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

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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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Two Sentence Summary: Bile duct loss during the course of drug induced liver injury is uncommon but can be an indication of vanishing bile duct syndrome. Bile duct loss during acute cholestatic hepatitis is an ominous early indicator of possible vanishing bile duct syndrome, and the severity of the loss is the best predictor of eventual adverse outcome..

Footnote Page

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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.

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Comment [HJ([1]: This is given above and I think that all of page 19-21 should be moved to the supplementary material or actually deleted and replaced by a reference where these are given such as 13, 14, 15 or 16. Another possibility is just to reference the DILIN website for a complete list of investigators. Note that these data are given yet again in Supplementary Table 1.

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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
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

Comment [HB2]: Jay: Please revise as you think best in light of Editors' suggestions.

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
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.

Titles and Legends for Figures

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).

Figure 1

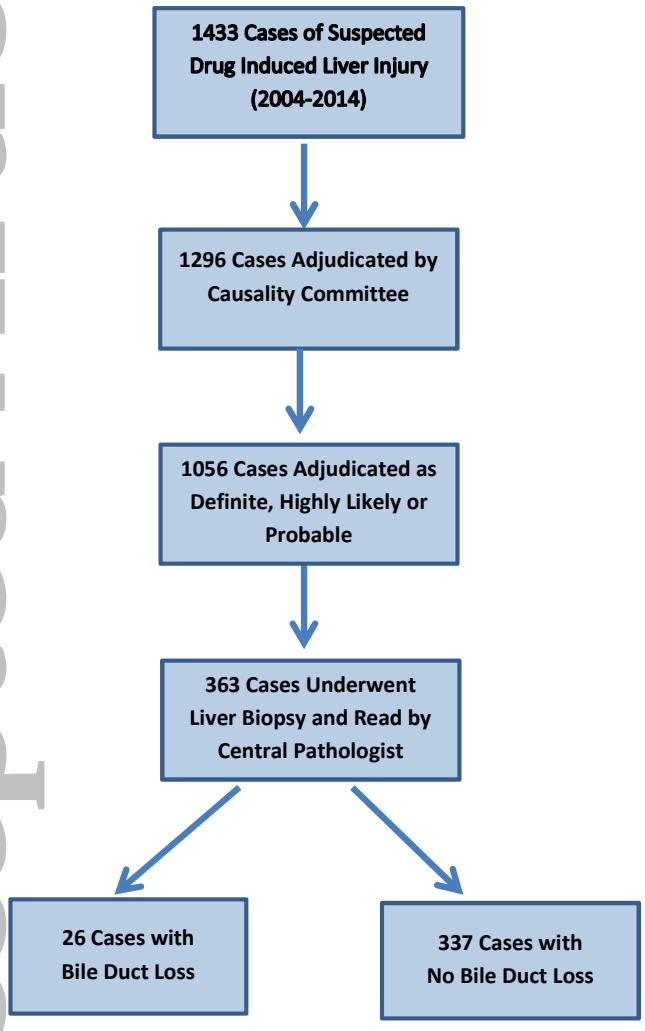


Figure 2:

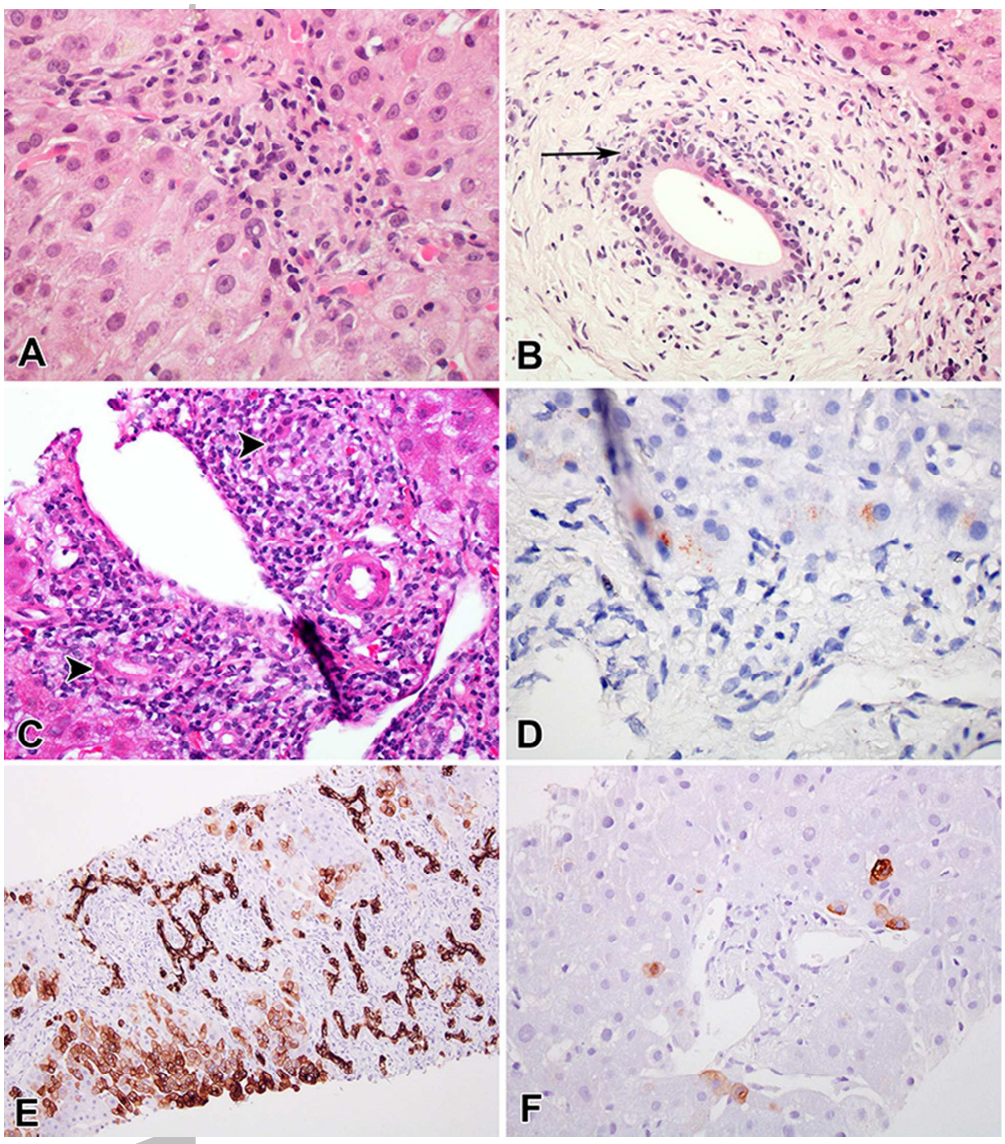
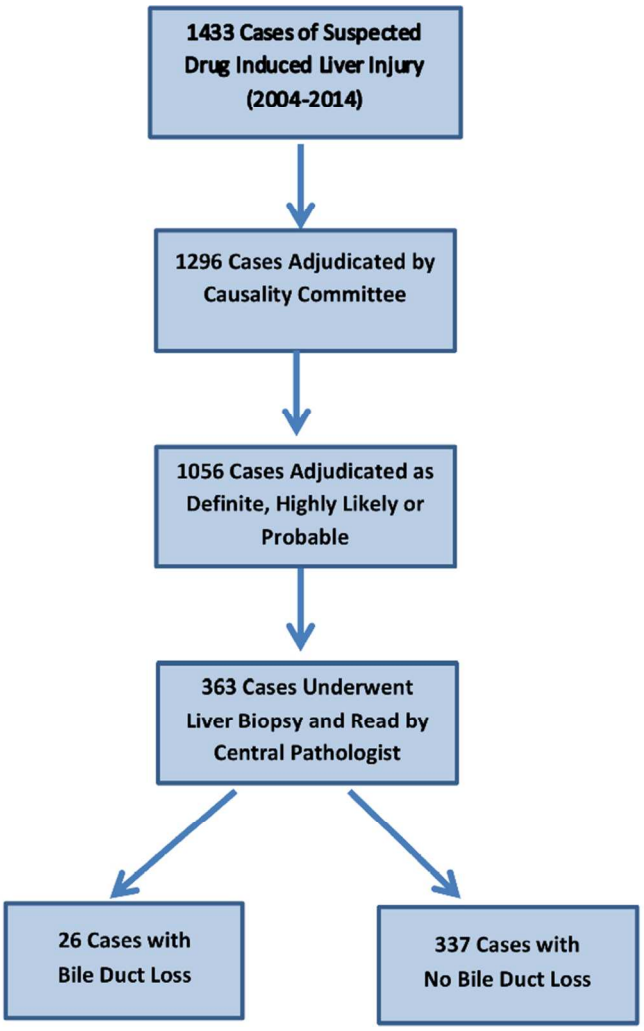
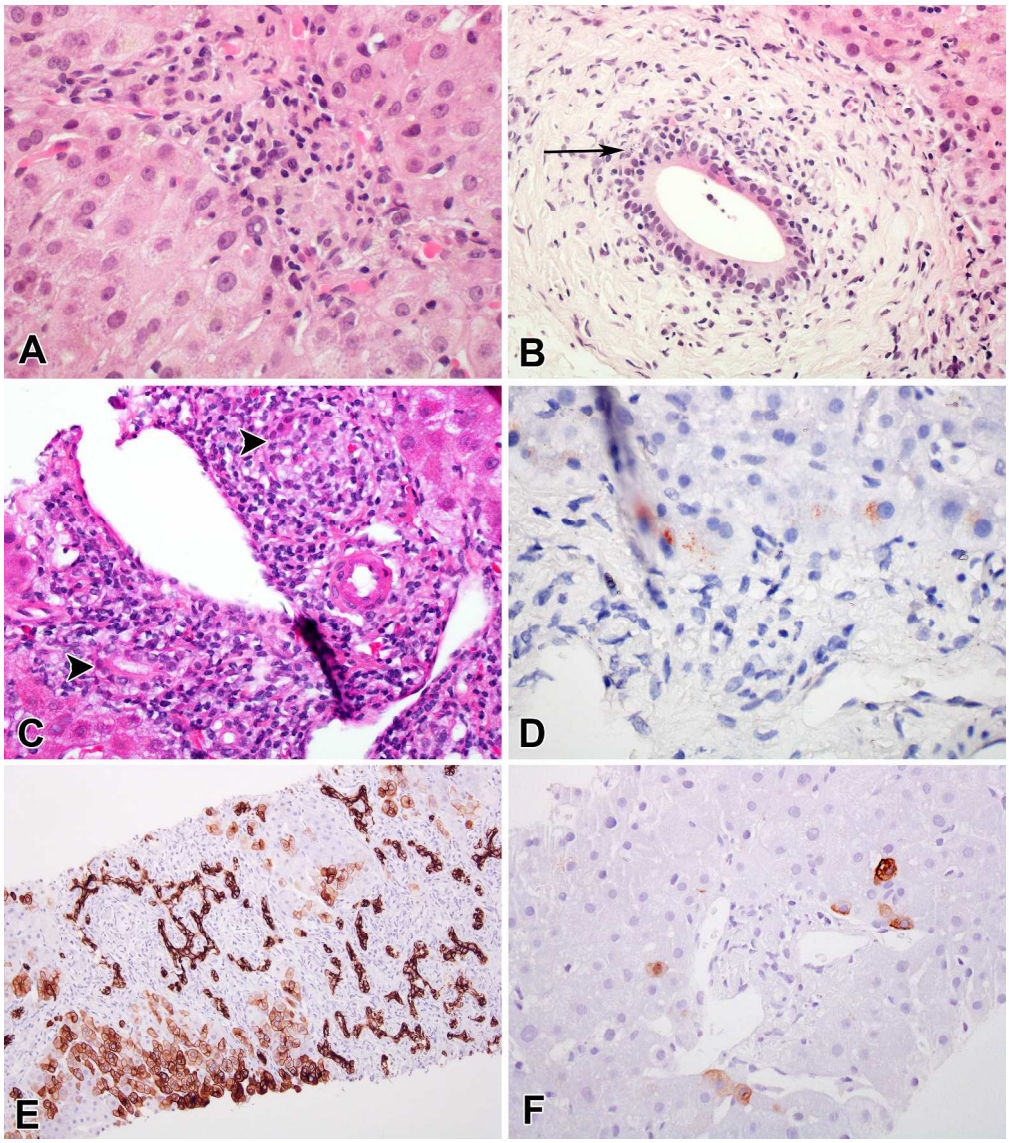


Figure 1





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Suppl Table 1

Clinical Centers Participating in DILIN

University of Connecticut Health Center [2003-2008]: Drs. Herbert Bonkovsky (PI) and James Freston, Robert Rosson, and George Wu

University of North Carolina Chapel Hill [2003-2016]: Drs. Paul Watkins (PI) and Paul Hayashi.

University of Michigan Ann Arbor [2003-2016]: Drs. Robert Fontana (PI), Hari Conjeevaram and Richard Moseley.

University of California, San Francisco, California Pacific Medical Center [2003-2013]: Drs. Timothy Davern (PI), and Mauricio Bonacini

Indiana University-Purdue [2003-2016]: Drs. Naga Chalasani (PI), Raj Vuppalanchi, Marwan Ghabril

Icahn School of Medicine at Mount Sinai [2013-2016]: Drs. Joseph A. Odin (PI), Jawad Ahmad (co-PI),

Mayo Clinic College of Medicine [2009-2013]: Dr. Jay Talwalkar (PI).

University of Pennsylvania [2009-2013]: Dr. Raj Reddy (PI).

University of Southern California and University of California-Los Angeles [2009-2016]: Drs. Andrew Stolz (PI), Neil Kaplowitz, Francisco Durazo

Albert Einstein Medical Center and University of Pennsylvania [2008-2016]: Dr. Vic Navarro (PI), Raj Reddy.

University of Texas-Southwestern [2008-2013]: Drs. William Lee and Lafaine Grant.

Suppl Table 2

Scoring System for Severity of Chronicity

Score	Description	ALT	Alk P	Total Bilirubin	INR
0	None	Normal	Normal	Normal [≤1.2 mg/dL]	< 1.5 [or missing]
1	Mild	ALT 1-3 x ULN or Alk P 1-2 x ULN or total Bilirubin 1.3-2.4 mg/dL [Any one or several of the above]*			< 1.5 [or missing]
2	Moderate	ALT > 3 x ULN or Alk P > 2 x ULN		< 2.5 mg/dL	< 1.5 [or missing]
3	Moderate-severe	ALT > 1 x ULN or Alk P > 1 x ULN		≥ 2.5 mg/dL	< 1.5
4	Severe	ALT > 1 x ULN or Alk P > 1 x ULN		≥ 2.5 mg/dL	≥ 1.5 **

Chronicity is scored at 6, 12 and 24 months using the results closest to the dates that are 182, 365 and 730 days after laboratory defined onset of DILI. The allowable range of dates (windows) is 182-300 days for 6

months, 300-600 days for 12 months and 600-900 days for 24 months. Dates for the 6 month specimen are preferred to be after 182 days, but if none are available before day 300, an earlier date after 160 days can be used. Missing values for INR can be replaced by values obtained within 60 days of the sample. Results that do not fit any of the patterns below or that have missing essential results are flagged and determined "manually" by a DILIN investigator after re-review of the case with all the laboratory results. Scores that still cannot be resolved and all cases without laboratory results beyond 160 days of onset are considered "unknown".

* Cases that are assigned a score of "mild" based upon bilirubin levels alone or normal ALT and Alk P but bilirubin ≥ 2.5 mg/dL require manual review by a DILIN investigator to verify that the bilirubin elevation is due to liver injury (and not hemolysis, Gilbert's syndrome, sepsis, and so forth).

** or other symptoms, signs or evidence of hepatic failure (ascites, encephalopathy)

Suppl Table 3: Demographic, Clinical and Laboratory Features of Subjects who Underwent Liver Biopsies with Initial R Values ≤ 8 with VBDS [n =26] vs those without VBDS [n= 193]

Feature	Bile Duct Loss (n=26)	No Duct Loss (n=193)	P values
Sex (Female)	14 (54%)	109 (57%)	0.84
Race			0.10
White	20 (77%)	160(83%)	
Black	6 (23%)	20(10%)	
Other	0	13 (7%)	
Age* (years)	54 (11-87)	53 (14-86)	0.49
Symptoms (any)	25 (96%)	184 (95%)	1.00
Jaundice	25 (96%)	158 (82%)	0.09
Itching	20 (77%)	125 (65%)	0.27
Fatigue	11 (42%)	99 (51%)	0.41
Abdominal Pain	11 (42%)	83 (43%)	1.0
Rash	10 (39%)	56 (29%)	0.36
Fever	12 (46%)	60 (31%)	0.18
Eosinophils >500/µL	4 (15%)	31/190 (16%)	1.00
ANA	5 (19%)	49/191 (26%)	0.63
SMA	4/25 (16%)	35/183 (19%)	1.00

Latency* (days)	38 (3-551)	40 (1-7046)	0.32
Initial: Bilirubin* (mg/dL)	7.2 (0.2-34.1)	5.6 (0.2-32.5)	0.40
ALT* (U/L)	296 (57-1,268)	276(6-3658)	0.61
Alk P* (U/L)	368 (71-1,1261)	273 (52-1952)	0.02
R ratio*	1.7 (0.6-8.0)	2.8 (0.1-7.9)	0.38
Peak: Bilirubin* (mg/dL)	21.5 (0.6-59)	13.9 (0.4-55.0)	0.003
ALT* (U/L)	497 (97-3,388)	381 (9.0-5831)	0.11
Alk P* (U/L)	804 (357-2,414)	379 (95-2865)	<0.001
INR*	1.6 (1.0-6.8)	1.2 (0.9-12.9)	0.032
Bilirubin peak to <2.5 mg/dL (median in days)	70 (n=23)	36 (n=165)	0.005
Corticosteroid therapy	20 (77%)	74/189 (39%)	<0.001
Ursodiol therapy	16 (62%)	63/189 (33%)	0.008
Severity Score *	3.5 (1-5)	3.0 (2-4)	0.02
Severity Score:			0.02
1 (mild)	1 (4%)	25 (13%)	
2 or 3 (moderate)	12 (46%)	112 (58%)	
4 or 5 (severe or fatal)	13 (50%)	56 (29%)	
Chronicity at 6 months	15/16 (94%)	59/125 (47%)	<0.001
Liver Transplantation ‡	2 (8%)	9 (5%)	0.62

Death, all causes ‡	7 (27%)	18 (9%)	0.02
Primary	2 (8%)	8 (4%)	
Contributory	3 (12%)	5 (3%)	
Unrelated	2 (8%)	5 (3%)	

Suppl Table 4

Results of early biopsies that did not show bile duct loss in patients in whom later biopsies did show duct loss compared to those of patients that showed bile duct loss on early biopsies

Feature	Early Biopsy Shows No Duct Loss n=6	Early Biopsy Shows Duct Loss n=19	p value
Time after onset to liver biopsy (days)	14 (5-88)	11 (2-377)	0.68
Histology Activity Index* (0-18)	4.0 (2-12)	5 (3-9)	0.52
Fibrosis score* (0-6)	2 (1-4)	1 (0-4)	0.03
Copper score (0-2)	0 (0-1)	0 (0-2)	0.38
Copper positivity (1 or 2) number (%)	1 (17%)	6/17 (35%)	0.62
Bile Duct Injury score (0-2)	0.5 (0-2)	2 (0-2)	0.10
Bile duct injury (1 or 2) number (%)	3 (50%)	16/17 (94%)	0.04

Number of Portal Areas seen	7 (4-13)	14 (7-28)	0.002
Number of Portal Areas with ducts	3 (3-9)	3 (0-15)	0.97
Fraction of Portal Areas with bile ducts (%)	65% (15%-75%)	40% (0%-75%)	0.14

Data given are median values (ranges).

Suppl Material: Detailed Overview of DILIN

The Network has consisted of multiple clinical centers, a central data coordinating center and a sample and data repository maintained by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The principal investigators and locations of the clinical centers have varied over the course of the study and are provided in **Supplementary Table 1**. The Duke Clinical Research Institute (DCRI: Durham, NC) has served as the data coordinating center since the inception of DILIN. The DILIN Prospective Protocol allowed the clinical centers to enroll all patients with liver injury suspected to be due to drugs or herbal and dietary supplements (HDS) seen at their institutions within six months of onset who agreed to participate. Extensive demographic, clinical, laboratory, imaging and histologic material was retrieved from the patients' medical records, and a medical histories and physical examinations were performed at the time of enrollment. In addition, serum, plasma and whole blood were taken to perform laboratory tests that might not have been done initially and to provide samples stored at the NIDDK repository. Patients were asked to return in six months and, if liver tests, physical examination or imaging studies remained abnormal, again at 12 and 24 months after enrollment. All data were entered into an electronic database maintained at the DCRI. A more nearly complete description of the Prospective Protocol has been published[13] and the full text is available on the DILIN public website at: www.DILIN.org.

All details of the Prospective Protocol and consent forms were approved by local Institutional Review Boards as well as by a central Data Safety Monitoring Board established for this study by the NIDDK. All patients provided written informed consent.

Suppl Material: List of Representative Case Summaries.

Case 1: 48 year old Hispanic woman treated with cephalexin, lamotrigine and moxifloxacin developed DRESS syndrome followed by severe progressive cholestatic liver injury resulting in death 71 days after onset 104-0054 [8], Cefalexin.

Case 2: 47 year old African American woman exposed to moxifloxacin developed cholestatic hepatitis followed by progressive cholestasis leading to liver transplantation 13 months after onset. 104-0121 [10], Moxifloxacin

Case 3: 19 year old Caucasian woman exposed to olanzapine and azithromycin developed fever, rash and cholestatic hepatitis with prolonged course and persistent liver test abnormalities for 2 years. 109-0004 [21], Olanzapine

Case 4: 20 year old African American woman developed toxic epidermal necrolysis and cholestatic hepatitis after exposure to azithromycin and still had symptoms, disability and abnormal liver tests 2 years later. 104-0123 [11], Azithromycin

Case 5: 56 year old African American man developed cholestatic hepatitis after use of Gluco-Ease plus and still had pruritus and abnormal liver tests two years later. 109-0017 [24], Gluco-Ease Plus

Case 6: 81 year old Caucasian man developed cholestatic hepatitis shortly after 10 day course of amoxicillin/clavulanate which resolved completely within 6 months. 102-0094 [3], Amoxicillin/Clavulanate

Case 7: 66 year old Caucasian woman developed jaundice while taking multiple herbal medications and mixtures of supplements with jaundice resolving within 3 months but prolonged liver tests abnormalities that did not resolve completely until two years after onset. 108-0061 [20], HDS

Clinical Summaries

Clinical Summary: Case #1
Cefalexin/Lamotrigine/Moxifloxacin
Vanishing Bile Duct Syndrome/DRESS

A 48 year old Hispanic woman had a long history of bipolar illness, depression and multiple suicide attempts. After an admission for another suicide attempt by overdosage with lorazepam, levothyroxine, dextropropoxyphene, acetaminophen, and venlafaxine, she was switched to a regimen of escitalopram, aripiprazole and lamotrigine (25 mg twice daily). During that admission for overdose, her liver tests were completely normal. After discharge she was treated with cefalexin for a superficial foot infection and was later given moxifloxacin. Approximately one month after starting lamotrigine, two weeks after starting cefalexin (and 6 days after stopping it), and 8 days after starting moxifloxacin (which she took for 4 days), she developed a diffuse erythematous rash and fever. The rash spread and was pruritic and slightly painful, and she was hospitalized for management and observation. On admission, she had fever and rash, but was not jaundiced. The serum bilirubin was 0.4 mg/dL, ALT 316 U/L [4.9 times ULN], AST 359 U/L [9 times ULN], alkaline phosphatase 108 U/L [normal], albumin 3.0 g/dL and INR 1.1. Over the next few days the liver enzymes worsened and she became jaundiced. While the cefalexin and moxifloxacin had been stopped, lamotrigine and her other psychotropic agents were continued. She had no previous history of liver disease or risk factors for viral hepatitis. She did not drink alcohol but had a history of drug rash in response to penicillin. Tests for hepatitis A, B, C and E were negative as were ANA and SMA. Ultrasound showed no evidence of biliary obstruction although she did have gallstones and a contracted gallbladder. She was treated with corticosteroids and eventually discharged, but returned to the hospital several days later with worsening rash and fever. At this point, lamotrigine and aripiprazole were stopped. The serum bilirubin had risen to 15.5 mg/dL, ALT 603 U/L, alkaline phosphatase 1008 U/L [R ratio = 1.8], and INR 1.1. A liver biopsy (done a month after initial presentation and when bilirubin reached 26.3 mg/dL) showed severe cholestatic hepatitis and paucity of bile ducts compatible with vanishing bile duct syndrome (among 6 portal areas none had identifiable bile ducts: duct fraction = 0%). She was treated with prednisone and ursodiol but became increasingly jaundiced and INR rose to 2.6. She was not considered a liver transplant candidate and was discharged to hospice care where she died two and a half months after initial presentation. This case was reviewed and judged to be highly likely drug induced liver injury but there was disagreement regarding the responsible agent: cefalexin was considered the probable cause, lamotrigine possible and moxifloxacin unlikely (RUCAM scores 6 to 7). The skin manifestations and fever were considered compatible with DRESS syndrome. The severity was scored as 5+ (fatal). The cause of death was considered hepatic failure due to vanishing bile duct syndrome.

Serial Laboratory Results (selected)

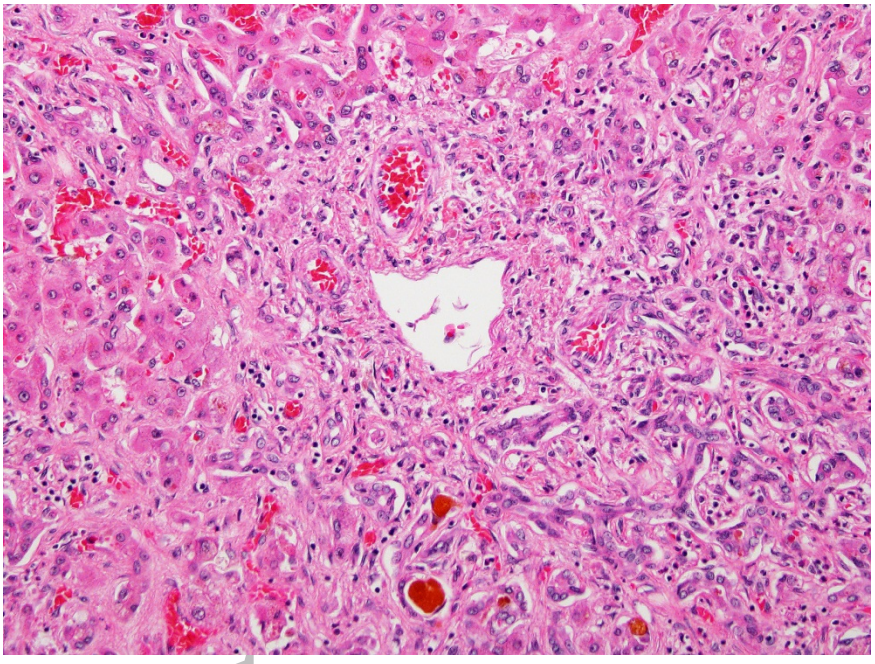
Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
5/2/2006	-34	28	102	0.1	Normal values
5/8/2006	-28				Lamotrigine started
5/22/2006	-13				Cefalexin started [7 days]
6/4/2006	-1				Onset of rash and fever
6/5/2006	0	316	108	0.4	Hospital admission, R = 6.1
6/6/2006	1	737	153	0.4	
6/7/2006	2	1505	313	1.4	
6/8/2006	3	1808	400	3.1	INR 1.2
6/13/2006	8	794	839	9.0	
6/15/2006	10	603	1008	15.5	Lamotrigine stopped, R = 1.8
6/20/2006	15	621	1123	17.9	
6/23/2006	18	488	1428	20.8	INR 1.5
7/5/2006	30	264	1493	28.0	R = 0.6
7/12/2006	37	337	2101	29.0	Liver biopsy: bile duct loss
7/17/2006	42	173	1429	36.1	
7/19/2006	44	128	1817	44.5	INR 2.2
7/24/2006	49	165	1948	48.6	Discharged to hospice care
8/6/2006	62	268	1737	56.6	
8/15/2006	71				Death
Normal values		**< 65**	**< 135**	**< 1.2**	

Summary Table

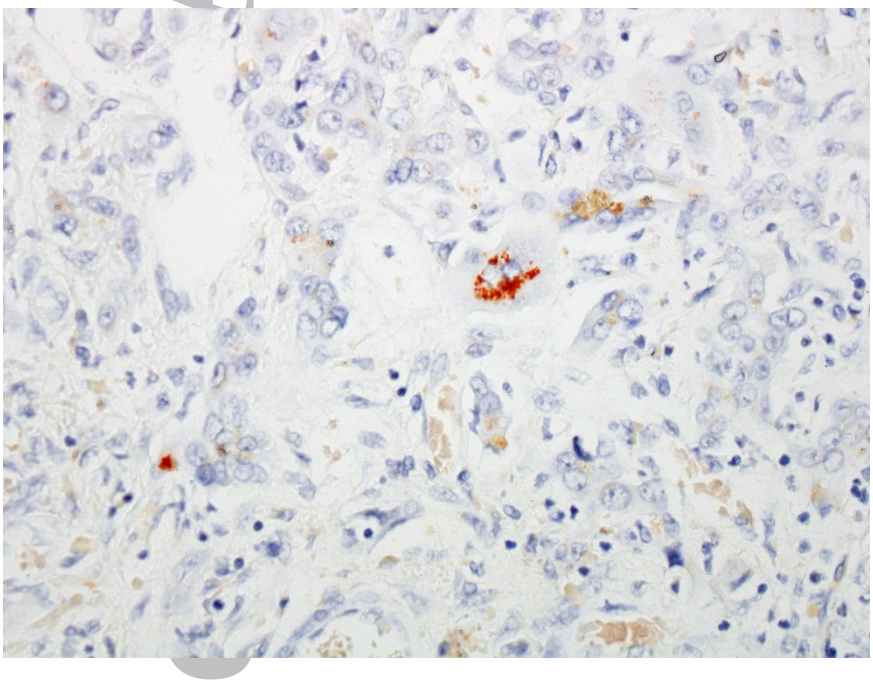
Feature	Result
Implicated Agent(s)	Lamotrigine, cefalexin, moxifloxacin
Time to onset	28 days, 14 days, 8 days
Enzyme pattern	Initially hepatocellular (R=6.1), later cholestatic (R<1.0)
HEV testing	Anti-HEV IgG and IgM neg
HCV testing	Anti-HCV and HCV RNA neg
Hospitalization	Yes
Prednisone	Yes
Ursodiol	Yes
Chronicity	Unknown, died within 3 months
Severity	5+ (Fatal)
Causality	Overall 2 (Highly likely), lamotrigine 4 (possible), cefalexin 3 (probable), moxifloxacin 5 (unlikely)
RUCAM	7 (Probable)
Concomitant medications	17 (meloxicam, aripiprazole, escitalopram)
Comment	Died of liver failure 71 days after onset

Clinical Summary: Case #2: Moxifloxacin
Vanishing Bile Duct Syndrome

A 47 year old African American woman with mild diabetes was hospitalized for 2 days with suspected pneumonia and treated with intravenous followed by oral moxifloxacin. She began to feel ill within days of starting the antibiotic and stopped it early. She then developed dark urine, pruritus and jaundice and sought medical attention again. She had no previous history of liver disease, alcohol abuse, risk factors for viral hepatitis or drug allergies. Her other medical conditions (and medications) included type 2 diabetes (metformin, fish oil) and allergic rhinitis (cetirizine, acetaminophen). On presentation 8 days after starting and 4 days after stopping moxifloxacin, she was jaundiced and had a mild rash. Laboratory tests showed a total bilirubin of 7.2 mg/dL, ALT 266 U/L (6.7 times ULN), AST 131 U/L (3.7 times ULN) and alkaline phosphatase 392 U/L (3.9 times ULN) yielding an R ratio of 1.7 (cholestatic). Serum albumin was 4.4 g/dL and INR 1.0. There was a slight eosinophilia (6%). Tests for hepatitis A, B, C and E were negative (she had IgG anti-HEV, but no IgM reactivity). Both ANA and SMA were negative and imaging of the liver by ultrasound and CT scan showed no evidence of biliary obstruction. She was monitored on no therapy but jaundice deepened and worsened. She subsequently remained jaundiced and was disabled from fatigue and weakness. Eleven months after onset of the liver disease, jaundice began to worsen with serum bilirubin rising to 33.3 mg/dL and she underwent successful liver transplantation 13 months after initial presentation. The explant showed chronic cholestatic liver injury with marked cholestasis and mild portal and lobular inflammation. There was profound ductopenia: in 20 consecutive portal areas, none had bile ducts (bile duct fraction = 0%). Even the largest portal areas lacked ducts. There was marked ductular reaction. This case was scored as severe (4+, jaundice, hospitalization and prolongation of INR). The causality score was 2 (highly likely) and RUCAM score was 6 (probable). The abrupt onset, immunoallergic features, severe cholestatic hepatitis and persistent cholestatic injury are typical of drug-induced vanishing bile duct syndrome, most likely due to moxifloxacin hepatotoxicity. The patient was "case 3" in the publication: Orman ES, Conjeevaram HS, Vuppalanchi R, Feston JW, Rochon J, Kleiner DE, Hayashi PH for the DILIN Research Group. Clinical and histological features of fluoroquinolone-induced liver injury. Clin Gastroenterol Hepatol 2011; 9: 517-523.



Photomicrograph showing portal area in explant liver lacking a bile duct. (H&E, 200x)



Copper stain with strong positive reaction in hepatocytes near portal area (Copper, 400x)

Serial Laboratory Results (Selective)

Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
6/25/2008	-8	62	99	0.7	Moxifloxacin started
6/29/2008	-4				Moxifloxacin stopped

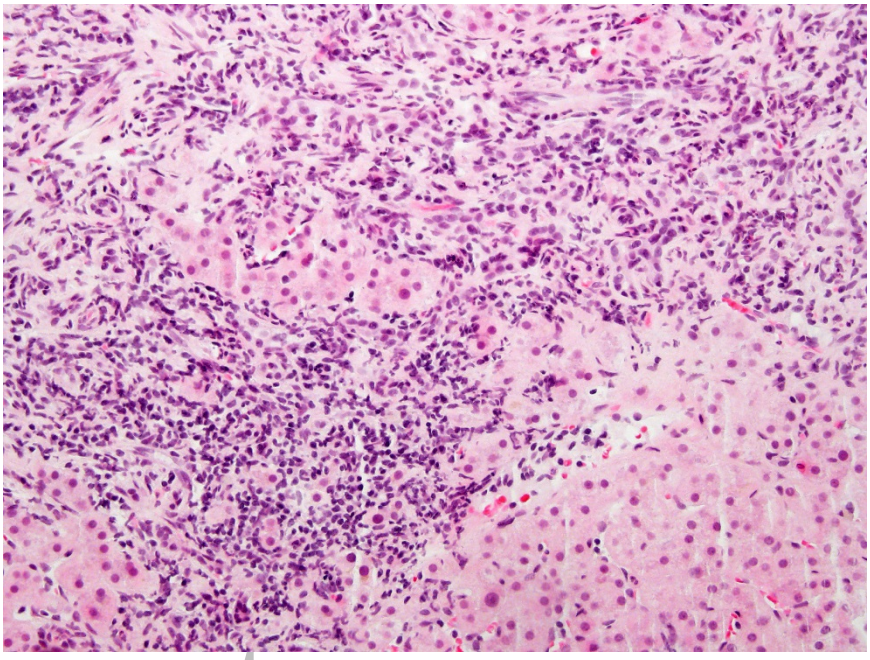
Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
7/3/2008	0	266	392	7.2	Symptom Onset, R = 1.7
7/5/2008	2	173	278	6.0	
7/9/2008	6	206	356	8.4	
7/14/2008	11	252	366	9.8	Liver Biopsy: Bile duct loss
7/28/2008	25	250	326	13.5	
8/4/2008	32	257	330	15.1	
8/19/2008	47	158	310	13.9	
9/3/2008	62	118	209	11.9	
9/18/2008	77	120	395	12.4	
0/14/2008	103	343	450	11.5	
11/5/2008	125	293	513	13.8	
3/13/2009	253	60	357	13.8	6 mo chronicity score = 4
4/22/2009	293	93	224	13.6	INR 2.2
5/28/2009	329	38	281	22.8	
6/15/2009	347	62	198	26.0	
7/14/2009	376	23	113	33.3	1 year chronicity score = 4
7/15/2009	377				Liver transplant
Normal Values		**<40**	**< 125**	**< 1.2**	

Summary Table

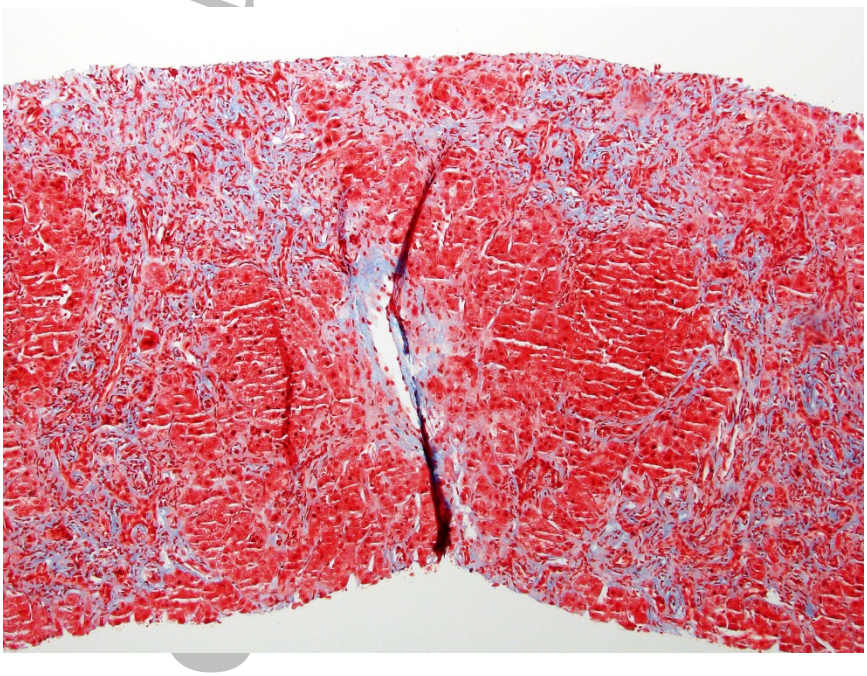
Feature	Result
Implicated Agent(s)	Moxifloxacin
Time to onset	8 days
Enzyme pattern	Cholestatic (1.7)
HEV testing	Anti-HEV IgG pos, IgM neg
HCV testing	Anti-HCV and HCV RNA neg
Hospitalization	Yes
Prednisone	Yes
Ursodiol	Not mentioned
Chronicity	Yes, chronicity score = 4+ at 6 mo and 1 year
Severity	4+ (Severe, jaundiced, liver failure)
Causality	2 (Highly likely)
RUCAM	6 (Probable)
Concomitant medications	4 (Metformin, cetirizine)
Comment	Liver transplantation 1 year after onset

Clinical Summary: Case #3
Olanzapine/Azithromycin:
DRESS Syndrome: Bile Duct Loss

A 19 year old Caucasian woman developed maculopapular rash followed by fever within 3 weeks of starting olanzapine (25 to 30 mg daily) for acute psychosis. She also complained of shortness of breath and eosinophilia and was thought to have pneumonia. She was treated with azithromycin (250 mg daily) but returned two days later with worsening symptoms, fatigue, nausea, itching and a maculopapular rash at which point olanzapine and azithromycin were stopped. She had no previous history of liver disease, alcohol abuse, risk factors for viral hepatitis or drug allergies. She had a fairly recent onset of psychosis and mania and had received multiple drugs in the previous two months including aripiprazole, clonazepam, haloperidol, lithium, lorazepam, oxcarbazepine, quetiapine and risperidone. Other medications included ibuprofen, acetaminophen, benzatropine, ceftriaxone, dexamethasone, enoxaparin, and senna. Laboratory testing initially showed a serum bilirubin of 1.2 mg/dL, ALT 255 U/L [3.6 times ULN], AST 194 U/L [4.9 times ULN] and alkaline phosphatase 306 [2.3 times ULN]. The R ratio was 1.6 (cholestatic). She was admitted for evaluation and despite discontinuation of olanzapine and azithromycin, serum bilirubin levels continued to rise and peaked at 54.8 mg/dL three weeks later. Serum INR rose to 1.7 but then fell into the normal range. Tests for hepatitis A, B, C and E were negative as was ANA while SMA was weakly positive (titer not available). Imaging of the liver showed hepatomegaly without evidence of gallstones or biliary obstruction. A liver biopsy done 20 days after onset showed mild cholestatic hepatitis. Only 3 portal areas were present in the biopsy sample available for review and all 3 had identifiable bile ducts. There was no fibrosis and no ductular reaction. A second biopsy was performed 107 days after onset showed severe cholestasis, but the recuts available for review lacked any portal areas. She received no specific therapy and remained jaundiced for 6 months, but serum bilirubin ultimately fell into the normal range. In follow up, she continued to have mild symptoms of fatigue and itching and serum enzyme levels remained moderately elevated. A repeat liver biopsy done almost 2 years after onset showed chronic cholestatic liver injury with moderate inflammation and bile duct injury with bile duct loss; among 6 portal areas in the biopsy sample, only 2 had identifiable bile ducts (bile duct fraction = 33%). Marked ductular reaction was present spanning between portal areas and accompanied by bridging fibrosis. The case was reviewed and considered to be very likely drug induced liver injury, probably due to olanzapine. The RUCAM score was 6 (probable). Azithromycin was considered only possibly responsible. The use of azithromycin and recent receipt of other psychotropic, potentially hepatotoxic agents, made more definitive assessment of causality difficult. The severity score was 4+ (severe). The initial clinical presentation was typical of DRESS syndrome. Inadequate sampling may account for the failure to observe bile duct loss on the first two biopsies.



Marked ductular reaction and focally severe inflammation in the portal tracts in the third biopsy (200x)



Masson stain showing bridging fibrosis associated with ductular reaction.

Serial Laboratory Results

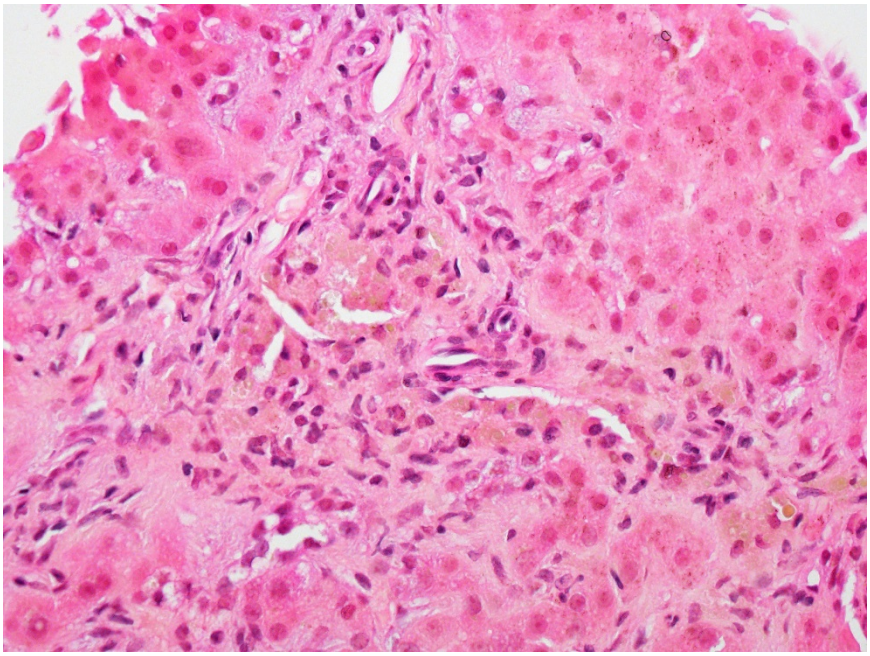
Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
1/21/2009	-21				Olanzapine started
1/22/2009	-20	37	71	0.2	Normal
2/7/2009	-4				Onset of symptoms
2/9/2009	-2				Azithromycin started
2/11/2009	0	255	306	1.2	Both drugs stopped
2/13/2009	2	237	317	2.0	CT scan
2/15/2009	4	275	517	5.0	
2/18/2009	7	268	655	7.8	
2/24/2009	13	184	384	18.1	
2/28/2009	17	220	433	25.8	
3/3/2009	20	339	547	35.5	Liver biopsy: cholestasis
3/10/2009	27	152	171	32.6	
3/31/2009	48	143	236	23.0	
4/13/2009	61	201	327	13.5	
4/27/2009	75	288	505	2.2	
5/29/2009	107	180	821	21.0	
6/19/2009	128	181	794	14.1	6 month chronicity score = 3
7/15/2009	154	110	639	9.3	
9/10/2009	211	282	569	2.7	
1/30/2009	292	172	441	1.3	
2/11/2010	365	214	441	0.9	1 year chronicity score = 2
8/17/2010	552	223	424	0.4	
1/24/2010	651	154	264	0.4	Liver biopsy: bile duct loss
3/24/2011	771	169	268	0.4	2 year chronicity score = 2
Normal Values		**< 35**	**< 135**	**< 1.2**	

Summary Table

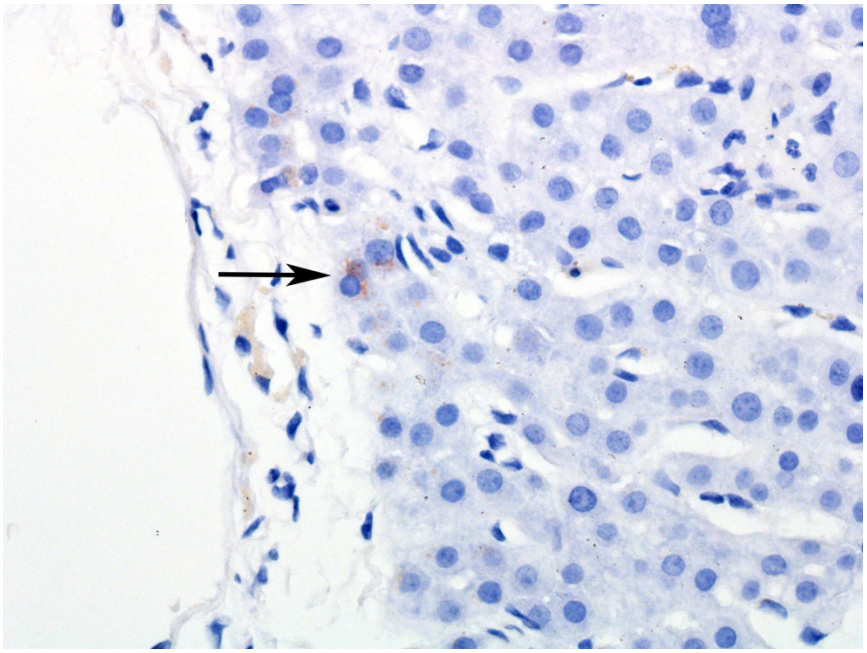
Feature	Result
Implicated Agent(s)	Olanzapine, Azithromycin
Time to onset	21 days, 2 days
Enzyme pattern	Cholestatic (R ratio = 1.6)
HEV testing	Anti-HEV IgG neg
HCV testing	Anti-HCV neg, HCV RNA neg
Hospitalization	Yes
Prednisone	No
Ursodiol	Not mentioned
Chronicity	Yes, chronicity score 3+ at 6 mo, 2+ at 1 and 2 yrs
Severity	4+ (Jaundiced, hospitalized, INR above 1.5)
Causality	Overall 2 (Highly likely), Olanzapine 3 (Probable), Azithromycin 4 (Possible).
RUCAM	6 (Probable)
Concomitant Medications	17 (Oxcarbazepine, Risperidone, Ceftriaxone)
Comment	DRESS, VBDS, prolonged cholestasis

Clinical Summary: Case #4 Azithromycin/Ibuprofen: VBDS, Toxic Epidermal Necrolysis

A 20 year old previously healthy, African-American college student developed sore throat, fever and cough and was given ibuprofen and later azithromycin without improvement whereupon she rapidly developed worsening throat pain, painful swallowing, progressively generalized rash, fever and jaundice. She was dehydrated and had blistering lesions over her body including mouth and vagina. She was hospitalized and both ibuprofen (6 days) and azithromycin (2 days) were stopped. She had no history of liver disease, alcohol abuse, risk factors for viral hepatitis or drug allergies. She was taking no other medications or herbal preparations. Physical examination was marked by jaundice, skin rash, fever, liver enlargement and splenomegaly. Initial laboratory results showed serum bilirubin 3.7 mg/dL, 140 U/L [4 times ULN], AST 269 U/L [9 times ULN], alkaline phosphatase 71 U/L [0.5 times ULN] and albumin 4 g/dL. Skin biopsies were consistent with toxic epidermal necrolysis. She became progressively more jaundiced and was transferred to an intensive care unit where she developed respiratory failure and required intubation. Tests for hepatitis A, B, C and E were negative as were tests for Ebstein Barr Virus, cytomegalovirus and herpes simplex infections. She received intravenous immunoglobulin and multiple antibiotics. She was positive for ANA but negative for SMA, double stranded DNA and anti-cardiolipin. Her ICU stay was complicated by pneumothorax, respiratory and cardiac arrest and she was treated with multiple courses of antibiotics and high doses of corticosteroids. She became intensely jaundiced and had persistent elevations in serum aminotransferase levels and alkaline phosphatase. Eventually, she recovered enough to leave the ICU and then the hospital but was slow to improve and still had liver test abnormalities when seen 6, 12 and 24 months after onset. An initial liver biopsy on day 37 (when serum bilirubin was 17.3 mg/dL) showed a mild acute hepatitis without cholestasis. The bile ducts were considered adequate and did not show injury. On re-review, bile ducts were present in 4 of 6 portal areas on the sample (bile duct fraction = 67%). A repeat liver biopsy done 11 months after onset (serum bilirubin ~ 3.0 mg/dL) showed chronic cholestatic liver injury with mild inflammation, zone 3 cholestasis and marked bile duct loss: among 13 portal areas only 2 had identifiable bile ducts (bile duct fraction = 15%). Ductular reaction was present, but not striking. In follow up, she suffered severe disability with neuropathy, fatigue, weakness, and difficulty maintaining her weight (falling to 75 lbs at one year). The clinical course and outcome were compatible with severe toxic epidermal necrolysis and cholestatic hepatitis with vanishing bile duct syndrome. The case was judged as highly likely drug-induced liver injury, highly likely due to azithromycin and only possibly due to ibuprofen.



Portal area from second biopsy with mild inflammation, lacking a bile duct (400x)



Copper was focally present (arrow) (Copper stain, 600x)

Serial Laboratory Results (Selected)

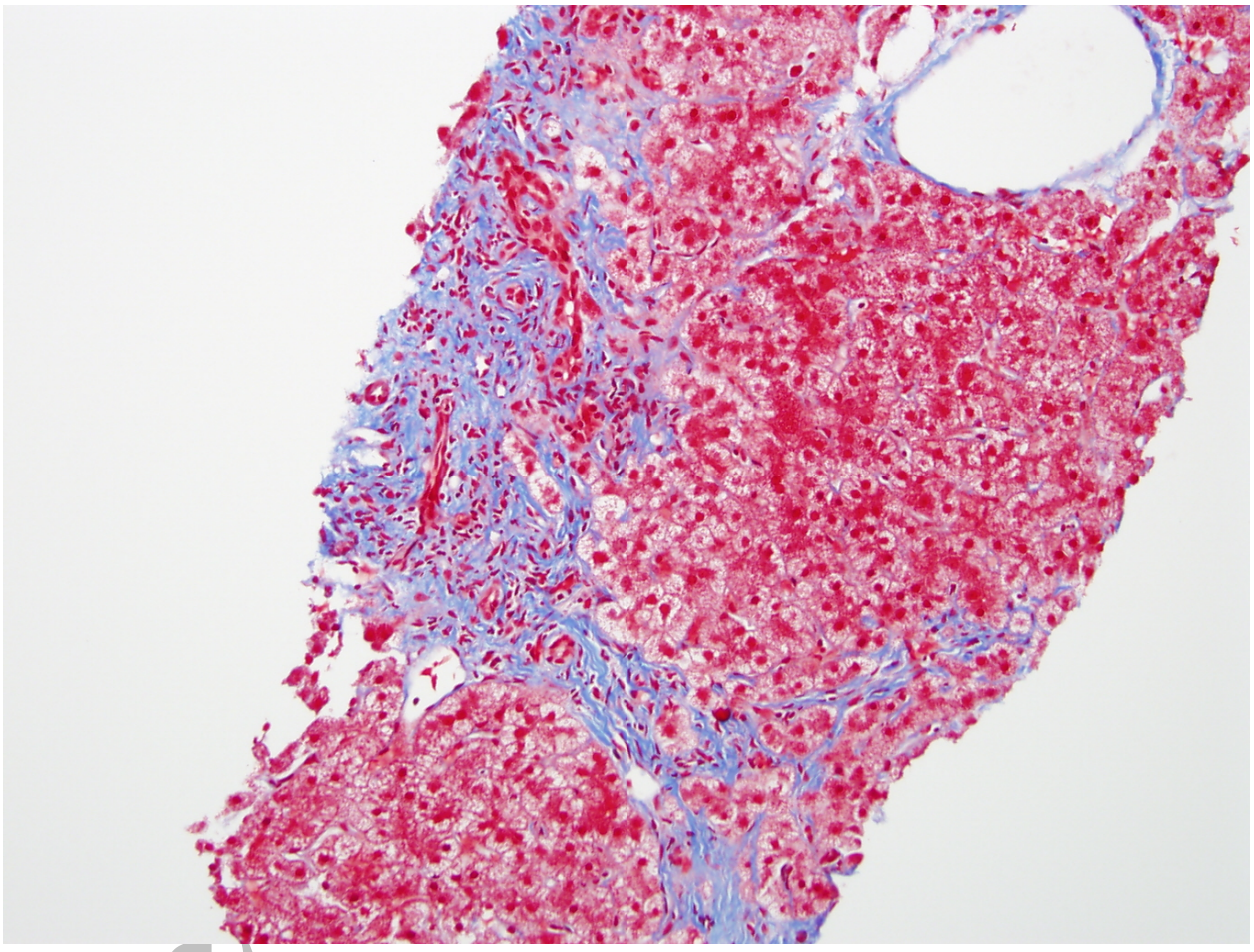
Date	Days	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
10/9/2008	-2				Azithromycin started
10/11/2008	0	140	71	3.7	Admission, R ratio 7.3
10/15/2008	4	247	141	4.8	INR 1.0
10/17/2008	6	177	190	9.3	
10/25/2008	14	89	424	11.7	R ratio = 0.8
11/1/2008	21	204	679	14.9	
11/10/2008	30	258	518	18.3	
11/14/2008	34	237	522	20.0	
11/16/2008	36	234	459	17.3	Liver biopsy: cholestasis, hepatitis
11/27/2008	47	175	323	13.4	
12/22/2008	72	431	460	12.7	
1/30/2009	111	529	328	13.1	
2/16/2009	128	221	294	11.1	
4/14/2009	185	351	363	8.1	6 month chronicity score = 3
6/16/2009	248	359	449	3.5	
8/27/2009					Liver biopsy: bile duct loss, copper
9/30/2009	354	318	261	1.3	1 year chronicity score = 2
11/23/2009	408	311	406	1.3	
10/25/2010	744	133	298	0.8	2 year chronicity score = 2
Normal values		**< 35**	**< 130**	**< 1.2**	

Summary Table

Feature	Result
Implicated Agent(s)	Azithromycin
Time to onset	2 days
Enzyme pattern	Hepatocellular (7.3), then cholestatic (<1.0)
HEV testing	Anti-HEV IgG and IgM neg
HCV testing	Anti-HCV and HCV RNA neg
Hospitalization	Yes
Prednisone	Yes
Ursodiol	Not Mentioned
Chronicity	Yes, chronicity score 3+ 6 mo, 2+ at 1 and 2 years
Severity	4+ (jaundice, hospitalization, INR >1.5)
Causality	2 (highly likely)
RUCAM	1 (unlikely)
Concomitant Medications	2 (Ibuprofen)
Comment	TEN

Clinical Summary: Case #5
HDS: Gluco-Ease Plus: Bile Duct Loss

A 56 year old African American man with diabetes on long term insulin therapy began an herbal product for glucose control called "Gluco-Ease Plus" and 3-4 months later he began to have itching, followed by jaundice and fatigue (the timing of onset of symptoms was somewhat unclear). He had no history of liver disease, alcohol abuse, drug allergies or risk factors for viral hepatitis. His only other medications were insulin, lisinopril, and low dose aspirin which he had been taking for several years. He had taken fish oil and opuntia Streptacantha briefly a few months before the onset of jaundice. On presentation he was jaundiced but had no fever or rash. When laboratory tests were taken, serum bilirubin was 1.6 mg/dL, ALT 276 U/L [6.1 times ULN], AST 200 U/L [5 times ULN], and alkaline phosphatase 679 U/L [5.7 times ULN]. The R ratio was 1.1 (cholestatic). Serum albumin was 2.9 g/dL and INR done two weeks later was normal (0.96). Tests for hepatitis A, B, C and E were negative and both ANA and SMA were unreactive. Imaging of the liver by ultrasound, CT and MRI suggested minor dilatation of intrahepatic bile ducts and he underwent ERCP which was normal. A liver biopsy showed a chronic hepatitis-like injury without obvious bile duct injury or loss. There was no bile accumulation. He had stopped the Gluco-Ease Plus shortly before hospitalization. His serum bilirubin rose to a peak of 9.5 mg/dL two weeks later and then began to fall. He was jaundiced for almost three months and serum enzymes decreased but remained elevated. One year after onset, the serum ALT was 63 U/L and alkaline phosphatase 479 U/L and he underwent a second liver biopsy, which showed chronic cholestasis with pseudoxanthomatous change and copper accumulation. There was only mild bile duct loss: among 12 portal areas, 9 had identifiable bile ducts (bile duct fraction =75%). There was no ductular reaction but there was definite periportal fibrosis. When seen more than two years after onset, his serum ALT was 109 U/L and alkaline phosphatase 739 U/L, but bilirubin was normal (0.8 mg/dL). He continued to complain of occasional pruritus and fatigue. This case was reviewed and judged to be probably drug induced liver injury from the Gluco-Ease Plus. The listed ingredients of this product include: Uva Ursi leaves, Couch Grass, Jambul seed, Shave Grass (Horsetail), Huckleberry leaves, Prince's Pine, Gymnema Sylvestre. The course and outcome are compatible with severe cholestatic hepatitis with partial vanishing bile duct syndrome. The ultimate prognosis is uncertain.



Masson trichrome stained section of second biopsy showing periportal fibrosis (200x).

Serial Laboratory Results

Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
3/15/2009	-136				Gluco-Ease Plus started
7/15/2009	-14				Symptom onset: Agent stopped
7/29/2009	0	276	679	1.6	
7/30/2009	1	267	715	1.7	
8/2/2009	4	374	706	1.7	Liver biopsy #1
8/14/2009	16	255	910	4.3	CT Scan
8/16/2009	18	200	938	9.5	
8/20/2009	22	233	816	5.7	ERCP: normal
8/28/2009	30	211	896	6.5	
9/9/2009	42	108	761	3.9	
10/20/2009	83	89	712	1.8	
11/23/2009	117	91	686	1.5	
12/21/2009	145	90	455	1.1	
3/25/2010	239	48	405	1.0	6 month chronicity score = 2
4/12/2010	257	50	371	0.9	
5/7/2010	282	49	332	1.0	
6/1/2010	307	62	439	0.9	
7/6/2010	342	63	479	1.2	1 year chronicity score = 2
10/26/2010	454	153	692	0.9	
1/14/2011	534	60	452	0.8	Liver biopsy #2: bile duct loss
4/11/2011	621	54	515	1.0	
5/31/2011	671	47	505	0.8	2 year chronicity score = 2
10/31/2011	824	109	739		3 year chronicity score = 2
Normal Values		**< 45**	**< 120**	**< 1,2**	

Summary Table

Feature	Result
Implicated Agent(s)	HDS: Gluco-Ease Plus
Time to onset	136 days
Enzyme pattern	Cholestatic (R ratio = 1.1)
HEV testing	Anti-HEV IgG neg
HCV testing	Anti-HCV neg
Hospitalization	Yes
Prednisone	No
Ursodiol	Not mentioned
Chronicity	Yes, chronicity score 2+ at 6 mo,1 and 2 years
Severity	3+ (Jaundiced, hospitalized)
Causality	3 (Probable)
RUCAM	6 (Probable)
Concomitant Medications	3 (Lisinopril, Aspirin, Insulin)
Comment	VBDS with chronic symptoms

Clinical Summary: Case #6
Amoxicillin/Clavulanate
Bile duct loss with prompt recovery

An 81 year old Caucasian man with mild dementia, benign prostatic hypertrophy and chronic obstructive pulmonary disease underwent minor outpatient surgery for excision of cysts on his face and back and was prescribed a 10 day course of amoxicillin/clavulanate (875/125 mg twice daily). Two weeks later he underwent repeat cyst excision and was given doxycycline (100 mg twice daily) for 7 days. During this second course of antibiotics he developed nausea, fatigue, abdominal discomfort, dark urine and pruritus followed by jaundice. He denied fever or rash. He had no history of liver disease, drug allergies, alcohol abuse or risk factors for viral hepatitis. His other medications included finasteride and tamsulosin for prostatism, alprazolam for sleep and trazodone for depression. He also was taking a multivitamin and potassium chloride. These he had taken for more than a year. He denied other over-the-counter or herbal medications. On initial examination, he was jaundiced but did not have fever, rash or other signs of chronic liver disease. Laboratory testing showed a total bilirubin of 16.6 mg/dL, ALT 221 U/L [3.4 times ULN], AST 144 U/L [3.6 times ULN] and alkaline phosphatase 482 U/L [3.6 times ULN. The R ratio was 1.0. Serum albumin was 3.3 g/dL and INR (taken 3 days later) 1.47. Tests for hepatitis A, B and C were negative; tests for anti-HEV were not available. Serum ANA and SMA were negative. Ultrasound and CT scan of the abdomen were normal, except for sludge in the gall bladder. A liver biopsy done 4 days after admission showed cholestatic hepatitis with mild inflammation, marked canalicular cholestasis, focal bile ductular reaction, bile duct injury and mild bile duct loss: among 14 portal areas, only 10 had identifiable bile ducts (bile duct fraction = 71%). Trazodone and doxycycline were stopped on admission. He began to improve and was discharged after one week with serum bilirubin of 12.2 mg/dL. In follow up 1, 2 and 6 months later he was asymptomatic and all liver tests were normal. This case was scored as 3+ in severity (jaundice and need for hospitalization). A review group judged the case as definite drug-induced liver injury from amoxicillin/clavulanate. The RUCAM score was 8 (highly probable). Typical of the course was the intense cholestasis with itching arising in an elderly man 1-2 weeks after a course of the antibiotic. The timing of onset was considered unlikely to be due to doxycycline induced liver injury which is quite rare and usually hepatocellular. The finding of bile duct loss on liver biopsy was concerning but did not seem to presage a subsequent prolonged cholestasis or development of vanishing bile duct syndrome.

Serial Laboratory Results

Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
2/7/2008	-21				Surgery, Amox/Clav started
2/16/2008	-12				Amox/Clav stopped
2/21/2008	-7				Doxycycline started
2/26/2008	-2				Symptom onset
2/28/2008	0	221	482	16.6	Admission, R ratio = 1.0
2/29/2008	1	183	411	21.9	Ultrasound, CT scan
3/1/2008	2	153	355	18.5	INR 1.47, albumin 2.8
3/2/2008	3	140	341	17.0	
3/3/2008	4	121	273	14.2	Liver biopsy: Bile duct loss
3/4/2008	5	121	311	14.1	INR 1.21
3/5/2008	6	136	418	12.2	Discharge
4/9/2008	41	66	146	1.3	Asymptomatic
5/8/2008	70	44	99	0.6	Normal
8/11/2008	165	36	94	0.7	Normal
Normal Values		**< 45**	**< 115**	**< 1.2**	

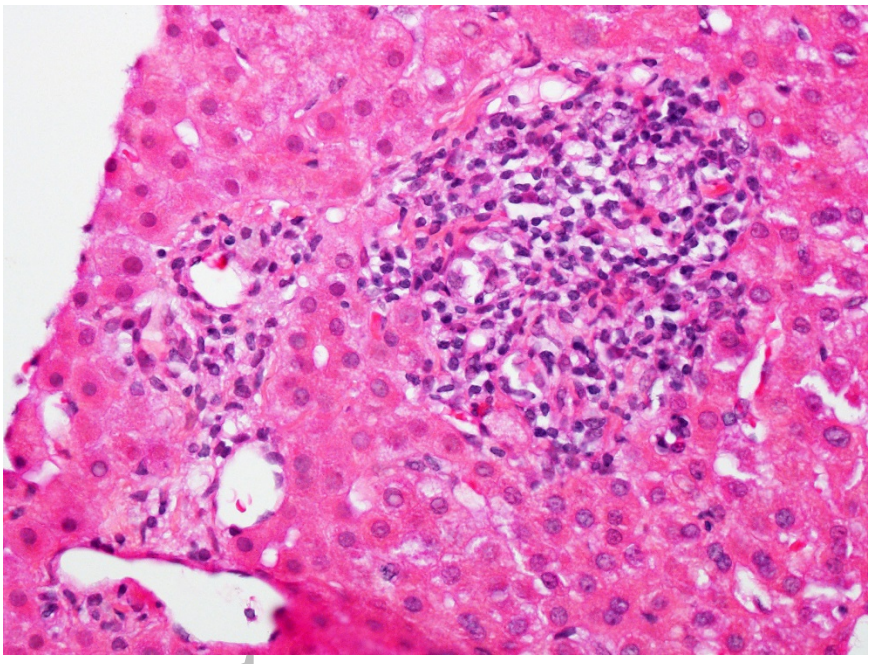
Summary Table

Feature	Result
Implicated Agent(s)	Amoxicillin/Clavulanate
Time to onset	21 days [12 days after stopping]
Enzyme pattern	Cholestatic (R ratio = 1.0)
HEV testing	Not tested
HCV testing	Anti-HCV neg
Hospitalization	Yes
Prednisone	No
Ursodiol	Yes
Chronicity	No
Severity	3+ (Jaundiced, hospitalized)
Causality	1 (Definite)

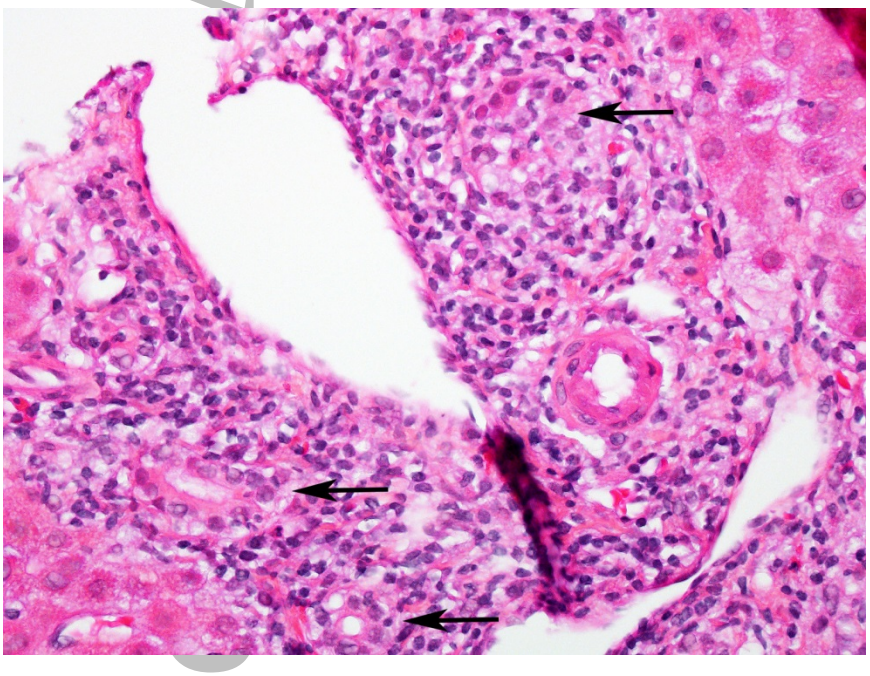
RUCAM	8 (Highly probable)
Concomitant Medications	7 (Doxycycline, trazodone)
Comment	Resolved within 3 months

Clinical Summary: Case
Herbal Supplement with Artemesinin
Bile Duct Loss

A 66 year old Caucasian woman with asthma, who was taking many over-the-counter supplements and herbal preparations for asthma and general health, developed fatigue, dark urine and jaundice approximately 2 months after starting a Chinese herbal preparation that contained artemesinin. She denied fever or rash. She had no history of liver disease, alcohol abuse or risk factors for viral hepatitis. She gave a history of multiple drug allergies and adverse reactions including jaundice after receipt of amoxicillin with clavulanic acid. Her medical conditions included asthma, hypertension, gastroesophageal reflux and irritable bowel syndrome. Her other prescription medications included montelukast, hydroxyzine, theophylline, valsartan and pantoprazole. She was also taking multiple vitamins and minerals as well as herbal preparations including cat's claw, "betafood", cranberry, candida, chamomile, choline, Enzycore, flax oil, fibers, Gastrex, ginger, gymnema, "liverplex", milk thistle, pro-gest, quercetin, Ultra Clear, and whole food fiber, On presentation she was mildly jaundiced and blood tests showed a serum bilirubin of 2.6 mg/dL, ALT 663 U/L [14.7 times ULN], AST 184 U/L [5.3 times ULN] and alkaline phosphatase 281 U/L [2.2 times ULN]. The R ratio was 7.9 (hepatocellular). These values had been normal when tested one month previously. The INR was 1.0 and albumin 3.8 gm/dL. Tests for hepatitis A, B, C and E were negative as were ANA and SMA. A CT of the abdomen showed evidence of diverticulosis, but no abnormality of the liver or biliary tree. She was admitted to the hospital and the various herbal and dietary supplements were stopped. Serum bilirubin levels rose to a peak of 11.9 mg/dL three weeks after presentation. A liver biopsy was done on day 14 that showed changes of chronic cholestasis, moderate inflammation, marked bile duct injury and mild bile duct: among 20 portal areas, only 15 had identifiable bile ducts (bile duct fraction = 75%). She received no specific therapy for the liver injury and gradually improved. Jaundice cleared within 2 months, but her serum ALT and alkaline phosphatase levels remained elevated for more than a year. When she was seen 1 year after onset, she was asymptomatic but serum enzyme levels were still mildly elevated. On repeat testing after 2 years, the values were in the normal range. This case was scored as moderately severe (jaundice and hospitalization). A causality review group adjudicated the case as probably HDS-induced liver injury. The RUCAM score was 4 (possible). Because of the many single- and multi-ingredient dietary supplements that she was taking, it is difficult to assign causality to a specific herbal component.



Portal area with no evident duct (H&E, 400x)



Portal area with several damaged ducts (arrows). The periportal hepatocytes show pallor consistent with pseudoxanthomatous change. (H&E, 400x).

Serial Laboratory Results

Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
3/5/2012	-67				Herbal agents started
4/19/2012	-22	39	87	0.2	Normal values
5/5/2012	-6				Onset of symptoms

Date	Days from Onset	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Event
5/9/2012	-2				Herbal agents stopped
5/11/2012	0	663	281	2.6	CT Scan: normal liver
5/13/2012	2	531	274	3.6	MRCP normal biliary tree
5/14/2012	3	473	278	4.3	
5/15/2012	4	405	280	4.8	
5/16/2012	5	332	262	5.1	Discharged
5/21/2012	10	86	280	9.0	Liver biopsy: bile duct loss
5/30/2012	19	63	275	7.7	
6/4/2012	24	45	247	11.9	
6/12/2012	32	86	254	7.2	
6/18/2012	38	134	259	4.9	
6/27/2012	47	149	262	3.2	
7/23/2012	73	101	243	0.9	
8/24/2012	105	112	357	0.6	
12/4/2012	207	53	176	0.8	6 month chronicity score = 1
1/10/2013	244	64	235	0.4	Ultrasound normal
3/25/2013	318	35	153	0.6	
7/17/2013	432	55	181	0.4	1 year chronicity score = 1
3/26/2014	684	31	87		Normal
Normal Values		**< 40**	**< 130**	**< 1.2**	

Summary Table

Feature	Result
Implicated Agent(s)	Herbal agent with artemesinin
Time to onset	67 days
Enzyme pattern	Hepatocellular (7.9), later cholestatic (<1.0)
HEV testing	Anti-HEV IgG pos, IgM neg
HCV testing	Anti-HCV neg, HCV RNA neg
Hospitalization	Yes
Prednisone	No
Ursodiol	Not mentioned
Chronicity	Yes, chronicity score 1 at 6 mo &1 year, 0 at 2 years
Severity	3+ (Jaundiced, hospitalized)
Causality	3 (probable)
RUCAM	4 (possible)
Concomittant medications	Several multi-ingredient nutritional supplements
Comment	Mild duct loss and eventual recovery

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