Laboratory and genetic markers for assessing liver function to predict drug metabolism and detect drug-induced liver injury

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Abstract

Introduction: To date, liver function is measured with general liver function tests and requires assessment prior treatment in vulnerable patient groups, as it influences drug metabolism. CYP450 enzymes in the liver play an important role in drug metabolism. An effective and safe treatment will only be achieved when plasma drug concentrations are within a therapeutic window. Therefore, assessing liver function prior treatment is needed to predict drug response. The available general liver function tests lack the ability to predict specific drug metabolism. Therefore, new biomarkers and genetic mutations for measuring the liver metabolic capacity to predict drug metabolism are needed for moving towards personalized medicine. In addition, biomarkers for early detection of drug-induced liver injury (DILI) to prevent further liver damage are of great importance.

Aim: The objective of this review is to investigate and identify laboratory and genetic markers that assess liver function to predict drug metabolism. Additionally, biomarkers for the early detection of DILI will be explored.

Method: A literature review was conducted in the Pubmed database, on May 3, 2024. Only English literature was reviewed, case studies and congress articles were excluded. No further exclusion criteria or date restriction were applied.

Results: First, biomarkers assessing liver injury in general include AST, ALT, bilirubin, HA, ADMA, RBP4, resistin, and XOR. However, these markers are not suitable for predicting an individual's drug response. Laboratory markers found for drug metabolizing enzymes in the liver, include miRNAs (CYP2B6), 4βOHC (CYP3A), bile acids (CYP3A), TG/AG (UGT2B17), ratios 2-PY and 4-PY to NMN (AOX), and plasma levels of CES1. Second, multiple polymorphisms are found for both regulatory genes and drug metabolizing enzymes. Third, early detection of DILI may be achieved via miRNA-122, K18, GLDH, HMGB1, MCSFR1 and OPN.

Conclusion: In this review, an overview is provided concerning currently known laboratory and genetic markers for predicting drug response and detecting early DILI. Biomarkers with greatest potential for predicting drug response are miRNAs for CYP2B6 activity, the 4βOHC:cholesterol ratio and bile acids for CYP3A activity. These biomarkers still need validation in future studies, and more endogenous biomarkers measuring CYP450 enzyme activities need to be found. Additionally, genotyping has great potential in finding polymorphisms in CYP450 enzymes itself as well as regulatory genes, to predict drug metabolism. Lastly, early detection of DILI both miRNA-122 and K18 have greatest potential, due to liver-specificity and earlier detection compared to ALT. Still, the ideal biomarker has not been found.

Summary

Introduction: To date, liver function is measured with general liver function tests. These tests need to be performed before drugs are being administered, because liver enzymes metabolize drugs which influences plasma concentrations. An effective and safe treatment will be achieved when plasma drug concentrations are within specific ranges. Drug plasma concentrations above this range will result in toxicity, whereas concentrations below this range cause an ineffective treatment. Therefore, measuring liver function is required to predict treatment efficacy and safety. However, the available tests measure liver injury in general and lack the ability to predict activity of specific liver enzymes, which can predict metabolism of drugs metabolized by that enzyme. Biomarkers can be used for that specific purpose being endogenous biological parameters as indicators to processes and responses to drug treatment. Genetic mutations causing altered enzyme expression and/or activity also have potential for clinical use. Assessing liver injury and predicting drug response is especially important for a large group of patients with liver impairment vulnerable to drug toxicity. Therefore, new accurate biomarkers and genetic mutations are needed to assess enzyme activity and predict drug metabolism. Also, biomarkers for early detection of drug-induced liver injury (DILI) to prevent further liver damage are of great importance.

Aim: Our objective was to identify endogenous molecules and genetic mutations that predict the enzymatic activity in the liver. Also, endogenous molecules for early detection of DILI were explored.

Method: A literature review was performed in the Pubmed database, on May 3, 2024. Only English literature was reviewed, case studies and congress publications were excluded. No further exclusion criteria or date restrictions were applied.

Results: Biomarkers that can't predict specific activity of enzymes, but can detect liver injury in general include AST, ALT, bilirubin, HA, ADMA, RBP4, resistin, and XOR. Biomarkers found to predict liver enzyme activity, include miRNAs (for CYP2B6 enzyme), 4βOHC (for CYP3A enzyme), bile acids (for CYP3A enzyme), TG/AG (for UGT2B17 enzyme), ratios 2-PY and 4-PY to NMN (for AOX enzyme), and plasma levels of CES1 for CES1 itself. Multiple genetic mutations were found for liver enzymes, including CYP2C9, CYP2D6, CYP3A4, UGT1, CDA, and CES1, and alter its expression and/or activity. Lastly, early detection of DILI may be achieved via the biomarkers miRNA-122, K18, GLDH, HMGB1, MCSFR1 and OPN.

Conclusion: Assessing enzymatic activity is the most effective and most accurate course of action to predict drug metabolism and individual's drug response. Biomarkers with greatest potential for predicting drug response are miRNAs for CYP2B6 activity, 4βOHC:cholesterol and bile acids for CYP3A activity. These biomarkers still need validation in future studies, and more endogenous biomarkers measuring CYP450 enzymes activities need to be found for other CYP450 enzymes as well. Additionally, finding genetic mutations in the metabolizing enzymes also has great potential to predict drug metabolism. Lastly, early detection of DILI with both miRNA-122 and K18 have both potential, due to liver-specificity and earlier detection compared to ALT. Still, the ideal biomarker has not been found.

Introduction

Liver disease is responsible for 4% of all deaths worldwide, and its absolute number of cases is estimated to be 1.5 billion worldwide (1,2). Liver disease is approximately two times more prevalent in men compared to women (1). Besides high mortality, there is a high global impact due to the amount of disability-adjusted life-years (DALYs), as liver disease is the 15th leading cause of DALYs globally (1). Acute liver failure causes high morbidity and mortality rates, with an estimated incidence of one per million (1). In the US and Europe, drug-induced liver injury (DILI) is the most common cause of acute liver failure (1).

To date, liver function is measured with general liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), the international normalized ratio (INR), blood urea nitrogen (BUN), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), 5'nucleotidase, prothrombin time (PT), lactate dehydrogenase, total protein, globulins, creatinine, platelets, alkaline phosphatase, bilirubin, and albumin (3–5). Furthermore, the Child-Pugh score is being used to categorize patients according to serum bilirubin, serum albumin, ascites, neurological disorder, and clinical nutrition status into categories for liver function (A=good hepatic function, B=mild hepatic dysfunction, C=advanced hepatic dysfunction) (6,7).

In general, liver function needs to be assessed prior treatment in vulnerable patient groups, as is established in the NHG guideline with a step-by-step protocol for medication assessment (8). Liver function is an important factor that influences drug disposition in the body, which is dependent on four processes: absorption, distribution, metabolism, and excretion (ADME) (9,10). The liver plays an important role in the metabolism of drugs, for example Cytochromes P450 (CYP450) enzymes which are abundantly present in the liver (9). They activate prodrugs into drugs and inactivate drugs into their inactive metabolites, which influences drug plasma concentrations (9). An effective and safe treatment will be achieved when plasma drug concentrations are within a drug-specific therapeutic window. Drug plasma concentrations above this window will result in toxicity, whereas concentrations below this window cause an ineffective treatment (9). Therefore, assessing liver function in vulnerable patients groups with suspected liver impairment prior treatment is needed to predict whether the treatment needs a dose adjustment to prevent toxicity and increase the chance of effectivity.

The available general liver function tests lack the ability to assess the metabolic capacity of the liver for individual enzymes and drugs (7), as liver injury is a different parameter than metabolizing enzyme activity. This is especially of relevance for a large group of patients with liver impairment, who might have altered enzyme activity and as a consequence need dose adjustments to optimize treatment safety and effectivity (11). It is thought that two thirds of drug related adverse events are preventable by dose adjustments in patients with liver disease (12). Biomarkers can be used for that purpose to predict drug response, being endogenous biological parameters as indicators to processes and responses to drug treatment (7,13). Usually biomarkers can be detected before clinical outcomes arise (7). Additionally, the ideal biomarker has multiple characteristics: specific, sensitive, predictive, fast, cost effective, stable, noninvasive, and relevant

(7). However, the ideal biomarker to predict drug metabolism still does not exist. Besides biomarkers, pharmacogenetics has great potential, as genetic mutations affect metabolizing enzymes in the liver. Genotyping individuals can find mutations that may result in altered enzyme expression and/or activity. This will classify subjects into poor (PM), intermediate (IM), normal (NM), and ultrarapid (UM) metabolizers, which can eventually predict drug response and toxicity (14). Lastly, biomarkers for early detection of DILI to prevent further liver damage are of great importance.

Currently, the evidence on these topics is very scattered. Therefore, the aim of this review is to investigate and identify laboratory and genetic markers that assess liver function to predict drug metabolism. Additionally, biomarkers for the early detection of DILI will be explored.

Method

A literature review was conducted in the Pubmed database, on May 3, 2024. Search terms used were liver, hepati, metaboli, drug, medic, biomarker, and predict (see Supplement 1 for complete syntax). Only English language articles were reviewed, and case studies as well as congress articles were excluded. No further exclusion criteria or date restriction were applied. First, articles were included or excluded based on scanning titles and abstracts. Subsequently, references from relevant articles were screened. As a next step, included articles were reviewed in detail. Data extraction was performed manually, extracting data concerning biomarkers and genetic markers for measuring liver function, as well as biomarkers for early detection of drug-induced liver injury as endpoints. In addition, the research design, sample size and conclusion were extracted.

Results

Systematic review results

The systematic search resulted in 353 articles, of which 40 were selected for inclusion in this review after title/abstract screening (see Supplement 2, Figure 1). The studies cover a wide range of laboratory and genetic markers used to measure liver injury and drug-induced liver injury. First, laboratory markers for liver function will be discussed. Then, genetic markers for enzyme expression and activity will be discussed, followed by laboratory makers for early detection of DILI.

1. Biomarkers for liver function

1.1 ALT and AST

Already for a long time and still one of the golden standards for measuring liver injury are serum levels of ALT and AST (5).

ALT is involved in the reversible reaction between alanine and 2-oxoglutarate to generate pyruvate and glutamate (5). ALT is localized in the cytosol, endoplasmic reticulum, and mitochondria (5,13). ALT is not liver-specific, as it is also present

in kidney, heart, muscle, fat and brain (5). It is suggested that increased plasma ALT levels reflect membrane disruption and mitochondrial dysfunction of hepatocytes (5). However, suggestions have been made that increased ALT concentrations might also reflect an adaptive response to overall metabolic demand changes (5). Normal ALT levels ranges between 4 and 36 IU/L (3).

AST enzymes are involved in amino acid metabolism, and are present in the cytoplasm and mitochondria (5). Therefore, higher AST levels are suggested to reflect membrane disruption and hepatocyte dysfunction (5). Besides present in hepatocytes, AST exist in adipocytes and is thus not liver-specific (5). Physiological AST level vary between 5 and 30 IU/L (3).

ALT is thought to measure liver injury more specifically than AST (13). Serum ALT and AST concentrations are variable, and depend on multiple factors, including sex, age, waist circumference and BMI, ethnicity and alcohol consumption (5,15). Additionally, serum ALT and AST levels are heritable (5). Only severe hepatocyte damage will significantly increase AST and ALT levels (5). ALT and AST have long half-lives, 47 hours and 17 hours respectively (5,16). Lastly, increased ALT and AST as indicators for hepatocyte membrane disruption, do not directly reflect hepatic drug metabolizing enzyme activity (5).

ALT and AST rank third and fourth place for their concentrations in the liver, with lactate dehydrogenase (LDH) and malate dehydrogenase (MD) ranked as first and second (5). Therefore, LDH and MD with higher abundance in liver tissue might better and accurately predict liver injury, suggesting these markers might add relevance to the current general liver function tests.

In summary, both AST and ALT are still being used as biomarkers for general liver injury, and LDH and MD may be added as biomarkers for measuring liver injury. However, these biomarkers do not directly reflect drug metabolism by the liver and are not liver-specific, making them less valuable as marker.

1.2 Bilirubin

Currently, bilirubin is one of the standard liver function tests to measure hepatic failure (3–5,7). Bilirubin is a product of heme catabolism in the liver (7). Physiological bilirubin plasma concentrations range from 2 to 17 µmol/L and bilirubin has a half-life of 2-4 hours (3,7). The Child-Pugh score categorizes patients by severity of liver impairment with 5 variables, including bilirubin level, however this score reflects liver injury in general (7).

Thus, bilirubin and the Child-Pugh score are still being used to assess general hepatic failure. The Child-Pugh score includes bilirubin in its assessment, making it more reliable compared to bilirubin alone.

1.3 GGT

To date, GGT is one of the standard liver function tests as a hepatobiliary biomarker, because it is quick and inexpensive (3–5,17). GGT, an enzyme in the cellular membrane, plays an important role in glutathione metabolism and their

metabolites are protective molecules against oxidative stress (17). However, the source of serum GGT is still unknown (17). To date there is no evidence that serum GGT derives from the liver, but it is still used as a biomarker for assessing hepatobiliary function (17). Physiological GGT plasma levels vary between 6 and 50 IU/L (3).

So, GGT is an easy and inexpensive biomarker for measuring hepatobiliary function. However, it is still unknown whether plasma GGT levels derive from the liver, making it a less reliable laboratory marker.

1.4 Hyaluronic acid

Recently, hyaluronic acid (HA) was suggested as early biomarker for liver impairment (7). HA has a half-life of approximately 2-5 minutes (7,18). Gudowska et al. investigated the association between the severity of liver disease and HA concentrations. An association was found between the Child-Pugh score and HA concentrations (18). Subgroup analyses revealed that patients with Child-Pugh class C, the highest level of impairment, had significantly higher HA concentrations compared to both Child-Pugh class B and A (18). In addition, Child-Pugh class B had significantly higher HA levels compared to Child-Pugh class A as well (18).

Thus, levels of HA are correlated with the Child-Pugh score and may have potential as additional marker in the standards tests for assessing liver injury.

1.5 ADMA

Lately, asymmetric dimethylarginine (ADMA) was suggested as biomarker for liver dysfunction (19). ADMA is an inhibitor of nitric oxide (NO) synthase, which will impair NO-dependent endothelial functioning and therefore is associated with endothelial dysfunction (19). As ADMA plays a role in endothelial dysfunction and organ failure, Koch et al. explored in 255 critically ill patients whether ADMA levels were associated with liver dysfunction. Their results showed that ADMA concentrations were significantly associated with markers reflecting hepatic biosynthetic capacity (INR, albumin) and cholestasis (bilirubin, GGT) (19).

Increased ADMA levels reflect endothelial dysfunction, and correlate with multiple standard liver function tests to predict liver dysfunction.

1.6 RBP4

Retinol binding protein 4 (RBP4) was suggested as biomarker for assessing the hepatic biosynthetic capacity (20). RBP4 is synthesized in the liver and functions as transporter protein for vitamin A (retinol) from the liver to target tissues (20). Koch et al. investigated the role of RBP4 and liver function in 123 critically ill patients. They found that serum RBP4 was closely associated with liver function, as RBP4 levels were significantly correlated with parameters for hepatic biosynthetic capacity (including albumin, PT, and bilirubin) (20).

In short, RBP4 might be a predictive biomarker for the synthesis of biomolecules in the liver as well as liver impairment.

1.7 Resistin

Resistin was suggested as biomarker for measuring hepatic failure (21). It is a hormone that regulates glucose metabolism and insulin sensitivity (21). Koch et al. studied the correlation between resistin levels and organ dysfunction in 170 critically ill participants compared to 60 healthy controls. Their study found that resistin levels were closely associated with liver impairment, because resistin levels were significantly correlated to markers reflecting hepatic dysfunctioning, such as pseudocholinesterase and bilirubin (21).

Thus, resistin might be a potential biomarker for assessing liver injury.

1.8 Cytochrome P450 enzymes

Drugs are mostly metabolized by hepatic enzymes, such as the CYP450 enzymes or phase I enzymes (9). Therefore, biomarkers that are able to measure CYP enzyme activities can estimate drug metabolism, and thus predict treatment effectivity and toxicity.

1.8.1 CYP2B6

This enzyme is estimated to metabolize 8% of prescribed drugs (22). The study of Ipe et al. investigated in 72 human individuals whether cell-free miRNAs may serve as biomarker for CYP2B6 activity, using efavirez as a probe drug. Circulating miRNAs are non-coding RNAs of 22 nucleotides long, which negatively regulates expression of genes including genes for drug metabolism (22). The linear model, consisting of information about 2510 miRNAs, CYP2B6 genotype, as well as demographics, showed that 7 miRNAs (miRNA-204–5p, miRNA-212–3p, miRNA-3649, miRNA-3941, miRNA-4254, miRNA-4442 and miRNA-6867–5p) were significant for predicting CYP2B6 activity (22). Using this model, at least 36% of CYP2B6 variability was explained, which was an improvement over other models not integrating miRNAs (22). The study of Benson et al. investigated in 4 participants the feasibility of using miRNAs as biomarker, by exploring the variability of miRNA-16 and miRNA-223 in blood stored for 0.5 to 12 hours under room temperature. They concluded that miRNA-16 and miRNA-223 are stable at room temperature for up to 12 hours, making them feasible and valuable as biomarker (23). Thus, these miRNAs that assess CYP2B6 activity have great potential predicting drug response of drugs metabolized by CYP2B6, and have the advantage to be specific, stable and can be extracted noninvasively.

1.8.2 CYP3A

This enzyme is the most abundant CYP450 enzyme in the liver, and it metabolizes 45-60% of all prescribed drugs (10,24). With inter-individual variability in CYP3A4 metabolism, there is a need for accurate biomarkers predicting CYP3A4 activity.

Kvitne et al. studied in 96 humans the correlations between 4β-hydroxycholesterol (4βOHC) and CYP3A4 expression and activity in both liver and intestines. Results showed that 4βOHC plasma concentrations were positively associated with CYP3A4 expression and activity in the liver, but not in the intestines (25). The review of

Mao et al. further discussed the use of 4βOHC as potential predictor for CYP3A activity. They showed that both plasma 4βOHC and normalized 4βOHC, which is the ratio of plasma 4βOHC concentration to total cholesterol concentration, might be a useful predictor of CYP3A activity (26). Importantly, they emphasize that the ratio might provide better predictions in patients with high variable cholesterol levels or when patients take medicines that impact cholesterol concentrations (26). The review of Tremmel et al. further confirmed that these metabolites in both urine and plasma correlated with the CYP3A activity (27). Zhang et al. created a physiologically-based pharmacokinetic (PBPK) model to investigate relationships between pyrotinib exposure, CYP3A genotypes and normalized 4βOHC as endogenous biomarker in patients with liver impairments. They found a linear correlation coefficient larger than 0.6 between pyrotinib exposure and normalized 4βOHC levels, as well as an increased pyrotinib exposure along with the progression of liver impairment (28). Regarding 4βOHC as biomarker for CYP3A4 activity, it has a practical advantage that only one noninvasive blood sample is needed (25). Besides noninvasive, this measurement is also cost effective as well as more ethical to perform, compared to using a probe drug to measure CYP3A activity (25,26,29). These endogenous markers can also be monitored in vulnerable groups, in which probe drugs are not desirable (29). However, the estimated half-life of 4βOHC ranges from 2.5 to 17 days, which challenges interpreting 4βOHC levels in response to altering CYP3A activity (26,28,29). The exact half-life needs to be verified by other studies, to provide more knowledge for interpreting studies about 4βOHC levels and CYP3A activity. This long half-life makes 4βOHC a stable and suitable biomarker with low variability over time, and has great potential to predict CYP3A4 activity and thus predict drug response of drugs metabolized by CYP3A4.These results suggest that 4βOHC plasma or urine concentrations might be a predictive and potential biomarker for liver CYP3A4 metabolism. Normalized 4βOHC might be even better to use as a biomarker.

Deoxycholic acid (DCA) is a secondary bile acid that is metabolized by CYP3A into tertiary bile acids, including DCA-1β-ol and DCA-5β-ol (30). Zeng et al. explored whether these tertiary bile acids can predict CYP3A activity in six beagle dogs, using midazolam concentrations in plasma and urine as reference. Results showed that ratios of both tertiary bile acids to DCA were significantly correlated with midazolam clearance, both in serum and urine (30). It was confirmed that Beagle dogs have bile acid metabolism that is comparable to that of humans, especially the conversion from secondary to tertiary bile acids, making the study feasible to extrapolate (30). However, this needs to be confirmed in human studies. An advantage of these bile acids is that these are endogenous molecules, thus avoiding invasive assessments and use of probe drugs (30). Therefore, it is suggested that the ratios of tertiary bile acids to DCA might be potential predictive biomarkers for CYP3A activity in beagles.

In short, 4βOHC levels, normalized 4βOHC, and the ratios of tertiary bile acids to DCA are potential biomarkers for predicting CYP3A activity. This will create opportunities to predict drugs response of drugs metabolized by CYP3A. These biomarkers are specific, stable, ethical, and noninvasive.

1.9 Other liver enzymes in drug metabolism

Metabolites of phase I CYP450 enzymes will be further metabolized by phase II enzymes, enhancing excretion. Phase II enzymes consist of uridine diphosphate-glucuronosyltransferases (UGTs), sulfotransferases (SULTs) and glutathione-S-transferases (GSTs) among others (9). For example, of the top 200 prescribed drugs about 10% are metabolized by UGTs (9). Zhang et al. explored in 63 children aged 7 to 18 years the possibility of testosterone glucuronide (TG) normalized by androsterone glucuronide (AG, TG/AG) as urinary biomarker for assessing UGT2B17 activity. The results showed that urinary TG/AG was significantly associated with copy number variants of different enzyme activities, suggesting that TG/AG is a potential biomarker for assessing UGT2B17 enzyme activity (31). Both hormones are easy measured in urine, making it an noninvasive assessment. However, this biomarker can't be used in steroid-related disorders, when production of TG and AG is altered (31). Therefore, using these hormones is not ideal, as altered hormone levels influences the reliability of this marker.

Next, aldehyde oxidase (AOX) is an enzyme that significantly contributes to non-CYP drug metabolism (32). Sanoh et al. reviewed literature about this enzyme, including biomarkers for inter-individual variability in AOX activity. It is suggested that the ratios of N1-methylnicotinamide (NMN) to its metabolites 2-PY and 4-PY in urine is a potential biomarker for AOX activity in humans (32).

Xanthine oxidoreductase (XOR) is a metabolizing enzyme for the formation of uric acid, and its activity has been shown to be associated with obesity, hyperuricemia, liver dysfunction and insulin resistance (33,34). Furuhashi et al. studied in 627 Japanese participants whether there was an association between metabolic parameters and XOR activity. Results showed that XOR activity was positively significantly correlated with levels of both ALT and AST, thus liver impairment (34).

Carboxylesterase 1 (CES1) is an enzyme for hydrolytic reactions in drug metabolism, and contributes to 80-95% of total hepatic hydrolytic activity (35,36). CES1 is important for metabolizing drugs, especially the ester-prodrugs (36). Shi et al. hypothesized that plasma concentrations of hepatic drug-metabolizing enzymes can reflect their abundance in the liver. Therefore, Shi et al. explored in 15 humans whether CES1 plasma concentrations were associated with its substrate exposure. The study demonstrated a significant inverse relationship between plasma CES1 concentration and the AUC of its substrate (37). It was suggested that CES1 concentrations explained about 50% of the variability of the substrates AUCs (37). However, the plasma levels were considerably lower compared to the liver levels (37). Therefore, plasma CES1 levels might be not significantly correlated to drug metabolism, due to its low abundance in plasma (37). In addition, plasma levels doesn't reflect enzyme activity, questioning the suitability of this marker.

In summary, urinary TG/AG and ratios NMN to its metabolites 2-PY and 4-PY are potential biomarker for assessing UGT2B17 and AOX enzyme activity respectively. Both are noninvasive to measure and specific for drugs metabolized by these enzymes. XOR activity was correlated with liver impairment, and thus may not be able to predict enzyme-specific metabolism. CES1 levels in plasma correlates with systemic clearance and may predict drug metabolism by CES1, however plasma

CES1 levels do not reflect the activity of the enzyme variants, making the prediction less valuable.

An overview of all discussed laboratory markers assessing liver function can be seen in Supplement 3, Table 1.

2. Genetic markers for hepatic enzyme function

Genetic variants of liver drug metabolizing enzymes, predominantly genetic polymorphisms, result in different expression and activity patterns of enzymes. Drugs metabolized by these enzymes can have altered metabolism, which results in individual variability. Therefore, when these polymorphisms are known and can be assessed individually, it might predict treatment response.

2.1 CYP450 enzymes

Wortham et al. investigated in twenty human liver samples the effects of variable expression of nuclear receptors and coregulators on basal expression level and activity of multiple CYP450 enzymes. They found that the nuclear receptors Constitutive Androstane Receptor (CAR) and Hepatic Nuclear Factor 4α (HNF4α) regulate mRNA expression of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, MRP2, OATP2, P450 oxidoreductase (POR), and UDP-glucuronosyltransferase 1A1 significantly (38). Furthermore, CYP450 enzyme activities were strongly associated with POR expression (38). These results suggest that these genes exert effects on the expression (CAR and HNF4α) or activity (POR) of CYP450 enzymes, and polymorphisms of these regulatory genes might affect CYP450 enzymes (38). The study of Hart et al. explored in 99 human liver samples whether polymorphisms in the POR gene affect POR expression, POR activity, and CYP450 activities. Eight CYP enzymes correlated significantly with POR (CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP3A4/5, and CYP4A9/11) (39). Four out of 34 polymorphisms studied changed an amino acid, which resulted in a structural change, followed by decreased POR activity and several CYP450 enzyme activities (39).

These results suggest that identifying polymorphisms in the CAR, HNF4α and POR genes might predict altered expression or activity of POR and CYP450 enzymes, thereby predicting drug metabolism by CYP450. However, the allele frequency needs to be explored, as that will give insight into its relevance in the population.

Besides assessing CYP450 enzyme expression and activity in general, it can also be measured in an enzyme-specific manner. This will result in more personalized medicines, when drugs are known to be metabolized by that specific enzyme.

2.1.1 CYP2C9

This enzyme metabolizes approximately 15-25% of prescribed drugs (40,41). The CYP2C9 has already 33 known polymorphism variants (*1B through *34) thus far (40). The polymorphisms CYP2C9*2 and *3 contribute to the largest inter-individual variability and are the most important (40,41). The review of Zhou et al. highlights the importance of the CYP2C9*2 and *3 polymorphisms on drug

clearance and therapeutic response, as both alleles have reduced enzymatic activity. For multiple drugs, CYP2C9 polymorphism gene-dose/effect relationships are observed (40). However, there might be differences in substrate specificity among the polymorphisms, which has to be investigated in more detail (40).

Besides genetic mutations in the enzyme itself, mutations in the CYP2C9 regulatory domains have potential as well. Wang et al. explored 87 human liver samples to study whether the presence of any regulatory CYP2C9 polymorphism would alter the CYP2C9 mRNA expression. They found a promoter Variable Number Tandem Repeat polymorphism (pVNTR), which was completely responsible for changes in CYP2C9 mRNA expression (41). pVNTR was available in three different forms: short (pVNTR-S), medium (pVNTR-M), and long (pVNTR-L) (41). pVNTR-S reduced CYP2C9 mRNA expression compared to reference variant pVNTR-M, but was found to be in linkage disequilibrium with CYP2C9*3 (41). As CYP2C9*3 reduces CYP2C9 activity and pVNTR-S reduces CYP2C9 mRNA expression, there may be synergistic effects when they coexist (41). In a multi regression model assessing all three variants, only CYP2C9*2 and *3 were significant predictors (41). The independent effects of pVNTR-S in vivo remain unclear and need further investigation.

Thus, CYP2C9 genotyping, especially variants *2 and *3, can be considered as potentially valuable for predicting response to drugs metabolized by CYP2C9.

2.1.2 CYP2D6

This is one of the most important CYP450 enzymes, because it is estimated to metabolize about 25% of currently prescribed drugs (14,42). CYP2D6 panels already exist to check for nine common alleles (*1, *2, *3, *4, *9, *10, *17, *29, and *41) and gene copy number variations (e.g., gene deletion (*5), gene duplication and multiplications) (42). Then, based on genotyping an activity score is assigned and CYP2D6 enzyme activity is predicted (42). Ray et al. explored in 122 human liver microsomes haplotype-phenotype relationships of rs5758550 and rs16947, including other common CYP2D6 alleles. Identified are the distal enhancer SNP rs5758550 that increases CYP2D6 expression, whereas rs16947 promotes an alternative unstable isoform and thus reduces full-length CYP2D6 expression (14,42). They concluded that adding both rs5758550 and rs16947 improved prediction of CYP2D6 activity, compared to the current CYP2D6 panel (14). These results were confirmed by Collins et al. in human liver samples and included even a third SNP rs1058164 G. This rs1058164 G was associated with an isoform of CYP2D6 with a deletion of exon 3, which results in lacking active site residues and decreased full-length CYP2D6 expression (42). With an expression score, the impact of adding the three SNPs to the current CYP2D6 panel was tested. A model that incorporates the current CYP2D6 panel accounted only for 36% of CYP2D6 variability, whereas this was 42% when including rs16947 and rs5758550 into the model and 59% when all three SNPs were included (42).

In short, these results suggest that including more specific SNPs into general genotype testing might predict CYP2D6 expression and activity more accurately. Genotyping CYP2D6 is of great relevance, as it is one of the most important CYP450 enzymes.

2.1.3 CYP3A

Genetic polymorphisms play an important role in predicting the most important CYP450 enzyme, CYP3A, activity as well. Wang et al. investigated in 76 human liver samples CYP3A4 heteronuclear RNA (hnRNA) as well as mRNA expression, searching for allelic expression imbalance (AEI) with at least one of eight CYP3A4 marker SNPs. AEI was found in 10 liver samples and was completely caused by an intron 6 SNP (rs35599367, C>T, CYP3A4*22), which decreased both CYP3A4 expression and activity (24). Additionally, patients carrying the T allele needed lower statin doses for therapeutic effect compared to individuals without the T allele, confirming that polymorphism CYP3A4*22 might predict CYP3A4 activity (24). Next, Wang et al. reviewed the genetic polymorphisms known to influence CYP3A4 activity. Few CYP3A4 regulatory polymorphisms are known, except for CYP3A4*22 and the promoter allele CYP3A4*1B (10). Some SNPs (*2, *3, *7, *9, *10, *15, *17, *19) have a minor allele frequency of >1%, but no change in activity or expression, whereas other SNPs (*4, *5, *6, *8, *11, *12, *13, *16, *20) alter activity or expression with a minor allele frequency of <1% (10). Therefore, they suggest that combining CYP3A4*22 with CYP3A5 polymorphisms *1, *3 and *7 have potential for predicting overall CYP3A activity (10).

In summary, not all known polymorphisms have equal relevance, due to varying prevalence and changes in expression/activity. Still, genotyping CYP3A has great potential, as 45-60% of all prescribed drugs are metabolized by this enzyme. The polymorphisms with greatest potential are CYP3A4*1B and *22, in combination with CYP3A5*1, *3 and *7 to predict CYP3A drug metabolism.

2.2 Other enzymes in drug metabolism

Some genetic polymorphisms have been shown to decrease UGT1A1 activity, which might alter drug metabolism (9). Her et al. described the common UGT1A1*28 polymorphism, which results in a decreased UGT1 enzyme activity (36).

Cytidine deaminase (CDA) is an enzyme responsible for metabolizing cytidine and 2’-deoxycytidine into uridine and 2’-deoxyuridine, which is an important step in metabolizing nucleoside analogs (cancer drugs) (43). CDA polymorphisms cause variabilities in metabolism, impacting pharmacokinetics and pharmacodynamics (43). Serdjebi et al. reviewed CDA status, of which up to 1000 CDA polymorphisms are already known, with the most important ones rs2072671 (CDA*2, 79A>C), rs60369023 (CDA*3, 208G>A), and rs1048977 (435C>T). Results for CDA*2 are still controversial, ranging from no effect on enzyme activity to decreased enzyme activity (43). CDA*3 is found to have a decreased enzyme activity, but is thought to have a clinical impact in Asian populations only (43). Other links between found polymorphisms and phenotype have not been found yet (43).

Genetic polymorphisms are responsible for the interindividual CES1 variability, thus Wang et al. investigated SNPs with a minor allele frequency of >0.5% in cell lines and 104 human liver tissues. Results showed that five SNPs (L40Ter (rs151291296), G142E (rs121912777), G147C (rs146456965), Y170D (rs148947808), and R171C (rs201065375) were a loss-of-function for CES1 (35). Additionally, other SNPs decreased CES1 enzyme activity with substrate specificity,

including A158V (rs202121317), R199H (rs2307243), E220G (rs200707504), and T290M (rs202001817) (35). CES1 pharmacogenetics was further reviewed by Her et al., and they described G143E as the only known CES1 loss-of-function. Furthermore, other SNPs discussed resulted in inconclusive results, including E220G (rs200707504), S75N (rs2307240), -816A>C (rs3785161), -75G>T (rs3815583), and 1168-33C>A (rs2244613) (36).

In short, genotyping phase II enzymes UGTs, SULTs and GSTs need to be considered as potential genetic markers for predicting drug metabolism. Inconclusive results (CDA and CES1) and low prevalence in ethnic groups (CDA) makes genotyping of these specific markers less valuable.

An overview of all discussed genetic markers assessing hepatic enzyme function can be found in Supplement 4, Table 2.

3. Biomarkers for early detection of drug-induced liver injury

DILI is one of the most important causes for drugs to be withdrawn from the market (44–46). Currently, DILI is diagnosed by ruling out other possibilities of liver injury, highlighting the need for specific DILI biomarkers (44–47). Multiple reviews were analyzed to discuss biomarkers detecting DILI.

First, in patients with DILI both miRNA-122 and miRNA-192 increase in a dose- and exposure-dependent manner, in correlation with serum ALT and AST levels and histopathology of liver degeneration (45,48). This suggests that both miRNAs correlate to DILI, with miRNA-122 levels to be more liver-specific (45,46,48). In addition to miRNA-122, glutamate dehydrogenase (GLDH, an enzyme present in the mitochondrial matrix of hepatocytes) as well as keratin-18 (K18, marker for necrosis and apoptosis) are more liver- and DILI-specific than ALT/AST (13,45–47). GLDH seems more valuable as biomarker for diagnosing DILI compared to miRNA-122, because miRNA-122 has substantial inter- and intra-subject serum variability whereas GLDH has stable serum levels and is unaffected by factors such as age and gender (13,44,46). High mobility group box 1 (HMGB1) is a nuclear protein that is released by most cell types during necrosis (45,46). HMGB1, together with miRNA-122 and K18, show elevated levels before ALT peaks, which is an advantage compared to ALT measurements (13,45,48). Additionally, HMGB1, GLDH, and K18 are markers that might give insight into the mechanism of liver impairment (13).

Other potential biomarkers for DILI include caspase-cleaved keratin18 (ccK18), osteopontin (OPN), and macrophage colony-stimulating factor receptor 1 (MCSFR1). First, K18 can be cleaved to its variant ccK18, which can be used as biomarker for apoptosis in liver injury (46). Second, high levels of OPN are found in patients with severe liver damage and DILI, suggesting its potential use for detecting DILI (45). Lastly, MCSFR1 (a cytokine-receptor) might be a potential biomarker for DILI, as MCSFR1 levels were higher in patients with DILI (45,46). However, MCSFR1 might be less valuable compared to ALT, as ALT levels were higher than MCSFR1 levels (45).

In summary, miRNA-122, GLDH, and K18 are potential biomarkers for early detection of DILI, with higher liver specificity compared to ALT and AST. Additionally HMGB1, miRNA-122, and K18 are biomarkers with an advantage to be detected earlier than ALT peaks.

An overview of all discussed laboratory markers for the early detection of DILI is presented in Supplement 5, Table 3.

Discussion and conclusion

This review provides an overview of currently known laboratory and genetic markers measuring liver function to predict drug metabolism and detect DILI, as the available tests lack this ability. At the moment, the ideal biomarker has not been found, but multiple potential biomarkers are addressed here.

The available general liver function tests measure liver impairment instead of predicting drug response. New potential biomarkers for assessing liver enzyme activity include miRNAs for CYP2B6 activity, 4βOHC:cholesterol ratio and bile acids for CYP3A activity. These biomarkers contain most of the characteristics required for a biomarker, including specific, stable and non-invasive. As these biomarkers are underrepresented in terms of number of studies and size/composition of test groups, more studies are needed for validation. Furthermore, additional studies are needed to explore more endogenous biomarkers reflecting different CYP450 enzyme activities, as they play an important role in drug metabolism and response. Eventually, a model exploring all biomarkers to assess which biomarkers are most accurate and the possibility of combining biomarkers is necessary.

The polymorphisms discussed in this review are mostly in CYP450 enzymes itself, and have great potential in predicting drug metabolism. However, the enzymes have regulatory mechanisms and cofactors required for their reactions (10). Therefore, polymorphisms in these molecules can also predict CYP450 expression and activity, and their feasibility need to be explored in future studies.

Evidence suggests that polymorphism variants have substrate specificity, as well as that phenotype prediction is multifactorial (27). Other factors influencing interindividual variability in drug and/or metabolite levels include patient-related factors such as age, sex, (co)morbidity, and drug-drug interactions (DDIs) (27). Therefore, focusing on single genes or polymorphisms may not be feasible as biomarkers and polygenic risk scores (PRS) have been proposed. Evidence of proof for these PRS is still lacking (27). The question remains to what extent genetic information alone can predict a patient's phenotype or whether other strategies are required (27). Both bioinformatic modeling and mechanistic PBPK modeling may provide tools for the future to incorporate multiple features, thereby simulating effects of gene mutations on the exposure of a drug (22,28). These models can be used both in the drug development process and prediction of drug response in vulnerable populations, such as patients with liver impairment (28). Also, models including genetic mutations and demographic characteristics to predict drug response might result in better prediction models. Eventually, known

biomarkers for predicting drug metabolism can be included as well, to predict drug response in a multivariate model.

Furthermore, polymorphisms have different occurrence among different ethnic groups. These different occurrences need to be explored in more detail, because this knowledge can be used in daily clinical practice for measuring specific polymorphisms in specific individuals. Sometimes this infrequent occurrence result in not including them in studies, or are often not detected in small studies (10,42). Therefore, their effects on phenotypes can only be assessed by in vitro studies (10). When these rare variants are studied in more detail in large cohorts, they can eventually be incorporated into predictive panels or predictive models (10).

Pharmacogenetic testing is already feasible in practice, and even results in reducing adverse drug reactions with 30% (27). This highlights the need for further investigations into pharmacogenetics and improve existing or fabricate new panels for genetic testing of liver enzymes, especially CYP450 enzymes.

In the future genetic testing might be conducted on an one-time basis early in life, to gain information about an individual's complete genetic profile and metabolizer status (40). This information can be contained in the medical record of that individual, stored for future use if and when needed (40). This genetic information can then be used in combination with laboratory markers measured in blood, for optimal prediction.

Regarding biomarkers for the early detection of DILI miRNA-122 and K18 have potential. Both are more liver-specific compared to ALT and AST, and can be detected before ALT peaks. However, these markers are not drug-specific and still need further validation.

More studies are needed that investigate vulnerable patients groups, including patients with liver dysfunction. Biomarkers that assess liver function and/or enzyme activity will categorize patients into enzyme phenotype, allowing for further and more detailed investigation of the required dose adjustments. At the moment, studies investigating dose adjustments in patients with liver impairments is very limited. PBPK models can be used for this purpose before investigating this invasively with patients (28).

The last 5 years, artificial intelligence (AI) created multiple models out of existing data in diagnostic, prognostic and predictive fields. Eventually, such AI models may be implemented into the clinic with broad applicability, such as prediction models for drug metabolism with biomarkers and demographic characteristics. Also, AI might be a tool that further can be used to screen proteins that may be of interest for assessing drug metabolism, thereby fueling developments in novel biomarkers. Currently, a few tools are already approved worldwide, but for hepatology more validation is still needed (49).

As long as no ideal and validated biomarkers are available to predict an individual's drug response, therapeutic drug monitoring (TDM) is important in the early phase of treatment. During TDM, drug concentrations will be measured in individual patients, aiming for an optimal drug dosage within therapeutic window (50).

Strengths and limitations

This review is one of the first exploring laboratory and genetic markers to predict drug metabolism and drug response. Furthermore, the articles used for this review were published not that long ago, most were published after 2015.

However, some limitations need to be addressed. Most studies used in this review had small sample sizes, thus the results of those studies should be approached with caution. Also, some markers were investigated in one study only, addressing the need for further investigation. Additionally, the laboratory and genetic markers were investigated separately, whereas the human body is a complex system. Furthermore, the method is based on a literature search from the Pubmed database only, a further opportunity is to explore other databases for other relevant research information.

Conclusion

In conclusion, the currently available liver functional tests detect liver injuries in general, lacking the ability to predict an individual's drug metabolism and response. Patients suffering from liver impairment require accurate pretreatment liver assessments to predict whether treatment or dose needs adjustment beforehand to optimize effectivity as well as safety. Biomarkers assessing liver injury in general include AST, ALT, bilirubin, HA, ADMA, RBP4, resistin, and XOR, but don't have potential to predict an individual's drug response. To take a step towards personalized medicines, specific markers need to be identified to assess specific enzyme expression and activity. Biomarkers with great potential are miRNAs for CYP2B6 activity, 4βOHC:cholesterol and bile acids for CYP3A activity. These biomarkers still need validation in future studies, and more endogenous biomarkers measuring CYP450 enzymes activities needs to be found. Genotyping also has great potential in finding polymorphisms in CYP450 enzymes itself as well as regulatory genes, to predict drug metabolism. As phenotype prediction with genes is multifactorial, generating multivariate models is an interesting subject for the future. Lastly, for early detection of DILI both miRNA-122 and K18 have potential, due to liver-specificity and earlier detection compared to ALT, but need further validation. The ideal biomarker still needs to be found, but potential biomarkers contributing to personalized medicines are addressed.

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Supplement

Supplement 1:

Complete syntax Pubmed search:

(liver*[tiab] OR hepati*[tiab]) AND (metaboli*[tiab] AND (drug*[tiab] OR medic*[tiab])) AND (biomarker*[tiab] AND predict*[tiab])

Supplement 2:

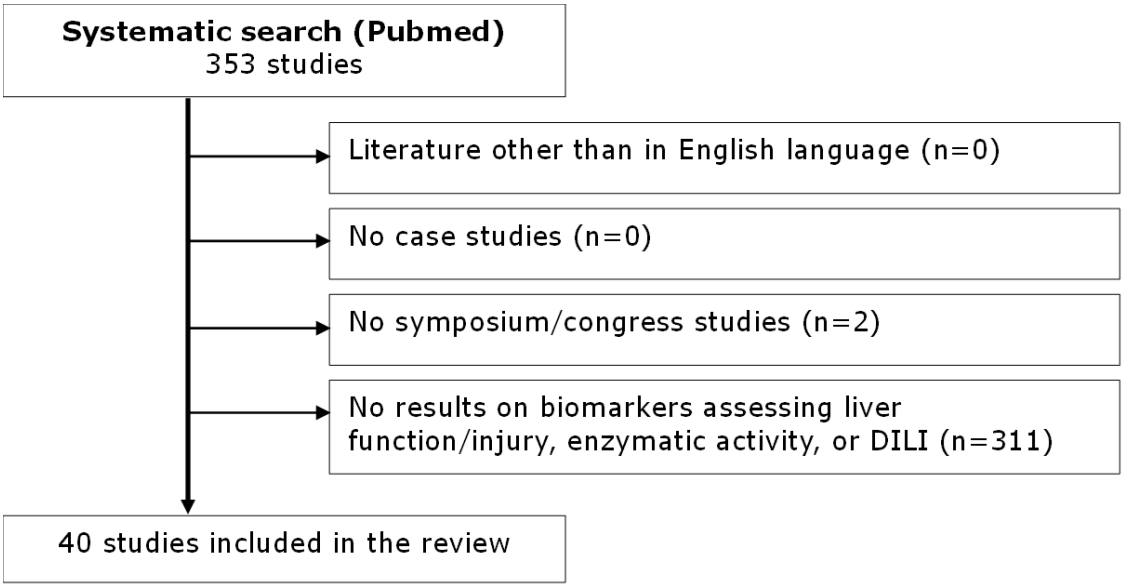

Figure 1: Flowchart with exclusion criteria for studies in this review.

Supplement 3:

Table 1: Overview laboratory makers assessing liver function.

Marker	**To assess**	**Note**
AST	Liver injury	Variable concentrations. Not liver-specific.
ALT	Liver injury	Variable concentrations. More specific than AST. Not liver-specific.
Bilirubin	Liver injury	Included in Child-Pugh score.
GGT	Hepatobiliary function	Unknown source.
HA	Liver injury	Correlates with Child-Pugh score.
ADMA	Liver injury Hepatobiliary function	Correlates with GGT.
RBP4	Hepatic biosynthesis Liver injury	Correlates with bilirubin.
Resistin	Liver injury	
XOR	Liver injury	Correlates with ALT and AST.
7 microRNAs (miRNA-204–5p, miRNA-212–3p, miRNA-3649, miRNA-3941, miRNA-4254, miRNA-4442 and miRNA-6867–5p)	CYP2B6 activity	Stable, specific, non-invasive.
4βOHC Ratio 4βOHC:total cholesterol	CYP3A4 activity	Ratio might be better for patients with variable cholesterol levels. Cost-effective, specific, ethical, stable, non-invasive.
Ratio DCA-1β-ol:DCA Ratio DCA-5β-ol:DCA	CYP3A activity	Tested in beagle dogs. Non-invasive, ethic, specific.
TG/AG	UGT2B17 activity	Altered hormone levels influences the reliability.
Ratios 2-PY and 4-PY to NMN	AOX activity	
Plasma CES1 levels	CES1 activity	Plasma concentration is not equal to enzyme activity. Lower compared to levels in liver.

Supplement 4:

Table 2: Overview genetic markers assessing liver function.

Gene/ polymorphism	Effect	Note
CAR and HNF4α	Regulate expression CYP450 enzymes.	No specific CYP enzyme.
POR	Regulates activity CYP450 enzymes.	No specific CYP enzyme.
CYP2C9*2 and *3	Decreased activity.	
pVNTR-S	Effects remain unclear.	Regulatory domain of CYP2C9.
rs5758550	Increased CYP2D6 expression.	
rs16947	Decreased full-length CYP2D6 expression.	Alternative unstable isoform.
rs1058164 G	Decreased full-length CYP2D6 expression.	Isoform with deletion exon 3.
CYP3A4*22	Decreased CYP3A4 expression & activity.	
UGT1A1*28	Decreased UGT1 activity.	
CDA*2	Controversial, no effect or decreased activity.	
CDA*3	Decreased activity.	Clinical impact in Asians only.
L40Ter, G142E, G147C, Y170D, R171C, G143E.	Loss-of-function variants for CES1.	
A158V, R199H, E220G, T290M	Decreased CES1 activity.	With substrate specificity.

Supplement 5:

Table 3: Overview biomarkers assessing early detection of DILI.

Marker	Note
miRNA-122	More liver- and DILI-specific than ALT and AST. High serum variability. Detected before ALT peaks.
miRNA-192	Correlates to ALT and AST.
GLDH	More liver- and DILI-specific than ALT and AST. Low serum variability. Give insight into DILI mechanism.
K18	More liver- and DILI-specific than ALT and AST. Detected before ALT peaks. Give insight into DILI mechanism.
HMGB1	Detected before ALT peaks. Give insight into DILI mechanism.
ccK18	Cleavage variant of K18.
OPN	
MCSFR1	MCSFR1 levels were lower compared to ALT.