Drug Induced Liver Injury (DILI) 2013

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This is to acknowledge that Dr. Getachew has disclosed no financial relationship and he will not be discussing any off-label uses in his presentation.

Biography

Dr. Getachew earned an M.D. from the University of Wisconsin School of Medicine in Madison. He completed his internal medicine training at University of Texas San Antonio Health Science Center. He went on further to complete a fellowship at UT Southwestern Medical Center as a T32 fellow. In 2008, he joined the faculty as an instructor and is now an assistant professor. His area of interest is studying liver injury and immune response to viral infections.

Learning Objectives

1. How to assess patients with potential drug induced liver injury (DILI)
2. How to assess the severity of drug induced liver injury using Hy's rule
3. How to use the livertox web site

Contents

Overview

Drug-Induced Liver Injury (DILI) is a multi-faceted problem in the general care of patients. It poses an enormous challenge to physicians, patients and pharmaceutical companies in drug development and application. Drug discontinuation due to DILI sometimes leaves patients hopeless in the face a life threatening disease. As physicians, our first obligation is to "do no harm." Thus, when faced with a potentially hepatotoxic agent that has a life saving application, we are forced to make a decision that is both ethically and medically challenging. The other aspect of this problem is the enormous financial burden DILI imposes on the pharmaceutical industry from the early stages of preclinical testing all the way to phase-3 clinical stage trial.

DILI is the leading cause of acute liver failure resulting in over 600 liver transplantations and accounts roughly for 0.1 to 1% of hospital admissions as well as 10% of outpatient consultations per year. [1] It has been cited as the primary reason for failed drug approvals, market withdrawals, usage restrictions, and warnings to practicing physicians in the United States. [2-4] Of all the adverse drug reactions related to use of medication, DILI is attributed in about 10 to 21% of the reported cases. [5, 6] It has been estimated that about $1/5^{th}$ of all drug withdrawals from the market are related to liver injuries. [7] This is due to recognition of these drugs' potential to cause liver damage once they are made available to the larger community. Drugs such as isoniazid had to be relabeled due to their toxic potential to the liver. [8]

One can cite a multitude of reasons for this. Severe DILI is a rare idiosyncratic adverse event that makes detection difficult. When severe DILI is first recognized, after a drug is launched, assigning causality is difficult resulting in catastrophic outcomes for some patients. In its citation of the troglitazone case, the FDA points out that nearly one hundred cases of acute liver failures were documented between recognition of its harmful effects and its withdrawal from the market. [9, 10]

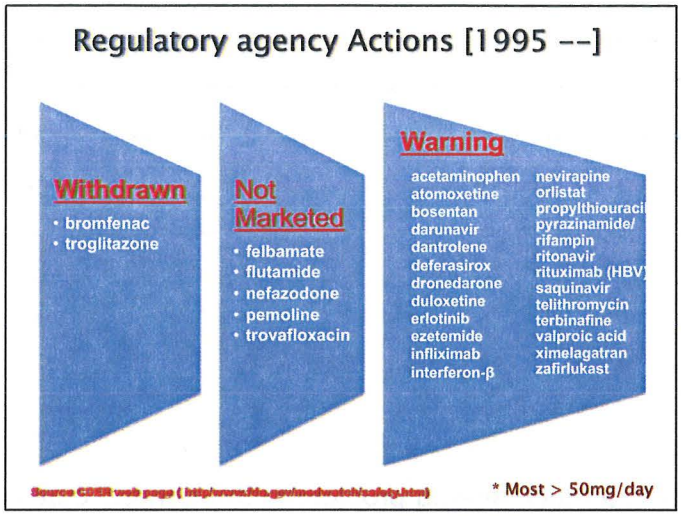


Figure 1. CDER database shows all the drugs that have been either withdrawn or have had warning labels on them since 1995 from DILI.

Definitions

Different classification categories have been used to define the pattern of drug induced liver injury. These definitions use either the pattern of liver injury or the effect of the drug following dosing. One broad classification of DILI separates drugs into predictable and unpredictable hepatotoxins. This classification has also been referred to as intrinsic versus idiosyncratic. Intrinsic or predictable is defined as toxicity that result from damage to the hepatocyte in a dose dependent and predictable manner. It affects the majority of the population at large enough doses with a short latency period. A good example of this type of reaction is overdoses of acetaminophen that lead to adverse drug reactions in virtually everyone at high enough doses. On the other hand, the unpredictable or idiosyncratic pattern of DILI shows a non-dose dependent pattern of adverse events. [11] It is rare and exhibits class effect. It could lead to a pattern of adaptation or to fulminant liver injury pattern. If patients are re-challenged with the offending drug, they will experience a more severe adverse event. Although idiosyncratic reactions are often not dose dependent, several studies have shown that daily doses greater 50 mg pose a higher idiosyncratic DILI risk. [12, 13]

The second form of classification is based on pattern of liver injury. If the primary driver of the injury is based with in hepatocytes, it will have a hepatocellular pattern. This is mainly reflected by an elevation of alanine aminotransferase (ALT) levels. If the injury is mainly to the bile duct canalicular cells, patients will experience an elevation in their alkaline phosphatase (Alk Phos). This pattern is referred to as a cholestatic pattern. Some people will have a mixture of both Alk phos and ALT elevation, which is termed a mixed injury pattern. The pattern of

injury has further been re-classified based on the ALT/ Alk Phos ratio as outlined in the graph below.

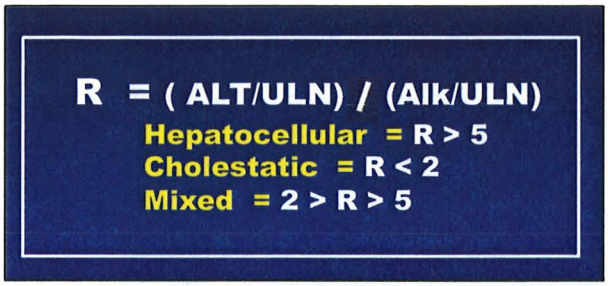


Figure 2. R is a formula that has been adapted to assess the nature of liver injury pattern. The formula takes into account alanine aminotransferase and alkaline phosphatase to be normalized to its upper normal limit. Values that show R levels greater than 5 have hepatocellular damage while values less than 2 are consistent with cholestatic pattern. Mixed patterns have values that fall between 2 and 5.

Epidemiology

Studying epidemiology of DILI has been a challenging task since most of the phenotype of this disease goes unnoticed due to its asymptomatic stage. Thus, there is very limited data regarding the true incidence of DILI. Most of the studies in this area are retrospective reports. Some reports estimate that up to 1/3 of the cases referred for acute hepatitis are from DILI. [14] According to the Physician Desk Reference over half the medications listed will cite associations with abnormalities in liver tests. [15] Some retrospective studies hint the incidence of DILI is about 40.6 per 100,000 persons per year. [16] This low incidence and the idiosyncratic nature of DILI make it very difficult to identify high-risk patients that are susceptible to injury. [2] In addition, the confirmatory test or lack thereof makes this estimation process complicated by both underestimating some cases and over-estimating others. [17]

The only study to date that has tried to analyze the true incidence of DILI in the general population in a prospective manner is a study done by Sgro et al in 2002. This group prospectively collected data in rural France over a 3-year period. They were able to capture data on 81,301 inhabitants over the age of 15 that included detailed demographic data and medication use. From a data bank they were able to assess the crude global annual incidence rate of around 13.9 ± 2.4 per 100,000 inhabitants. No statistical difference was noted in DILI incidence between the sexes. The drugs most often implicated were antibiotic, psychotropic, hypolipidemic and non-steroidal anti-inflammatory drugs [NSAIDs]. [18]

One recent study, by Lucena et al in 2009, has also tried to shade some light on the incidence of DILI by gender and age. [19] Prior to this study, there was a

general assumption that old age and being female were risk factors for increased DILI. [2, 5, 18]. However, the Lucena et al study has shown that neither sex nor old age puts individuals at increased risk from DILI.

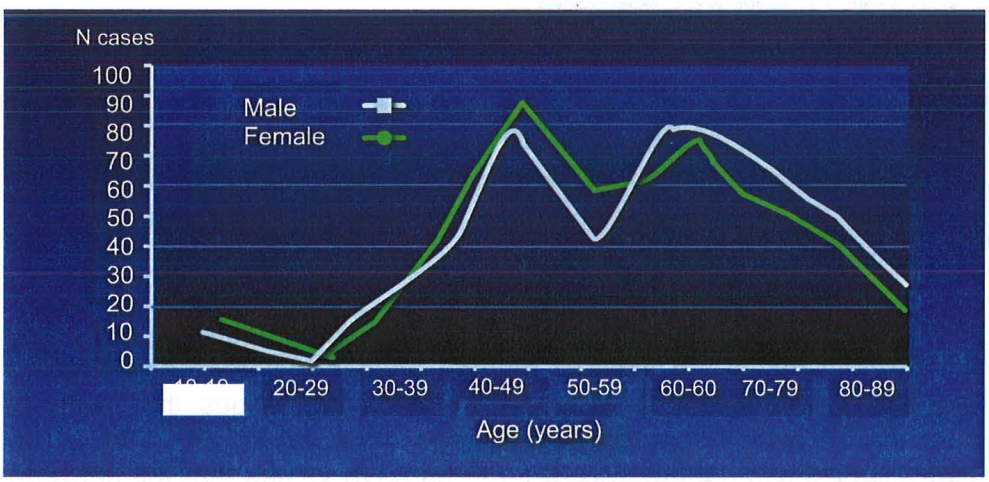


Figure 3. This graph depicts the age and gender distribution in Spanish drug induced liver registry study. Despite long held assumption that older age and female gender predisposes one to DILI, this graph shows similar gender and a non-specific age pattern.

In this Spanish study, more than 650 cases were evaluated in a retrospective manner from 1994-2007. Based on patterns of liver injury, the authors were able to conclude that cholestatic liver injury was more predominant in older males while hepatocellular injury was seen in a younger cohort with female predominance. Their study also showed anti-infective and NSAID to be the predominant drugs implicated in DILI. The VigiBase™ data resource, one of the largest database assembled from three DILI registries including the Spanish, Swedish and US/DILIN network, were able to confirm the finding seen by Lucena et al. [20]

Risk Factors for DILI

In assessing risk factors, it is important to review the data from both a genetic as well as a non-genetic cause of risk factors. A recent review by Chalasani et al discussed these risk factors in detail. [21]

Non-genetic Risk Factors

Age

The traditionally held belief that older individuals are more susceptible to DILI comes from the notion that pharmacokinetic factors will be altered, as we get older. The only well supported evidence, which links age and drugs, limits these to very specific drugs. It is a well-known fact that children are at higher risk for developing ASA sensitivity at a much higher rate than adults in what is termed as

"Rye syndrome" phenomenon. Another example of a childhood restricted phenomenon is related to valproate and erythromycin use. [22] It is also well documented that isoniazid hepatotoxicity increases with age, increasing by a factor of four from age 30 to age 60. The reasons that age affects DILI phenotypes are not well defined. [23]

Gender

Two recent studies did not show gender as a risk factor for DILI. Both, Shapiro and Lewis, and Lucena et al reported similar gender DILI incidence. However, it is prudent to point out that being female has been associated with higher degree of severity and poor outcome as a result of DILI. This finding is also consistent with Lucena et al's finding that hepatocellular pattern with a younger female predominance results in poorer prognosis than its cholestatic counterpart. [17, 19] Liver transplantation data from Russo et al also corroborates this finding in that more women have undergone liver transplantation secondary from DILI. [24]

Drug Interactions

The evidence behind one drug causing or enhancing another drug's hepatotoxic potential has been studied. Multiple drugs that are used to treat tuberculosis have been associated with some form hepatotoxicity. In a recent Meta analysis the use of combination drugs has been shown to increase liver injury as opposed to administration of individual drugs. The role of rifampin in accelerating and amplifying isoniazid hepatotoxicity is a classic example. Other examples of enhanced toxicity are seen in dual use of trazadone and thioridazine. Hull et al reported a case of acute liver failure with these lethal combinations due to CYP2D6 inhibition. [25]

Alcohol Consumption

The story behind the effect of alcohol is less clear. In some settings such as acetaminophen or CCL4 poisoning, it has been shown that alcohol increases toxicity by induction of CYP2E1. However, the impact alcohol has on over all idiosyncratic DILI is not well established. It is also important to point out that the causality assessment scale method that is used as an instrument for diagnosing DILI assigns alcohol consumption as an important risk factor. [23] The DILIN causality assessment system also uses " significant alcohol use " defined as greater than 14 drinks/week as a risk factor. [2] Further studies are warranted to delineate the exact role of alcohol and its culpability for causing DILI.

Underlying Disease States

The FDA points out drug pharmacokinetics are altered in patients with underlying liver disease. However, it also points out the fact that individual drugs are unpredictable and no correlation can be made definitively with liver damage and severity. Tarantino et al has also shown that liver damage and drug correlation is hard to predict. [26] The general consensus from different studies is that no

universal rule can guide us to modify dosing regimens in patients with different chronic liver diseases. [27] However, in a set of specific liver diseases this might be different. Wong et al and several other investigators have shown increased risk of DILI in chronically infected HBV and HCV patients from TB and HAART medication. [28] HIV and HAART therapy poses another challenge in the arena of DILI association. Hepatitis from DILI ranges from 2% - 18% in patients that are treated with HAART. [27] The presence of HCV (especially in genotype 3) or HBV confection has also been shown to increase DILI incidence independently. [29, 30] Patients with Obesity and Nonalcoholic Fatty Liver Disease do not appear to be at increased risk for developing DILI.

Pathogenesis

Lessons learned from APAP pathogenesis

The pathogenesis of DILI is complicated due to the fact that the drugs responsible for the clinical phenotype are numerous in number. Thus, the hypothesis that has been generated for its pathogenesis comes from understanding gained from toxicity of drugs such as acetaminophen. The pathogenesis of acetaminophen was worked out in detail in the late 90's and early 2000. It is well accepted that this drug is a dose dependent toxin and its toxicity is driven by its highly reactive metabolite termed N-Para-aminoquinonimine (NAPQI) that is directly related to the level of glutathione, a rescue molecule produced by the liver. [31] Once NAPQI depletes glutathione the fate of a hepatocyte depends in its ability to counter balance damaging reactive oxygen species and rescue elements of downstream nuclear response. Activation of Nrf-2 due to depletion of GSH leads to the activation of antioxidant response element (ARE) in the nucleus that in turn activates protective genes such as glutamyl cysteine ligase (rate-limiting enzyme of GSH synthesis) and heme oxygenase. [32] The flip side of this equation is ATP depletion and necrosis due to loss of the permeability transition (MPT) of the cell mediated by the collapse of mitochondrial function. In the case of APAP, necrosis can switch to an apoptotic mode if the cell can maintain ATP via anaerobic mode because of release of cytochrome c and ATP-dependent activation of caspases. Additional studies have also shown that once a cell is damaged by NAPQI adducts, the innate immune system amplifies the damage; it does this by initiating downstream signaling cascades to start the recovery process by removing the weak and damaged cells by the process of the so-called " danger hypothesis of immune surveillance." [33, 34]

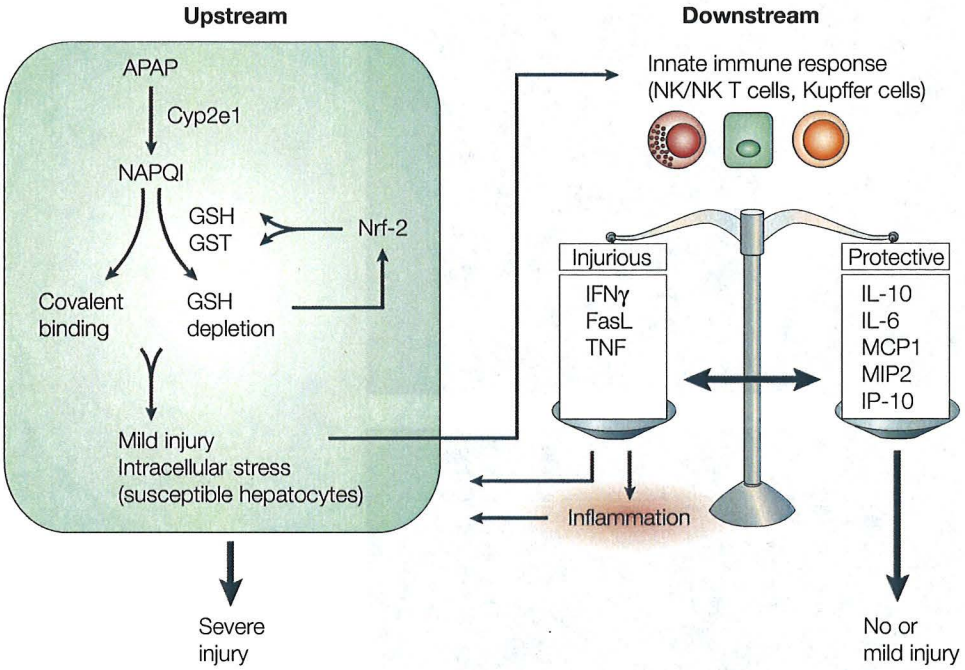
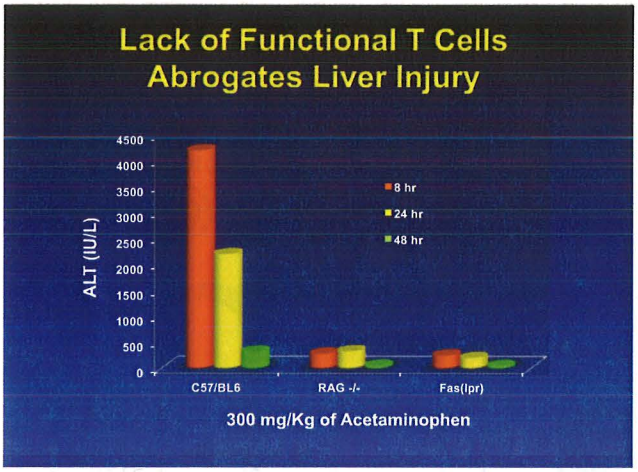


Figure 5. Upstream events will lead to the recruitment and activation of protective intracellular response by up regulating factors such as transcription factor NRF2. Additional responses include activation of the innate immune system, which activates the downstream innate immune system. The ultimate outcome of this cascade of response depends on the pro verses anti protective cytokine responses as depicted in the figure. Adapted from Kaplowitz, N Hepatology, February 2006:S235.

Knockout or mutant mice that lack interferon-γ (IFNγ) or Fas are known to be APAP resistant. [34, 35] Our laboratory has replicated this finding either by using functional T cell knocks-outs or mice that lack CTL regulation. RAG -/- mice lack the ability to rearrange T and B cell receptors; thus, they will not be able to mount effective T cell immune response. As shown in the graph below, these mice were resistant to acetaminophen toxicity. On the other hand, mice that lack granzyme B (GrB -/-) or serpinb6 (SPI6 -/-) were sensitive to acetaminophen toxicity. Both these results demonstrate the importance of immune arm in the toxicity of acetaminophen.



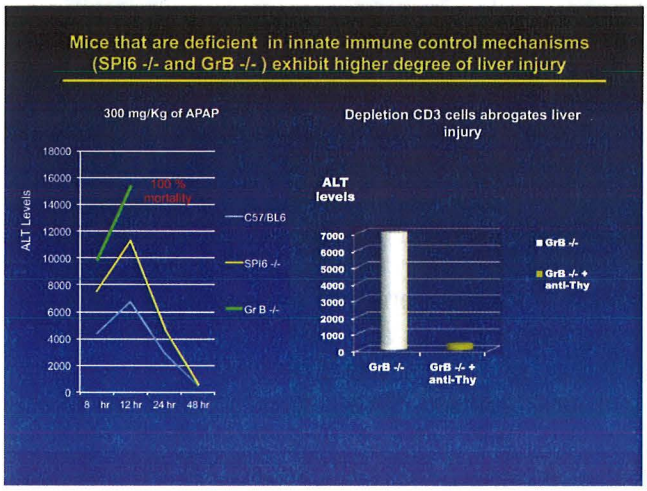


Figure 6. Models to test liver toxicity includes knock out animals that either enhances T cell response or depress its activity. Rag (-/-) and FAS^{LPR} has been shown to impact this outcome by resisting APAP injury. Granzyme B (-/-) and SPI6 (-/-) show enhanced toxicity due to enhanced T and NKT cell activity and/ or cell death protection mediated by GrB arm of the effector immune arm in the case of SPI6 (-/-).

In idiosyncratic DILI, the immune system is believed to be involved as the result of a concept called "haptenization". In this process a covalent bond is formed between the drug and cellular protein, which ultimately is presented by antigen presenting cells leading to cytotoxic T cell recognition or a natural killer cell response. [36] The fact that some people are susceptible to some drugs while the majority is spared remains a mystery. Different theories have been proposed including risk factors that include genetic and non-genetic risk factors.

Diagnosis

Diagnosing DILI has been a daunting task to physicians due to lack of specific

biomarkers and the rarity of the phenotype along with a very diverse range of clinical presentation. [37] There are a variety of ways a diagnosis could be made for a specific disease entity. In the case of DILI, different modalities have been attempted to arrive at a better sensitivity and specificity. These include biochemical tests, liver biopsy, genetic tests, causality assessment scales and re-challenging patients with the same drug to recapitulate similar clinical scenario. But at the end of the day, in 2013, DILI is, in part, a diagnosis of exclusion.

Biochemical tests (ALT/AST/ BILI)

The biochemical tests that are routinely used to test for liver injury include AST, ALT, Alk Phos and bilirubin among others. These biomarkers indicate that a hepatocyte or biliary canalicular cell has been injured, but they do not implicate the cause of this abnormality. The possibilities in the rise of the liver test range from gallstone disease to viral hepatitis to pregnancy related conditions. In addition, the fact that the prevalence of clinically significant DILI is rare adds a poor positive predictive value to these tests. In light of this fact, different investigators have raised a fundamental question regarding utility of periodic monitoring of ALT for prevention of DILI. McNeill et al addressed this issue in 2003 and concluded that the evidence to suggest periodic monitoring is very scant at best. [38] The current suggested guidelines from the FDA and other societies point to the need to monitor LFT periodically. If ALT levels more than 3 times of the upper normal limit is noted within the time frame of 5 to 30 days after initiating a drug, DILI should be considered highly in the differential diagnosis. If the ALT drops by more than 50% within a week after cessation of the suspected drug the index of suspicion for DILI should be even higher. [39]

The question of how to monitor patients for potentially early diagnosis and/ or prevention of DILI remains unanswered. [40] In recent troglitazone risk assessment, the reviewers concluded that despite the recommended monthly LFT monitoring less than half of the patients were compliant for various reasons. [41] Even more worrying is the fact that some of the cases that resulted in acute liver failure had a monthly LFT monitoring with full compliance, and the progression of liver injury from normal ALT to liver failure took place in less than one month. [9, 10] Despite these uncertainties, FDA's final suggestion of this conundrum is to continue monthly monitoring since no other alternative is available and monitoring is better than not doing anything at all.

Liver Biopsy

The role of liver biopsy in DILI diagnosis is a controversial issue. This is because there is no pathognomonic hallmark one could follow that is specific to DILI. However, liver biopsy is performed in many cases of suspected DILI to either exclude other liver diseases or determine the extent of the injury. [42, 43] The abnormalities that are seen on liver biopsies include eosinophilic infiltrates and

granulomas, micro vesicular steatosis, balloon degeneration, hepatocyte necrosis as well as zone 3 necrosis. Even though these findings could be suggestive of DILI, they are by no means specific to this entity. [44] Probably the use of liver biopsy should be used when autoimmune hepatitis (AIH) is suspected and the drug also mimics an AIH like syndrome. A recent review by Suzuki et al showed that liver biopsies were able to distinguish the above entities by using a combination of liver biopsy features including intra-acinar lymphocyte infiltrate or canalicular cholestasis in the absence of portal inflammation, rosette formation, plasma cell portal inflammation and intra-acinar eosinophil infiltration. [45].

CAM (Causality Assessment Methods)

In light of the fact that no gold standard methodology is available for diagnosing DILI, a causality assessment scale has been proposed over the last few decades. A group of international experts convened in France in 1989 to suggest causality assessment scale under the umbrella of Council for International Organizations of Medical Sciences (CIOMS); this was summoned by Danan and Benichou of the Drug Safety Department of the French pharmaceutical maker Roussel Uclaf.[46] They used both clinical and laboratory data to assess the causality of a drug and its potential implication for causing DILI. These parameters included time to onset of symptoms, time to normalization of liver test after drug discontinuation, risk factors, concomitant drug use, alternative non-drug-related causes of liver injury, previous information on hepatotoxicity of the drug, and response to re-administration. A scale of scoring category was assigned from "definite to exclude" based on a scoring system that ranged from -8 to +14.

Ever since its inception, this scale named RUCAM, has come under criticism for its limitations and applicability, which include lack of clear definition and what time point to use to assess liver test abnormality. It also lacks guidance for a patient that is known to have chronic liver disease such as hepatitis C or B infection and a concomitant drug is implicated in causing further liver injury. The use of multiple drugs as a potential culprit here poses another dilemma in the applicability of this scoring system.

Modifications have been made to address some of these concerns in recent years. These include the Maria and Victorino method and the Naranjo scale, which modify the RUCAM scoring method. When these different scales were compared to each other, RUCAM has been found to perform best for diagnosing hepatotoxicity. Despite this, RUCAM has not been adopted in general clinical practice due to its time-consuming nature. However, it still remains the most commonly used diagnostic tool for DILI adjudication. A recent study done by the DILIN network compared the RUCAM scale to a structured expert opinion. The investigators from this study reported that a structured expert opinion had a better inter-rater and re-test reliability. However, they also point out that the universal applicability of the use of structured expert opinion in daily clinical

practice is not a realistic scenario since these experts will not be available at all times. [47]

Method for causality assessment of adverse drug reactions (RUCAM)

Criteria		Score
1. TIME TO ONSET OF THE REACTION		
	Highly suggestive	*+ 3*
	Suggestive	+ 2
	Compatible	+ 1
	Inconclusive	*0*
If incompatible, then case "unrelated"		
If information not available, then case "insufficiently documented"		
2. COURSE OF THE REACTION		
	Highly suggestive	+ 3
	Suggestive	+ 2
	Compatible	+ 1
	Against the role of the drug	- 2
	Inconclusive or not available	0
3. RISK FACTOR(S) FOR DRUG REACTION	Presence	+ 1 to + 2[a]
	Absence	0
4. CONCOMITANT DRUG(S)[c]	Time to onset incompatible	0
	Time to onset compatible but unknown reaction	- 1
	Time to onset compatible and known reaction	- 2
	Role proved in this case	- 3
	None or information not available	0
5. NON DRUG-RELATED CAUSES[c]	Ruled out	+ 2
	Possible or Not investigated[b]	+ 1 to - 2
	Probable	- 3
6. PREVIOUS INFORMATION ON THE DRUG		
	. Reaction unknown	0
	. Reaction published but unlabelled	+ 1
	. Reaction labelled in the product's characteristics	+ 2
7. RESPONSE TO READMINISTRATION	Positive	+ 3
	Compatible	+ 1
	Negative	- 2
	Not available or Not interpretable	0
or PLASMA CONCENTRATION of the drug known as toxic		+ 3
or VALIDATED LABORATORY TEST with high specificity, sensitivity and predictive values	Positive	+ 3
	Negative	- 3
	Not interpretable or not available	0

[a] one additional point for every validated risk factor (maximal value + 2)
[b] depending on the nature of the reaction
[c] Sum of negative values of criteria 4 and 5 cannot be lower than - 4

Figure 7. Depicts the original RUCAM scale adapted by CIOMS for the assessment of DILI. The scale uses seven predefined clinical and historical information categories to assess the likelihood of a drug as a causality agent in

the liver injury. Adapted from Danan and Benichou et al. J Clin Epidemiol: 46: 1993: 1323-1330

Challenge/Re-challenge

The gold standard of DILI diagnosis should rely on identifying cases that are re-challenged and result in a confirmation of repeat injury once the initial insult has subsided. However, studies have shown us that this kind of practice has tremendous risk to the patient. In one of the largest retrospective studies performed by GSK, data from 1958 to 2007 was reviewed in a post-marketing surveillance adverse event report. Papay et al reviewed over 36,000 cases and found 88 cases that met the requirement of a positive re-challenge case. Of the 88 patients that were re-challenged 2 patients (>3%) had a fatal outcome. [48] A similar finding has been cited about the fate of re-challenged patients from a Spanish group where they had 4% near-fatal outcomes that were rescued by liver transplantation. [49] Lee et al also warn us in their 2003 review by stating that "re-challenge is typically met with a more severe reaction regardless of whether the initial reaction was severe or mild" and "cautious re-challenge should be considered only if the diagnosis of drug-induced toxicity was highly questionable and only if no other drug is available to treat a serious problem." [50] The FDA has also weighed in on the concept of re-challenge in its 2007 concept paper where it emphasized re-challenging not be considered if the initial damage has shown ALT levels >5 x UNL.

This is not to say that a re-challenge should not be implemented at all. Whenever a patient's conditions mandate we treat the clinical condition and/or alternative therapies are not an option re-challenge has been a reasonable option. This has been done with patients that have pulmonary tuberculosis who would warrant therapy to save their lives. In a recent study done by Sharma et al drug re-introductions were performed without significant DILI. In a prospective trial from 2004-2009 they were able to re-challenge patients using three-drug regimen of isoniazid, rifampicin, and pyrazinamide at either full dose of all drugs from the start or gradual introduction of the three drugs at different doses. They had an 89% success rate while only 11% had recurrence of severe DILI and treatment had to be stopped. No death or liver failures were noted in this study showing successful re-introduction of medication. [51] The only other study that tried to address the re-introduction of anti-TB drugs was conducted in Turkey where they reported a 24% DILI rate after reintroduction of medication including pyrazinamide. [52]

Diagnosis of Exclusion

Currently, we do not have a definitive test for diagnosing DILI. The lack of specific testing for detection of this clinical entity hampers us from assigning causality with precision. Thus, at this point we largely rely on making this diagnosis after we rule out other potential causes of abnormal liver test in patients with this presentation. This is important clinically since DILI has a wide

spectrum of manifestation that can simulate viral hepatitis, acute AIH, PBC or gallstone disease. [4] Exclusion of these diseases requires serological studies of viral markers and AIH, US evaluation to exclude gallstone disease, appropriate testing to exclude hemochromatosis and hemolysis. [39]

In a recent update, cases assigned to DILI from the DILIN registry had to be revised after acute hepatitis E has been implicated as the culprit. [53] In this study, patients that were enrolled in DILIN prospective study had their serum data reanalyzed for the presence of HEV RNA and IgM levels. Among the 318 patients that were tested about 16% tested positive for anti-HEV IgG conferring history of exposure. Further analysis showed that 4 patients had positive HEV RNA confirming the diagnosis of acute HEV despite the previous assertion that a drug was implicated for the liver injury. The study concluded that routine testing for HEV should be considered when adjudicating for DILI.

However, in some cases despite our best effort we might not be able to implicate a drug as cause for the liver injury. This is true when drugs such as nitrofurantoin or minocycline cause the injury due to the fact that the liver injury pattern by these drugs closely mimics AIH. [54] In their analysis, these authors have concluded that the use of steroids could help to differentiate the DILI caused by these drugs versus AIH; virtually all cases of DILI in this setting had no relapse after the cessation of steroid while AIH had greater than 90% relapse potential.

If a diagnosis of DILI is considered, it is important to consider its hepatotoxic potential and a cross-reference for its past history should be established using the NIH website
http://livertox.nih.gov.

Genetics of DILI

Studying the genetics of DILI is a difficult task because this disease expresses a rare phenotype. Over the last decade progress has been made in addressing the potential role genetics could play in this process. The initial approach the science took was to identify and study sets of candidate genes to spot susceptible genes. The method had some drawbacks in that people were only investigating only the "obvious" genes that they deemed were important. This kind of study limits one's choice to a biased approach based on an arbitrary method. [55]

However, despite its limitation important associations have been established between certain drugs and gene polymorphisms. This was well demonstrated between the drug isoniazid and N-acetyltransferease 2 (NAT2) where individuals termed slow acetylators were found to be in higher risk category. Other associations have been implicated in smaller studies that found a relationship between INH and human leukocyte antigen (HLA) class II DRB1*0701-DQB1*02 haplotype. Despite this suggestion the only consistent result that has linked antituberulosis drug to DILI has been NAT2 gene.

Similar pattern have emerged for other drugs as well linking a candidate gene to a specific drug. Amoxicillin/Clavulanate, a widely used antibiotic, has been linked to HLA class II (DRB1*1501) genotype in its association with DILI.[49, 56] Perhaps the strongest association using a candidate gene method comes from the link that was made between Flucloxacillin and HLA-B*5701 allele. In the study preformed by Daly et al an association linkage with a 80-fold increased risk was implicated.[55] However, when general DILI cohorts were looked at from different groups no clear association were established except for a few genes where HLA's such as DRB1*07 and DQB1*02 showed a potential protective effect against amoxicillin/clavulanate hepatotoxicity. [57] Cytokine genotypes also showed no clear associations with general DILI with an exception of an IL-10 haplotype group that showed poor clinical outcome in the setting of DILI. [58]

Candidate gene studies have been replaced by Genome-wide association studies (GWAS), especially now with the availability of comprehensive data on variability in human genes from the Hap Map Projects. This method of investigation has also yielded a very fruitful understanding of DILI and its relationship to genetics. The table below summarizes all the possible association that has been made to different drugs using this methodology.

HLA alleles in DILI

Phenotype	Drug	HLA
Cholestatic	Flucloxacillin	B* 5701
	Ticlopidine	A*3303
Hepatocellular	Ximelagatran	DRB1*0701 DQA1*0201
	Nevirapine	DRB*0101
	Lumiracoxib	DRB1*1501 DQB1*0602 DRB5*0101 DQA1*0102
	All DILI	STAT4
Mixed	Amoxicillin - Clavulanate	DRB1*1501 DGB1*0602 DQA1*0201
	Lapatinib	DQB1*0202 DRB1*0701

Figure 8. HLA association has been sited by several GWAS studies implicating specific drugs to specific HLA regions. The spectrum of liver injury could vary from cholestatic to hepatocellular but the ultimate association was made to an HLA region implicating the association of the immune system and its importance in the pathogenesis of DILI.

Like the candidate gene method, GWAS has several limitations, which include limited structural variants in the genome of investigation, lack of its ability to account for the impact of the environment and inherited factors (so called epigenomics and epigenetics). [59] In the largest GWAS studies to date done on DILI, Urban et al tried to assess the overall ability of GWAS to detect DILI as a disease entity. They were able to review over 800 patients and 200 drugs. [60]

This study concluded that no SNP had a clear association with DILI events. The only potential association was linked to STAT4 gene and common DILI. STAT4, a transcription factor that is restricted to myeloid cells, thymus and testis, is required for the development of Th1 cells from naive CD4+ T cells and IFN-γ production in response to IL-12. Thus, the only real linkage that has been made to DILI has been only to immune response genes despite the previous held assumptions that drug metabolizing or drug transporter genes were involved. These recent findings also highlight the complexity of rare phenotypes that have low genetic penetrance. It also points out despite its promising results GWAS has very low predictive value in these rare disease phenotypes.

So, the question at hand is where this leaves the use of genetics and personalized medicine that has been much talked about in the lay press. The good news is the next generation of genetic sequencing is here where the whole genome can be interrogated at a reasonably cheaper price. However, the amount of information that has to be processed to assess the result in a meaningful and timely manner is going to require the collaboration of multiple centers with the ability to process this massive information. The role of epigenetics and epigenomics will have to be further studied once whole genome sequencing becomes the norm to study DILI and other diseases.

Outcomes of DILI

The majority of DILI cases have a favorable outcome. This is true because most liver injuries from drugs only have mild transaminitis that resolve without clinical sequelae a pattern termed "adaptation". However, some drugs have been implicated in causing long-term complications. It has long been recognized that DILI can be associated with the development of ductopenia, progressive liver fibrosis and cirrhosis if treatment with the implicated drug is not discontinued. [61] Aithal et al have reported, in a retrospective review, that after a median follow up of 5 years close to 40% of the 33 patients followed had persistent liver test or imaging abnormality. [62] Even though most of the chronic changes in liver injury are results of long-term use of medication, some drugs have been implicated to cause chronic injury from an acute episode. An example of such a drug is methotrexate that has been implicated in causing cirrhosis in rare individuals. [63] A number of drugs associated with cholestatic (CS) type of injury have been associated with the development of vanishing bile duct syndrome with long-term persistent CS injury. A number of different drugs have been reported as a suspect causes of chronic liver disease, including the development of liver cirrhosis. [64]

The other spectrum of DILI is the feared outcome of acute liver failure (ALF). Dr. Zimmerman, a giant in this field, had observed early on that patients that were jaundiced with high levels of transaminase had a fatality rate that ranged from 10-50%. [65] FDA later coined the term "Hy's rule" to show this association and prognosis of DILI. [66] However, despite the FDA endorsement of this association the rule was never validated until groups from the Swedish DILI

registry and the Spanish DILI network confirmed this finding. [67, 68]

The concept of using these biomarkers have been revisited recently by Maddery et al in assessing liver injury in the premarketing arena. This group has been able to show that using the eDISH (evaluation of Drug-Induced Serious Hepatotoxicity) methodology, it is possible to evaluate liver safety in drug development with better sensitivity and specificity. [69] The eDISH 4 quadrant theory obeys the rule that has been proposed by Dr. Zimmerman decades ago where ALT greater than 3x UNL and TB greater than 2x UNL posses greater toxicity to the patient. Using the 4 quadrants on could predict the potential hepatic adverse event in phase 3 trial since eDISH helps to capture all data in systematic fashion and reliable assign a drugs potential for severe DILI.

The hallmark of ALF caused by DILI includes a rise in liver synthetic laboratory values including serum bilirubin, prothrombin time (or the international normalized ratio), and a decrease in serum albumin. As the injury progresses hepatic encephalopathy ensues signaling a poor outcome, which is associated with 70% mortality in cases of idiosyncratic DILI and 40% in acetaminophen without liver transplantation. [44, 70] Lee et al have reported that acetaminophen is the number one drug that causes ALF. [71]

Treatment

The crucial aspect of DILI treatment is arriving at a correct diagnosis, prompt discontinuation of the "offender" and avoiding re-exposure to the implicated drug. The outcome of a good majority of patients, which either progressed to chronic form of DILI or ended up with acute liver failure, is by far most dependent upon recognition of DILI. [36] It is important to note that only very few drugs have an antidote to treat their toxicity. In case of acetaminophen DILI, the use of N-acetyl cysteine (NAC- Mucomyst) has proven to be effective if patients present before the late stages of liver failure. When given within 24 hours of overdose, NAC clearly decreases the risk of liver injury. [72, 73] However, once the patient has progressed to fulminant hepatic failure liver transplantation is the only potential therapy to save a patient's life. The other potential antidote is the use of intravenous L-carnitine in valproate DILI.[74] In the case of leflunomide (Arava) DILI the use of cholestyramine (Questran) has been recommended. [75]

These are few examples where a specific antidote is available or suggested. In the rest of the cases, as mentioned earlier, discontinuation of the drug and monitoring is warranted. If the patients' biochemical tests suggest that they are meeting the "Hy's rule" definition of high mortality risk, referral to a hepatologist is warranted in the eventuality that the patient requires liver transplantation. The use of corticosteroids is of unproven benefit in the setting of fulminant failure. However, in patients that exhibit features of DILI hypersensitivity and persistently elevated LFTs for 3 month, some authorities have advocated its use despite lack

of any controlled trial. [22, 24, 76] If hepatocellular liver injury evolves to ALF, liver transplantation may be the only life saving treatment in 2013. Therefore, the limited available therapeutic option for subjects with severe drug-induced liver injury warrants a heightened attention to physician education and prevention of re-exposure of affected patients.

Summary

In summary DILI, intrinsic or idiosyncratic, presents a large burden on the health care system with significant morbidity and mortality. It is the major reason for medication withdrawal from the market. DILI is difficult to diagnose and study. Thus, clinical judgment is the cornerstone of its recognition and DILI should always be on the differential diagnosis of patients presenting with acute or chronic liver dysfunction. Patients that fulfill HY's rule have poor prognosis, thus, prompt discontinuation of the offending medication is essential to avoid disease progression. If patients progress to hepatic failure they should be referred to a liver transplant center for further management.

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