Biomarkers of Liver Injury due to Toxic Agents: Progress, Current Applications, and Emerging Directions

12

Mitchell R. McGill

Contents

M. R. McGill (✉)
Department of Environmental Health Sciences, Fay W. Boozman College of Public Health, Little Rock, AR, USA

Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA
e-mail: mmcgill@uams.edu

V. B. Patel et al. (eds.), *Biomarkers in Toxicology*, Biomarkers in Disease: Methods, Discoveries and Applications, https://doi.org/10.1007/978-3-031-07392-2_14

Abstract

The liver is unusually susceptible to toxicant-induced injury due to its unique physiology and biochemistry, but current liver injury biomarkers have limited value beyond detection of the injury and the resulting impact on liver function. Better biomarkers are needed for (1) diagnosis and determination of etiology, (2) prognosis, and (3) preclinical assessment of the hepatotoxic liability of new drugs and xenobiotics. A number of biomarker candidates that may meet these needs have been identified over the last 10 years. In addition, several biomarkers with mechanistic importance have been proposed. In this chapter, we will briefly review current liver biomarkers and discuss the most popular emerging biomarker candidates. We will also discuss strengths and weaknesses of each and what work remains to be done to move the field forward.

Keywords

Acylcarnitines · Alanine aminotransferase · Acetaminophen · Acute liver failure · Bile acids · Biomarkers · Drug-induced liver injury · Factor V · Glutamate dehydrogenase · Hepatotoxicity · High-mobility group box 1 · Keratin 18 · Lactate dehydrogenase · MicroRNA-122 · Pyrrolizidine alkaloids

Abbreviations

ADH2	Aldehyde dehydrogenase 2
AFP	Alpha-fetoprotein
ALDH1A1	Alcohol dehydrogenase 1A1
ALF	Acute liver failure
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APAP	Acetaminophen
AST	Aspartate aminotransferase
DILI	Drug-induced liver injury
FABP1	Fatty acid-binding protein 1
FBP1	Fructose-1,6-bisphosphatase 1
FV	Factor V
GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase
HMGB1	High-mobility group box 1 protein
INR	International normalized ratio
K18	Keratin 18
LDH	Lactate dehydrogenase
Lect2	Leukocyte cell-derived chemotaxin 2
miR-122	MicoRNA-122
OPN	Osteopontin
PT	Prothrombin time

Introduction

The liver is highly susceptible to injury caused by xenobiotics owing to its unique blood supply and biochemistry (Fig. 1). Most ingested compounds absorbed from the intestinal lumen travel directly to the liver vasculature through the hepatic portal vein and enter the parenchymal cells of the liver – the hepatocytes – through transporters in the cell membranes. Once inside, enzymes highly expressed in the hepatocytes process those compounds, storing useful nutrients (e.g., glucose) or packaging them for distribution to the body (e.g., triglycerides), while simultaneously preparing potentially harmful substances (e.g., drugs or toxins) for elimination through renal and intestinal excretion. This phenomenon of "first-pass metabolism" is critical for proper nutrient utilization and elimination of toxicants before they can reach the rest of the body. However, it also exposes the liver to higher concentrations of exogenous compounds than those seen by cells in other organs. In addition, some of the enzymes that process those xenobiotics actually make the compounds more reactive, resulting in collateral damage to the cells through reactions with proteins and DNA. As a result, numerous toxins and toxicants, ranging from the amatoxins and phallotoxins in certain species of fungi, to carbon tetrachlo-

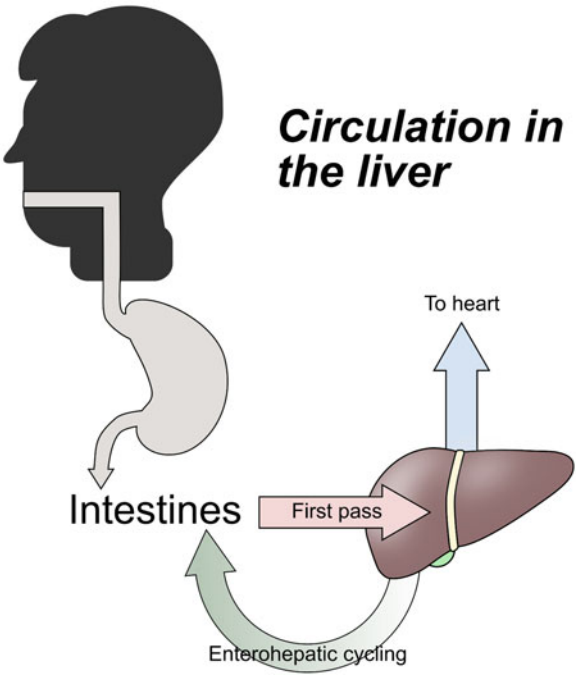

Fig. 1 Hepatic circulation. Ingested compounds travel to the small intestines, where they are absorbed into the blood. Venous blood from the intestines then carries those compounds to the liver, where they are metabolized. Some compounds or their metabolites are then excreted back into the small intestines via bile, where they can be eliminated in the feces or reabsorbed and taken back to the liver ("enterohepatic cycling"). Others enter the systemic circulation. Blood flow from the intestines to the liver is indicated by the red arrow. Blood flow from the liver to the systemic circulation is indicated by the blue arrow. Enterohepatic cycling is indicated by the light green arrow

ride used in industrial applications, to widely used drugs like acetaminophen (APAP), effectively target the liver.

Toxic liver injury is a challenge for clinicians, regulators, and public health practitioners. Clinically, it is difficult to diagnose it in patients, to determine the cause, and to predict its outcome. From a regulatory perspective, it is challenging to identify drugs with potential to cause hepatotoxicity during both preclinical and clinical development before they can reach the market. Finally, from a public health perspective, it is known that some chemicals in the environment can cause chronic liver disease and liver cancer, but it is difficult to determine the significance of those exposures and to monitor them in the real world. One possible approach to address these and other challenges is the development of biomarkers of exposure, diagnosis, prediction, and prognosis in toxic liver damage. Indeed, many investigators have focused their efforts in this area of research over the last 10 years. In this chapter, we will briefly review conventional liver injury biomarkers, discuss the challenges of diagnosis and prediction in toxic liver injury in more detail, and discuss the state-of-the-art liver injury biomarker research.

Current Liver Injury Biomarkers

Although the term "liver function tests" commonly refers to all of the conventional liver-centered biomarkers measured in serum or plasma, it is more accurate to divide them into separate categories: markers of (1) injury, (2) function, (3) proliferation, and (4) infection (Table 1). The major markers of liver injury are the

Table 1 Current liver injury biomarkers

Category	Biomarker	Mechanism of release/elevation/decrease
Biomarkers of injury	Alanine aminotransferase (ALT)	Release: cell death, membrane blebbing, increased expression (?)
	Aspartate aminotransferase (AST)	Release: cell death, membrane blebbing, increased expression (?)
	Alkaline phosphatase (ALP)	Release: cell death, increased expression (?)
	γ-Glutamyl transferase (GGT)	Release: increased expression, cell death
	Lactate dehydrogenase (LDH)	Release: cell death (?)
Biomarkers of function	Total and/or direct bilirubin	Elevation: impaired biliary excretion
	Prothrombin time (PT) or international normalized ratio (INR)	Elevation: impaired synthesis of coagulation factors
	Albumin and other serum proteins	Decrease: impaired synthesis
Biomarkers of proliferation	Alpha-fetoprotein (AFP)	Decrease: impaired proliferation and synthesis
Biomarkers of infection	Viral antigens and antibodies	Infection

aminotransferases, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Elevations in serum ALT and AST in patients with hepatitis were first identified by Arthur Karmen and Fernando De Ritis independently ca. 1955 (De Ritis et al. 1955; Karmen et al. 1955). It is generally thought that these enzymes are passively released from damaged or dying cells due to loss of plasma membrane integrity. The latter is supported by the observation that serum ALT values remain normal or relatively low during early TNF signaling-mediated apoptotic liver injury in mice – a carefully controlled process of cell implosion – but later increase with progression to secondary necrosis (Lawson et al. 1998; Leist et al. 1995). Additional mechanisms have also been proposed, such as membrane blebbing in which protrusions off the plasma membrane grow and burst, and increased expression (McGill 2016). The most common methods to measure ALT and AST today use a coupled enzyme reaction in which their pyruvate or oxaloacetate products, respectively, are further metabolized by lactate dehydrogenase or malate dehydrogenase, consuming NADH in the process. The loss of NADH in the reaction is then measured by absorbance (Karmen et al. 1955; McGill 2016). Other markers in this group include serum alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT), though it should be noted that elevated ALP and GGT activity in circulation in liver disease may be due in part to induction of hepatic expression instead of or in addition to passive release due to injury (Pike et al. 2013; Teschke et al. 1977; Wu et al. 1976). In fact, this is widely accepted in the case of GGT due to reports in the 1970s that serum values for GGT correlate well with hepatic levels (Teschke et al. 1977). However, it should be noted that not all studies have been able to reproduce those findings and there is still some disagreement (Selinger et al. 1982).

The major markers of liver function are bilirubin and prothrombin time (PT). Bilirubin is a product of erythrocyte degradation (Erlinger et al. 2014). Aging erythrocytes are phagocytosed by macrophages, where the heme group of hemoglobin dissociates as a result of low lysosomal pH. Heme is then converted to biliverdin by heme oxygenase, and the biliverdin is reduced to bilirubin via bilirubin reductase. Bilirubin can then circulate in the blood in a complex with serum albumin. At the liver, bilirubin is taken up by hepatocytes, where it is conjugated with glucuronic acid. Finally, the conjugated bilirubin is transported into bile and excreted in feces via the intestines. Elevations in serum conjugated bilirubin (also called "direct" bilirubin because it reacts quickly in commonly used bilirubin tests without addition of reaction accelerants that are necessary to measure hydrophobic free bilirubin) are often observed in obstructive liver diseases (e.g., gallstones) due to impaired excretion as a result of the obstruction (Dufour et al. 2000). These elevations are sometimes also seen in severe hepatocellular damage (Dufour et al. 2000). Apparently, even the severely damaged liver retains some capacity to take up and conjugate bilirubin but cannot excrete it properly, resulting in elevated serum values. The liver is also the site of synthesis of all but one of the major coagulation factors, including the critical components fibrinogen (factor I), prothrombin (factor II), and factors V and X that are essential for the common pathway of coagulation. Thus, liver damage leads to reduced coagulation factor synthesis and therefore increased PT. PT is measured by mixing citrated plasma with calcium and thromboplastin

(a mixture of phospholipids and tissue factor) and measuring the time required to form a clot. The international normalized ratio (INR), a normalized value calculated from PT, is also increased in severe liver damage.

Alpha-fetoprotein (AFP) is unique as it is the sole marker of hepatocyte proliferation in use. Serum AFP is commonly measured as a tumor marker to diagnose, monitor, and prognosticate in hepatocellular carcinoma and some other cancers (Lai et al. 2017; Mizejewski 2004). It is also a critical part of birth defect screening, as maternal serum AFP is one of the tests used in the triple and quad screens (Crandall 1981; Mizejewski 2004). Recent studies have also demonstrated that it has prognostic value as a marker of liver regeneration and recovery in acute liver failure (ALF) (Schiødt et al. 2006; Schmidt and Dalhoff 2005; Singh et al. 2019; Varshney et al. 2017). However, it is not yet widely used for that purpose due to limitations including the fact that differences between transplant-free survivors and non-survivors are not clear until late in the progression of injury.

Finally, the markers of infection consist primarily of viral hepatitis antigens and antibodies (Peeling et al. 2017). These include IgM anti-hepatitis A virus antibodies (anti-HAV), IgM hepatitis B core protein antibodies (anti-HBc) and hepatitis B antigens (e.g., HBsAg), and, finally, hepatitis C antibodies (anti-HCV). PCR tests to detect and quantify viral load are also helpful in some cases (Peeling et al. 2017).

Although the focus of the remainder of this chapter will be markers of injury, it is useful to keep in mind that these other biomarkers of liver function, hepatocyte proliferation, and infection can complement investigation of liver injury by allowing one to probe the causes and predict outcomes of injury. We will now cover major issues with current liver injury biomarkers and recent developments in novel markers.

Limitations of the Current Biomarkers

The current biomarkers of liver injury, ALT and AST, are useful for detection and diagnosis of liver injury once a patient is symptomatic. However, they suffer several limitations. First, ALT and AST are not etiology-specific and therefore cannot be used to diagnose the cause of liver injury, excepting the modest utility of the AST/ALT ratio in identification of alcohol-induced liver disease. Second, these tests have very poor prognostic utility. ALT and AST values do not correlate with outcome after acute liver injury (Christensen et al. 1984; Dufour et al. 2000; Karvellas et al. 2017; Kuroda et al. 2021; McGill et al. 2014a; Tygstrup and Ranek 1986) and only weakly correlate in chronic liver diseases (Dufour et al. 2000). In addition, there is evidence that ALT at presentation is a relatively poor predictor of later liver injury in patients who present early after an insult such as APAP overdose (Dear et al. 2018). And finally, ALT and AST lack specificity for liver damage. Both enzymes are present in other tissues, particularly muscle and kidney (LaDue and Wroblewski 1956), limiting specificity for the liver in general. In addition, there are numerous reports of minor to moderate nonprogressive serum ALT elevations due to certain drugs in the absence of other evidences of liver injury

(Harrill et al. 2012; Singhal et al. 2014; Watkins et al. 2006), demonstrating less-than-desirable specificity for damage. Over the last two decades, a number of novel serum biomarkers have been discovered and proposed by research laboratories to address these limitations, and in some cases, their clinical value is just now being realized. These biomarkers are summarized in Fig. 2 and discussed in detail in the following sections.

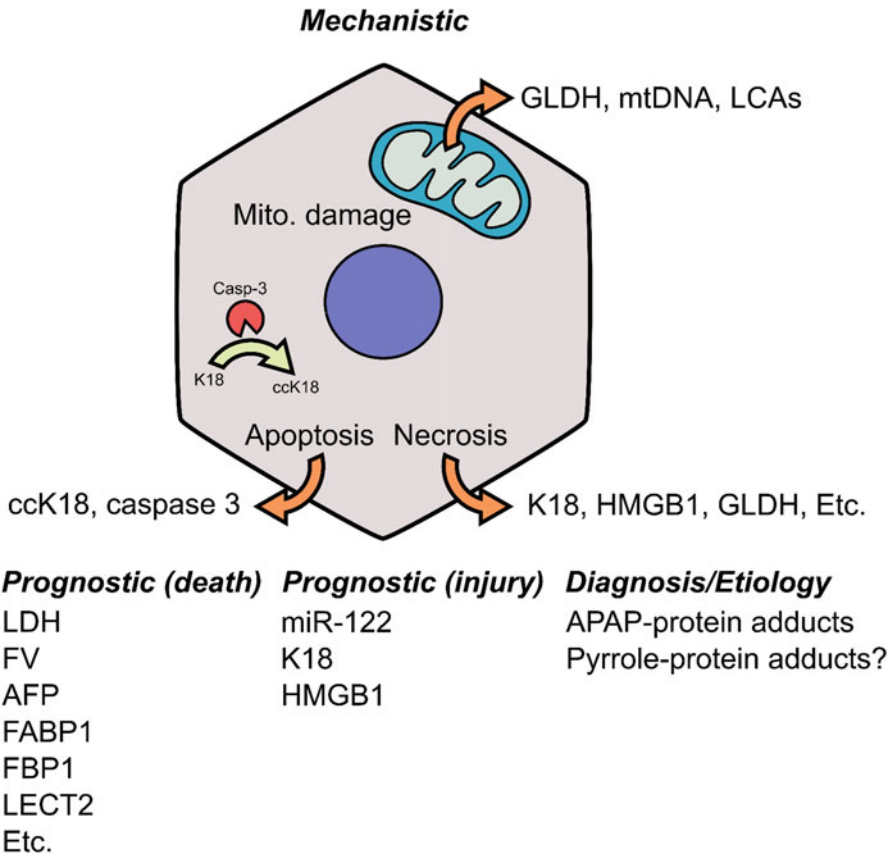

Fig. 2 Popular emerging biomarkers of liver injury. A number of biomarkers of liver injury have been developed with evidence to support various uses. Mechanistic/translational biomarkers indicate mitochondrial damage (glutamate dehydrogenase (GLDH), mitochondrial DNA (mtDNA), and long-chain acylcarnitines (LCAs)), apoptosis (caspase-cleaved keratin 18 (ccK18) and caspase-3 activity), and necrosis (full-length K18, total high = mobility group box 1 protein (HMGB1), and others). Some of these biomarkers also have prognostic utility, as indicated, in addition to lactate dehydrogenase (LDH), factor V (FV), alpha-fetoprotein (AFP), fatty acid-binding protein 1 (FABP1), fructose-1,6-bisphosphatase 1 [FBP1], leukocyte cell-derived chemotaxin 2 (LECT2), and others. Finally, emerging biomarkers for diagnosis/etiology include APAP-protein adducts for APAP overdose and pyrrole-protein adducts for pyrrolizidine alkaloids

Emerging Biomarkers of Etiology and Exposure

Currently, there is only one commonly used biomarker with sufficient specificity to diagnose the cause of toxic liver injury, aside from routine therapeutic drug monitoring to identify drug plasma concentrations outside normal ranges. We have known since the 1970s that the drug APAP is converted to a reactive metabolite that binds to proteins (Jollow et al. 1973) (Fig. 3). This fact has been exploited to develop APAP-protein adducts as a serum biomarker of APAP exposure and overdose. The earliest methods to measure APAP-protein adducts were immunoassays using antibodies against APAP or an APAP-cysteine conjugate (Roberts et al. 1987). Using this approach, APAP-protein adducts were initially measured in liver tissue and serum from APAP-treated mice and roughly a decade later in serum from APAP overdose patients (James et al. 2001; Pumford et al. 1989). Shortly after the first measurements in humans, an HPLC-based method was developed with electrochemical detection (Muldrew et al. 2002) followed later by mass spectrometry detection (Cook et al. 2015; McGill et al. 2011; Xie et al. 2015). Values $\geq$1 μM in the context of elevated ALT are considered specific for APAP overdose (Alonso et al. 2015; James et al. 2009; Khandelwal et al. 2011). Currently, only one Clinical Laboratory Improvement Ammendements (CLIA)-licensed laboratory offers serum APAP-protein adducts as part of their test menu (Acetaminophen Toxicity Diagnostics, LLC, in Little Rock, AR, United States), but expansion to other laboratories is possible in the coming years. In addition, the same company has developed a lateral flow immunoassay calibrated to the 1 μM cutoff (Roberts et al. 2017) and is currently seeking approval for the device from the US Food and Drug Administration. Thus, APAP-protein adduct testing for clinical use may become more common in the near future.

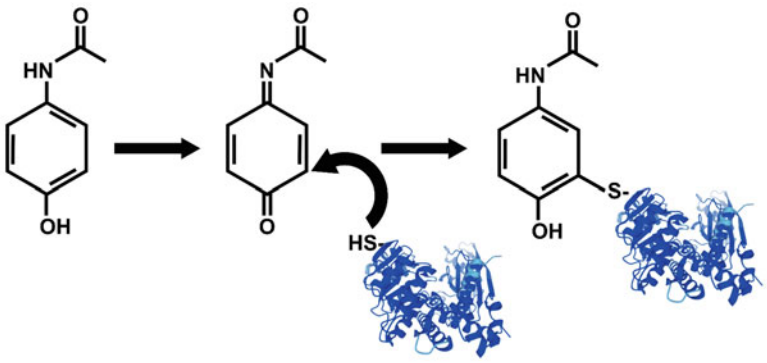

Fig. 3 Formation of APAP-protein adducts. Acetaminophen (APAP, left structure) is converted to N-acetyl-*p*-benzoquinone imine (NAPQI, middle structure), which has a partial positive charge on the meta carbon. The electrophilic carbon reacts with nucleophilic sulfhydryl groups (usually cysteine residues) on proteins (example protein shown in blue), reverting the structure of the drug back to APAP and forming the APAP-protein adduct (right structure)

For reasons described elsewhere, it will be a challenge to develop other biomarkers to determine etiology in the context of conventional drug-induced liver injury (DILI) (McGill and Jaeschke 2018, 2019). However, biomarkers of exposure may be useful in some cases of hepatotoxicity due to environmental chemicals. For example, there is growing interest in the measurement of pyrrole-protein adducts. Pyrrolizidine alkaloids are structural derivatives of pyrrolizidine produced by numerous plant species. Hundreds of these alkaloids have been identified in the wild and have long been recognized as a challenge in production of grazing livestock (Prakash et al. 1999). In recent years, concern regarding entry into the human food supply has intensified with recognition that these alkaloids may be a more common cause of human cancer than previously realized (He et al. 2021). As a result, a number of laboratories have developed and tested analytical methods to measure pyrrole-protein adducts (He et al. 2021; Gao et al. 2012; Ma et al. 2019, 2021; Ruan et al. 2015). Interestingly, these adducts may one day find clinical application as well, as pyrrolizidine alkaloids may be a cause of hepatotoxicity in some cases of herb-induced liver injury (Ruan et al. 2015).

Emerging Predictive Biomarkers

It is frequently suggested that it may be possible for clinicians to predict if a patient is likely to develop hepatotoxicity before writing a prescription for a DILI-associated drug for them based on a genetic marker or some other types of biomarker. This would not be an ideal approach due to the vanishingly low incidence of DILI among users of most DILI-causing drugs resulting in exceedingly low positive predictive values for most potential biomarkers (especially most genetic associations) (McGill and Jaeschke 2018, 2019; Stephens et al. 2021). One prominent exception is the association between human leukocyte antigen (HLA) B*5701 and abacavir hepatotoxicity (Mallal et al. 2002), which is useful due in part to the fact that abacavir causes other idiosyncratic reactions in addition to DILI leading to high overall incidence of adverse effects. Nevertheless, these genetic markers may be useful as a way to retrospectively identify a drug as a likely cause of hepatotoxicity after the fact. Thus, such biomarkers may have some real-world utility. A number of genetic associations with DILI are known, and many more continue to be identified. The affected genes encode products ranging from HLAs, to drug-metabolizing enzymes, to drug transporters (Stephens et al. 2021; Urban et al. 2014). At this point, however, few have clear potential for application in the near future.

Emerging Prognostic Biomarkers

There is an urgent need for improved biomarkers of prognosis in toxic liver injury in order to guide liver transplantation. Currently, although *N*-acetyl-*l*-cysteine is an effective treatment for APAP-induced liver injury when administered early after APAP overdose, few other specific treatments are available for toxic liver damage.

The major lifesaving treatment for liver injury patients who progress to liver failure is a liver transplant. However, donated liver are in limited supply, and those who do receive a transplant face serious postoperative challenges, including the possibility of graft rejection and development of infection due to the immunosuppressant drugs often required to stave off rejection. Better biomarkers could make the clinician's job easier when identifying which patients need a new liver to survive. To that end, a number of recent studies of varying quality have evaluated the prognostic potential of novel liver biomarkers over the past two decades, mostly using samples from APAP overdose patients because they are more widely available than samples from patients with other forms of toxic liver injury. A veritable alphabet soup of potential biomarkers has been described. These include full-length and caspase-cleaved keratin 18 (K18 and ccK18) (Bechmann et al. 2010; Church et al. 2019; Craig et al. 2011), high-mobility group box 1 protein (HMGB1) (Basta et al. 2015; Craig et al. 2011), glutamate dehydrogenase (GLDH) (Church et al. 2019; McGill and Jaeschke 2014), fatty acid-binding protein 1 (FABP1) (Karvellas et al. 2017), miR-122 (Church et al. 2019), fructose-1,6-bisphosphatase 1 (FBP1) (Wang et al. 2017), osteopontin (OPN) (Church et al. 2019; Srungaram et al. 2015), and leukocyte cell-derived chemotaxin 2 (LECT2) (Slowik et al. 2019). One of the more exciting recent reports found that a liver-regeneration-associated microRNA signature can also predict poor outcomes (Tavabie et al. 2021). So far, however, none have emerged as clear contenders for real-world use. Clinical adoption of these biomarkers has likely been impeded by (1) lack of FDA-approved reagents for their measurement and (2) lack of motivation to seek FDA approval on the part of commercial partners due to the relatively small market that exists for acute liver injury and liver failure patients. These issues may be circumvented by identification of biomarkers that already have approved reagents and are commonly measured in patients with other conditions. Two such "recycled" biomarkers that fulfill that criterion and have recently been shown to have prognostic value in acute liver injury are coagulation factor V (FV) and LDH. On one hand, admission FV values seem to correlate with positive outcomes (Patidar et al. 2021), while early LDH values seem to predict death (Vazquez et al. 2022). Nevertheless, further validation of both biomarkers is required.

In addition to biomarkers to predict death and therefore transplant need in severe injury, biomarkers have been tested to predict the development of later liver injury in patients who present early after a hepatotoxic exposure – before a rise in ALT. Among these, the most promising appear to be microRNA-122 (miR-122), K18, and HMGB1, with perhaps strongest performance from miR-122. All three displayed specificity >80% at 95% sensitivity to predict elevated peak ALT values (>100 U/L) in a validation cohort of APAP overdose patients who presented with ALT values in the normal range (Dear et al. 2018). In addition, miR-122 was shown to increase after moderate alcohol consumption with no change in ALT (McCrae et al. 2016), and although the data were preliminary in nature, miR-122 and K18 appeared to increase somewhat prior to ALT in two patients with hepatotoxicity caused by antitubercular drugs (Rupprechter et al. 2021). Finally, a recent study found that

GLDH and K18 were significantly elevated in serum from subjects with compensated cirrhosis compared to non-cirrhotic volunteers, while there was no difference in ALT between groups (McGill et al. 2021). Based on the prevalence of liver injury among early presenters after APAP overdose and the sensitivity and specificity achieved with these biomarkers (particularly miR-122), it seems likely that these markers are already approaching the limits of what is possible in this respect (McGill and Jaeschke 2018, 2019), and the pursuit of more widespread clinical adoption of one or more of these markers to predict injury in early-presenting APAP-induced liver injury patients could be appropriate at this time.

Emerging Biomarkers with Greater Specificity

There are two major challenges with the use of ALT and/or AST to detect and monitor liver injury during clinical trials, and both could be considered issues of specificity. First, these aminotransferases have poor utility to discriminate between liver and muscle damage in clinical trials involving patients with musculoskeletal diseases (Schomaker et al. 2020). Both ALT and AST are highly expressed in muscle and kidney tissue in addition to hepatocytes (LaDue and Wroblewski 1956), so both increase in the context of muscle damage. Currently, the combination of creatine kinase (CK) and ALT may be used to explore the source of the aminotransferases. For example, if a patient in a clinical trial has minor to modest ALT elevations with extremely high CK values and no major risk factors for liver damage, then one may assume that the ALT is elevated secondary to muscle injury. On the other hand, if ALT is much greater than CK, then the liver is a likely source of the ALT. However, a better approach would be to compare with a biomarker that is almost solely expressed in the liver. It is thought that GLDH is highly localized to mitochondria in the liver or at least that it is much more abundant there than in most other tissues (Schmidt and Schmidt 1988). Indeed, recent evidence has demonstrated that GLDH has clear utility to help differentiate liver and muscle damage (Schomaker et al. 2020). To that end, the GLDH subsection of the Hepatotoxicity Working Group of the Critical Path Institute (a public-private collaboration between the US FDA, pharmaceutical companies, and academic researchers) is currently working toward qualification of GLDH for use in clinical trials.

In addition to tissue specificity, there is the issue of specificity for injury. The US FDA recognizes that modest ALT elevations frequently do not indicate clinically significant, progressive liver damage (FDA 2009). Some drugs are known to cause transient ALT elevations in a significant proportion of patients who take them without leading to a single case of serious injury, liver failure, or death (Gracon et al. 1998; Harrill et al. 2012; Singhal et al. 2014; Watkins et al. 1994). A recent study using an unbiased, untargeted proteomics approach to compare serum between a model of benign ALT elevations and toxic ALT elevations revealed a number of potential biomarkers with greater specificity for injury, which were then confirmed to be elevated in serum from patients with APAP

hepatotoxicity (Vazquez et al. 2020). Chief among the candidate biomarkers was alcohol dehydrogenase 1A1 (ALDH1A1) and aldehyde dehydrogenase 2 (ADH2) (Vazquez et al. 2020). The authors of that manuscript propose a screen-and-confirm algorithmic approach in which ALT is used to screen for liver injury during clinical trials, and one of the candidate biomarkers is used to confirm it (Vazquez and McGill 2021). There is still much more work to be done to validate these novel injury-confirmation markers, but it appears to be a promising future direction based on the available data.

Mechanistic Biomarkers

Another potential use of novel biomarkers is investigation of liver injury mechanisms. A "mechanistic" biomarker is one that depends upon and therefore provides insight into a process that drives the pathophysiology of a disease at a fundamental level (i.e., molecular, cellular, or tissue). The term frequently refers to biomarkers intended for use as a way to monitor response to cancer treatments with specific therapeutic actions (De Haas et al. 2008; Lopez-Girona et al. 2011; Keen et al. 2014; Sorensen et al. 2009; Ueno et al. 2005) but has been applied to other contexts including liver injury in recent years (McGill and Jaeschke 2014). Several promising mechanistic biomarkers have been identified in patients with liver injury (Fig. 2). McGill et al. demonstrated that elevated serum levels of GLDH and mitochondrial DNA (mtDNA) in APAP hepatotoxicity likely reflect mitochondrial damage (McGill et al. 2012; McGill and Jaeschke 2021). In addition, nuclear DNA fragments in serum could reflect release of mitochondrial intermembrane endonucleases as a result of mitochondrial dysfunction (McGill and Jaeschke 2021). Similar data were reported for serum long-chain acylcarnitines (Bhattacharyya et al. 2014; McGill et al. 2014b), which are normally metabolized in mitochondria and therefore accumulate when mitochondria are damaged. On the other hand, ccK18 and the ratio of ccK18 to full-length K18 are markers of caspase-dependent apoptosis (Caulín et al. 1997; Leers et al. 1999) that are elevated in serum from some patients with toxic ALF (Craig et al. 2011; Woolbright et al. 2017). Direct measurement of caspase activity in serum also appears to be a useful measure of apoptosis in liver injury (McGill et al. 2012), while total HMGB1 may more commonly represent necrosis (McGill and Jaeschke 2014). Finally, a number of cytokines increase in serum during toxic liver damage and likely reflect inflammation that may affect injury or recovery (McGill and Jaeschke 2014). A new direction in mechanistic biomarkers in liver disease is those that reflect liver regeneration and therefore may be useful for prognosis as well. Two examples are Lect2 (Slowik et al. 2019), which is involved in inflammation, and phosphatidic acid, which appears to promote liver regeneration by inhibiting glycogen synthase kinase 3β (Clemens et al. 2019; Lutkewitte et al. 2018). The latter has been shown to increase in liver tissue and serum from mice with APAP hepatotoxicity and in serum from humans with APAP-induced liver injury, but its prognostic value remains to be determined.

Summary and Conclusions

Recent years have brought the discovery and preliminary evaluation of numerous novel biomarkers of liver injury. Measurement of serum APAP-protein adducts has clear value to diagnose APAP-induced liver injury and is already being measured clinically in some parts of the United States. Other biomarkers (factor V, LDH, alpha-fetoprotein, FABP1, FBP1, etc.) hold promise for prediction of death in severe liver injury but require further validation, while others (miR-122 and K18) may predict later liver injury in early presenters after APAP overdose. Finally, some (K18 and ccK18, GLDH, mtDNA, long-chain acylcarnitines, caspase activity, and regeneration markers) appear to have mechanistic value for translational research. Future work should focus on validating more of these biomarkers for clinical use. In addition, identification of more and potentially better biomarkers may be achieved through the use of novel tools, such as artificial intelligence approaches (Umbaugh and Jaeschke 2021).

Applications to Prognosis

In this chapter, we reviewed a selection of biomarkers that appear to predict (1) later injury in early-presenting patients with acetaminophen-induced hepatotoxicity (e.g., microRNA-122) (Dear et al. 2018) and (2) poor outcomes in severe toxic liver injury and/or acute liver failure (e.g., alpha-fetoprotein, osteopontin, factor V, lactate dehydrogenase) (Schmidt and Dalhoff 2005; Church et al. 2019; Patidar et al. 2021; Vazquez et al. 2022). MicroRNA-122, in particular, appears to be approaching the maximum predictive value for the former. The latter biomarkers require further validation in larger studies.

Applications to Other Diseases or Conditions

The focus of this chapter was on biomarkers of toxic liver injury. However, many of the biomarkers presented here are likely elevated and have clinical value in other forms of liver disease. Indeed, a few of these biomarkers are known to be elevated in fatty liver disease (Lee et al. 2020), cirrhosis (McGill et al. 2021), and other chronic hepatic diseases. They may also be useful in acute-on-chronic liver failure.

Mini-dictionary of Terms

- **Acute liver failure:** A condition in which liver function is rapidly compromised as a result of liver injury, leading to coagulopathy and encephalopathy within a short time frame and without evidence of prior liver disease
- **Drug-induced liver injury:** Liver injury caused by drugs that may present with a dose-response pattern characteristic of either intrinsic or idiosyncratic hepatotoxicity

- **Etiology:** The original cause of a disease or condition
- **Mechanistic biomarker:** A biomarker that provides some kind of insight into the mechanism(s) of disease
- **Positive predictive value:** The percentage of patients with a positive biomarker result who actually have the condition of interest
- **Predictive biomarker:** A biomarker that can predict the onset of an illness or condition before the illness or condition has developed
- **Prognostic biomarker:** A biomarker that can predict the outcome of an illness or condition after the illness or condition has developed
- **Sensitivity:** The percentage of patients with a condition that have a positive biomarker result
- **Specificity:** The percentage of patients without a condition that have a negative biomarker results

Key Facts of Acetaminophen

Acetaminophen was first synthesized and accidentally discovered to be an effective fever reducer in the late 1800s.

Due to unwarranted concerns that it can cause methemoglobinemia, it was not widely available to consumers until the 1950s.

Acetaminophen is now the most commonly used drug in the United States.

The first reports of acetaminophen-induced toxic liver injury appeared in the 1970s.

Today, acetaminophen is the single most commonly implicated cause of acute liver failure in the United States, the United Kingdom, and several other countries.

Key Facts of Acute Liver Injury

Acute liver injury is the sudden onset of severe liver damage.

Circulating alanine aminotransferase values >300 U/L are highly specific for acute liver injury, though they do not provide any insight into the etiology and have little prognostic value.

The most common causes in the United States are hypoxic hepatitis, drug-induced liver injury (especially acetaminophen hepatotoxicity), and pancreatobiliary diseases.

Outcomes are generally good unless the patient progresses to acute liver failure.

Outcomes are generally better for hypoxic hepatitis than for other causes, such as drug-induced liver injury.

Key Facts of Acute Liver Failure

Acute liver failure is defined as coagulopathy and encephalopathy developing within days to weeks of acute liver injury in the absence of prior chronic liver disease.

Despite recent progress toward improved outcomes, acute liver failure remains highly fatal with overall mortality around 25–30%.

Drug-induced liver injury is the single most common cause of acute liver failure and related deaths in most countries.

Acetaminophen is the single most commonly implicated agent in toxic acute liver failure.

Key Facts of Drug-Induced Liver Injury

Drug-induced liver injury is one of the most common causes of acute liver injury and acute liver failure in the United States and several other countries.

There are two forms: intrinsic and idiosyncratic.

Intrinsic drug-induced liver injury is characterized by a clear dose-response, with high predictability, meaning that all or nearly all individuals who consume a dose greater than some threshold will experience liver damage.

Idiosyncratic drug-induced liver injury is challenging to predict because it occurs in only a small proportion of individuals exposed to commonly used pharmacologic doses, and most cases appear to involve an immune system component.

Altogether, drug-induced liver injury is by far the most common cause of acute liver failure and one of the most common causes of acute liver injury.

Key Facts of Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids are a class of naturally occurring toxins present in many plants of agricultural significance.

These compounds are known to cause acute liver damage in livestock and humans and are likely also carcinogenic.

Recent data indicate that dietary exposure to these compounds may be more common than previously thought, with potential clinical significance.

Men may be more sensitive to their toxic effects than women.

Some insects feed on plants that produce pyrrolizidine alkaloids, accumulate the compounds within their own tissues, and use them as either a poisonous deterrent or as a precursor for pheromone synthesis.

Summary Points

- The liver is more susceptible to toxic damage than most other organs due to its unique anatomy and physiology.
- Current liver injury biomarkers can be grouped into markers of injury, function, infection, and proliferation.
- Current liver injury biomarkers are nonspecific and lack prognostic value either because their values do not correlate with outcomes or because they increase too late in the disease to be useful.
- Numerous studies over the last two decades have identified biomarkers that may be useful for determination of etiology in patients with liver injury, prediction of

drug-induced liver injury in patients before they begin taking a drug, diagnosis or determination of prognosis once injury occurs, and exploration of injury mechanisms.

- Low incidence or prevalence of idiosyncratic drug-induced liver injury is a major challenge in identification of biomarkers for diagnosis and prediction, but biomarkers for prognosis are ripe for further exploration and development.

Cross-References

► Biomarkers of Alcohol Toxicity
► Drug-Induced Nephrotoxicity and Use of Biomarkers

References

Alonso EM, James LP, Zhang S, Squires RH. Acetaminophen adducts detected in serum of pediatric patients with acute liver failure. J Pediatr Gastroenterol Nutr. 2015;61(1):102–7.

Basta G, Del Turco S, Navarra T, Lee WM. Circulating levels of soluble receptor for advanced glycation end products and ligands of the receptor for advanced glycation end products in patients with acute liver failure. Liver Transpl. 2015;21(6):847–54.

Bechmann LP, Jochum C, Kocabayoglu P, Sowa JP, Kassalik M, Gieseler RK, et al. Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. J Hepatol. 2010;53(4):639–47.

Bhattacharyya S, Yan K, Pence L, Simpson PM, Gill P, Letzig LG, et al. Targeted liquid chromatography-mass spectrometry analysis of serum acylcarnitines in acetaminophen toxicity in children. Biomark Med. 2014;8(2):147–59.

Caulín C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol. 1997;138(6):1379–94.

Christensen E, Bremmelgaard A, Bahnsen M, Andreasen PB, Tygstrup N. Prediction of fatality in fulminant hepatic failure. Scand J Gastroenterol. 1984;19(1):90–6.

Church RJ, Kullak-Ublick GA, Aubrecht J, Bonkovsky HL, Chalasani N, Fontana RJ, et al. Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: an international collaborative effort. Hepatology. 2019;69(2):760–73.

Clemens MM, Kennon-McGill S, Apte U, James LP, Finck BN, McGill MR. The inhibitor of glycerol 3-phosphate acyltransferase FSG67 blunts liver regeneration after acetaminophen overdose by altering GSK3β and Wnt/β-catenin signaling. Food Chem Toxicol. 2019;125:279–88.

Cook SF, King AD, Chang Y, Murray GJ, Norris HRK, Dart RC, et al. Quantification of a biomarker of acetaminophen protein adducts in human serum by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry: clinical and animal model applications. J Chromatogr B Anal Technol Biomed Life Sci. 2015;985:131–41.

Craig DGN, Lee P, Pryde EA, Masterton GS, Hayes PC, Simpson KJ. Circulating apoptotic and necrotic cell death markers in patients with acute liver injury. Liver Int. 2011;31(8):1127–36.

Crandall BF. Alpha-fetoprotein: a review. Crit Rev Clin Lab Sci. 1981;15(2):127–85.

De Haas EC, Di Pietro A, Simpson KL, Meijer C, Suurmeijer AJH, Lancashire LJ, et al. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. Neoplasia. 2008;10(10):1041–8.

De Ritis F, Coltori M, Giusti G. Transaminase activity of human serum in viral hepatitis; preventive note. Minerva Med. 1955;46(34):1207–129.

Dear JW, Clarke JI, Francis B, Allen L, Wraight J, Shen J, et al. Risk stratification after paracetamol overdose using mechanistic biomarkers: results from two prospective cohort studies. Lancet Gastroenterol Hepatol. 2018;3(2):104–13.

Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 2000;46(12):2050–68.

Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. Gastroenterology. 2014;146:1625–38.

FDA: Guidance for industry. Drug-induced liver injury: premarketing clinical evaluation. 2009.

Gao H, Li N, Wang JY, Zhang SC, Lin G. Definitive diagnosis of hepatic sinusoidal obstruction syndrome induced by pyrrolizidine alkaloids. J Dig Dis. 2012;13(1):33–9.

Gracon SI, Knapp MJ, Berghoff WG, Pierce M, DeJong R, Lobbestael SJ, et al. Safety of tacrine: clinical trials, treatment IND, and postmarketing experience. Alzheimer Dis Assoc Disord. 1998;12(2):93–101.

Harrill AH, Roach J, Fier I, Eaddy JS, Kurtz CL, Antoine DJ, et al. The effects of heparins on the liver: application of mechanistic serum biomarkers in a randomized study in healthy volunteers. Clin Pharmacol Ther. 2012;92(2):214–20.

He Y, Shi M, Wu X, Ma J, Ng KTP, Xia Q, et al. Mutational signature analysis reveals widespread contribution of pyrrolizidine alkaloid exposure to human liver cancer. Hepatology. 2021;74(1): 264–80.

James LP, Farrar HC, Sullivan JE, Givens TG, Kearns GL, Wasserman GS, et al. Measurement of acetaminophen-protein adducts in children and adolescents with acetaminophen overdoses. J Clin Pharmacol. 2001;41(8):846–51.

James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA, et al. Pharmacokinetics of acetaminophen-protein adducts in adults with acetaminophen overdose and acute liver failure. Drug Metab Dispos. 2009;37(8):1779–84.

Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen induced hepatic necrosis. II. Role of covalent binding in vivo. J Pharmacol Exp Ther. 1973;187(1):195–202.

Karmen A, Wróblewski F, LaDue JS. Transaminase activity in human blood. J Clin Invest. 1955;34 (1):126–33.

Karvellas CJ, Speiser JL, Tremblay M, Lee WM, Rose CF. Elevated FABP1 serum levels are associated with poorer survival in acetaminophen-induced acute liver failure. Hepatology. 2017;65(3):938–49.

Keen HG, Ricketts SA, Maynard J, Logie A, Odedra R, Shannon AM, et al. Examining changes in [18F]FDG and [18F]FLT uptake in U87-MG glioma xenografts as early response biomarkers to treatment with the dual mTOR1/2 inhibitor AZD8055. Mol Imaging Biol. 2014;16(3):421–30.

Khandelwal N, James LP, Sanders C, Larson AM, Lee WM. Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver failure. Hepatology. 2011;53(2):567–76.

Kuroda H, Abe T, Fujiwara Y, Nagasawa T, Suzuki Y, Kakisaka K, et al. Contrast-enhanced ultrasonography-based hepatic perfusion for early prediction of prognosis in acute liver failure. Hepatology. 2021;73(6):2455–67.

LaDue J, Wroblewski F. Serum glutamic pyruvic transaminase SGP-T in hepatic disease: a preliminary report. Ann Intern Med. 1956;45(5):801–11.

Lai Q, Iesari S, Melandro F, Mennini G, Rossi M, Lerut J. The growing impact of alpha-fetoprotein in the field of liver transplantation for hepatocellular cancer: time for a revolution. Transl Gastroenterol Hepatol. 2017;2:72.

Lawson JA, Fisher MA, Simmons CA, Farhood A, Jaeschke H. Parenchymal cell apoptosis as a signal for sinusoidal sequestration and transendothelial migration of neutrophils in murine models of endotoxin and Fas-antibody-induced liver injury. Hepatology. 1998;28(3):761–7.

Lee J, Vali Y, Boursier J, Duffin K, Verheij J, Brosnan MJ, et al. Accuracy of cytokeratin 18 (M30 and M65) in detecting non-alcoholic steatohepatitis and fibrosis: a systematic review and meta-analysis. PLoS One. 2020;15(9):e0238717.

Leers MPG, Kölgen W, Björklund V, Bergman T, Tribbick G, Persson B, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. J Pathol. 1999;187(5):567–72.

Leist M, Gantner F, Bohlinger I, Tiegs G, Germann PG, Wendel A. Tumor necrosis factor induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. Am J Pathol. 1995;146(5):1220–34.

Lopez-Girona A, Heintel D, Zhang LH, Mendy D, Gaidarova S, Brady H, et al. Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response. Br J Haematol. 2011;154(3):325–36.

Lutkewitte AJ, Schweitzer GG, Kennon-McGill S, Clemens MM, James LP, Jaeschke H, et al. Lipin deactivation after acetaminophen overdose causes phosphatidic acid accumulation in liver and plasma in mice and humans and enhances liver regeneration. Food Chem Toxicol. 2018;115:273–83.

Ma J, Ruan J, Chen X, Li D, Yao S, Fu PP, et al. Pyrrole-hemoglobin adducts, a more feasible potential biomarker of pyrrolizidine alkaloid exposure. Chem Res Toxicol. 2019;32(6):1027–39.

Ma J, Zhang W, He Y, Zhu L, Zhang C, Liu J, et al. Clinical application of pyrrole–hemoglobin adducts as a biomarker of pyrrolizidine alkaloid exposure in humans. Arch Toxicol. 2021;95(2):759–65.

Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet. 2002;359(9308):727–32.

McCrae JC, Sharkey N, Webb DJ, Vliegenthart ADB, Dear JW. Ethanol consumption produces a small increase in circulating MIR-122 in healthy individuals. Clin Toxicol. 2016;54(1):53–5.

McGill MR. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI J. 2016;15:817–28.

McGill MR, Jaeschke H. Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. Expert Opin Drug Metab Toxicol. 2014;10(7):1005–17.

McGill MR, Jaeschke H. Biomarkers of drug-induced liver injury: progress and utility in research, medicine, and regulation. Expert Rev Mol Diagn. 2018;18(9):797–807.

McGill MR, Jaeschke H. Biomarkers of drug-induced liver injury. Adv Pharmacol. 2019;85:221–39.

McGill MR, Jaeschke H. Biomarkers of mitotoxicity after acute liver injury: further insights into the interpretation of glutamate dehydrogenase. J Clin Transl Res. 2021;7(1):5.

McGill MR, Yan HM, Ramachandran A, Murray GJ, Rollins DE, Jaeschke H. HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology. 2011;53(3):974–82.

McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest. 2012;122(4):1574–83.

McGill MR, Staggs VS, Sharpe MR, Lee WM, Jaeschke H. Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. Hepatology. 2014a;60(4):1336–45.

McGill MR, Li F, Sharpe MR, Williams CD, Curry SC, Ma X, et al. Circulating acylcarnitines as biomarkers of mitochondrial dysfunction after acetaminophen overdose in mice and humans. Arch Toxicol. 2014b;88(2):391–401.

McGill MR, James LP, McCullough SS, Moran JH, Mathews SE, Peterson EC, et al. Short-term safety of repeated acetaminophen use in patients with compensated cirrhosis. Hepatol Commun. 2021; https://doi.org/10.1002/hep4.1810.

Mizejewski GJ. Biological roles of alpha-fetoprotein during pregnancy and perinatal development. Exp Biol Med. 2004;229(6):439–63.

Muldrew KL, James LP, Coop L, McCullough SS, Hendrickson HP, Hinson JA, et al. Determination of acetaminophen-protein adducts in mouse liver and serum and human serum after hepatotoxic doses of acetaminophen using high-performance liquid chromatography with electrochemical detection. Drug Metab Dispos. 2002;30(4):446–51.

Patidar KR, Davis BC, Slaven JE, Ghabril MS, Kubal CA, Lee WM, et al. Admission factor V predicts transplant-free survival in acute liver failure. Dig Dis Sci. 2021;66(2):619–27.
Peeling RW, Boeras DI, Marinucci F, Easterbrook P. The future of viral hepatitis testing: innovations in testing technologies and approaches. BMC Infect Dis. 2017;17:699.
Pike AF, Kramer NI, Blaauboer BJ, Seinen W, Brands R. A novel hypothesis for an alkaline phosphatase "rescue" mechanism in the hepatic acute phase immune response. Biochim Biophys Acta Mol Basis Dis. 2013;1832(12):2044–56.
Prakash AS, Pereira TN, Reilly PEB, Seawright AA. Pyrrolizidine alkaloids in human diet. Mutat Res Genet Toxicol Environ Mutagen. 1999;443(1–2):53–67.
Pumford NR, Hinson JA, Potter DW, Rowland KL, Benson RW, Roberts DW. Immunochemical quantitation of 3-(cystein-S-yl)acetaminophen adducts in serum and liver proteins of acetaminophen-treated mice. J Pharmacol Exp Ther. 1989;248(1):190–6.
Roberts D, Pumford N, Potter D, Benson R, Hinson J. A sensitive immunochemical assay for acetaminophen-protein adducts. J Pharmacol Exp Ther. 1987;241(2):527–33.
Roberts DW, Lee WM, Hinson JA, Bai S, Swearingen CJ, Stravitz RT, et al. An immunoassay to rapidly measure acetaminophen protein adducts accurately identifies patients with acute liver injury or failure. Clin Gastroenterol Hepatol. 2017;15(4):555–562.e3.
Ruan J, Gao H, Li N, Xue J, Chen J, Ke C, et al. Blood pyrrole-protein adducts – a biomarker of pyrrolizidine alkaloid-induced liver injury in humans. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2015;33(4):404–21.
Rupprechter SAE, Sloan DJ, Oosthuyzen W, Bachmann TT, Hill AT, Dhaliwal K, et al. MicroRNA-122 and cytokeratin-18 have potential as a biomarkers of drug-induced liver injury in European and African patients on treatment for mycobacterial infection. Br J Clin Pharmacol. 2021;87(8):3206–17.
Schiødt FV, Ostapowicz G, Murray N, Satyanarana R, Zaman A, Munoz S, et al. Alpha-fetoprotein and prognosis in acute liver failure. Liver Transpl. 2006;12(12):1776–81.
Schmidt LE, Dalhoff K. Alpha-fetoprotein is a predictor of outcome in acetaminophen-induced liver injury. Hepatology. 2005;41(1):26–31.
Schmidt ES, Schmidt FW. Glutamate dehydrogenase: biochemical and clinical aspects of an interesting enzyme. Clin Chim Acta. 1988;173(1):43–55.
Schomaker S, Potter D, Warner R, Larkindale J, King N, Porter AC, et al. Serum glutamate dehydrogenase activity enables early detection of liver injury in subjects with underlying muscle impairments. PLoS One. 2020;15(5):e0229753.
Selinger MJ, Matloff DS, Kaplan MM. γ-Glutamyl transpeptidase activity in liver disease: serum elevation is independent of hepatic GGTP activity. Clin Chim Acta. 1982;125(3):283–90.
Singh S, Hynan LS, Rule JA, Lee WM. Changes in alpha-foetoprotein and Gc-globulin in relation to outcomes in non-acetaminophen acute liver failure. Liver Int. 2019;39(12):2368–73.
Singhal R, Harrill AH, Menguy-Vacheron F, Jayyosi Z, Benzerdjeb H, Watkins PB. Benign elevations in serum aminotransferases and biomarkers of hepatotoxicity in healthy volunteers treated with cholestyramine. BMC Pharmacol Toxicol. 2014;15(1):42.
Slowik V, Borude P, Jaeschke H, Woolbright BL, Lee WM, Apte U. Leukocyte cell derived chemotaxin-2 (Lect2) as a predictor of survival in adult acute liver failure. Transl Gastroenterol Hepatol. 2019;4:17.
Sorensen AG, Batchelor TT, Zhang WT, Chen PJ, Yeo P, Wang M, et al. A "vascular normalization index" as potential mechanistic biomarker to predict survival after a single dose of cediranib in recurrent glioblastoma patients. Cancer Res. 2009;69(13):5296–300.
Srungaram P, Rule JA, Yuan HJ, Reimold A, Dahl B, Sanders C, et al. Plasma osteopontin in acute liver failure. Cytokine. 2015;73(2):270–6.
Stephens C, Lucena MI, Andrade RJ. Genetic risk factors in the development of idiosyncratic drug-induced liver injury. Expert Opin Drug Metab Toxicol. 2021;17(2):153–69.
Tavabie OD, Karvellas CJ, Salehi S, Speiser JL, Rose CF, Menon K, et al. A novel microRNA-based prognostic model outperforms standard prognostic models in patients with acetaminophen-induced acute liver failure. J Hepatol. 2021;75(2):424–34.

Teschke R, Brand A, Strohmeyer G. Induction of hepatic microsomal gamma-glutamyltransferase activity following chronic alcohol consumption. Biochem Biophys Res Commun. 1977;75(3):718–24.

Tygstrup N, Ranek L. Assessment of prognosis in fulminant hepatic failure. Semin Liver Dis. 1986;6(2):129–37.

Ueno T, Toi M, Linder S. Detection of epithelial cell death in the body by cytokeratin 18 measurement. Biomed Pharmacother. 2005;59(Suppl 2):S359–62.

Umbaugh DS, Jaeschke H. Biomarkers of drug-induced liver injury: a mechanistic perspective through acetaminophen hepatotoxicity. Expert Rev Gastroenterol Hepatol. 2021;15(4):363–75.

Urban TJ, Daly AK, Aithal GP. Genetic basis of drug-induced liver injury: present and future. Semin Liver Dis. 2014;34(2):123–33.

Varshney A, Gupta R, Verma SK, Ahmad S. Alpha-fetoprotein as a prognostic marker in acute liver failure: a pilot study. Trop Dr. 2017;47(3):202–5.

Vazquez JH, McGill MR. Redrawing the map to novel DILI biomarkers in circulation: where are we, where should we go, and how can we get there? Livers. 2021;1:286–93.

Vazquez JH, Clemens MM, Allard FD, Yee EU, Kennon-McGill S, MacKintosh SG, et al. Identification of serum biomarkers to distinguish hazardous and benign aminotransferase elevations. Toxicol Sci. 2020;173(2):244–54.

Vazquez JH, Kennon-McGill S, Byrum SD, Mackintosh SG, Jaeschke H, Williams KD, Lee WM, Dranoff JA, McGill MR, Acute Liver Failure Study Group. Proteomics indicates that lactate dehydrogenase is prognostic in acetaminophen-induced acute liver failure patients and reveals altered signaling pathways. Toxicol Sci. 2022;187(1):25–34.

Wang J, Sun Z, Jiang J, Wu D, Liu X, Xie Z, et al. Proteomic signature of acute liver failure: from discovery and verification in a pig model to confirmation in humans. Mol Cell Proteomics. 2017;16(7):1188–99.

Watkins PB, Zimmerman HJ, Knapp MJ, Gracon SI, Lewis KW. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. JAMA. 1994;271(13):992–8.

Watkins PB, Kaplowitz N, Slattery JT, Colonese CR, Colucci SV, Stewart PW, et al. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. J Am Med Assoc. 2006;296(1):87–93.

Woolbright BL, Bridges BW, Dunn W, Olson JC, Weinman SA, Jaeschke H. Cell death and prognosis of mortality in alcoholic hepatitis patients using plasma keratin-18. Gene Expr. 2017;17(4):301–12.

Wu A, Slavin G, Levi AJ. Elevated serum gamma-glutamyl-transferase (transpeptidase) and histological liver damage in alcoholism. Am J Gastroenterol. 1976;65(4):318–23.

Xie Y, McGill MR, Cook SF, Sharpe MR, Winefield RD, Wilkins DG, et al. Time course of acetaminophen-protein adducts and acetaminophen metabolites in circulation of overdose patients and in HepaRG cells. Xenobiotica. 2015;45(10):921–9.