Biomarkers for drug-induced liver injury

New biomarkers are needed in the clinic to distinguish drug-induced liver injury from other types of liver injuries, and to identify which specific drug is the culprit when drug-induced liver injury occurs in the setting of polypharmacy. There is also a need for new biomarkers that can be used in clinical trials to identify which new drugs in development have serious liver liabilities. When there are few alternative therapies available, biomarkers are needed to be able to identify patients susceptible to drug-induced liver injury from drugs known to have this liability. Developing and validating such biomarkers will require the establishment of well-annotated serum and urine banks from prospective clinical trials of drugs that have serious liver safety liabilities as well as those that do not.

KEYWORDS: biomarkers ■ drug-induced liver injury ■ genetics ■ metabolomics ■ proteomics ■ transcriptomics

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Drug-induced liver injury (DILI) remains a major problem for patients, physicians and those involved in the development of new drugs. There are many reasons for this. Firstly, there are many drugs in use today that can cause serious liver injury, even life-threatening liver injury in a small subset of treated patients. The physician prescribing these drugs generally cannot identify which patients are susceptible to DILI until they develop it. Once a patient develops liver injury, a confident diagnosis of DILI can usually only be attained once other possible causes of liver injury have been excluded. Such an evaluation may be costly and delay stopping the implicated medication. Furthermore, in a patient treated with multiple medications, it may not be possible to confidently identify the specific culprit when DILI is suspected and this may force the physician to stop multiple medications unnecessarily. The situation is compounded by the fact that potentially hepatotoxic drugs continue to enter the market place because serious liver liabilities may not be evident, even on submission of a new drug application. These problems exist largely because of the limitations with currently available biomarkers of DILI, which have not changed in over four decades.

The clinical and histological presentation of DILI can mimic most types of liver disease. Hepatocellular liver injury is generally the DILI of greatest concern as it can evolve quickly and be life threatening before the development of jaundice. For this reason, this review will address promises and challenges in developing and validating new biomarkers needed for hepatocellular DILI.

Current status of DILI biomarkers

The biomarkers most commonly used to detect and manage hepatocellular injury are alanine aminotransferase (ALT) and bilirubin, although others have been proposed (Table 1). Serum ALT is a very sensitive detector of hepatocellular necrosis and is more liver specific than aspartate aminotransferase [1]. However, serum ALT cannot distinguish liver cell necrosis due to DILI from necrosis resulting from other causes, such as viral hepatitis. In addition, even high serum ALT elevations can occur in situations other than hepatic necrosis, including hepatic glycogen accumulation in poorly controlled diabetes [2,3], hepatocyte autophagy in anorexia nervosa [4] and hepatic steatosis [5]. Moreover, frequent and high ALT elevations can be caused by drugs that have little or no potential to cause clinically important liver injury, including some lipid-lowering drugs [6] and anticoagulants [7]. These ALT elevations will generally resolve with continued drug treatment. It is unclear if the elevations reflect true liver injury; however, even when ALT elevations are observed in patients treated with medications capable of clinically important liver injury, treatment can also often be continued with resolution of the elevations. In this instance, it appears that hepatocyte injury, including necrosis, is occurring but resolves in a process termed 'adaptation' [8]. Examples of adaptation that have been observed with drugs capable of causing acute liver failure include ALT elevations caused by isoniazid [9], troglitazone [10] and ximelagatran [11]. It is probable that hepatic necrosis is transiently occurring in these patients, and there

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Table 1. Some promising but nonvalidated serum biomarkers for detecting or assessing hepatocellular injury in humans.

Biomarker	Advantages relative to ALT	Disadvantages relative to ALT	Ref.
GLDH	Released during hepatocellular injury and more sensitive, elevations persist longer after injury. Not present in skeletal muscle	Activity tracks serum ALT so unlikely to distinguish adaptors from susceptible individuals	[55]
PON-1	Decreases observed due to reduced physiologic secretion, so a fall in level may precede a rise in ALT or other leakage enzymes	Low dynamic range, polymorphic expression confounds establishment of normal range, reductions observed in nonliver disease states	[56]
MDH	Released during hepatocellular injury and may be more sensitive	Present in heart and skeletal muscle	[57]
PNP	Also present in Kupffer and endothelial cells so may detect toxicity to these cells	Present in heart and skeletal muscle	[58]
SDH	May translate better across multiple preclinical species to humans	Poor stability in plasma	[59]
GST-α	More sensitive to centrilobular injury	Polymorphic expression confounds establishment of normal ranges	[60]
CK-18 fragments	Released during hepatocyte apoptosis	Small study in drug-induced liver injury	[61]
HMGB1	Released during hepatocyte necrosis	Small study in drug-induced liver injury	[61]
Bile acids	Rises may mean loss of liver function that precedes rises in serum bilirubin or INR	Influenced by diet and fasting. Diurnal variation	[62]

ALT: Alanine aminotransferase; CK: Cytokeratin; GLDH: Glutamate dehydrogenase; GST: Glutathione S-transferase; HMG: High-mobility group; INR: International normalized ratio; MDH: Malate dehydrogenase; PNP: Purine nucleoside phosphorylase; PON: Paraoxonase; SDH: Sorbitol dehydrogenase box.

exists at least one animal model of drug-induced hepatic necrosis that shows reversal of DILI with continued drug exposure [12]. It appears that this adaptation involves altered regulation of multiple gene products including drug metabolizing enzymes [13] and transporters [14]. Changes in transporter regulation have been observed in human livers during recovery from DILI [15], suggesting that similar mechanisms of adaptation may be operative in rodents and humans. A widely accepted theory is that the patients who develop clinically important DILI represent a subset of those with ALT elevations who cannot adapt to the injury. There are currently no biomarkers that can distinguish ALT elevations that are benign from those that can portend progressive liver injury. There are two isozymes of ALT that appear to have different tissue distributions, with ALT2 being more specific in the rat [16]. It has been reported that transcription of the *ALT1* gene is activated in cultured human hepatocytes by peroxisome proliferator-activated receptor-α agonists, and this was proposed as a mechanism underlying serum ALT elevations observed in a clinical trial of an investigational drug (AZD4619) [17]. However, there have been no published studies on the clinical advantages of distinguishing the two isozymes and such tests are not commercially available.

Zimmerman first noted that a patient who presents with jaundice as a result of hepatocellular DILI has at least a 10% chance of developing acute liver failure regardless of which drug has caused the hepatocellular injury [18]. Recent reports have confirmed this observation [19,20], which has been termed 'Hy's Law'. Concomitant elevations in serum ALT and bilirubin is, therefore, a much greater predictor of patient outcome during a hepatocellular injury than serum ALT alone. However, bilirubin together with ALT are not optimal biomarkers because, during a hepatocellular injury, there must be a very substantial loss of functional hepatocytes before the bilirubin rises. Therefore, the patient is in danger of developing liver failure (Figure 1). The ideal biomarker would distinguish the patient who will adapt from those who are in danger of liver failure before the health of the patient is seriously jeopardized.

Road to new DILI biomarkers

Genetics

Genetic factors, at least partially, account for inter-individual differences in susceptibility to DILI from some drugs [21]. For example, it has been demonstrated that people with the HLA B5701 haplotype have an 80-fold greater risk of developing DILI if they receive treatment with flucloxacillin [22]. However, only approximately one in 500 individuals with the HLA B5701 haplotype (which has a prevalence of greater than 5% in Caucasian populations) will develop liver injury when treated with flucloxacillin. In this case, pretreatment

screening to identify susceptible patients may not be cost effective. On the other hand, knowing genotyping for the HLA haplotype could be very helpful in establishing the diagnosis of flucloxacillin DILI and in identifying the culprit medication in a patient with DILI who is receiving multiple drugs.

The extent to which susceptibility to DILI reflects genetic factors is uncertain. The limitation to progress in this research has been the absence of well-annotated gene banks from patients who have experienced DILI. To address this need, the DILI Network (DILIN) [23] and the Severe Adverse Events Consortium [101] have been developing genebanks from patients who have experienced DILI. Analysis of these genebanks has begun.

Serum protein adducts

It is assumed that many drugs cause DILI through the formation of reactive metabolites that bind to specific intrahepatocyte proteins and may interfere with their function. During liver injury, these adducts should enter the circulation along with other hepatocyte proteins such as ALT. Since these adducts would be drug-specific, it may be possible to use these as biomarkers to aid in the diagnosis of DILI and to identify which drug is the cause. To date, the only 'proof-of-principle' of this approach is acetaminophen:protein adducts; detection of these adducts in circulation has been proposed to be pathonomonic for DILI and to confidently identify acetaminophen as the cause for the DILI [24–26]. Acetaminophen:protein adducts have a much longer half-life in serum than acetaminophen or its primary metabolites and may, therefore, have diagnostic value well after other acetaminophen-derived products are no longer detectable [26]. However, serum acetaminophen–protein adducts have been detected in low concentrations in the blood of healthy volunteers receiving therapeutic doses of acetaminophen and in the absence of ALT elevations [James L, Pers. Comm.], suggesting that adducts may be forming within hepatotocytes during therapeutic dosing. Therefore, it is possible that an individual consuming therapeutic doses of acetaminophen would develop high levels of circulating adducts if he/she experienced a liver injury unrelated to acetaminophen, such as acute viral hepatitis.

It should be noted that finding serum protein-adduct biomarkers for most types of DILI may not be feasible. Acetaminophen behaves as a dose-dependent hepatotoxin, liver injury generally occurs only after a very large dose is consumed (>15 g), and the protein-binding metabolite (*N*-acetyl-p-benzoquinone imine) may reflect well more than 5% of total metabolism. With most other drugs capable of causing DILI, the reaction is 'idiosyncratic' and occurs at much lower daily doses of the drug. Lower levels of circulating adducts, if they exist, may be technically challenging to detect. However, the amount of adduct generated might be sufficient to generate an immune response that could be detected.

Antiliver antibodies

Patients with liver injury associated with several drugs, including tienilic acid [27], dihydralazine [28] and halothane [29] characteristically have circulating antibodies to liver proteins. The antigenic proteins are frequently cytochromes P450 but may also be other drug-metabolizing enzymes [30,31]. The current concept is that a highly reactive metabolite covalently binds to, or otherwise damages, the enzyme that produced it [32,33]. It seems most likely that the antibodies are produced only after liver injury has occurred, with release of these predominantly intracellular enzymes. Therefore, these antibodies are probably epiphenomenon not connected

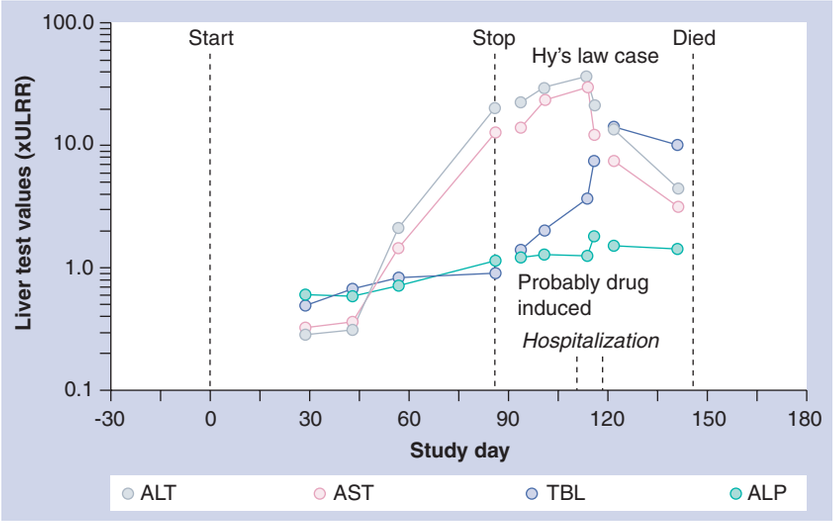

Figure 1. Patient who experienced fatal hepatocellular injury in a clinical trial of a new drug. The patient had normal liver chemistries for the first 45 days of treatment. At 60 days, modest elevations in serum ALT and AST were noted. Experimental drug treatment was continued until 90 days, at which point the serum transaminases were very elevated but the serum bilirubin remained within normal limits. The patient was withdrawn from treatment at this time. Nonetheless, the liver injury progressed to liver failure and the subject expired. It was not until after stopping treatment that the patient satisfied Hy's law criteria (serum ALT > three-times upper limits of normal and serum bilirubin > two-times upper limits of normal). This clinical trial subject points out the danger of continuing to treat in the face of ALT elevations to determine if bilirubin will rise. In addition, it highlights the need for new biomarkers.
ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBL: Total bilirubin; ULRR: Upper limits of reference range.
Image courtesy of John Senior (US FDA).

to the mechanism of liver injury. However, characterizing the circulating antibodies may yield useful biomarkers for the diagnosis of DILI, since different drugs may be bioactivated by different enzymes. There have been few published investigations of the potential for antiliver antibodies as DILI biomarkers, largely because the requisite bank of well phenotyped serum does not exist. The DILIN is creating such a serum bank that should soon be available to investigators [23].

Lymphocyte transformation test

In the lymphocyte transformation test, the patient's lymphocytes are isolated from fresh blood samples and cultured in the presence of the drug suspected to have caused the adverse reactions [34]. A positive test is proliferation of a subset of T lymphocytes measured as the incorporation of radionucleotides or the release of certain cytokines [35,36]. The test is most frequently positive when DILI is accompanied by fever, rash and/or eosinophilia [36,37], consistent with an immunological mechanism underlying DILI. However, lymphocyte transformation has been observed in some patients who have experienced DILI in the absence of hypersensitivity findings [37]. This is consistent with recent reports of associations between HLA haplotypes and susceptibility to DILI, which does not have hypersensitivity features, including hepatocellular injury caused by xymelagatran [38], flucloxacillin [22] and ticlopidine [39]. In Japan, the lymphocyte transformation test is commercially available and is widely used to establish causality links between drugs and liver injury. The lymphocyte stimulation test was performed in 60% of 1676 DILI cases occurring in Japan between 1977 and 2006, and the test was positive in 33% [40]. However, this test is only rarely used in other countries. The DILIN is proceeding with an ancillary study to critically assess the validity of lymphocyte transformation and its potential role in causality.

Proteomics

Techniques are rapidly developing to identify and quantify thousands of serum proteins and to identify patterns of proteins that may yield useful biomarkers for DILI. During treatment with isoniazid, ALT elevations accompanied by hepatitis symptoms (fatigue, nausea and right upper quadrant pain) appear to be more predictive of progressive liver injury (i.e., inability to adapt) than asymptomatic ALT elevations [41]. It seems likely that these symptoms may be mediated by cytokines or other endogenous proteins that may appear in serum long before symptoms appear.

Metabolomics

High-resolution nuclear magnetic resonance and mass spectral techniques can now be used to determine the 'finger print' of thousands of endogenous metabolites present in urine or serum. It is possible to use these technologies to simultaneously quantitate thousands of metabolites in serum or urine obtained before, during and after liver injury. Changes in serum or urine metabolome could potentially distinguish DILI from other types of liver injury, since it seems likely that the initiation and progression of DILI should influence the liver's metabolism of endogenous substances. Such changes could well precede abnormalities in currently available biomarkers. For example, progressive mitochondrial injury has been demonstrated to possibly account for DILI caused by multiple drugs, including fialuridine [42], nefazadone [43] and other drugs [44]. This process could lead to characteristic changes in serum or urine metabolome long before mitochondrial function has deteriorated to the point of hepatocyte damage and ALT release. Some metabolic changes would be expected to be drug or drug-class specific, particularly those that result from 'upstream' processes such as reactive metabolite accumulation. Therefore, metabolomics could be useful in identifying which specific drug is the culprit in the patient with DILI. However, other changes in the metabolome might reflect 'downstream' processes that would be common to most or all forms of DILI, such as those related to inflammation.

The potential role of metabolomics in DILI has been suggested by three studies. In the first, the urinary metabolome was analyzed before and after rats were administered a toxic single dose of acetaminophen [45]. Using a nuclear magnetic resonance approach, these investigators were able to identify a pattern of endogenous metabolites in the baseline (pretreatment) urinary metabolome that correlated with the extent of liver injury observed in each rat after treatment with acetaminophen. The concept of using the serum or urine metabolome to predict outcome from exposure to a drug was termed 'pharmacometabonomics' [45]. In the first published human study of pharmacometabonomics, the urine metabolome was characterized in 24-h urine samples collected from healthy adult volunteers before and after they were

treated with acetaminophen (4 g/day × 7 days) [46]. This regimen produced elevations in serum ALT exceeding two-times baseline value in approximately a third of the subjects. The baseline (pretreatment) urine metabolome did not predict who would develop the ALT elevations as might have been anticipated from the earlier rat study [45]. However, changes in the urinary metabolome determined soon after the start of acetaminophen dosing (but before development of ALT elevations) did correlate with the ALT elevations subsequently observed. Predictive models derived from the urine metabolome contained acetaminophen metabolites, and the urinary excretion products derived from the known reactive metabolite, *N*-acetyl-p-benzoquinone imine, did tend to be higher in those who would subsequently develop ALT elevations. However, the aggregate changes in the endogenous metabolome resulting from the acetaminophen treatment were far more predictive than the acetaminophen metabolites. The concept of predictive changes in the endogenous metabolome occurring soon after starting drug treatment was termed 'early intervention pharmacometabonomics' [46]. This approach may have promise for identifying patients who will develop liver injury during treatment with drugs that cause DILI.

In the third study, unbiased analysis of the serum metabolome revealed that patients who are susceptible to xymelagatran DILI tended to have lower serum pyruvate than nonsusceptible patients, and that treatment with xymelagatran tended to cause a further drop in serum pyruvate [47]. Mechanisms linking this observation to DILI have not been reported. These studies suggest promise for metabolomics approaches in the diagnosis and management of DILI.

As a final note, one recent study suggested that inter-individual variation in colonic flora could influence the metabolic paths taken by acetaminophen in humans [48]. In a large group of healthy adult volunteers, the extent of sulfation of a test dose of acetaminophen was inversely correlated with the urinary elimination of *p*-cresol sulfate [48], *p*-cresol is produced by colonic flora so it appeared that this bacterial product was successfully competing with acetaminophen for sulfation in the liver. The conclusion was that variation in colonic flora may significantly contribute to variable responses to medications, including susceptibility to DILI. This source for variation in susceptibility, which may be more affected by diet than host genetics, may be readily assessable by urinary metabolomics.

Transcriptomics

Hepatotoxicants can produce characteristic changes in mRNA transcripts in the rodent liver [49] and it is reasonable to assume that drug-specific changes in transcriptome occur in humans during DILI. It has been assumed that mRNAs released during hepatocyte necrosis are quickly degraded and not measurable in serum. An alternative approach has been to examine changes in whole-blood transcriptome, which reflects changes in mRNAs expressed in the cellular elements of blood, particularly lymphocytes. In one rodent study, changes in whole-blood transcriptome were a more sensitive and specific predictor of the extent of liver injury than serum ALT [50]. In the same study, transcript changes were also observed in whole blood obtained from five patients experiencing severe DILI caused by acetaminophen overdose. In a recent study, many of these same investigators demonstrated characteristic changes in whole-blood transcriptome (and metabolome) after healthy volunteers received a single 4-g dose of acetaminophen [51]. Although these studies are intriguing, the relationship between the liver and lymphocyte transcriptome is unknown. An exciting and recent finding is that during DILI, liver-derived mRNAs, both miRNA [52] and mRNA [53] are detectable in circulating cell-free plasma. A recent study has shown that the liver-derived mRNA in serum is contained in microparticles secreted from hepatocytes, accounting for their survival in the circulation [54]. In this same study, it was demonstrated that much of the liver transcriptome is present in these circulating particles, and that the pattern of these transcripts during liver injury caused by acetaminophen differed from the pattern observed during liver injury due to galatcosamine. It remains to be determined to what extent of the full liver transcriptome is expressed in circulating plasma and the extent to which these observations in rats translate to patients suffering from DILI. Needless to say, the idea of serum yielding a 'virtual liver biopsy' is intruiging and could represent a major advance in biomarkers for DILI.

Biomarkers for DILI in clinical trials

Clinical trials of new drugs could obviously benefit from new biomarkers capable of confidently making the diagnosis of DILI, identifying the implicated drugs and identifying patients susceptible to liver injury if this is a liability of the drug in development. There is an additional and related need for biomarkers that are specific to clinical trials of new molecular entities: the need

for biomarkers that can confidently identify and, ideally, quantify the risk a given drug has to produce clinically important liver injury. Current preclinical testing has not appreciably reduced the incidence of drugs discovered to be capable of producing rare, serious liver toxicities. Such events are often only appreciated in late Phase III clinical trials or after the drug enters the market place. As discussed, serum ALT elevations alone are not reliable indicators of such liability; the current gold-standard 'biomarker' for serious liver liability is hepatocellular injury with elevation in serum bilirubin (Hy's Law, discussed previously). This has been recently defined in a US FDA guidance document as elevation in serum ALT exceeding three-times the upper limits of normal and a serum bilirubin exceeding two-times the upper limit of normal [55]. Liver chemistry data from a Hy's Law case observed in a clinical trial are presented in Figure 1. It should be noted that this patient satisfied Hy's Law only days after stopping treatment with the implicated drug. Moreover, the patient succumbed to liver failure despite stopping treatment before meeting this criterion. The combined elevation of serum ALT and bilirubin reflects serious dysfunction of the liver and is, therefore, not really a biomarker of potential to cause liver injury – it is the drug causing clinically important and potentially life-threatening liver injury. New biomarkers are clearly needed to detect liver liabilities in drugs before (and hopefully well before) the subject becomes ill. In the absence of such biomarkers, there will remain the dilemma of when to stop treatment in a clinical trial when a subject develops isolated elevations in serum ALT. If the drug is stopped before the patient satisfies Hy's Law, a potentially serious liver liability may go unrecognized. To continue to treat until the bilirubin rises may place the subject in harm's way.

Of the approaches outlined in this article, the most promising avenues to identify the needed biomarkers may be plasma analysis of liver-derived mRNA, whole-blood transcriptome analysis, plasma proteomics, and urine and plasma metabolomics. One approach may be to perform these analyses in subjects who experience ALT elevations from drugs capable of causing clinically important liver injury (such as isoniazid) and compare the results with those obtained in patients with ALT elevations from drugs that have little or no liver liability. Although the likelihood of success is greatest when liver injury is occurring, an exciting possibility is that characteristic changes could be observed very early in treatment before onset of liver injury.

Future perspective

The bottleneck in developing new biomarkers for DILI is no longer the required technology, but the existence of appropriate and well-annotated tissue banks. The DILIN has been collecting urine, serum and lymphocytes from all subjects in their registry and this will soon be a rich resource for biomarker discovery. However, since subjects are enrolled only after the diagnosis of DILI is established, blood or urine is not collected from patients before the start of treatment or during treatment. Such prospectively collected specimens will be required for discovery and validation of nongenetic markers capable of predicting whether a given patient will develop DILI and what the outcome of continued treatment will be. Prospectively collected specimens will also be required to identify and validate biomarkers that can replace Hy's Law as a reliable indicator of a drug's potential to cause serious liver injury. The Institute of Medicine convened a workshop in October 2008 to begin to identify the steps that will be required to develop better biomarkers of drug safety. The recommendations include prospective blood and urine collections in patients treated with drugs known to be capable of severe liver injury, as well as drugs that cause frequent ALT elevations but rarely cause severe liver injury [102]. When a potential liver signal is detected in clinical development it is also recommended that the pharmaceutical industry adopt standard protocols for biospecimen collection and link the specimens to the important phenotypic data. It may not always be necessary to analyze these specimens at any time during the lifecycle of the drug. However, it would be highly advantageous to have this resource should a problem arise at any point. An example of this is the research performed after ximelagatran was removed from worldwide markets owing to rare but severe liver injury. Subsequent research identified a genetic association only because DNA had been collected in a small fraction of the clinical trial recipients [38]. In addition, the finding that serum low pyruvate was associated with DILI susceptibility was possible because serum had been saved in a subset of patients participating in clinical trials. However, it was not possible to examine the combined ability of both the genetic variation and pyruvate levels to predict susceptibility because both serum and DNA was not available from the same individuals [47]. It is important that the Institute of Medicine recommendations be enacted.

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Executive summary

Drug-induced liver injury remains a major problem for patients, physicians & drug development

- The potential of a drug to cause drug-induced liver injury (DILI) is often not recognized until late in clinical trials or once the drug is marketed.
- The major type of concern in idiosyncratic hepatocellular DILI.

Available biomarkers for DILI are suboptimal

- Patients that will be susceptible to clinically important DILI can not be identified.
- They are not useful in distinguishing DILI versus other types of liver injury.
- In clinical trials, concomitant elevation of serum alanine aminotransferase and bilirubin ('Hy's Law') can identify drugs with potential for serious DILI, but use of these biomarkers can place subjects at risk.

Genetic testing

- Patients with HLA B5701 haplotype have an approximately 80-fold risk of developing DILI caused by flucloxacillin and genotyping could be useful in diagnosing this form of DILI.
- The DILI Network and the Severe Adverse Events Consortium are in the process of creating and analyzing genebanks from patients who have experienced DILI.

Drug-protein adducts, antiliver antibodies & proteomics

- Acetaminophen-protein adducts in serum appear to be useful in distinguishing DILI caused by this drug from other causes of injury.
- Antiliver antibodies may also be useful in the diagnosis of some forms of DILI.
- Certain proteins, particularly cytokines, may rise early in the course of DILI and identify those likely to develop serious liver injury.

Lymphocyte transformation test

- Widely used in Japan to aid in the diagnosis of DILI, but not elsewhere.
- Most frequently positive when signs of hypersensitivity accompany DILI.

Metabolomics

- Can potentially account for nongenetic factors that can influence DILI susceptibility, including variation in diet and colonic flora.
- Changes in the urine or serum metabolome might identify patients susceptible to DILI.

Transcriptomics

- Changes occur in whole-blood transcriptome occur during some forms of DILI and predominantly reflects changes in lymphocyte gene expression.
- Liver-derived miRNAs and mRNAs can be detected in plasma and this is a promising new line of biomarker research.

Conclusion

- Technologies exist to identify and validate better biomarkers for DILI but the limiting factor in progress is the paucity of well phenotyped biospecimens.
- Establishing such prospectively collected biobanks should be high priority.

Bibliography

Papers of special note have been highlighted as:
- of interest

1 Green RM, Flamm S: AGA technical review on the evaluation of liver chemistry tests. *Gastroenterology* 123(4), 1367–1384 (2002).

2 Sayuk GS, Elwing JE, Lisker-Melman M: Hepatic glycogenosis: an under recognized source of abnormal liver function tests? *Dig. Dis. Sci.* 52(4), 936–938 (2007).

3 Olsson R, Wesslau C, William-Olsson T, Zettergren L: Elevated aminotransferases and alkaline phosphatases in unstable diabetes mellitus without ketoacidosis or hypoglycemia. *J. Clin. Gastroenterol.* 11(5), 541–545 (1989).

4 Rautou PE, Cazals-Hatem D, Moreau R *et al.*: Acute liver cell damage in patients with anorexia nervosa: a possible role of starvation-induced hepatocyte autophagy. *Gastroenterology* 135(3), 840–848, 848.e1–3 (2008).

5 Browning JD, Szczepaniak LS, Dobbins R *et al.*: Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 40(6), 1387–1395 (2004).

6 Kaplowitz N: Idiosyncratic drug hepatotoxicity. *Nat. Rev. Drug Discov.* 4(6), 489–499 (2005).

7 Monreal M, Lafoz E, Salvador R, Roncales J, Navarro A: Adverse effects of three different forms of heparin therapy: thrombocytopenia, increased transaminases, and hyperkalaemia. *Eur. J. Clin. Pharmacol.* 37(4), 415–418 (1989).

8 Watkins PB, Seeff LB: Drug-induced liver injury: summary of a single topic clinical research conference. *Hepatology* 43(3), 618–631 (2006).

- **Summaries of presentations by experts in many different aspects of drug-induced liver injury, including potential biomarkers.**

9 Mitchell JR, Long MW, Thorgeirsson UP, Jollow DJ *et al.*: Acetylation rates and monthly liver function tests during one year of isoniazid preventive therapy. *Chest* 68(2), 181–190 (1975).

10 Watkins PB, Whitcomb RW: Hepatic dysfunction associated with troglitazone. *N. Engl. J. Med.* 338(13), 916–917 (1998).

11 Lee WM, Larrey D, Olsson R *et al.*: Hepatic findings in long-term clinical trials of ximelagatran. *Drug Saf.* 28(4), 351–370 (2005).

12 Torti VR, Cobb AJ, Everitt JI, Marshall MW, Boorman GA, Butterworth BE: Nephrotoxicity and hepatotoxicity induced by inhaled bromodichloromethane in wild-type and p53-heterozygous mice. *Toxicol. Sci.* 64(2), 269–280 (2001).

13 Shayiq RM, Roberts DW, Rothstein K *et al.:* Repeat exposure to incremental doses of acetaminophen provides protection against acetaminophen-induced lethality in mice: an explanation for high acetaminophen dosage in humans without hepatic injury. *Hepatology* 29(2), 451–463 (1999).

14 Aleksunes LM, Campion SN, Goedken MJ, Manautou JE: Acquired resistance to acetaminophen hepatotoxicity is associated with induction of multidrug resistance-associated protein 4 (Mrp4) in proliferating hepatocytes. *Toxicol. Sci.* 104(2), 261–273 (2008).

15 Shayiq RM, Roberts DW, Rothstein K *et al.*: High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. *J. Pathol.* 200(5), 553–560 (2003).

16 Yang RZ, Park S, Reagan WJ: Alanine aminotransferase isoenzymes: molecular cloning and quantitative analysis of tissue expression in rats and serum elevation in liver toxicity. *Hepatology* 49(2), 598–607 (2009).

17 Thulin P, Rafter I, Stockling K, Tomkiewicz C *et al.*: PPAR-α regulates the hepatotoxic biomarker alanine aminotransferase (*ALT1*) gene expression in human hepatocytes. *Toxicol. Appl. Pharmacol.* 231(1), 1–9 (2008).

18 Zimmerman HJ: The spectrum of hepatotoxicity. *Perspect. Biol. Med.* 12(1), 135–161 (1968).

19 Bjornsson E, Olsson R: Outcome and prognostic markers in severe drug-induced liver disease. *Hepatology* 42(2), 481–489 (2005).

20 Andrade RJ, Lucena MI, Kaplowitz N *et al.*: Outcome of acute idiosyncratic drug-induced liver injury: long-term follow-up in a hepatotoxicity registry. *Hepatology* 44(6), 1581–1588 (2006).

21 Pachkoria K, Lucena MI, Molokhia M *et al.*: Genetic and molecular factors in drug-induced liver injury: a review. *Curr. Drug Saf.* 2(2), 97–112 (2007).

22 Daly AK, Donaldson PT, Bhatnagar P *et al.*: HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat. Genet.* 41(7), 816–819 (2009).

▪ **Documents impressive success of a genome wide association study to identify the most highly predictive genetic marker of drug-induced liver injury susceptibility to date.**

23 Hoofnagle JH: Drug-induced liver injury network (DILIN). *Hepatology* 40(4), 773 (2004).

24 Davern TJ 2nd, James LP, Hinson JA *et al.*: Measurement of serum acetaminophen-protein adducts in patients with acute liver failure. *Gastroenterology* 130(3), 687–694 (2006).

25 James LP, Capparelli EV, Simpson PM *et al.*, Acetaminophen-associated hepatic injury: evaluation of acetaminophen protein adducts in children and adolescents with acetaminophen overdose. *Clin. Pharmacol. Ther.* 84(6), 684–690 (2008).

26 James LP, Capparelli EV, Simpson PM *et al.*: Pharmacokinetics of acetaminophen protein adducts in adults with acetaminophen overdose and acute liver failure. *Drug Metab. Dispos.* 84(6), 684–690 (2008).

27 Homberg JC, Andre C, Abuaf N: A new anti-liver-kidney microsome antibody (anti-LKM2) in tienilic acid-induced hepatitis. *Clin. Exp. Immunol.* 55(3), 561–570 (1984).

28 Bourdi M, Larrey D, Nataf J *et al.*: Anti-liver endoplasmic reticulum autoantibodies are directed against human cytochrome P-450IA2. A specific marker of dihydralazine-induced hepatitis. *J. Clin. Invest.* 85(6), 1967–1973 (1990).

29 Nguyen C, Rose NR, Njoku DB: Trifluoroacetylated IgG4 antibodies in a child with idiosyncratic acute liver failure after first exposure to halothane. *J. Pediatr. Gastroenterol. Nutr.* 47(2), 199–202 (2008).

30 Obermayer-Straub P, Strassburg CP, Manns MP: Target proteins in human autoimmunity: cytochromes P450 and UDP-glucuronosyltransferases. *Can. J. Gastroenterol.* 14(5), 429–439 (2000).

31 Manns MP, Obermayer-Straub P: Cytochromes P450 and uridine triphosphate-glucuronosyltransferases: model autoantigens to study drug-induced, virus-induced, and autoimmune liver disease. *Hepatology* 26(4), 1054–1066 (1997).

32 Watkins PB: Antimicrosomal antibodies: what are they telling us? *Hepatology* 13(2), 385–387 (1991).

33 Beaune P, Dansette PM, Mansuy D *et al.*: Human anti-endoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis are directed against a human liver cytochrome P-450 that hydroxylates the drug. *Proc. Natl Acad. Sci. USA* 84(2), 551–555 (1987).

34 Pichler WJ, Tilch J: The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy* 59(8), 809–820 (2004).

35 Merk HF: Diagnosis of drug hypersensitivity: lymphocyte transformation test and cytokines. *Toxicology* 209(2), 217–220 (2005).

36 Schreiber J, Zissel G, Greinert U, Schlaak M, Müller-Quernheim J: Lymphocyte transformation test for the evaluation of adverse effects of antituberculous drugs. *Eur. J. Med. Res.* 4(2), 67–71 (1999).

37 Maria VA, Victorino RM: Diagnostic value of specific T cell reactivity to drugs in 95 cases of drug induced liver injury. *Gut* 41(4), 534–540 (1997).

38 Kindmark A, Jawaid A, Harbron CG *et al.*: Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. *Pharmacogenomics J.* 8(3), 186–195 (2008).

39 Hirata K, Takagi H, Yamamoto M *et al.*: Ticlopidine-induced hepatotoxicity is associated with specific human leukocyte antigen genomic subtypes in Japanese patients: a preliminary case-control study. *Pharmacogenomics J.* 8(1), 29–33 (2008).

40 Takikawa H, Murata Y, Horlike N, Fukui H, Onji M: Drug-induced liver injury in Japan: analysis of 1676 cases between 1997 and 2006. *Hepatology Res.* 39, 427–431 (2009).

41 Nolan CM, Goldberg SV, Buskin SE: Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. *JAMA* 281(11), 1014–1018 (1999).

42 McKenzie R, Fried MW, Sallie R *et al.*: Hepatic failure and lactic acidosis due to fialuridine (FIAU), an investigational nucleoside analogue for chronic hepatitis B. *N. Engl. J. Med.* 333(17), 1099–1105 (1995).

43 Dykens JA, Jamieson JD, Marroquin LD *et al.*: *In vitro* assessment of mitochondrial dysfunction and cytotoxicity of nefazodone, trazodone, and buspirone. *Toxicol. Sci.* 103(2), 335–345 (2008).

44 Xu JJ, Henstock PV, Dunn MC *et al.*: Cellular imaging predictions of clinical drug-induced liver injury. *Toxicol. Sci.* 105(1), 97–105 (2008).

45 Clayton TA, Lindon JC, Cloarec O *et al.*: Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* 440(7087), 1073–1077 (2006).

46 Winnike JH, Li Z, Wright FA, Macdonald JM, O'Connell TM, Watkins PB: Early prediction of acetaminophen-induced hepatotoxicity in humans with pharmaco-metabonomics. *Clin. Pharmacol. Ther.* (2010) (Epub ahead of print).

47 Andersson U, Lindberg J, Wang S *et al.*: A systems biology approach to understanding elevated serum alanine transaminase levels in a clinical trial with ximelagatran. *Biomarkers* 14(8), 572–586 (2009).

48 Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK: Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proc. Natl Acad. Sci. USA* 106(34), 14728–14733 (2009).

49 Blomme EA, Yang Y, Waring JF: Use of toxicogenomics to understand mechanisms of drug-induced hepatotoxicity during drug discovery and development. *Toxicol. Lett.* 186(1), 22–31 (2009).

50 Bushel PR, Heinloth AN, Li J *et al.*: Blood gene expression signatures predict exposure levels. *Proc. Natl Acad. Sci. USA* 104(46), 18211–18216 (2007).

51 Fannin RD, Russo M, O'Connell TM *et al.*: Acetaminophen dosing of humans results in blood transcriptome and metabolome changes consistent with impaired oxidative phosphorylation. *Hepatology* 51(1), 227–236 (2010).

52 Wang K, Zhang S, Marzolf B *et al.*: Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc. Natl Acad. Sci. USA* 106(11), 4402–4407 (2009).

53 Miyamoto M, Yanai M, Ookubo S, Awasaki N, Takami K, Imai R: Detection of cell-free, liver-specific mRNAs in peripheral blood from rats with hepatotoxicity: a potential toxicological biomarker for safety evaluation. *Toxicol. Sci.* 106(2), 538–545 (2008).

54 Wetmore BA, Brees DJ, Singh R *et al.*: Quantitative analyses and transcriptomic profiling of circulating mRNAs as biomarkers of liver injury. *Hepatology* (2010) (Epub ahead of print).

55 Watkins PB, Seligman PJ, Pears JS, Avigan MI, Senior JR: Using controlled clinical trials to learn more about acute drug-induced liver injury. *Hepatology* 48(5), 1680–1689 (2008).

56 Rodrigo L, Hern´andez AF, L´opez-Caballero JJ, Gil F, Pla A: Immunohistochemical evidence for the expression and induction of paraoxonase in rat liver, kidney, lung and brain tissue. Implications for its physiological role. *Chem. Biol. Interact.* 137, 123–137 (2001).

57 Kawai M, Hosaki S: Clinical usefulness of malate dehydrogenase and its mitochondrial isoenzyme in comparison with aspartate aminotransferase and its mitochondrial isoenzyme in sera of patients with liver disease. *Clin. Biochem.* 23, 327–334 (1990).

58 Fukuda M, Yokoyama H, Mizukami T: Kupffer celldepletion attenuates superoxide anion release into the hepatic sinusoids after lipopolysaccharide treatment. *J. Gastroenterol. Hepatol.* 19, 1155–1162 (2004).

59 Horney BS, Honor DJ, MacKenzie A, Burton S: Stability of sorbitol dehydrogenase activity in bovine and equine sera. *Vet. Clin. Pathol.* 22, 5–9 (1993).

60 Giffen PS, Pick CR, Price MA, Williams A, York MJ: α-glutathione-*S*-transferase in the assessment of hepatotoxicity – its diagnostic utility in comparison with other recognized markers in the Wistar Han rat. *Toxicol. Pathol.* 30, 365–372 (2002).

61 Antoine DJ, Williams DP, Kipar A: High-mobility group box-1 protein and keratin-18, circulating serum proteins informative of acetaminophen-induced necrosis and apoptosis *in vivo*. *Toxicol. Sci.* 112(2), 521–531 (2009).

62 Thompson MB: Bile acids in the assessment of hepatocellular function. *Toxicol. Pathol.* 24(1), 62–71 (1996).

Websites

101 Holden A: Detecting and investigating drug induced adverse events: the International Serious Adverse Event Consortium's experience to date www.fda.gov/downloads/Drugs/ScienceResearch/Research Areas/ucm077482.pdf

102 Olson S, Robinson S, Giffin R: Accelerating the development of biomarkers for drug safety: workshop summary www.iom.edu/Reports/2009/BiomarkersDrugSafety.aspx.

- **Outlines key questions to be answered and an agenda for development of new biomarkers for drug-induced liver injury as formulated by a workshop conducted by the Institute of Medicine.**