



REVIEW ARTICLE

Open Access

Molecular Genetic Markers of Hepatocyte Destruction

Bon LI^{1*}, Maksimovich N Ye², Gubarevich I Ye¹, Malenovskaya M Y¹ and Kukolovich E V¹¹Grodno State Medical University Republic of Belarus, Grodno²Doctor of Medical Sciences, Professor, and researcher in Grodno, Belarus

ABSTRACT

The clinical standard for assessing liver injury is to measure serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), reflecting damage to hepatocytes leading to the release of these enzymes into the blood. Although these tests are widely used in clinical practice, they have important limitations. First, these enzymes are not completely specific to hepatocytes. AST is expressed in cardiac and skeletal muscle, kidneys, brain, pancreas, lungs, white blood cells, and red blood cells, whereas ALT is predominantly present in the liver and kidneys, with small amounts in the heart and skeletal muscle. Second, liver enzymes do not always reflect the full severity of the disease and have been shown to be deficient in various liver pathologies. A possible reason for this is that enzymes can be released from both dying and reversibly damaged cells, and therefore do not indicate the exact nature of liver damage, as well as the number of cells damaged. Third, ALT and AST have long half-lives (approximately 47 and 17 hours, respectively), which limits the ability to monitor rapid changes in liver damage [1-3].

Recent advances in molecular biology have identified a new generation of biomarkers that exploit the genetic and epigenetic signatures of hepatocytes themselves. These markers offer the prospect of tissue-specific, mechanistically informative, and analytically sensitive assessment of hepatocyte destruction. This review focuses on molecular genetic markers that are either released directly from dying hepatocytes or reflect hepatocyte-specific molecular alterations, with emphasis on their biological rationale, analytical characteristics, and clinical evidence. We examine circulating cell-free DNA (cfDNA) with hepatocyte-specific methylation patterns, cytokeratin-18 (K18) and its caspase-cleaved fragment (ckK18), microRNA-122 (miR-122), high-mobility group box 1 (HMGB1), and additional emerging markers, before considering integrated multi-marker approaches [4-6].

ARTICLE HISTORY

Received October 07, 2026

Accepted October 12, 2026

Published October 19, 2026

Keywords: Liver Injury, Biomarkers, ALT, AST

Circulating Cell-Free DNA with Hepatocyte-Specific Methylation

Biological Rationale

When hepatocytes undergo apoptosis or necrosis, their genomic DNA is fragmented and released into the bloodstream as cell-free DNA. However, cfDNA in circulation represents a mixture of DNA from multiple cell types—hematopoietic cells contribute the majority under baseline conditions—making total cfDNA quantification non-specific for liver injury. The solution to this specificity challenge lies in DNA methylation: hepatocytes exhibit distinctive methylation patterns at specific genomic loci that differ from all other cell types, enabling selective identification and quantification of hepatocyte-derived cfDNA fragments [7].

Clinical Evidence

The clinical validity of hepatocyte cfDNA measurement has been demonstrated across multiple contexts. In liver transplant

recipients, hepatocyte cfDNA was elevated within the first postoperative month, and sustained elevation correlated with allograft injury. Strikingly, in 5 of 6 patients with biopsy-proven acute rejection, hepatocyte cfDNA was elevated, and in 3 patients, this elevation preceded clinically detectable rejection—suggesting potential utility for earlier intervention.

In patients with sepsis, hepatocyte cfDNA levels were markedly elevated and correlated with ALT and AST, despite the presence of extensive cell death in multiple organ systems. This finding underscores the tissue specificity of the methylation-based approach even in complex clinical scenarios.

Perhaps the most compelling demonstration of specificity comes from Duchenne muscular dystrophy (DMD) patients, who frequently present with elevated ALT and AST of muscle origin rather than hepatic origin. In 10 DMD patients with elevated transaminases, hepatocyte cfDNA concentrations remained within the normal range, confirming that methylation-based detection correctly attributed the enzyme elevations to muscle rather than liver [8].

Contact Elizaveta I Bon, Associate Professor of the Department of Pathological Physiology named D.A. Maslakova Grodno State Medical University, Grodno, Republic of Belarus.

© 2026 The Authors. This is an open access article under the terms of the Creative Commons Attribution NonCommercial ShareAlike 4.0 (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

Cytokeratin-18 and its Caspase-Cleaved Fragment

Hepatocyte-Specific Expression

Cytokeratins 8 and 18 (k8/k18) are the major intermediate filament proteins of adult hepatocytes, where they serve essential cytoprotective functions against apoptosis and necrosis. Their abundance in hepatocytes and epithelial specificity make k18 an attractive biomarker target. During apoptosis, caspases cleave k18 at a conserved aspartate residue (asp396), generating a neoepitope recognized by the m30 antibody. In contrast, total k18 (detected by m65 antibody) is released during both apoptotic and necrotic cell death [9-11].

CK18 aggregates are the main components of Mallory bodies, a hallmark in the diagnosis of alcoholic liver disease, which made CK18 seem to be a promising biomarker. Indeed, serum levels of CK18 in heavy drinkers were higher than those of healthy controls, with serum CK18 levels also being higher in cases of alcoholic hepatitis when compared to cases of fatty liver. Interestingly, urinary levels of full-length CK18 are increased in alcoholics. A recent study by Lavallard and colleagues evaluated CK18 and CK18-fragments in serum samples of 143 heavy alcoholics. They confirmed the findings of previous studies by showing a strong correlation of CK18 and CK18 fragment with Mallory bodies, hepatocyte ballooning, and fibrosis. Elevated levels of serum hepatocyte death and apoptotic markers were independent risk factors in predicting severe fibrosis. The authors concluded that CK18 and CK18-fragment serum levels could be useful to rapidly evaluate liver injuries and the efficacy of therapies in alcoholic liver disease [12].

The mechanisms of acute liver failure (ALF) in humans are complex, etiology-dependent, and are thought to be influenced by the balance between various cell death modes, mainly necrosis and apoptosis, as well as by cellular regeneration. Therefore, measuring biomarkers that can help in the distinction of cell death modes are attractive tools that may help in the understanding of mechanistic pathways of ALF and serve as potential prognosis indicators. In an early study in a group of 68 patients with ALF, Bechmann et al. found a strong correlation of non-spontaneous remission and total CK18 levels as measured by M65 determination. They implemented a CK18 (M65)-based MELD (model of end-stage liver disease) score by replacing bilirubin with M65 in the MELD score calculation. They were able to demonstrate a high sensitivity and specificity in predicting survival of ALF patients. However, due to the low number of patients studied, and the lack of validation cohort, the accuracy of this new score for the different etiologies of ALF could not be assessed. The study however provided evidence supporting the concept that measuring different CK18 markers could help in the distinction between the different types of cell death involved in various forms of ALF. Subsequently, the largest study conducted to date by the Acute Liver Failure Study Group (ALFSG) included a total of 500 patients with ALF and demonstrated that M30 rather than M65 proved to be the most effective measurement to predict which patient would require liver transplantation or die. Based on these results the authors developed the ALFSG index which includes coma grade, INR, levels of bilirubin and phosphorus, and log10 M30 value, and subsequently validated these findings in a separate group of 250 patients with ALF [13,14].

M30 and m65 as Markers of Apoptosis and Necrosis

The m30 and m65 assays thus provide mechanistically distinct information: M30 specifically detects caspase-cleaved K18 and serves as an apoptosis marker, while M65 detects the total soluble K18 pool and reflects both apoptotic and necrotic death

. The M30:M65 ratio has been proposed as an index of the relative contribution of apoptosis versus necrosis to hepatocyte destruction.

In a comparative evaluation of DILI versus other forms of acute liver injury, ckK18 (M30) achieved an area under the receiver operating characteristic curve (AUC) of 0.79 for distinguishing DILI from non-DILI acute liver injury, the highest among the biomarkers tested. The discriminative performance improved substantially (AUC 0.98) when comparing DILI to autoimmune hepatitis, suggesting particular utility in this diagnostic dilemma [15,16].

In metabolic dysfunction-associated fatty liver disease (MAFLD), CK18 M65 showed superior performance in distinguishing healthy individuals from MAFLD patients compared to M30, consistent with the mixed apoptotic-necrotic cell death characteristic of steatohepatitis. Both markers correlated positively with ALT and AST.

Clinical Context

A limitation of K18-based markers is their release from other epithelial tissues, including cholangiocytes and gastrointestinal epithelium. However, the conserved caspase cleavage site and hepatocyte abundance render M30/M65 useful in clinical contexts where hepatic injury is suspected. Keratin variants that predispose carriers to liver disease have also been identified, adding a genetic dimension to keratin biology.

MicroRNA-122: A Liver-Enriched Molecular Marker

Biology and Release

MicroRNA-122 (miR-122) is the most abundant microRNA in the liver, accounting for approximately 70% of total hepatic miRNA content, and is highly enriched in hepatocytes relative to other tissues. Unlike enzymes, which are released passively upon membrane compromise, miR-122 is actively exported in extracellular vesicles and protein-bound complexes, conferring remarkable stability in circulation [17-19].

Diagnostic Performance

MiR-122 has consistently demonstrated utility as a sensitive marker of hepatocyte injury across diverse etiologies. In DILI, miR-122 elevation precedes or parallels ALT elevation, and several studies have reported that miR-122 may detect hepatocyte injury at earlier timepoints than conventional enzymes. The marker differentiates DILI and acute liver injury from healthy controls with AUC values exceeding 0.75 and correlates well with traditional transaminases.

The mechanistic information provided by miR-122 is complementary to that of K18: while K18 distinguishes apoptotic from necrotic death, miR-122 provides a quantitative measure of hepatocyte-specific miRNA release that may be more sensitive than enzyme leakage at low levels of injury.

The potential of various miRNAs as biomarkers of disease severity in various chronic liver conditions have been recently explored. Yamada and colleagues revealed that serum levels of miR-122, as well as miR-21, miR-34a, and miR-451 were increased in patients with NAFLD, when diagnosis was based on ultrasound determination of liver fat, and that miR-122 levels correlated with the severity of liver steatosis. Furthermore, miR-103/107 levels were increased in liver biopsies from patients with ALD, NAFLD, and NASH, and those levels were closely associated with insulin resistance. In addition, Bala and colleagues found that inflammation related miRNAs (miR-155, miR-125b, and miR-146a) were increased in the plasma of patients with CHC. Another study

by the same group using various experimental mouse models, demonstrated that circulating levels of these inflammation related miRNAs are different in response to the type of liver injury, such as APAP, ALD, and TLR9 and TLR4 ligand-induced liver damage. The authors also explored mechanism of miRNA release and found that in ALD and in inflammatory liver injury, serum/plasma miR-122 and miR-155 were predominantly associated with the vesicles, whereas in DILI/APAP injury these miRNAs were present mainly in the protein-rich fraction. Consistent with these results we recently found that in murine models of NASH, microvesicles containing miR-122 are released by hepatocytes and can be measured in circulation.

Several studies showed that liver fibrosis caused by hepatic stellate cell (HSC) activation could be monitored by miRNAs. Lakner and coworkers recently showed that miR-19b is reduced in rodent and human liver fibrosis. A similar study found reduced miR-29 family members in a carbon tetrachloride-induced mouse model of hepatic fibrogenesis (CCl₄), as well as in patients with advanced liver cirrhosis. Using a bile duct-ligation fibrosis model in rats, Venugopal et al. described reduced levels of miR-150 and miR-194. Similarly, serum levels of miR-122 inversely correlated with severity of fibrosis in patients with CHC. The levels were decreased in patients with severe fibrosis, while patients with early stages of fibrosis and high inflammatory activity showed increased levels of miR-122. On the other hand, increased levels of miR-214-5p and miR-221/222 have been observed in rodent and human liver fibrosis. Several key areas for future investigation include assessing the mechanisms and kinetics of liver microRNA release into blood and other potential biofluids reservoirs, as well as establishing the different compartments, such as extracellular vesicles, that comprise the predominant source of circulating miRNAs in various liver disease processes. Future prospective and longitudinal biomarker studies in APAP and non-APAP acute liver injury, as well as in various chronic liver diseases, will be required to determine whether miR-122 works in isolation, or in combination with other miRNAs, in order to create miRNA profiles that can provide added clinical utility and prognostic value to currently available tools [20].

Microparticles

Microparticles (MPs) are small membrane vesicles released in a highly regulated manner from dying or activated cells. MPs range between 100 and 1000nm in size and are generated through cell membrane shedding. This process involves a sorting of membrane proteins into the shed MP and the inversion of phosphatidylserine from the inner to the outer membrane during cellular activation, or early apoptosis. MPs can be classified as cell-to-cell communicators. They carry specific signatures such as surface receptors, integral membrane, cytosolic and nuclear proteins, and RNAs including miRNAs from parental cells and deliver these signatures to other cells. Notably, MPs not only stay in the original tissue, but may also circulate in the bloodstream. The first report of MP release into circulation during liver injury came from the AM Diehl group where they demonstrated an increase in blood and bile MPs using a bile duct ligation (BDL) rat model. The Diehl group went on to show that these MPs contain biologically active Hedgehog (Hh) ligands and provided evidence suggesting that activation of Hh signals on hepatic sinusoid endothelial cells may be an important mechanism by which MPs contribute to tissue remodeling during chronic cholestatic liver injury. More recently, our group identified circulating MPs released in mice with diet-induced NASH as potential and novel biomarkers for the diagnosis of NASH. We found that hepatocytes exposed to excess amounts of saturated fatty acids released membrane-bound microparticles that induced angiogenesis when

administered to mice. Microparticles from the blood of mice with diet-induced steatohepatitis originated from the liver and triggered migration and tubular structure formation when applied to an endothelial cell line. The angiogenic effects of microparticles generated by hepatocytes exposed to saturated fatty acids, or of those from mice with diet-induced steatohepatitis, involved the uptake of the microparticles by endothelial cells, a process that required Vanin-1, an enzyme located on the surface of the microparticles. Thus, the pathological angiogenesis that can occur in steatohepatitis could be reduced by preventing endothelial cells from internalizing Vanin-1–positive microparticles from hepatocytes. The relevance of MP determination in human disease has been studied in recent pilot studies. Rautou et al. reported elevated circulating levels of leuko-endothelial-derived CD31(+)/41(–) MPs, pan-leukocyte-derived CD11a (+) MPs, lymphocyte-derived CD4(+) MPs, and erythrocyte-derived CD235a (+) MPs in patients with liver cirrhosis. Two recent studies by Kornek and Schuppan showed that MPs positive for various inflammatory cell markers such as CD4(+) or CD8(+) T cells, CD14(+) monocytes, and iNK cells can be detected in the plasma of patients with various liver conditions. Notably, circulating MPs positive for monocyte-marker CD14, or iNK cell marker, were higher in a small group of patients with NASH when compared to those with hepatic steatosis, while patients with chronic hepatitis C infection had increased levels of MPs positive for CD4 and CD8 T cell markers. Thus, extracellular vesicles, in particular MPs, are evolving as attractive biomarkers to diagnose and monitor liver injury in various acute and chronic liver conditions. MPs not only carry a specific signature of the cell of origin, but may also be important in the pathogenesis and progression of liver damage.

Molecular Markers for Hepatocellular Diseases

Malignant Hepatocellular Tumors

Hepatocellular carcinoma (HCC)

More than 90% of HCCs are correlated to a known etiology, and hepatocarcinogenic mechanisms can be classified as etiologically specific and nonspecific mechanisms. Specific mechanisms include hepatitis B via viral integration, with the constant cis- and trans-activation of oncogenic factors, hepatitis C via the oncogenic effects of the core antigen and NS5A protein, and aflatoxin via direct genotoxic effects, leading to TP53 codon 249 mutations. Nonspecific mechanisms accumulate the abnormalities imposed by chronic liver diseases. HCC usually develops from chronic liver disease to a dysplastic nodule, prior to progression into HCC. The molecular markers for high-grade dysplasia include telomere shortening, telomerase reverse transcriptase (TERT) activation, and cell-cycle checkpoint inactivation. Early HCC accumulates mutations in CTNNB1, which encodes β -catenin, and progressed HCC further presents with TP53 mutations, DNA amplification, alterations in methylations, and other genetic abnormalities. Multiple immunohistochemical markers are used to assist in the HCC diagnosis: polyclonal CEA, CD10, HepPar, arginase-1, and albumin in situ hybridization (ISH) are used as hepatocellular markers, while glypican-3, glutamine synthetase (GS), HSP70, CD34, alpha-fetoprotein (AFP) and clusterin are used to identify hepatocellular malignancy. HSP70, glypican-3 and GS have been recommended in international guidelines. Molecular testing is used for DNAJB1-PRKACA translocation, in order to diagnose fibrolamellar variant HCCs.

Serologically, due to the low sensitivity (20%) in early HCC and fluctuating levels in cirrhosis, AFP was removed from the present screening assessment guidelines of the Canadian Association for the Study of the Liver (CASL), and the European Association for the Study of the Liver (EASL). However, AFP is still presently

used with other serological biomarkers, such as Lens culinaris-agglutinin-reactive fraction of AFP, and protein-induced by vitamin K absence or antagonist-II (PIVKA-II), for high-risk populations. Furthermore, studies have determined the des-gamma-carboxy prothrombin in patients with negative AFP. The results revealed that AFP was positive in 67% of HCCs, while AFP was negative in 66% of small HCCs and 20% of all HCCs. However, none of the serologic markers were accepted by clinical practice guidelines for HCC screening due to cost-effectiveness, challenges in availability, and study result variations.

Numerous markers are under investigation. Autophagy-related genes and their regulatory proteins are associated with HCC, including Beclin-1, ATG5 and ATG7, and these control a large number of molecular pathways in HCC oncogenesis, such as phosphatidylinositol-4,5-bisphosphate 3-kinase PI3K/AKT/mTOR, ERK/mitogen-activated protein kinase (MAPK), and apoptosis p53 pathways. For example, the ATG-4B mRNA expression controlled by autophagy-related genes may contribute to HCC development via the noncoding of miRNA-661, and this has been proven to be clinically useful, with 100% sensitivity, in a clinical validation, especially in early-stage HCC. Furthermore, HBV-related HCC is associated with mutated TP53, which involves the genetic integration with host genomes. HCV-related HCC overexpress the Kinesin family member 20A, Cyclin B1, Hyaluronan-mediated motility receptor, and other genes. In addition, these markers are linked to lower survival in patients with HCV-associated HCC. A study on IL-28 genetic polymorphisms revealed the association of the T allele with higher risk of HCC development. Another study revealed that two genotypes of certain single nucleotide polymorphisms (SNPs) of IL-28 were associated with lower risk of HCC development. Thus, the role of IL28 in diagnosing and prognosticating HCC appears unclear, if not contradictory.

Molecular factors are also used for the prognosis. Cytokeratin 19 (CK19) positivity is associated with increased recurrence rates, nodal metastasis, and more resistance to trans-arterial chemoembolization and percutaneous radiofrequency ablation. The expression of miR-1180-3p increases in HCC, and is linked to tumor proliferation and poor survival. A study conducted on KEGG pathways revealed that this epigenetic marker is associated with the regulation of the MAPK pathway, cell proliferation, apoptosis, and cell differentiation [1,4].

Immune checkpoint proteins drive signaling pathways that suppress T-cell function, including PD-1, PD-L1 and CTLA-4. Nivolumab was the first US Food and Drug Administration (FDA)-approved anti-PD-1 antibody for treating HCC. In addition, in 2020, the FDA granted the accelerated approval to nivolumab, in combination with ipilimumab, which targets CTLA-4 for the treatment of patients with HCC, who were previously treated with sorafenib. Furthermore, a study has recommended the anti-PD-1 antibody agent for PD-L1 positive HCC patients. Tumor mutation burden and microsatellite instability (MSI)/mismatch repair (MMR) are used to guide the immunotherapy for several cancers. These may play an important role in HCC immunotherapy in the future.

Hepatoblastoma (HB)

Approximately 80% of HBs exhibit genetic alternations in the Wnt/ β -Catenin signaling pathway. These alterations include the deletion of CTNNB1 exon 3, AXIN genes, and the APC gene. Overexpressed targets for Wnt signaling were also observed, such as cyclin D1, survivin and MYC. In addition, MYC further activates the Wnt signaling as a positive feedback mechanism. The genomic profiling of HB can be classified into two subtypes, based on genetic instability (gains of chromosomes 8q and 2p):

the overexpression of hepatic progenitor cell markers (AFP, CK19 and EpCAM), and the upregulation of MYC. Tumors with genetic instability are more aggressive, with a higher grade, and are more likely to metastasize. Histopathologically, HBs can be classified as epithelial or mixed epithelial, and mesenchymal. Epithelial HB may consist of fetal, embryonal, small cell undifferentiated, cholangioblastic and macrotrabecular components. β -catenin and glutamine GS are expressed in mesenchymal and fetal components. Furthermore, AFP highlights less-differentiated epithelial components, and HepPar1 can be observed in more differentiated epithelial components. Moreover, glypican-3 is expressed in epithelial fetal and embryonal components. In addition, CK7 and CK19 are positive in cholangioblastic components. SMARCB1 (INI1) highlights all HB components, except for small cell undifferentiated components.

Benign Hepatocellular Tumors

Focal Nodular Hyperplasia (FNH)

The pathogenesis of FNH has not been fully explored. The presence of large vessels and vascular anomalies suggest the etiology of focally elevated blood flow. Studies have revealed the altered expression of angiopoietin genes, ANGPT1 and ANGPT2, with an elevated ANGPT1:ANGPT2 ratio in FNH. The activation of the β -catenin pathway would result in a “map-like” GS expression, without mutations in CTNNB1 or AXIN1. The immunohistochemistry for FNH revealed that LFABP retained its normal expression, β -catenin was negative for nuclear expression, and serum amyloid A and C-reactive protein (CRP) were usually negative. Furthermore, patchy serum amyloid A or peri-septal CRP staining may be observed in some FNH cases.

Hepatocellular Adenoma (HCA)

HCAs are clonal benign neoplasms of four common subtypes: hepatocyte-nuclear-factor-1 α mutated (H-HCA), β -catenin-mutated type with the upregulation of GS (b-HCA), inflammatory type (IHCA) with the serum-amyloid-A overexpression, and unclassified type. H-HCA demonstrates biallelic HNF1A and CYP1B1 inactivation mutations. Liver fatty-acid binding protein is the characteristic for this group. IHCA activates IL-6/JAK/STAT due to mutations of the IL6ST gene, which codes gp130, FRK, STAT3, GNAS and/or JAK1. C-reactive protein/serum amyloid A is usually diffuse positive, with a well-defined demarcation. The b-HCA- and β -catenin-activated IHCA (b-IHCA, having features of both IHCA and b-HCA) presents with CTNNB1-activated genomic abnormalities, leading to β -catenin pathway activation. Immunohistochemical marker GS is a good surrogate for genetic abnormality. GS diffuse homogeneous overexpression indicates the exon 3 mutation, GS heterogeneous staining with a starry-sky pattern indicates the exon 3 S45 mutation, and a GS faint expression indicates the exon 7/8 mutation. The exon 3 mutation is usually associated with high risk of HCC. [5]

The term, “borderline lesion” or “atypical hepatocellular neoplasm,” has been used for b-HCA with cytologic atypia, but this remains insufficient for the diagnosis of HCC. This type has a high likelihood of HCC development. The TERT promoter mutation, as a typical genetic change in HCC, is usually identified in b-HCA/b-IHCA, with malignant transformations. Since surgical resection is recommended for b-HCA/b-IHCA and borderline lesion, it is crucial for β -catenin activation to be detected for CTNNB1 mutations. Molecular testing for CTNNB1 genomic abnormalities, TERT promoter mutations, and chromosomal gains (1, 7 and 8) may be warranted when GS immunostaining is equivocal [1].

In addition to the common HCA subtypes, sonic hedgehog HCA (shHCA) has been reported to present with somatic deletions

of INHBE, leading to the fusion of INHBE and GLI1, and this special group may be identified by PTGDS immunostaining. Argininosuccinate synthase 1 (ASS1) overexpression has been reported in another subtype of HCA (ASS1-positive HCA), and both subtypes are associated with high risk of hemorrhage.

Non-neoplastic hepatocellular diseases

Autoimmune Hepatitis (AIH)

Autoimmune Hepatitis (AIH) is an inflammatory liver disease in patients of all ages, and has female dominance. The key diagnostic criterion for all AIH scoring systems is the detection of autoantibodies. AIH type 1 can affect both adults and children, with characteristic positive anti-nuclear and/or anti-smooth muscle antibodies. On the other hand, AIH type 2 mostly affects children with characteristic positive anti-liver-kidney microsomal-1 and/or anti-liver cytosol-1 antibody. The autoantigens for type 2 AIH include cytochrome P4502D6 (CYP2D6) and formiminotransferase cyclodeaminase (FTCD), while those for type 1 AIH remain unclear. The genomic predisposition has been studied in AIH. Type 1 AIH presents with MHC class II HLA DRB1*03, which can be observed in all age groups, and DRB1*04, which is a late onset disease. Type 2 AIH presents with changes in DRB1*07 and DRB1*03. It has been reported that the serologic parameters of lymphocyte-to-platelet ratio (LPR) and immunoglobulin-to-platelet ratio (IGPR) are independently linked to the liver fibrosis stage in AIH patients without prior treatment.

Metabolic-Associated Fatty Liver Disease (MAFLD)

MAFLD, which was previously termed as, non-alcoholic fatty liver disease, is defined by the presence of >5% steatosis and metabolic risk factors, especially type-2 diabetes, obesity and metabolic syndrome, with the exclusion of excessive alcohol use. The reasons for the interindividual variability may be attributable to the different genetic backgrounds, epigenetic modifications and epitranscriptomics, and these are the recently described biological determinants. Genetic variants involved in liver lipid-metabolism are the major genetic risk factors for MAFLD, which include PNPLA3, TM6SF2, GCKR, MBOAT7, and HSD17B13. Furthermore, epitranscriptomics is an emerging field, which helps understand how chemical RNAs and their modifications control RNA structures and functions, without changing the sequences. A large number (>100) of chemical RNA modifications have been described. Among these, N6-methyladenosine (m6A) plays an important role in glucose and lipid homeostasis, and is involved in the progression of MAFLD. In light of the gut-liver crosstalk, gut-specific PPAR α may be applied as a novel target and predictive biomarker of NAFLD treatment.

Conclusion

Molecular genetic markers of hepatocyte destruction represent a significant advance over conventional serum enzymes. Hepatocyte-specific cfDNA methylation offers unprecedented tissue specificity, with demonstrated utility in transplant monitoring, sepsis, and discrimination of hepatic from muscle injury. Cytokeratin-18 and its caspase-cleaved fragment provide mechanistic insight into cell death modality, with M30/M65 ratio informing the apoptosis-necrosis balance. MicroRNA-122 extends sensitivity for hepatocyte injury, while HMGB1 and mtDNA integrate hepatocyte destruction with innate immune activation. The future of hepatocyte destruction assessment lies not in replacing ALT and AST but in complementing them with molecular markers that answer questions the conventional enzymes cannot: Which cells are dying? By what mechanism? With what immunological consequences? Integrated biomarker panels incorporating these molecular genetic markers are positioned to transform clinical

hepatology, enabling earlier diagnosis, more precise prognosis, and mechanistically informed therapeutic decisions.

References

- [1] Liang Y, Guo GL, Zhang L. Current and Emerging Molecular Markers of Liver Diseases: A Pathogenic Perspective. *Gene Expr* 2022; 21: 9-19.
- [2] Thakur S, Kumar V, Das R, Sharma V, Mehta DK. Biomarkers of Hepatic Toxicity: An Overview. *Curr Ther Res Clin Exp* 2024; 100: 100737.
- [3] Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. *J Hepatol* 2014; 60: 1063-1074.
- [4] Antropova EA, Khlebodarova TM, Demenkov PS, Venzel AS, Ivanisenko NV, et al. Computer analysis of regulation of hepatocarcinoma marker genes hypermethylated by HCV proteins. *Vavilovskii Zhurnal Genet Selektzii* 2022; 26: 733-742.
- [5] Cai J, Han T, Nie C, Jia X, Liu Y, et al. Biomarkers of oxidation stress, inflammation, necrosis and apoptosis are associated with hepatitis B-related acute-on-chronic liver failure. *Clin Res Hepatol Gastroenterol* 2016; 40: 41-50.
- [6] Fu S, Wu D, Jiang W, Li J, Long J, et al. Molecular Biomarkers in Drug-Induced Liver Injury: Challenges and Future Perspectives. *Front Pharmacol* 2020; 10: 1667.
- [7] Church RJ, Kullak-Ublick GA, Aubrecht J, Bonkovsky HL, Chalasani N, et al. Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: An international collaborative effort. *Hepatology* 2019; 69: 760-773.
- [8] Thulin P, Nordahl G, Gry M, Yimer G, Aklillu E, et al. Keratin-18 and microRNA-122 complement alanine aminotransferase as novel safety biomarkers for drug-induced liver injury in two human cohorts. *Liver Int* 2014; 34: 455-465.
- [9] Mills PR, Spooner RJ, Russell RI, Boyle P, MacSween RN. Serum glutamate dehydrogenase as a marker of hepatocyte necrosis in alcoholic liver disease. *Br Med J (Clin Res Ed)* 1981; 283: 754-755.
- [10] O'Brien PJ, Slaughter MR, Polley SR, Kramer K. Advantages of glutamate dehydrogenase as a blood biomarker of acute hepatic injury in rats. *Lab Anim* 2002; 36: 313-321.
- [11] van Golen RF, Reiniers MJ, Heger M, Alles LK, van Rooyen DM et al. The damage-associated molecular pattern HMGB1 is released early after clinical hepatic ischemia/reperfusion. *J Hepatol* 2019; 70: 1142-1151.
- [12] Xi D, Duan G, Liu B, You Y, Zhuang Y, et al. Recent advances of small-molecule fluorescent probes for sensing and imaging biomarkers of experimental intrinsic drug-induced liver injury. *Bioorg Chem* 2025; 164: 107515.
- [13] Howell LS, Ireland L, Park BK, Goldring CE. MiR-122 and other microRNAs as potential circulating biomarkers of drug-induced liver injury. *Expert Rev Mol Diagn* 2018; 18: 47-54.
- [14] Jia K, Zhang Y, Li X. Acteoside ameliorates hepatic ischemia-reperfusion injury via reversing the senescent fate of liver sinusoidal endothelial cells. *Phytomedicine* 2023; 112: 154708.
- [15] Antoine DJ, Jenkins RE, Dear JW, Williams DP, McGill MR, et al. Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity. *J Hepatol* 2012; 56:

1070-1079.

- [16] Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci USA* 2009; 106) :4402-4407.
- [17] Antoine DJ, Dear JW, Lewis PS, Platt V, Coyle J, et al. Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. *Hepatology* 2013; 58: 777-787.
- [18] McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, et al. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *J Clin Invest* 2012; 122: 1574-1583.
- [19] Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DG, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. *Hepatology* 2011; 54: 1767-1776.
- [20] Lehmann-Werman R, Magenheimer J, Moss J, Neiman D, Abraham O, et al. Monitoring liver damage using hepatocyte-specific methylation markers in cell-free circulating DNA. *JCI Insight* 2018; 3: e120687.