1.5 (0.4–3.1)	0.51
Therapy			
Corticosteroids	6 (86%)	10 (77%)	1.00
Ursodiol	4 (57%)	7 (54%)	1.00
Liver biopsy results			
HAI Score (0–18) [*]	7.0 (3–9)	4.5 (3–5)	0.23
Fibrosis Score (0–6) [*]	0 (0–2)	0 (0–1)	0.56
Bile Duct Injury Score (0–2) [*]	2.0 (0–2)	2.0 (1–2)	0.67
Bile Duct Loss score (0–2) [*]	1 (1–1)	2 (1–2)	<0.001
Mod-severe bile duct loss [*]	0 (0%)	12 (92%)	<0.001
Portal areas (PA) (n) [*]	14.0 (7–21)	9 (6–18)	0.30
Percent PA with Bile Ducts [*]	64% (43%–75%)	17% (0%–50%)	0.003

Abbreviations: Alk P, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibody; HAI, histology activity index; PA, portal areas; SMA, smooth muscle antibody

[*] For those with biopsy done within 3 months of onset (benign =6, poor = 9)

Poor outcome is defined as death with liver injury the primary or a contributory cause, liver transplantation or persistent evidence of at least moderate liver injury at the time of the last visit. Primary implicated agents in subjects with poor outcomes: azithromycin in 2, herbals in 2, thalidomide/lenalidomide in 2; and one each for infliximab, lamotrigine, olanzapine, metoclopramide, montelukast, moxifloxacin, olanzapine and temozolomide.

Benign outcome is defined as evidence of no or only mild liver injury at the time of the last visit at least 6 months after onset (includes patients who died of unrelated causes). Primary implicated agents: one case each for amoxicillin/clavulanate, enalapril, herbals, lansoprazole, mesalamine, omeprazole and temozolomide.