Drug-Induced Liver Injury
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Drug-induced liver injury (DILI) represents a diverse set of responses following exposure to any manufactured or naturally occurring chemical compound. The absolute incidence of DILI is difficult to determine because a significant number of cases are asymptomatic, and assigning causality of liver injury to a drug is fraught with many uncertainties. Most instances of DILI consist of a mild elevation of serum transaminase levels detected on routine, biochemical laboratory testing that resolves after removal of the offending chemical agent. Although rare, symptomatic cases of DILI are a prominent cause of liver dysfunction that leads to coagulopathy and hepatic encephalopathy within 26 weeks of the onset of illness.

Epidemiologic studies from community hospitals and tertiary care referral centers estimate that ALF affects approximately 1600 to 2000 people per year in the United States. Acute liver failure is a devastating disease as it is 1 of 12 participating sites in the National Institutes of Health–funded Drug-Induced Liver Injury Network.

Conclusions.—Drug-induced liver injury due to prescription, over-the-counter, and herbal products is a major cause of liver disease in the United States and around the world. Diagnosis of DILI is challenging because there is no single clinical, laboratory, or histologic feature specific to DILI. Accurate diagnosis requires establishing a causal relationship with the suspected agent and excluding competing causes of liver injury. The liver biopsy is an essential component in the management of DILI by offering clues to the underlying pathogenesis, providing prognostic information, and guiding therapy.


MECHANISMS OF DRUG INJURY

Drugs may cause liver injury in a predictable dose-dependent manner in most human and animal settings (intrinsic DILI) or in an unpredictable, non–dose-dependent manner (idiosyncratic DILI).

Intrinsic Drug-Induced Liver Injury

Acetaminophen toxicity is the most common cause of severe intrinsic DILI in the United States, representing approximately 50% of all ALF cases. Deaths due to acetaminophen toxicity are largely preventable, as approximately half of these cases are unintentional overdoses caused by a variety of patient misconceptions. Additionally, the deleterious course of an acute overdose can be mitigated by administration of N-acetyl-cysteine, which is a very effective antidote provided it is administered within 8 hours of ingestion.

During the mid-1970s, it was discovered that metabolism of acetaminophen by hepatocytes leads to production of a reactive metabolite (N-acetyl-p-benzoquinone imine [NAPQI]) that is reduced and detoxified by intracellular glutathione. Excessive accumulation of this metabolite by an overdose overwhelms the reducing capacity of glutathione, leading to covalent modification of numerous intracellular structures by NAPQI, causing a zone 3, centrilobular hepatocyte necrosis (Figure 1). The consistent and well-defined nature of acetaminophen-induced hepatotoxicity allowed for the introduction in 1977 of N-acetyl-cysteine as...
specific treatment for its toxicity. N-acetyl-cysteine acts by facilitating regeneration of glutathione, leading to detoxification of accumulated metabolites of acetaminophen.

The dose-related manner in which acetaminophen causes liver damage has facilitated the development of specific recommendations for safe usage of this very widely used medication. Within the United States, 20 billion doses of nonprescription acetaminophen are sold and the health care system spends $87 million dollars treating the complications of overdose. Recommendations released by the US Food and Drug Administration (FDA; Silver Spring, Maryland) in 2009 intend to limit the number of accidental overdoses by identifying combination medications as a common cause of nonintentional acetaminophen overdosing. The FDA recommendations also reduced the maximum daily dose to 3250 mg from 4000 mg, encouraging manufacturers to reduce the amount of acetaminophen to 325 mg per dose and clarifying warning labels and nomenclature to highlight the potential for severe hepatotoxicity.

Several other drugs can cause intrinsic DILI but are far less frequently prescribed than acetaminophen.

**Idiosyncratic Drug-Induced Liver Injury**

The relatively predictable course of intrinsic drug injury contrasts dramatically with idiosyncratic DILI, which is characterized by hepatotoxicity that occurs in a very low percentage of individuals who are exposed to a drug at the same dose for the same duration. In the United States, antimicrobials (amoxicillin-clavulanate, nitrofurantoin, sulfamethoxazole-trimethoprim, ciprofloxacin, isoniazid) lead the list of etiologic compounds causing DILI. Newer agents introduced to treat cancer (such as the tyrosine kinase inhibitors) and autoimmune disorders (such as the tumor necrosis factor α [TNF-α] inhibitors) also cause idiosyncratic drug reactions. The incidence of idiosyncratic DILI is hard to estimate as most cases are a mild, self-limited injury that reverses completely when the offending agent is identified and withdrawn. However, idiosyncratic DILI accounts for 13% to 16% of life-threatening acute/fulminant liver failure episodes in the United States. Additionally, idiosyncratic DILI is a common cause of the removal of drugs from the market by the FDA. Idiosyncratic DILI can be broadly divided between hypersensitivity (also called immunologic) and metabolic mechanisms of injury. Hypersensitivity-type reactions are characterized by fever, rash, granulomas, and eosinophilia in the peripheral blood or tissue biopsy sample and represent 23% to 37% of all idiosyncratic DILIs. The remaining cases are thought to be metabolic in nature and

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**Figure 1.** Acetaminophen toxicity is characterized by zone 3 (perivenular) coagulative necrosis (asterisks). Surviving hepatocytes are present in zone 1 around portal tracts (arrows). Note absence of any inflammation (hematoxylin-eosin, original magnification ×4).

**Figure 2.** A, Autoimmune hepatitis triggered by nitrofurantoin in an 80-year-old woman, showing marked portal and lobular inflammation consisting predominantly of (B) plasma cells with severe interface hepatitis (hematoxylin-eosin, original magnifications ×10 [A] and ×20 [B]).

**Figure 3.** Several drugs cause a pattern known as bland cholestasis, characterized by extensive canalicular bile plugs (arrows), which are present mostly around the central venules (asterisk) and are not accompanied by inflammation or necrosis (hematoxylin-eosin, original magnification ×40).
The 2000 or fewer cases of symptomatic DILI are distributed throughout the United States in small and less-than-ideal hospital settings, keeping the number of cases at any one institution relatively low. Furthermore, these cases represent hundreds of different chemical compounds, further precluding a single hospital from collecting a series of similar cases. Early information about DILI was therefore initially obtained from case reports, consisting of small numbers of patients, collected at a single or few institutions. Although case reports have been a crucial source for DILI, their impact is limited by the rare nature of idiosyncratic drug reactions. Large national registries have been created to centralize and standardize the analysis in order to gain a more complete understanding of DILI. Within the United States, the Drug-Induced Liver Injury Network (DILIN), funded by the National Institutes of Health (Bethesda, Maryland), began enrolling patients in prospective analyses in 2004; as of now, the network has collected more than 600 cases at 12 participating US sites. The importance of having a network specific to the United States is important, as drug usage varies dramatically by local medical practices and country-specific drug approval and regulations. Formal analyses between national registries have confirmed the varying distribution of offending agents between countries.

**Risk Factors for the Development of Idiosyncratic DILI.**—The existence of thousands of different chemical compounds with variable properties in combination with genetic and nongenetic host factors creates a diverse array of idiosyncratic DILIs. Poorly defined individual genetic components are thought to greatly contribute to the development and injury pattern for a given chemical compound. Human leukocyte antigen (HLA) genes encode the components of the major histocompatibility complexes, which are involved in antigen recognition and immune function and are thought to play a role in idiosyncratic DILI. Although studies have failed to provide strong evidence that HLA genotypes alter the risk of developing DILI, many associations between specific HLA genotypes and damage patterns in response to specific drugs are starting to emerge. The combination of amoxicillin-clavulanate is a common cause of idiosyncratic DILI and its association with HLA haplotypes is one of the best studied associations. Several HLA genotypes, such as HLA-DRB1*15 and HLA-DRB1*06, have been shown to be more common in humans with amoxicillin-clavulanate–induced DILI, although this association is still strongly influenced by additional confounders, notably ethnicity. The low positive predictive value of having any HLA allele, even one thought to predispose to DILI, is still too small to aid in the clinical prevention of DILI.

Genes involved in drug metabolism have also been studied in an attempt to understand the pathogenesis of idiosyncratic DILI. Polymorphisms within drug-metabolizing enzymes, such as cytochrome P450, N-acetyltransferase 2, UDP-glucuronosyltransferases, and glutathione S-transferases, have been found to be associated with DILI in relation to specific drugs, but many of the linkages are weak and nonreproducible.

Studies of the mitochondrial DNA polymerase γ gene (POLG) have discovered that certain genetic variations are associated with liver toxicity due to sodium valproate, a medication widely used for treatment of epilepsy, migraine, and a variety of other disorders that has long been known to cause idiosyncratic liver injury. These variant genes encode for enzyme isoforms that demonstrate compromised function in yeast and prevent human cellular proliferation in response to sodium valproate.

Age has been shown to be a risk factor for the development of DILI, but only for specific drugs. Although children are generally at a lower risk for DILI, they are at increased risk of developing hepatotoxicity from valproate and aspirin used for treatment of viral illnesses. In contrast, adults older than 50 years have an increased incidence of DILI with isoniazid and amoxicillin-clavulanate. Biological sex also plays a role in idiosyncratic DILI. Several studies have shown that although women are not at increased overall risk for DILI, they are more likely than men to progress to acute/fulminant liver failure from idiosyncratic DILI.

Although idiosyncratic DILI is not directly related to drug concentration, most instances do occur at higher concentrations. Large registries have shown that drugs that are commonly taken at a dose of 50 mg or above per day represent 77% of all idiosyncratic DILIs. Additionally, there have been small reports showing that decreasing the dose of a drug below 50 mg, but not stopping it entirely, has led to the resolution of DILI symptoms. Furthermore, it has been shown that the latency period is shorter with daily dosage of 50 mg or more.

Several investigators are actively studying the association of lipophilic properties and drug-metabolizing pathways with the likelihood of developing DILI. A study of the highly standardized DILIN database suggests that for most drugs, a threshold level of parent drug, metabolite, and/or adducts is a prerequisite for the DILI event, which is usually determined by chemical properties such as solubility and hepatic metabolism. Drugs with low permeability and solubility were found to be associated with lower peak serum aminotransferase levels and in some instances DILI occurred after prolonged exposure to the drug, such as several years of exposure to nitrofurantoin. Furthermore, most cases of liver injury were caused by drugs that undergo extensive hepatic metabolism. These cases also demonstrated higher peak alanine aminotransferase (ALT) values. Drug-induced autoimmune hepatitis was caused mainly by drugs that undergo extensive hepatic metabolism, suggesting formation of intermediates metabolites, which covalently bind to intracellular antigens, thus creating neoantigens that trigger a host immune response. However, methyldopa and nitrofurantoin (Figure 2, A and B), which are well-known to cause autoimmune hepatitis, are not extensively metabolized by the liver, suggesting an important role of host-related factors in the occurrence of a DILI event.

The DILIN data underscore an important paradigm, namely, that host reaction to drug exposure is an indelible part of the DILI equation. Thus, although drug properties could be correlated to the occurrence of a DILI event, there was no correlation to clinical severity and outcome. For the present, it seems that an essentially infinite number of unique chemicals can be created, and current knowledge does not permit definitive prediction of their toxicity. In practice, the specter of DILI should always be borne in mind and patients must be carefully monitored to see if symptoms develop after addition of a new drug.
BIOCHEMICAL CLASSIFICATION OF DILI

Drug-induced liver injury has been classified as hepatocellular, cholestatic, or mixed on the basis of its biochemical pattern of injury, which is determined by the ratio (R value) of elevation of serum level of ALT to that of serum alkaline phosphatase (ALP). A hepatocellular damage pattern is characterized by an increase in ALT greater than 2 to 5 times the upper limit of normal (ULN) and/or an ALT/ALP ratio also greater than 5. A cholestatic damage pattern is characterized by an increase in ALP greater than 3 times the ULN and/or an ALT/ALP ratio less than 2. A mixed hepatocellular/cholestatic pattern is characterized by an increase in ALT greater than 2 to 5 times the ULN and an increase in ALP greater than 3 times the ULN and/or an ALT/ALP ratio between 2 and 5.

DIAGNOSIS OF DILI

There is no specific or diagnostic clinical presentation, laboratory test, or histologic pattern to aid in the diagnosis of DILI. Signs and symptoms of DILI vary with the pattern and severity of injury, which themselves vary with the drug and the individual patient. Similarly, DILI may present a wide variety of histologic patterns depending upon the drug and the host. Assigning causality to a drug is therefore a meticulous process that requires carefully linking administration of a drug to onset of disease on the one hand and excluding competing causes of liver diseases on the other.

In the late 1980s, meetings sponsored by the pharmacovigilance division of Roussel Uclaf (Paris, France) in conjunction with the Council for International Organizations of Medical Sciences (Geneva, Switzerland) led to the creation of the Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM). The CIOMS/RUCAM was the first attempt to create standard definitions of liver injury and define criteria suggestive of DILI that were assigned points; higher scores translated into increased likelihood of DILI. The scores are then translated into categories of suspicion: definite or highly probable (score 6–8), probable (score 4–5), possible (score 3–5), unlikely (score 1–2), excluded (score 0). The CIOMS/RUCAM scale has been rigorously evaluated over time, demonstrating great interobserver agreement and the ability to correctly identify DILI and is now commonly referred to as the CIOMS/RUCAM scale. However, the CIOMS scale is felt to be cumbersome by practitioners and has not been readily adapted into most clinical practices. Additional assessment methods, such as the Naranjo Adverse Drug Reactions Probability Scale (NADRPS) and the Maria and Victorino (MV) clinical scale, have been developed in hope of creating a simpler method to define causality for DILI but have not been as rigorously evaluated. Although there is great interobserver reliability in the MV clinical scale, there is little agreement with the other scales. A head-to-head comparison between the CIOMS and MV scales reveals surprisingly poor agreement between the 2 methods, and the COIMS scale was concluded to be much more reflective of the presence of true DILI. A comparison of the CIOMS and NADRPS scales yielded similar conclusions. The NADRPS suffers from a low sensitivity (54%) and poor negative predictive value (29%) that greatly diminishes the ability to correctly identify DILI. The CIOMS continues to be the most reliable and widely used scale for determining causality in DILI.

Diagnosis of DILI by Expert Consult System of the DILIN

The US DILIN created a structured expert opinion approach to assess causality in DILI. A 65-page case report form was created from the patients’ charts to include complete clinical history, serial laboratory tests, history of previous liver disease, alcohol ingestion, serology for viral and autoimmune hepatitis, and serum levels of ceruloplasmin, α-1 antitrypsin, ferritin, and iron. From this detailed report, an abbreviated case report form (summary case report form) was created, which along with a detailed case summary (clinical narrative) was presented for review. The review panel consisted of 3 hepatologists who were experts in the field of DILI. Each hepatologist reviewed the case individually and assigned a causality score on a 5-point scale (definite, highly likely, probable, possible, unlikely). Each reviewer also assessed the case on RUCAM scale.

It was found that the DILIN structured expert opinion process was more likely to assign causality to a drug than the RUCAM scale. Although there was greater interobserver agreement with the DILIN expert consult system than with the RUCAM scale, interobserver variation was considerable within both systems and correlation between the 2 adjudication methods was weak.

THE ROLE OF LIVER BIOPSY IN IDIOSYNCRATIC DILI

Liver biopsy is not required in the clinical evaluation of severe, idiosyncratic DILI and is performed in less than half of suspected cases, including those enrolled in formal studies. However, when performed, the liver biopsy provides valuable information on the nature and severity of liver injury, the possible pathogenesis, and the expected clinical outcome. In addition, the liver biopsy may guide therapy, for example, the finding of drug-induced autoimmune hepatitis may call for treatment with steroids. Above all, a liver biopsy helps to rule out competing causes of liver injury, an essential component in the diagnosis of DILI. In our tertiary care referral center (also a DILIN study site), we receive approximately 25 clinical cases per year that carry a primary diagnosis of DILI, and approximately 40% of those cases were subjected to biopsy as part of the clinical evaluation.

Histologic Patterns of Liver Injury

The US DILIN recognizes 18 distinct histologic damage categories: acute hepatitic, chronic hepatitic, acute cholestatic (Figure 3), chronic cholestatic (Figure 4, A and B), cholestatic-hepatitic, granulomatous, macrovesicular steatotic, microvesicular steatotic, steatohepatitic (Figure 5), zonal necrosis (Figure 1), nonzonal necrosis, vascular injury (Figure 6, A and B), hepatocellular alteration, nodular regenerative hyperplasia (Figure 7), mixed or unclassified injury, minimal nonspecific changes, absolutely normal, and massive necrosis. The most common patterns are detailed in the Table. Acute and chronic hepatitic, acute and chronic cholestatic, and mixed hepatitis-cholestatic patterns are by far the most common patterns. Defining the histologic pattern of liver injury allows narrowing of the differential diagnosis to a small number of drugs that cause DILI and causes of non-DILI that can potentially be excluded by additional clinical testing. The pathologist and clinicians must work together to further rule out as many diseases as possible to increase the likelihood of an accurate diagnosis of DILI.
Figure 4. A, A pattern of chronic cholestasis characterized by severe ductular reaction consisting of numerous bile ductules at the edge of the portal tract (long arrows). The interlobular bile duct is preserved (short arrow) and is accompanied by an arteriole (arrowhead). B, Bile infarcts are present (arrows). The drug involved was a tricyclic antidepressant (hematoxylin-eosin, original magnification ×20 [A and B]).

Figure 5. Drug-induced injury due to amiodarone shows a distinctive pattern of balloononed hepatocytes containing numerous, well-formed Mallory-Denk bodies (arrowheads). These are typically present around the portal tract; long arrow points to hepatic arteriole and short arrow points to bile duct (hematoxylin-eosin, original magnification ×20).

Figure 6. A, Veno-occlusive disease showing extensive centrilobular necrosis with hemorrhage. Hepatocytes around the portal tract (dotted line) are viable and show macrovesicular steatosis. B, A central venule with thickened and edematous wall containing reactive fibroblasts. This injury was caused by a chemotherapeutic agent for treatment of leukemia (hematoxylin-eosin, original magnifications ×10 [A] and ×20 [B]).
Histologic Patterns Suggest Pathogenic Mechanisms.—Cases of DILI with a predominantly inflammatory phenotype show a predominance of CD8 cytotoxic T cells indicating activation of the host immune response, consistent with the hypothesis that the intrahepatic generation of neoantigens by the drug or its metabolite is responsible for DILI. An injury pattern that shows zonal necrosis suggests the involvement of zonally distributed enzymes in the pathogenesis. Bland cholestasis is probably due to toxicity at the level of the canalculus, probably involving transporter molecules. Microvesicular steatosis, as seen with tetracycline and valproate, results from acute impairment of β oxidation of fatty acids in mitochondria. Macrovesicular steatosis, as occurs with tamoxifen or antivirals used for treatment of human immunodeficiency virus, is thought to be due to partial but chronic impairment of mitochondrial function. Drugs, such as oxiplatin, cause veno-occlusive disease/sinusoidal obstructive syndrome by causing damage to endothelium lining hepatic sinusoids and central venules.

Histologic evaluation is an important component in safety assessment of newly discovered or experimental drugs, often providing essential information on underlying pathogenetic mechanisms. During the development of nucleoside analogues for FDA approval, it was determined that fialuridine, zidovudine, stavudine, and 2′,3′-dideoxyinosine lead to hepatotoxicity.16–30 Examination of liver biopsy specimens revealed microvascular steatosis signifying mitochondrial damage that was later confirmed in experimental models.31,32 The FDA approves approximately 20 to 40 new drugs per year, and many-fold more start the approval process but fail during phase 1 to 3 clinical trials.33 Hepatotoxicity is a leading cause of drug failure as well as of removal from the market during postapproval surveillance.

Correlation of Histologic Pattern With Biochemical Parameters.—Histologic patterns of DILI do not perfectly correlate with the biochemical pattern of injury. Biochemical testing underestimates the degree of cholestasis and bile duct damage, as more cases of cholestasis and duct injury are identified on biopsy than indicated with serum testing alone.11 However, biochemical testing does a better job predicting the presence of hepatocellular damage, as there is good correlation between elevations of ALT and/or R values predicting the presence of hepatocellular damage, as there is good correlation between elevations of ALT and/or R values and histologically confirmed hepatocellular damage.11 Furthermore, histologic examination of a mixed hepatocellular/cholestatic biochemical pattern reveals a greater degree of cholestasis compared to hepatocellular damage than would be predicted from biochemical assessment.13

Prognostic Information on Liver Biopsy

Liver biopsy can provide prognostic information that can assist in patient management. The Spanish DILI network demonstrated that patients with pure hepatocellular necrosis on biopsy had a higher rate of death when compared to cholestatic or mixed cholestatic/hepatocellular damage patterns.16 The poor prognostic implication of necrosis on biopsy have been appreciated in additional studies.13,32 More formal analysis has additionally shown that the degree of necrosis, fibrosis stage, microvesicular steatosis, panacinar

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**Figure 7.** Nodular regenerative hyperplasia due to a chemotherapeutic agent, showing compressed liver cell plates (arrows) alternating with a nodular area (asterisk) consisting of thickened plates (Gomori reticulin, original magnification x10).
steatosis, cholangiolar cholestasis, ductular reaction, neutrophils, and portal venopathy were all associated with a higher degree of severity of DILI. Patients who had histologic evidence of a hypersensitivity-type reaction, notably the presence of eosinophils and/or granulomas, had a greater propensity for survival and relatively mild course of damage with a better chance of recovery.11,15

Chronic DILI is defined as biochemical evidence of liver damage that persists for 3 to 6 months from the beginning of confirmed liver damage, depending on the pattern of liver injury observed, and can lead to persistent disease, including cirrhosis.11,15 Patients who had a mixed hepatocellular/cholestatic pattern of DILI on biopsy were more likely to progress to chronic DILI.52 Patients who had loss of bile ducts on biopsy had a prolonged course to recovery, but this finding alone did not indicate that the condition would progress to chronic liver injury.46 The ability to predict chronic injury is important with regard to long-term monitoring of liver functions after an episode of DILI, as a second liver biopsy is rarely justified.

The presence of jaundice in patients with DILI has been found to indicate a higher chance of death, which has been estimated to be 9% to 12%.16,47,53,54

CHALLENGES OF HERBAL PRODUCTS IN CAUSATION OF DILI

Use of herbal products in health supplements and alternative medicine is widespread around the globe. Nearly 50% to 70% of the general population in the United States uses a dietary supplement either for their purported benefits in maintaining good health or for treatment of various diseases.55,56 Twenty-three percent of patients enrolled in a hepatitis C treatment trial reported use of health and dietary supplements for treatment of their liver disease.57 In many parts of Asia and Africa, the general population relies on herbal and alternative medicines as their primary means of medical care.58 Not surprisingly, herbal medications accounted for 71% of cases of DILI in Singapore where they form the mainstay of medical treatment.59 It is also noteworthy that many herbal products are adulterated with active drugs.60 In the US-based DILIN study, herbal products accounted for 16% of the total number of cases of DILI; one-third of these were due to products intended for bodybuilding and one-quarter were due to products purported to facilitate weight loss.61 Hydroxycut (iovate Health Sciences Inc, Oakville, Ontario, Canada) and Oxy-Elite Pro (USP Labs LLC, Dallas, Texas) are examples of popular weight loss and/or bodybuilding supplements that cause DILI.

The diagnosis of DILI due to herbas follows the same principles as that for prescription and over-the-counter medicines (ie, an association between exposure and onset of liver disease has to be established and competing causes of liver disease have to be excluded). However, establishing these 2 criteria may pose special challenges with herbal medications. First, products may be consumed over long periods of time (months to years) before toxicity appears owing to cumulative damage from accumulation of threshold levels of the offending metabolite. Secondly, herbal products may change over time in terms of their concentration, purity, or potency either owing to change in formulation or change in the botanical products themselves due to variability in harvest conditions.62–65 Since herbal products have been shown to lead to chronic, self-perpetuating injury, improvement on withdrawal of the product cannot be easily demonstrated for establishing causality.62 On the other hand, in contrast to the situation with prescription medicines, which does not readily lend itself to rechallenge, incidental reexposure is not uncommon with herbal medicines, leading to recrudescence of liver injury, which helps to establish a causal relationship. The diagnosis of DILI due to herbal products thus depends first and foremost on a healthy level of suspicion that such a product might be involved in the liver disease. This is especially relevant owing to increasing popularity and availability of an ever increasing number of unregulated products with purported health benefits.

SOME SELECT AGENTS THAT CAUSE DILI

The Tried and Tested

Amoxicillin-Clavulanate.—Amoxicillin-clavulanate is the most frequent cause of DILI in several series from different countries.16,56,66–68 Amoxicillin is widely used around the world to treat a wide variety of infections. A semisynthetic β-lactam, it is often combined with clavulanic acid to inhibit bacterial β-lactamases, which would otherwise degrade the amoxicillin and render it ineffective. The incidence of DILI is much higher with the amoxicillin-clavulanic acid combination (1.7 per 10 000 prescriptions) than with amoxicillin alone (0.3 per 10 000 prescriptions).69,70 Similarly, rechallenge with amoxicillin-clavulanate is followed more often by recurrence than with amoxicillin alone.71

Liver injury due to amoxicillin-clavulanate is idiosyncratic; risk increases with age, multiple prescriptions, and longer duration of treatment. The risk for older patients with multiple prescriptions increases by 5.2 per 10 000 prescriptions.66,69,70 Males are at slightly greater risk. Genetic factors that increase the risk of amoxicillin-clavulanate toxicity include presence of DRB1*15 and DQB1*06 of the HLA class II antigens and double-null heterozygosity for glutathione S-transferase (GSTT1/GSTM1).20,72 The latter observation suggests that this enzyme is involved in detoxification of the drug, as its absence leads to lengthened exposure to intermediate metabolites of the drug.73

Signs and symptoms of liver disease, which usually begin within 6 weeks of initiating treatment, include a combination of nausea, abdominal pain, jaundice, fever, pale stools, dark urine, pruritus, or a viral illness-like syndrome.31,66,71 A significant number of patients show features of hypersensitivity such as eosinophilia and skin rash; Stevens-Johnson syndrome may occur.16,70

Laboratory tests may show a predominantly cholestatic, predominantly hepatocellular, or a mixed hepatocellular-cholestatic pattern of injury.16,66 Cholestatic and mixed patterns of injury appear to occur in older people who have undergone longer treatment regimens, whereas hepatic damage occurs more commonly in younger individuals who have undergone shorter durations of treatment.31,74 Histologic patterns also vary over time so that an initial hepatic or mixed pattern of injury may progress to a cholestatic profile.31,74

Histologically, amoxicillin-clavulanate liver injury appears most often as a cholestatic hepatitis (Figure 8, A and B), although very early biopsies may show acute cholestasis.74 Several cases with prominent bile duct injury have been reported. These show extensive infiltration of the biliary epithelium with inflammatory cells accompanied by nuclear

irregularity, cytoplasmic vacuolization, and eosinophilia of the biliary epithelium. The degree of bile duct injury may correlate with serum levels of alkaline phosphatase but there is usually no correlation with histologic cholestasis or the duration of jaundice. Rare cases of bile duct loss and granulomatous hepatitis have been reported after exposure to amoxicillin-clavulanate.

Most patients recover within 4 to 6 months following DILI due to amoxicillin-clavulanate. Rare patients may progress to ductopenia or to acute liver failure and death; severe outcomes were reported in 2.9% of cases in one series. Because amoxicillin-clavulanate hepatotoxicity may cause decompensation of cirrhosis, it should be administered with caution in cirrhotic patients, especially those who are elderly or have had previous exposure to the drug.

Fluoroquinolones.—Fluoroquinolones are the most commonly used antibiotics, often empirically, because of their wide antimicrobial coverage and effectiveness. They are also the most common cause of idiosyncratic drug injury in the DILIN study.

The clinical presentation and pattern of injury are similar for the various fluoroquinolones, consistent with a “class effect.” Liver injury due to fluoroquinolones is characterized by a short latency period of 2 to 9 days with an abrupt onset that begins while the patient is still taking the antibiotic. Immunoallergic features such as rash, fever, and eosinophilia are common. The pattern of injury is spread across the entire spectrum of hepatocellular, cholestasis, and mixed patterns of injury (Figure 9, A and B).

Patients with prominent features of hypersensitivity benefit from use of corticosteroids. Most patients with fluoroquinolone-associated hepatotoxicity recover, but the period of illness can be prolonged. Acute and chronic hepatic failure and death, although rare, are known to occur. Acute liver failure from levofloxacin, moxifloxacin, and gatifloxacin (2.1, 6.6, and 6.1 cases per 10 million prescriptions, respectively) is however less frequent than that due to amoxicillin-clavulanate (10 per 10 million prescriptions).
The hepatotoxicity of fluoroquinolones appears to be a hypersensitivity reaction as evidenced by the short latency period, frequent presence of eosinophilia and skin rash, and heightened injury on reexposure. Patients who have sensitivity to one fluoroquinolone will develop a reaction to other fluoroquinolones, usually with a heightened reaction. Furthermore, a significant number of patients have an allergy to nonfluoroquinolone medications, suggesting a generalized heightened sensitivity in such individuals. This tendency makes it important to take a proper drug history before prescribing fluoroquinolones, and such patients should be cautioned to avoid reexposure to other fluoroquinolones.

New Kids on the Block
Tumor Necrosis Factor α Antagonists.—Tumor necrosis factor α antagonists are a class of biological response modifiers that are essential for the treatment for a variety of inflammatory and autoimmune diseases. To better define toxicity of the TNF-α antagonists, 34 cases of liver injury were described in detail in a recent review. Most cases were due to infliximab, reflecting the fact that this drug has been in clinical use the longest and that it is the most commonly used TNF-α antagonist. The remaining cases were due to etanercept and adalimumab (4 cases each).

The most common pattern is an acute hepatocellular injury followed by a mixed pattern of injury; 1 case was characterized by prolonged cholestasis. In two-thirds of cases, the hepatocellular injury was autoimmune in nature as evidenced by presence of serum antinuclear and/or smooth muscle antibodies. Of these cases, almost 90% of those that were subjected to biopsy showed histologic features of autoimmunity. Although the median latency period for all patients was 13 weeks, 20% of patients showed latency periods longer than 24 weeks. Patients with autoimmune features had disease later than those without these features (median latency period, 16 weeks versus 10 weeks). Those who did not have autoimmune features showed a mixed pattern of injury with levels of alanine aminotransferase that were lower than for those who had autoimmune features (784 versus 528 U/L). Bilirubin was significantly elevated (>3 mg/dL) in approximately one-third of the patients.

Except for 1 patient who needed a liver transplant, all patients recovered from DILI on discontinuation of the drug. A little more than half the patients who developed an autoimmune hepatitis required steroids, which resulted in both clinical improvement as well as disappearance of antibodies from the serum. Furthermore, these patients did not require continued treatment with steroids after resolution of DILI, confirming that the autoimmune hepatitis was induced by the drug.

An interesting aspect of toxicity due to TNF-α inhibitors is that with all 3 commonly used agents, patients who experience DILI due to one agent (usually infliximab) can successfully switch their therapy to another agent (usually etanercept) without recurrence of liver injury. Thus, although there is a class effect, there also appears to be enough differences among the agents so that if one agent causes DILI, it does not mean that the patient is intolerant to the entire class. Etanercept appears to be least associated with liver enzyme elevations as compared to infliximab or adalimumab. Patients with rheumatoid arthritis who take TNF-α antagonists seem to have a low risk of liver enzyme elevations (ALT or aspartate aminotransferase elevation 3 times upper limit of normal), and anti-TNF agents are safe for use in most patients with chronic hepatitis C, including those with underlying liver disease.

The mechanism of injury of the TNF-α antagonists appears to be idiosyncratic as it can occur only after 1 infusion. The presence of autoantibodies suggests involvement of the immune system in the pathogenesis.

TYROSINE KINASE INHIBITORS

Small-molecule tyrosine kinase inhibitors (TKIs) are recent new additions to the armamentarium for cancer treatment and their efficacy has expedited their FDA approval. Although TKIs are a structurally heterogeneous group of chemical compounds, they appear to be associated with a relatively high incidence of DILI as a group. However, the incidence of DILI appears to be independent of their molecular target as evidenced by lack of cross-reactivity between epidermal growth factor receptor inhibitors, erlotinib, and gefitinib. Small reports of patients who developed severe hepatotoxicity to erlotinib or gefitinib indicate that their therapy was successfully switched to the other agent without signs of recurrent hepatotoxicity.

The onset of hepatotoxicity typically occurs within 8 weeks of initiation of therapy and appears to fall into 2 categories. The first category is low-grade toxicity defined by a mild, asymptomatic transaminitis that occurs in approximately 25% to 35% of patients and resolves without complication after the withdrawal of the medication. The second category consists of high-grade hepatotoxicity defined by a dramatic transaminitis, with or without cholestatic injury, that occurs in approximately 2% of patients and includes rare fatal events for several different agents. Severe TKI-induced hepatotoxicity appears to have a predominantly hepatocellular damage profile based on biochemical and histologic assessment. Although biochemical data have been collected on many cases, only a few drugs, such as imatinib and sorafenib, have been in use long enough for multiple reports of liver biopsy findings to be published. Case reports from imatinib-induced DILI demonstrate a consistent pattern of immune-mediated DILI that took weeks to months to develop. Biopsy revealed predominantly hepatocellular necrosis with some reports of concomitant cholestatic injury. However, only 1 case proved to be fatal and the other patients slowly recovered after removal of imatinib. Reports for sorafenib-induced hepatotoxicity are rare and demonstrate consistent hepatocellular necrosis, but have variation in timing and type of inflammatory infiltrate, thus obscuring a clear understanding of the nature of injury.

The first report in 2010 demonstrated hepatocellular necrosis with an inflammatory infiltrate that contained eosinophils, which occurred within 5 days of the initiation of treatment and resolved quickly after drug withdrawal, consistent with a hypersensitivity DILI. This report is consistent with a second case without liver biopsy in which the patient became symptomatic within 4 days of treatment; symptoms resolved quickly after drug withdrawal. However, for 3 patients symptoms developed within 3 to 8 weeks with persistence of symptoms after drug withdrawal, which led to death of all 3 patients. The 1 fatal case with a biopsy showed hepatocellular necrosis with a lymphocytic infiltrate consistent with immune-mediated DILI. The low incidence of DILI and small number of patients in clinical trials of TKIs highlight the importance of postmarket surveillance.
as it continues to define the prevalence of hepatotoxicity for this burgeoning class of targeted molecular therapeutics.

**Ones on the Fringe**

**Hydroxycut.**—Hepatotoxicity due to the weight-loss supplement Hydroxycut appears to be idiosyncratic, as it occurred in a small minority of people using the daily recommended dosage and occurred in most patients in less than 8 weeks, which is the standard time quoted by the product manufacturers in their studies of weight loss.\(^\text{106}\) Symptoms of DILI from Hydroxycut included a combination of nausea, abdominal pain, fever, and jaundice.\(^\text{106–109}\) Biochemical testing revealed a predominantly hepatocellular pattern of injury, but some cases also demonstrate a cholestatic injury.\(^\text{106–109}\) Liver biopsy was consistent with the biochemical testing and demonstrated a predominantly hepatocellular pattern of injury.\(^\text{106}\) The most severe cases also demonstrated hepatocyte necrosis and many went on to liver transplant or death.\(^\text{106}\) The mild cases had mixed hepatocellular/cholestasis or predominantly cholestatic patterns of injury and had spontaneous recovery of liver function.\(^\text{106}\) The histologic findings suggest a prognostic role for liver biopsy in cases of suspected Hydroxycut DILI that can help decide between liver transplant and supportive therapy.

High prevalence of Hispanic ethnicity has been observed in the largest case review, but there are no definitive links to specific HLA genotypes and it is unclear whether this association is due to nongenetic epidemiologic factors.\(^\text{106}\) The causative agent within Hydroxycut has been difficult to determine, as the product contains a variety of combinations that contain a mix of plant extracts, caffeine, and vitamin and amino acid supplements. Additionally, Hydroxycut has been reformulated after concerns of liver toxicity were raised publicly in 2004 and 2009, and now contains a variety of products containing new combinations that are available for purchase with minimal evidence to support their safety.

**OxyElite Pro.**—Recently, hepatotoxicity issues have been raised about the weight loss/bodybuilding supplement OxyElite Pro, leading to its withdrawal from the market in 2013 by the FDA. The first generation of OxyElite Pro contained geranium extract specifically containing 1,3-dimethylamylamine, which has been linked to liver damage. A second, newer formulation containing aegeline ([N-[2-hydroxy-2-(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide] was released to the market and was linked by the Centers for Disease Control and Prevention to 29 cases of acute liver failure, including 1 death and 2 liver transplants in Hawaii.\(^\text{110}\) The most consistent symptoms were loss of appetite, light-colored stools, dark urine, and jaundice.\(^\text{110}\) Biochemical testing reveals dramatic elevation in transaminase levels and jaundice with little evidence of bile duct damage.\(^\text{110}\) The initial evaluation of liver biopsies from 10 patients appears to be consistent with idiosyncratic DILI, but no formal report has been published. OxyElite Pro and additional supplements that contain aegeline, such as VERSA-1 (USP), have been banned by the FDA owing to the failure of the companies to provide safety data 75 days before releasing the new products.

**References**