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Detection to Prediction: Biomarkers in Severe Drug-Induced Liver Injury

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Abstract

Drug-induced liver injury (DILI) remains a leading cause of acute liver failure, contributing to approximately 10% of cases and posing substantial challenges in drug development and patient care. Modern diagnostic tools, despite their sophistication, have not fully solved the challenge of predicting severe outcomes. Nevertheless, emerging biomarker technologies show remarkable potential for early risk detection and tailored treatment strategies.

This literature review comprehensively evaluates predictive biomarkers for severe DILI outcomes, utilizing a rigorous methodology across PubMed, Scopus, and Web of Science databases from 2010 to 2024. The research focused on biomarkers associated with hepatocyte injury, immune system activation, mitochondrial dysfunction, and bile acid dysregulation, employing both qualitative and quantitative analytical approaches. The investigation revealed sophisticated biomarker landscapes with promising diagnostic potential. Hepatocyte damage markers, specifically microRNA-122, demonstrated sensitivity ranging from 70-80% in early liver injury detection, with most studies showing consistent performance in distinguishing hepatocellular damage. Immune activation markers like soluble CD163 showed moderate specificity in predicting inflammatory cascades, with literature reporting ranges between 60-75% in clinical studies.

Multi-omics approaches emerged as a valuable methodology, showing potential for increased predictive accuracy compared to traditional single-marker assessments. Advanced computational modeling enhanced risk stratification precision, offering a more nuanced understanding of hepatotoxicity mechanisms. However, significant challenges remain, including inter-population variability, lack of standardized diagnostic protocols, and complex regulatory approval processes. Predictive biomarkers represent a transformative approach to DILI management, with substantial potential to revolutionize personalized hepatotoxicity risk assessment. Strategic future research imperatives include multicenter longitudinal validation studies, development of standardized comprehensive biomarker panels, advanced machine learning integration for predictive modeling, and extensive validation across diverse genetic populations. The clinical implications are profound, providing a critical framework for enhanced early detection of hepatotoxicity risks, personalized pharmaceutical intervention strategies, proactive patient management protocols, and refined drug development safety assessments.

This research synthesizes cutting-edge scientific insights, bridging the gap between molecular understanding and clinical application. By meticulously examining the intricate landscape of DILI biomarkers, the study offers a comprehensive roadmap for future research, potentially transforming our approach to drug-induced liver injury. The integration of advanced diagnostic technologies, computational modeling, and personalized medicine principles promises a future where hepatotoxicity risks can be anticipated, managed, and mitigated with unprecedented precision.

Core tip: Drug-induced liver injury (DILI) is a major cause of acute liver failure, yet characterizing the condition and predicting its severity has remained challenging, especially when relying solely on traditional biomarkers such as serum transaminase and bilirubin levels. Novel biomarkers have shown more reliability in detecting and predicting severe DILI outcomes, such as microRNA-122, CD163, high mobility box protein-1 (HMGB1), and keratin 18 (k18), or drug-specific biomarkers such as protein-derived acetaminophen-cysteine (APAP-CYS). Incorporation of these biomarkers, especially in conjunction with one another, into the clinical assessment of DILI can augment the quality and effectiveness of the clinical care provided.

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Introduction

The liver is the primary site of drug metabolism, playing a crucial role in processing and detoxifying medications. When a drug is taken orally, it undergoes first-pass metabolism where hepatic enzymes, particularly cytochrome P450, mediate phase I reactions, generating reactive metabolites. If these metabolites are not effectively neutralized by phase II conjugation or antioxidant mechanisms, they can become hepatotoxic. A well-studied example is acetaminophen, which is metabolized into the toxic compound N-acetyl-p-benzoquinone imine (NAPQI). Excessive NAPQI production depletes glutathione stores, impairing detoxification and leading to oxidative hepatic injury

[1]. If untreated, this can result in hepatocellular necrosis, acute liver failure, and the need for liver transplantation.

The liver's central role in drug metabolism makes it particularly vulnerable to drug-induced liver injury (DILI), which occurs when toxic drug intermediates accumulate and overwhelm detoxification pathways. DILI is defined as liver damage caused by medications, herbal supplements, or other chemical substances and is the leading cause of acute liver failure in the United States, accounting for 13% of cases [2]. This underscores the serious impact of DILI on public health and the importance of early detection, risk assessment, and effective management strategies.

DILI is broadly classified into intrinsic and idiosyncratic forms. Intrinsic DILI is dose-dependent and predictable, occurring when a drug directly induces hepatotoxicity, as seen in acetaminophen overdose. Idiosyncratic DILI, on the other hand, is unpredictable, influenced by genetic predisposition, immune responses, or metabolic differences and is not linked to a drug's pharmacological mechanism [3]. Given its potential to cause severe liver damage, including acute liver failure, and its implications for patient safety and pharmaceutical regulation, DILI is a major concern in clinical practice and drug safety. However, diagnosing DILI is challenging due to nonspecific symptoms and the absence of definitive biomarkers, making it a diagnosis of exclusion. As a result, delayed recognition and underreporting are common, which complicate efforts to accurately assess its incidence and impact.

A key limitation in DILI diagnosis is the lack of specific biomarkers. Liver function tests (LFTs), such as ALT, AST, and bilirubin, are commonly used to assess liver injury, but they lack specificity and cannot reliably distinguish DILI from other liver diseases [4]. Similarly, imaging techniques such as ultrasound, CT, and MRI often fail to provide distinctive features for DILI, further complicating diagnosis. Another challenge is the difficulty in predicting severe outcomes. While acute liver failure, liver transplantation, and mortality are recognized complications, there are no early predictive markers to identify which patients are at the highest risk. This uncertainty often results in undertreatment or unnecessary discontinuation of beneficial medications. Given these limitations, an effective predictive biomarker should not only detect early liver injury but also correlate with disease severity and improve risk stratification, ultimately enhancing DILI diagnosis and management.

To improve DILI management, novel biomarkers are being explored to enhance early detection, risk stratification, and prognosis. Emerging candidates such as miR-122, CK-18, and GLDH show greater specificity and earlier detection than traditional LFTs [5]. MiR-122, a liver-specific microRNA, surpasses ALT in detecting early hepatocyte injury, while CK-18 correlates with disease severity, and GLDH provides higher liver specificity. HLA polymorphisms (e.g., HLA-B*5701) have also been linked to idiosyncratic DILI risk, supporting the role of genetic screening in identifying high-risk individuals [5]. The integration of validated biomarkers into routine clinical practice could lead to earlier diagnosis, improved patient outcomes, and enhanced drug safety evaluations, ultimately reducing DILI-related morbidity and mortality.

Methodology

The methodology for this manuscript involved a comprehensive literature review to assess and synthesize the role of biomarkers in diagnosing, predicting, and managing drug-induced liver injury (DILI). A systematic search of peer-reviewed journals and reputable biomedical databases, including PubMed and Scopus, was conducted using the keywords and Medical Subject Headings (MeSH) terms; "DILI", "microRNA-122", "keratin-18", "HMGB1", "sCD163", "FABP-1", "bile acid", "glutamate dehydrogenase", "multi-omics", "liver-specific biomarkers", "GSH", and "computational modeling". The inclusion criteria focused on studies evaluating biomarker sensitivity, specificity, predictive value, and clinical applicability in human populations or translational models published within the past 15 years. Excluded articles included publications not written in English, case reports, and articles without adequate detail of their methodological approach or questionable reproducibility. Furthermore, studies unrelated to DILI, lacking primary data, or published before 2010 were excluded. The search was then refined by applying filters in accordance with our inclusion criteria. Key biomarkers were then categorized into liver-specific (e.g., ALT, AST, miR-122), mechanistic (e.g., GLDH, K18), inflammatory (e.g., HMGB1, soluble CD163), or bile acid profiles, assessing their diagnostic and prognostic utility. Biomarkers were evaluated qualitatively for their ability to detect DILI and predict clinical outcomes in the disease process. Findings were synthesized narratively to identify strengths, limitations, and future opportunities for integrating biomarkers into clinical practice, emphasizing the potential to shift DILI management from reactive to predictive care.

Pathophysiology of DILI and Rationale for Biomarker Development

Drug-induced liver injury is a clinical presentation consistent with acute or chronic hepatic injury in response to an offending xenobiotic, most notably manufactured medications or herbal compounds. Understanding the pathophysiology of DILI as an adverse drug reaction is crucial for accurate diagnosis and prevention of its progression to liver failure. As a major form of hepatotoxicity, DILI is a primary contributor to acute liver failure in the United States with a 10-50 % case fatality rate [6]. Proper identification of DILI is critical as similar clinical manifestations including jaundice or elevated aminotransferases can be seen in other types of hepatic insult such as viral hepatitis. Furthermore, appropriate management is dependent on correct diagnosis and allows for informed drug monitoring and pharmacovigilance. This approach allows for clinicians to improve patient safety and outcomes as well as reassess drug efficacy.

DILI can occur in a dose-dependent, predictable manner, classified as intrinsic DILI. Within this classification, exogenous hepatotoxicants and their pharmacological properties themselves lead to direct liver injury. Drugs that are substrates of the cytochrome P450 liver enzyme system (CYP) are metabolized commonly forming ions, oxygen-free radicals, and other reactive or toxic substances. These byproducts can have the potential to be more harmful than the parent compound. An imbalance between the formation of reactive byproducts and their detoxification or inactivation by the phase I-III metabolic pathways allows for substance accumulation. Reactive metabolites may irreversibly bind to hepatocyte structures, such as mitochondrial proteins or cell membranes, leading to cellular damage or death. [7,8]. The metabolic detoxification and

biotransformation processes that are carried out by the CYP system are at great risk of unfavorable effect, commonly due to genetic polymorphisms, drug-drug interactions, or other underlying liver dysfunctions. In phase I, the chemical structure of drugs are modified through the integration of reactive or polar groups, generating reactive oxygen species as a byproduct. This process typically transforms drugs into active metabolites or, in the case of prodrugs, converts them into their pharmacologically active forms. During Phase II, modified drug molecules become coupled to polar compounds via conjugation reactions that are catalyzed by transferase enzymes to produce water-soluble compounds capable of being excreted via efflux transporters during Phase III [9].

Reactive metabolic byproducts from DILI-associated drugs such as acetaminophen, can react with molecular oxygen to form reactive oxygen radicals. Excessive generation of reactive oxygen species (ROS) overwhelms redox buffers, resulting in an oxidative burden. The primary mechanisms for redox balance involve reduced glutathione (GSH) which participates in Phase II neutralization of reactive electrophiles, aiding in their excretion [10]. In addition to cytosolic GSH mechanisms, the GSH reserve pool, at the level of the mitochondria, functions as an oxidative defense against mitochondrial ROS produced as byproducts of the electron transport chain (ETC). GSH synthesis primarily occurs in the liver with GCLC and GCLM genes encoding GCL (glutamylcysteine), the first rate-limiting enzyme in GSH synthesis. Consequently, DILI and subsequent depletion of GSH disrupts redox homeostasis at cellular organelles [11] particularly affecting the mitochondria and endoplasmic reticulum, key sites of ROS generation [12]. Additionally, detection of mitochondrial GSH (mtGSH) declines may serve as a potential biomarker for early detection of DILI. Hepatic mtGSH stores are tightly regulated and disruptions indicate intracellular degradation, a process that typically only occurs extracellularly under normal conditions. Furthermore, GSH transport from the cytoplasm into the mitochondria is essential for the maintenance of optimal concentrations. This occurs via SLC25A39 transport activity which is dependent upon GSH availability. The upregulation of mitochondrial transporters in response to GSH depletion may therefore aid as an additional indicator of early mitochondrial dysfunction in DILI [13].

Acetaminophen toxicity is a notable example of DILI as well as the most common cause of liver transplantation in the United States. Drug metabolism within the liver involves glucuronidation and sulfonation, processes dependent upon adequate glutathione stores to reduce N-acetyl-p-benzoquinone imine (NAPQI), a reactive intermediate. Overdose leads to excessive accumulation of NAPQI, a result mediated by cytochrome P450, ensuring mtGSH depletion. This depletion allows NAPQI to form protein adducts triggering oxidative stress and initiating mitochondrial apoptosis pathways [14]. In the context of acetaminophen toxicity, NAPQI protein adduct concentrations may serve as a potential indirect marker of early DILI detection alongside mtGSH.

Mitochondrial function is dependent on the maintenance of membrane integrity. Disruptions in permeability such as through mitochondrial permeability transition pore (MPTP) allow for calcium influx and subsequent membrane potential dissipation. This collapse in membrane potential alters mitochondrial function leading to halted ATP synthesis as well as structural changes causing water influx resulting in mitochondrial swelling and rupture. After mitochondrial membrane rupture, mitochondrial pro-apoptotic factors such as cytochrome c and intracellular damage-associated molecular patterns (DAMPs) trigger cell death [15]. Drugs such as troglitazone and nimesulide are often associated with DILI and mitochondrial oxidative stress and have been shown, in vitro, to induce changes in the mitochondrial permeability transition (MPT) signaling apoptotic cascade and necrotic cell death. Mitochondrial membrane potential collapse resulting from calcium influx warrants further investigation as a biomarker for mitochondrial dysfunction following DILI. However, insufficient research exists discussing MTP as a predictive biomarker for DILI. Future studies investigating mitochondrial membrane potential changes during MTP, the associated release of cytochrome c, and assays measuring MTP pore size can close this gap. Other mitochondrial dysfunction markers, such as glutamate dehydrogenase (GLDH) and mitochondrial DNA, which are elevated in DILI, have been proposed as prognostic and mechanistic biomarkers. GLDH is a mitochondrial matrix enzyme essential in various processes including the urea and Krebs cycle and exhibits high distribution within hepatic tissue making it an important potential biomarker for determining DILI outcomes and assessing mitochondrial damage severity [12].

Hepatocyte insult during DILI leads to the eventual disintegration of the cell membrane allowing for the release of DAMPs. These DAMPs stimulate innate immune responses, leading to cytokine release and activation of hepatic Kupffer cells, which secrete inflammatory cytokines such as TNF-α and hyperacetylated high mobility group box-1 (HMGB1). In mice models, total serum HMGB1 levels have been shown to rise earlier than ALT and rapidly return to baseline, occurring concurrently with the appearance of hepatic inflammatory cells and demonstrating concentration patterns consistent with acute injury processes. DAMPs have been proposed as potential DILI biomarkers due to their higher sensitivity for detecting liver toxicity compared to alanine aminotransferase (ALT) in clinical studies. Additionally, certain HLA variants are associated with higher susceptibility to cytotoxic T-cell mediated destruction of hepatocytes in response to drug neoantigens that are also capable of releasing DAMPs [16]. Further investigation to evaluate DAMPs and their role in potentiating inflammatory responses and further hepatocyte injury during DILI can allow for the identification of early DILI and its progression but also guide personalized treatment based on HLA predisposition for hepatotoxicity. Additionally, therapeutic interventions targeted towards DAMPs such as through toll-like receptor blockade preventing DAMP binding or neutralizing antibodies directed towards them may stop inflammatory processes further exacerbating hepatic insult and preventing adverse outcomes.

The wide-reaching pathophysiology of DILI provides ample sources of further discovery and investigation into novel biomarkers to better characterize the disease process. Careful selection of biomarkers for diagnosis, prediction, and treatment can accelerate progression to clinical implementation with utilization to improve patient outcomes. Not only can further research in this area further laboratory testing, but new treatment modalities can also be elucidated.

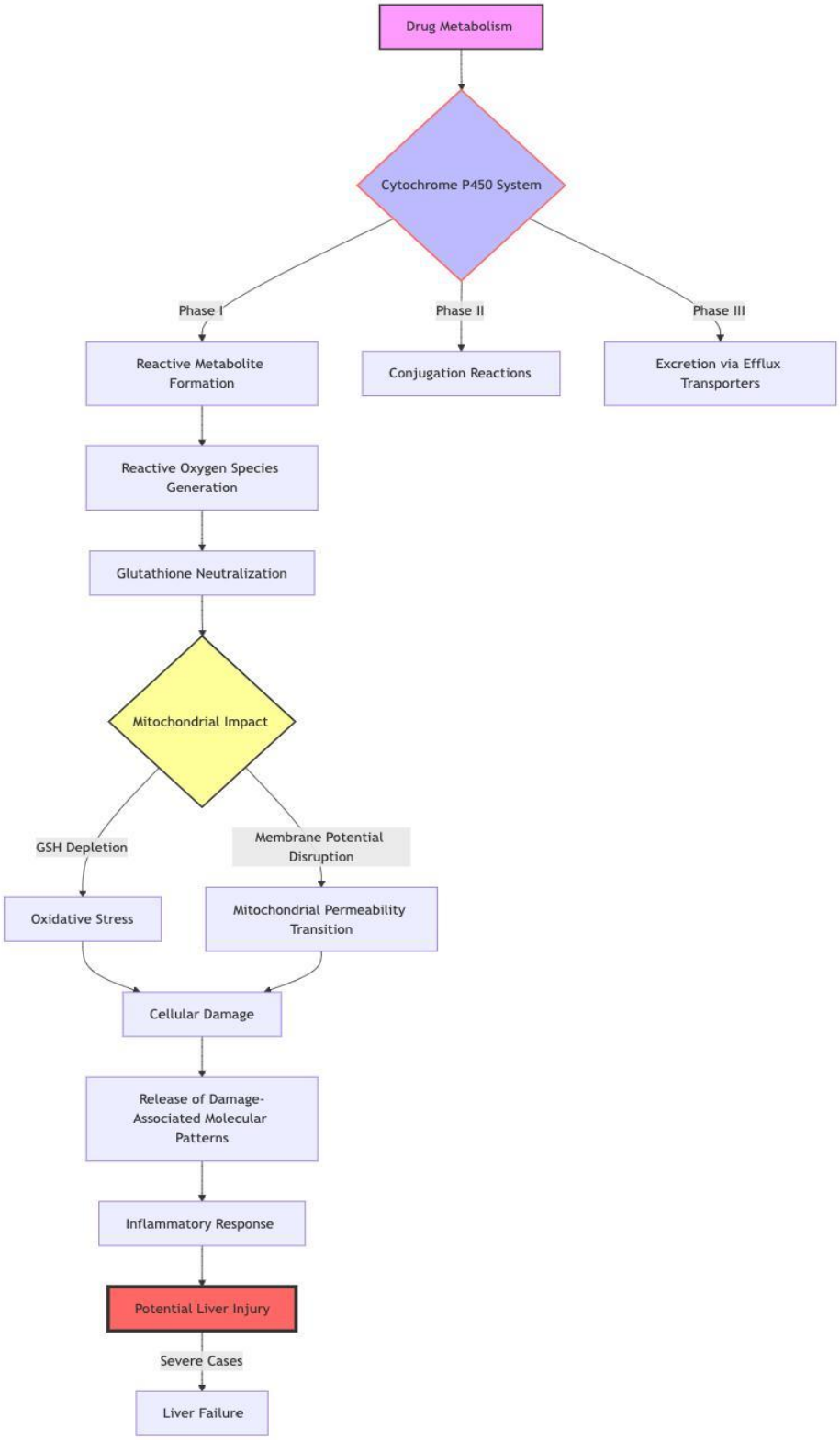

Figure 1: Highlights the pathways involved in drug-induced liver injury mediated by the Cytochrome P450 system.

- **Phase I** leads to the formation of reactive metabolites, generating reactive oxygen species (ROS). These ROS are initially neutralized by glutathione; however, excessive ROS may impact mitochondria, causing either glutathione (GSH) depletion leading to oxidative stress or disruption of mitochondrial membrane potential. Both outcomes ultimately contribute to mitochondrial permeability transition.
- **Phase II** involves conjugation reactions to facilitate detoxification.
- **Phase III** consists of the excretion of metabolites through efflux transporters.

The mitochondrial impact from Phase I metabolism leads to cellular damage, subsequent release of damage-associated molecular patterns (DAMPs), and an inflammatory response. Collectively, these events result in potential liver injury, which, in severe cases, can progress to liver failure.

Liver specific enzymes

Characterization of drug-induced liver injury (DILI) and limitations of transaminases

Traditionally, DILI has been defined and characterized by severity based on elevated transaminases, total serum bilirubin, INR, hospitalization status, evidence of organ failure, and patient death [6]. Transaminases are often evaluated utilizing the ratio (R ratio) of the serum lab values of alanine aminotransferase (ALT) to alkaline phosphatase (ALP) with respect to their upper limits of normal (ULNs); an R ratio of >5 indicates hepatocellular origin of liver injury, and an R ratio <2 denotes cholestatic origin of liver injury [17]. In conjunction with the clinical symptom profile as well as bilirubin, aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) lab values, the R ratio can help indicate the diagnosis and causality of the DILI, but due the to the broad ranges many pathologies can present with, its clinical utility is limited [17]. Additionally, utilizing the R ratio for determining cause of DILI may be problematic because a shift in the pattern of elevated transaminases may occur during the clinical course of DILI. Determining causality with reliance on numerical thresholds or algorithms is likely to be insufficient without clinical judgement . Thus, the time at which serum is sampled from the patient during the course of their disease can potentially confound the ability of the R ratio to indicate the causality of the drug-induced liver injury, reducing its effectiveness as a standalone biomarker for severe DILI. Additionally, there may be some genetic determination and variation for plasma liver enzymes, indicating a potential need for more individualized interpretation of plasma liver test values when diagnosing and monitoring drug-induced liver injury [19]. Furthermore, ALT and AST values may lack specificity for liver injury: the cytosolic and mitochondrial forms of AST is present in tissues of the liver, heart, skeletal muscle, kidneys, brain, pancreas, and lungs, and in white and red blood cells [20]. Likewise, in the case of the two isoforms of ALT, ALT1 and ALT2, ALT2 is restricted to non-hepatic tissue, such as the heart, skeletal muscle, and pancreas, but may still contribute to elevated ALT levels in the plasma [20]. However, the standard ALT activity lab assay does not differentiate between ALT from different organs, or between the ALT1 and 2 isoforms [20]. Thus, elevations of liver enzymes can have extrahepatic etiologies, and therefore these values cannot be considered in isolation for the diagnosis and surveillance of drug-induced liver injury, nor for early prediction of severe drug-induced liver injury outcomes.

Limitations of bilirubin as a marker of liver function

Drug-induced liver injury is also often characterized by an elevation in total bilirubin that is correlated with severity of liver injury, with total bilirubin (TBIL) values being higher in those with severe liver injury, and values also increasing during the course of the disease, peaking at an average of 1 week after DILI recognition, regardless of patient age, pattern of liver injury, and the specific drug or prescription causing the disease [21]. Additionally, some patients can have higher levels of plasma bilirubin at baseline, which is associated with a higher risk of developing fulminant hepatic failure from DILI [21]. However, while elevation of serum liver enzymes is taken as an indicator of liver injury, an increase in the levels of both total and conjugated bilirubin are considered markers of overall liver function [22]. Hence, TBIL must be considered in conjunction with other biomarkers for the diagnosis and monitoring of DILI. Additionally, although TBIL levels are liver-specific, they are relatively insensitive and elevate later in the course of the disease, reducing its standalone impact in prognosis following DILI diagnosis [23]. Moreover, elevated TBIL levels are not isolated to DILI; elevations in TBIL levels can occur due to extrahepatic etiology such as hemolytic anemia, exercise, or diet, due to liver or bile duct disease that is not drug-induced, or due to mild and common genetic conditions such as Gilbert syndrome [22]. Thus, TBIL levels, like transaminase lab values, do not suffice in standalone identification or prediction of severe drug-induced liver injury.

Acetaminophen-cysteine as a promising drug-specific biomarker

Thus far, the only drug-specific biomarker for DILI appears to be protein-derived acetaminophen-cysteine (APAP-CYS) [24]. APAP overdose is likely responsible for at least half of all DILI cases in Western countries, and thus the APAP-CYS value can be useful in diagnosing acetaminophen-induced liver injury [24]. Notably, literature analysis of various APAP-CYS studies indicates that a cutoff value of >1 nmol/mL of APAP-CYS is sensitive and specific for severe hepatotoxicity after APAP overdose [24]. Therefore, the acetaminophen-cysteine lab value may have utility in predicting severe hepatotoxicity and thereby severe drug-induced liver injury outcomes following acetaminophen overdose.

Considerations and interpretative barriers of microRNA-122 as a DILI biomarker

MicroRNA (miR-122) shows some promise as a prognostic biomarker for drug-induced liver injury [24]. MiR-122 is derived from a liver-specific non-coding polyadenylated RNA [25], compared to alanine aminotransferase (ALT), which, as discussed previously, can have extrahepatic sources. MiR-122 has been demonstrated to elevate in parallel with serum ALT levels and other liver histopathology degenerative changes in the context of acetaminophen-induced liver injury in murine models [25]. Additionally, miR-122 changes appeared to occur earlier in the disease progression than traditional biomarkers [25]. Additionally, admission miR-122 values in patients with early presentations that still had normal to minimal ALT values had predictive values in the range of 70-90% for the development of later liver injury [24]. However, it is not yet evident whether there is a significant enough difference between miR-122 values of survivors and non-survivors of DILI. Thus, it is not clear whether miR-122 is a robust indicator for severe or fatal DILI outcomes, or simply the eventual development of DILI [24]. MiR-122 elevation may also have causes that are not drug-induced; miR-122 is released actively from the liver in response to stress, and not solely from passive leakage from damaged hepatocytes, which is potentially responsible for the significant between- and within-subject variability that is observed with miR-122 values [26]. This therefore lessens miR-122's potential utility as a DILI biomarker, as it may be influenced by physiologic processes unrelated to hepatic damage [26].

Mechanistic biomarkers of cellular injury

Limitations of non-specific cellular damage mechanisms and high mobility group box protein (HMGB1)

Following hepatocyte damage by the action of drugs, cellular damage involving oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and bile salt export pump inhibition, among other disease processes, occurs [12]. These processes can lead to cell death and release of intracellular content, such as high mobility group box protein (HMGB1), which then stimulates a strong inflammatory response [12]. This

disease process highlights HMGB1 as a potential biomarker for severe DILI, which has already been demonstrated with the release from human hepatocytes following APAP DILI [27]. However, the mechanism by which HMGB1 mediates hepatic injury, the receptors involved, and the signaling pathways activated remain largely unknown [28], although a potential feed-forward mechanism signifies HMGB1's potential contribution to amplification of hepatocyte necrosis following APAP-induced liver injury through a RIPK3-dependent pathway [27]. In addition, diagnostic assays for HMGB1 are lacking in their clinical utility, due to either being too time-consuming, or unable to differentiate between HMGB1 isoforms [29]. In addition, HMGB1 is not a liver-specific biomarker [29]. Thus, HMGB1 would be best used in conjunction with a liver-specific DILI biomarker, such as microRNA-122.

Challenges in interpreting keratin 18 and apoptosis-necrosis index in DILI

Another proposed biomarker following hepatocyte injury is the cytoskeletal protein circulating keratin 18 (K18), which leaks passively into circulation following necrosis (full-length K18) and apoptosis (caspase-cleaved K18 [ccK18]) [26]. Calculating the ccK18: K18 ratio (AI) is believed to reflect the proportion of cell death that can be attributed to apoptosis versus necrosis, with lower AIs (i.e. more necrosis than apoptosis) occurring more frequently in DILI patients that died or needed a liver transplant following DILI [26]. However, despite its high specificity and sensitivity for detecting liver injury among patients with DILI diagnosis, K18 is also dramatically increased in plasma from patients with hypoxic hepatitis, alcohol-associated liver disease, nonalcoholic steatohepatitis, and other diseases [24]. Thus, elevated K18 levels are not isolated to DILI, and can have non-drug-induced hepatic etiologies, which should be considered when utilizing K18 as a potential indicator for severe DILI outcomes.

Assessing the limited value of measuring mitochondrial damage and glutamate dehydrogenase (GLDH)

Along with broad hepatocyte damage, different drugs can cause mitochondrial damage through a variety of mechanisms; for instance, some medications such as ciprofloxacin and tacrine can inhibit mtDNA replication and translation, inducing mtDNA depletion [30], and still, such as tacrine, ganciclovir, and diclofenac are known to trigger mtDNA damage [12]. Glutamate dehydrogenase (GLDH) is a mitochondrial matrix enzyme that is highly expressed in liver tissue and is a useful indicator of liver-specific mitochondrial damage and subsequent DILI outcome prediction [12]. GLDH levels showed a strong correlation with ALT levels in the context of DILI [26]. However, while MiR-122, previously discussed for its potential as a prognostic biomarker for DILI, appears to be a useful indicator for predicting acetaminophen-induced acute liver injury following overdose in patients with normal ALT levels at presentation, GLDH is not useful in this context [31]. GLDH is only a useful biomarker in DILI when ALT is elevated, and thus it is not a sensitive diagnostic biomarker for drug-induced liver injury, although it is a helpful metric.

High applicability of measuring glutathione depletion in acetaminophen toxicity

Another potential biomarker for DILI is glutathione (GSH) depletion, as its involvement in the initiation and progression of acetaminophen toxicity is well-defined [27]. GSH is a highly sensitive marker for oxidative stress and will likely be included in screening assays to assess for meeting DILI treatment endpoints [12]. GSH becomes significantly depleted due to the overwhelming load of toxic NAPQI that must be reduced into non-toxic metabolites [12]. Therefore, glutathione depletion may have significant utility as a biomarker of DILI to determine if a patient may be at risk of continued hepatocellular damage and determine alternative treatment strategies.

Fatty acid binding protein (FABP-1) as a potential biomarker

Fatty acid binding protein (FABP-1) has also been proposed as a potential biomarker for drug-induced liver injury, as it is found abundantly in the liver and is involved in fatty acid metabolism, storage, and transport [6], and significantly elevates with DILI. FABP-1 has been shown to have some utility as a DILI biomarker, as it elevates more rapidly than ALT, allowing for earlier detection of potential DILI, but it likely lacks predictive value for the development of severe DILI. Additionally, variations in FABP-1 level are not limited to DILI, and can be influenced by cancer, diabetes, and metabolic disorders [6]. Thus, although FABP-1 may have some utility in screening for drug-induced liver injury, particularly earlier in the disease state, more data is needed regarding its sensitivity and specificity as a biomarker for severe DILI outcomes.

Inflammatory mediators of cellular injury

Immune Activation and Inflammation in DILI

Drug-induced liver injury involves the release of a cascade of pro-inflammatory cytokines such as IL-6, interferons (IFNs), and tumor necrosis factor-α (TNF-α) [32, 33, 34]. In the case of acetaminophen-induced liver injury, NK cells were shown to have a role in the increased progression and severity of liver injury due to their secretion of IFN-γ, modulation of chemokine production and recruitment of neutrophils into the liver [35]. Additionally, several drugs associated with idiosyncratic DILI have been noted to synergize with TNF and kill human hepatocytes [36]. This association of biomarkers of immune activation and inflammation with DILI disease states suggests the potential for immunoassay studies to predict severe DILI outcomes. In a study of 27 immune analytes in 78 subjects with acute DILI, low levels of four (interleukin [IL] 9, IL 17, platelet-derived growth factor bb, and RANTES) were shown to be associated with low survival after an acute DILI event [37], indicating the potential for a serum immunoassay to predict severe DILI outcomes. However, these serum immune analyte profiles are not specific to or correlated with the underlying drug causes of DILI, the pattern of DILI (i.e. hepatocellular, cholestatic, or mixed nature), or the R ratio [37]. Therefore, while serum immunoassays can be utilized in predicting worse drug-induced liver injury outcomes, they lack specificity for drug-induced liver injury.

Soluble CD163 (sCD163) as a Marker of Macrophage Activation

Following DILI-related inflammation, the macrophage activation marker soluble CD163 (sCD163) may be released from tissue macrophages and monocytes by a metalloprotease-dependent pathway associated with the inflammation-inducible enzyme TNF-α converting enzyme (TACE/ADAM17) [38]. Yang et al. corroborated these findings, noting that the amount of CD68+ cell infiltration in DILI liver and plasma sCD163 levels are correlated with the severity of liver injury, and elevated CD163 serum levels in patients with DILI were also positively correlated with ALT and total bilirubin levels [39].

However, increased plasma levels of sCD163 have been linked to states of low-grade inflammation such as diabetes, obesity, liver disease, tuberculosis, and atherosclerosis [38]. While an association between sCD163 levels and severity of liver disease is evident, sCD163 elevation is not specific to liver injury [38]. Therefore, like serum immunoassay testing, sCD163 levels may be useful in predicting drug-induced liver injury severity, but they lack specificity in diagnosing drug-induced liver injury.

Bile acid profiles and liver function

Bile acid profiles suggest early detection and severity prediction of DILI

Another proposed biomarker for the hepatocellular and hepatobiliary damage associated with drug-induced liver injury is bile acid profiles. Bile acids are water-soluble steroids that are formed during cholesterol catabolism and are synthesized in hepatocytes [40]. In a methapyrilene-induced liver injury murine model, 20 specific bile acids were shown to elevate significantly, along with histopathological signs of hepatocellular and hepatobiliary damage, as well as standard clinical chemistry parameters (ALT, AST, AP, γGT, TBIL) [40]. The bile acids with the highest reliability as biomarkers for hepatocellular/hepatobiliary damage were cholic acid (CA) and chenodeoxycholic acid (CDCA) due to their consistent dose- and time-dependent changes in this DILI model, followed by glycocholic acid (GCA), taurocholic acid (TCA) and deoxycholic acid (DCA) [40]. However, bile acid metabolism and homeostasis in murine models have been shown to have significant differences from bile acid metabolism in humans, and thus the utility of murine models when assessing the effects of drugs on bile acid homeostasis to screen for DILI is limited [41]. However, more recent data in human DILI patients shows that the levels of serum bile acid metabolites palmitic acid, taurochenodeoxycholic acid, glycocholic acid (GCA), and tauroursodeoxycholic acid (TUDCA) were significantly higher in DILI patients than controls [42]. Furthermore, selected reaction monitoring assay of bile acids revealed that the increase in GCA, taurocholic acid (TCA), TUDCA, glycochenodeoxycholic acid (GCDCA), glycochenodeoxycholic sulfate (GCDCS), and taurodeoxycholic acid (TDCA) corresponded to a higher degree of liver damage, while serum concentrations of chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and lithocholic acid (LCA) were significantly lower in patients with severe DILI, when compared to healthy controls [42]. These findings were corroborated by Zhao et al., who found that bile acid metabolism is correlated with DILI severity, and levels of GCA, GCDCA, TCA, and TCDCA are potentially potent biomarkers in evaluating DILI severity. Moreover, increased serum fibroblast growth factor (FGF19) levels were found in DILI patients, potentially due to an alteration in gut microbiota, leading to activation of farnesoid X receptor-fibroblast growth factor (FXR-FGF19) signaling in an attempt to suppress BA accumulation in the liver of DILI patients [43]. This elevation of FGF19 did appear to have an association with DILI severity, with the serum level of FGF19 being 2-fold higher in critical DILI patients than non-critical DILI patients [43]. Thus, bile acid profiles and fibroblast growth factor (FGF19) levels do appear to have a relationship with the severity of drug-induced liver injury and thus could serve as potential biomarkers for early diagnosis of DILI, as well as prediction of severe DILI outcomes.

Advancing clinical diagnosis with integration of biomarkers into clinical practice

Potential predictive enhancement when combining biomarkers for improved DILI diagnosis

The biomarkers miR-122, HMGB1, and K18 do all show promise as biomarkers in DILI, but the discussed challenges reduce each marker's predictive value for DILI outcomes alone. However, a combined model of miR-122, HMGB1, and K18 has been shown to predict acute liver injury better than ALT alone following acetaminophen overdose, with miR-122 being the best predictor of ALT increase [31]. Additionally, adding GLDH, current biomarkers (ALT, INR, plasma acetaminophen concentration), and all recorded patient clinical characteristics (e.g. dose ingested and time to treatment) did not improve the sensitivity or specificity of the model in predicting acute liver injury [31]. Thus, these biomarkers may be most useful when considered in combination with one another when diagnosing and monitoring DILI, with miR-122, HMGB1, and K18 showing perhaps the most promise in predicting DILI, although more data are needed on whether these markers used in this combined model can help predict severe DILI outcomes.

Refining biomarker interpretation for DILI assessment

Traditional biomarkers such as the R factor proteins, ALT, AST, ALP, and TBIL levels have been a mainstay in the detection and determination of liver damage. However, these markers can be elevated due to extrahepatic pathology or fail to elucidate a mechanism of injury [19]. Measuring an increase in a marker directly related to the mechanism of injury would be most useful in determining the progression of injury. For example, a proteomic approach identified integrin beta-3 protein to be upregulated in patients with diclofenac-induced DILI [44]. This measure would likely have a higher predictive ability than traditional biomarkers as the measured protein is directly a result of the drug-induced injury. Using a metabolomic approach, biomarkers such as total keratin-18 (K18) and fragment K18, which increase during cell necrosis and apoptosis, can aid in predicting the severity of liver injury, particularly in cases of acetaminophen-induced damage [45]. Further investigation into additional drugs and their associated protein or metabolite markers could facilitate the development of a comprehensive, patient-specific biomarker panel, enabling the identification of the offending agent among a patient's medications. Furthermore, genetic testing associated with susceptibility to liver injury should be undergone in at-risk populations as there have been many links identified between human leukocyte antigen (HLA) and DILI [46]. This can be individualized as well, testing for specific genes of interest associated with a drug to develop predictions concerning the risk of DILI. By utilizing biomarkers that are directly linked to the underlying mechanism of injury, we can better assess the extent and predictability of liver damage.

Although there is a lack of a standardized definition and criteria for diagnosing DILI and often relies on excluding other causes, there are validated models available to assess its likelihood [47]. The Roussel Uclaf Causality Assessment Method (RUCAM) is commonly used as the gold standard to interpret traditional biomarkers and diagnose DILI. It was developed and based on the results of expert international consensus meetings and studies in the late 1980s [47]. The RUCAM has been updated to be clear and specific but has still been criticized for some ambiguity in a few of the descriptions used to interpret how scores are assigned when obtaining a result [48]. Other models

have been developed including the Drug-Induced Liver Injury Network (DILIN) criteria and the Council for International Organizations of Medical Sciences scale (CIOMS) which have had varying opinions on their accuracy, some noting that expert opinion/consensus based on clinical presentation may be the best approach as prospective validation of these scales is still incomplete [3]. While already difficult to diagnose, differentiating DILI becomes even more complex when in a setting of underlying metabolic dysfunction-associated fatty liver disease (MASLD), viral hepatitis, or other chronic liver disease [3]. Despite advancements in diagnostic models, the complexity of accurately diagnosing DILI, especially in the presence of comorbid liver conditions, highlights the ongoing need for more precise and universally accepted assessment tools.

To overcome these shortcomings, there is a growing interest in discovering new biomarkers that are directly associated with the mechanism of injury [19]. Although emerging biomarkers show promising potential in predicting the risk of DILI, the substantial variability in patient characteristics presents a significant challenge in relying on one or a few measures for risk assessment. Researchers have been utilizing published data in conjunction with machine learning to assess the risk of new compounds based on their chemical structure [49]. However, limitations often evolve from the lack of data that can be utilized to make predictions. New compilations of larger training datasets such as DILIRank, a reference list of compounds by DILI severity and potential, can be used to improve predictions [49]. A comprehensive approach integrating data on novel biomarkers, compound structure, and known hepatotoxicity events could lead to the development of a valuable repository for assessing individual patient risk. This system would allow for more accurate predictions regarding the likelihood of DILI, enabling personalized risk assessments. However, known DILI biomarkers are relatively scarce and there is a lack of functional animal models to validate new candidates [23]. Additionally, a significant challenge to implementation lies in the stability of the biomarkers in body fluids for detection [23]. Collecting data using experimental biomarkers in addition to traditional measures will provide avenues to further investigate and develop more accurate tests that are stable for routine testing to diagnose DILI.

Discussion

Challenges in technical and methodological approaches slowing novel DILI biomarker development

Genomics, transcriptomics, proteomics, metabolomics, lipidomics, cell-based assays, and more recently, multiomics and advanced computational modeling, have discovered a wide array of DILI biomarkers, but there are still several obstacles to overcome. The complexity of DILI, the lack of standardized diagnostic criteria, and the challenges that come with regulatory processes still present as challenges in the biomarker development of DILI. DILI is categorized by its pathogenesis and type of liver injury; intrinsic DILI comes from a drug's properties or metabolites, while idiosyncratic DILI depends on the patient's response. Both intrinsic and idiosyncratic DILI can present as hepatocellular injury, a biliary phenotype, or a mix of both [50]. The variability of drug properties, low prevalence of idiosyncratic DILI and its unpredictability contribute to the difficulty of biomarker validation and ultimately the standardization of diagnostic criteria. Future standardization of protocols and diagnostic criteria can enhance the reliability, reproducibility, and clinical applicability of DILI biomarkers, improving early diagnosis and patient care. DILI accounts for most cases of acute liver failure in the western world and classification has been defined by an international DILI Expert Working Group of clinicians and scientists, but standardization of the diagnostic criteria and terminology surrounding DILI is still needed to improve reliability and applicability in clinical practice [51]. Standardization would further align DILI biomarker research with regulatory requirements, expediting the approval process for clinical use and enabling large-scale collaboration. However, a major hurdle of large-scale collaboration is the lack of regulatory-qualified safety biomarkers and the overall low prevalence of idiosyncratic DILI [52].

Research gaps and identification of opportunities in biomarker development and predictive modeling

Future studies will be directed at the development of a standardized set of comprehensive biomarker panels, completion of longitudinal studies with diverse populations, and utilization of advanced technology to further enhance prediction models. Standardizing DILI biomarker research, such as methods of biomarker collection and analysis and clear definitions of diagnostic criteria, would significantly strengthen the consistency and accuracy of results. A proposed method of data collection is to have a central governing body managing the collection and quality control of specimens; this method has been successfully implemented by Acute Stroke Ready Hospitals but due to DILI's rarity and unpredictability, execution of this model will be more difficult [53]. Nonetheless, this would help ensure that findings are reproducible across different studies, leading to more reliable and applicable biomarkers. Standardization is a crucial step for longitudinal data collection and results comparison across research centers. Longitudinal data collection can improve biomarker validation, and foster collaboration, and implementation in clinical practice. Because of the low prevalence of DILI, continuous data collection from diverse patient populations will allow researchers to observe the onset of DILI, monitor biomarker fluctuations with severity of liver damage, help identify predispositions to DILI and use the data for prediction models[53]. Animal and cell-based models often fail to replicate human DILI, particularly idiosyncratic reactions, limiting the use of these findings in clinical settings. With advanced computer-based algorithms, long-term data collection and research can further advance DILI prediction models to more accurately mimic human DILI and predict clinical outcomes [51].

Potential for Translational Research and Collaboration

The emerging field of DILI biomarker research offers numerous pathways into pharmaceutical trials, clinical trials, and clinical practice. Once standardized and validated, biomarkers can monitor liver safety in new drug trials, predict patient susceptibility to DILI, and link biomarkers to clinical outcomes. Compared to traditional liver function tests, biomarkers can enhance diagnostic accuracy and better identify at-risk patients. Bridging the gap between discovery and clinical use requires interdisciplinary collaboration among researchers, clinicians, industry partners, and regulatory agencies to focus on identifying and validating biomarkers through real-world clinical outcomes and setting guidelines for development clinical use [51]. To advance DILI biomarker applications, enhanced replicative models must be developed in conjunction

with a database of shared, longitudinal findings, clinical findings, and outcomes [52]. Clinicians must be thoroughly educated on the risks and benefits of DILI biomarker use and DILI biomarkers can be integrated into workflows for ease of use. By fostering such collaboration, the transition from research to clinical practice can be streamlined, ultimately improving drug safety and patient care.

Results

This comprehensive evaluation offers an overview of the significant advancements in DILI biomarkers and their potential role in clinical care. Thus far, many biomarkers have shown promise for potential for clinical implementation with further characterization and validation needed before they can gain widespread acceptance. The use of APAP-CYS as a biomarker for hepatotoxicity, specifically after acetaminophen overdose, is an excellent example of a successful implementation of a targeted liver test. Biomarkers such as miR-122, HMGB1, and K18 have been identified as formidable indicators of hepatocyte injury, demonstrating a promising potential for early liver damage and severity detection [17, 24]. Individually, these biomarkers lack the mechanistic specificity to provide a binary determination of whether DILI is present or not. Detailing the landscape of commonly involved biochemical pathways by measuring miR-122, HMGB1, and K18 together, can provide clinicians with a strong foundation for assessing liver injury with greater accuracy. The development of a panel containing these markers may be on the horizon pending a movement to standardization of DILI diagnosis, not accounting for possible difficulties in manufacturing or cost.

Mitochondrial dysfunction biomarkers like GLDH and bile acid profiles such as glycocholic acid and taurocholic acid provide a deeper understanding of the mechanical processes of DILI and its progression [54]. Bile acid profiles are a unique way to identify hepatic pathology and there is much to continue to discover concerning their ability to predict disease. Further characterization of these profiles are needed before implementation can be made to aid in diagnosis but with time, this avenue has the potential to enhance diagnostic precision dramatically. Similarly, immune activation markers, like sCD163 and inflammatory cytokines, highlight immune pathways in liver injury and complement other biomarkers for a fuller understanding as well [38]. Inflammatory mediators, while useful in many pathological states, may be of less clinical applicability for diagnosing DILI due to the nonspecific pathophysiology underlying their elevation.

Integrating these biomarkers into pre-existing clinical tools can significantly improve diagnostic accuracy and patient outcomes. Risk assessment models like the RUCAM and DILIN criteria may benefit from integrating DILI-specific biomarkers to further characterize liver injury severity [19, 47]. Inclusion of meaningful biomarkers along with traditionally measured criteria can strengthen diagnostic ability, help assess treatment outcomes, and enable earlier identification of high-risk patients. Greater screening may also enable targeted interventions downstream in the recovery process, minimizing poor outcomes such as acute liver failure and transplantation.These findings highlight the transformative potential of biomarkers to shift DILI management from reactive care to a more preventative and predictive approach.

Conclusion

Multiple biomarkers have been studied to better understand their roles in risk stratification and management of DILI. Since the liver is the primary site of GSH synthesis, DILI-induced GSH depletion disrupts the homeostasis of antioxidative and oxidative processes in the mitochondria leading to mitochondrial dysfunction and ultimately cellular damage. Thus, abnormally low levels of mitochondrial GSH may serve as a potential biomarker for early detection of DILI. Other mitochondrial dysfunction markers, including GLDH and mitochondrial DNA, show promise for assessing DILI severity and prognosis due to their high hepatic distribution and involvement in metabolic processes. Additionally, recent studies show that serum levels of specific bile acid metabolites such as GCA, TCA, TUDCA, and GCDCA are significantly elevated in DILI patients and correlate with greater liver damage, while others like CDCA, DCA, and LCA are reduced in severe cases. These changes in bile acid profiles suggest their potential or use as biomarkers for assessing DILI severity. Moreover, high levels of FGF19 were observed in DILI patients and found to be higher in those with more critical disease. Together, bile acid metabolites and FGF19 may serve as valuable biomarkers for early diagnosis and prognosis of severe DILI. Biomarkers such as miR-122, HMGB1, and K18 show the most promise for predicting DILI, but their greatest utility may come when used in combination.

Though useful, both liver enzymes and bilirubin are nonspecific and limited in early detection and prognostication.
These markers must be interpreted in context and combined with clinical evaluation and potentially more specific biomarkers for accurate DILI diagnosis and monitoring. These findings improve our approach to managing DILI by offering a more precise and earlier method of detection, risk stratification, and prognosis beyond traditional liver function tests. Identifying mitochondrial dysfunction through markers like mitochondrial GSH, GLDH, and mitochondrial DNA provides insight into early cellular stress and damage, allowing for timely intervention. Bile acid profiles and FGF19 levels further enhance the ability to gauge disease severity and progression, offering a dynamic view of liver function during DILI. The use of emerging biomarkers such as miR-122, HMGB1, and K18 may improve diagnostic accuracy and allow for individualized treatment strategies. Altogether, this biomarker-driven approach shifts DILI management toward more predictive, personalized, and proactive care.

These emerging biomarkers can offer insight into molecular and cellular dysfunction well before clinical symptoms or traditional lab values rise. This would allow clinicians to identify at-risk patients sooner, adjust or discontinue offending drugs earlier, and monitor response to treatment more effectively. Additionally, biomarkers can help uncover genetic predispositions (e.g. through HLA typing) and mechanistic pathways specific to DILI, potentially guiding tailored therapy and preventative strategies. As these markers are further studied and integrated into clinical practice, they hold promise for transforming DILI management from reactive to preventive, ultimately reducing the incidence of severe outcomes such as liver failure or death.

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