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Hepatocellular carcinoma risk stratification by genetic profiling in patients with cirrhosis

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Abstract

Prediction of future hepatocellular carcinoma (HCC) risk in the sizable chronic liver disease population is an urgent unmet need to enable regular HCC screening for early detection. Germline DNA polymorphisms likely represent etiology-specific host factors that determine HCC susceptibility, including single nucleotide polymorphisms in *EGF*, *IFNL3*, *MICA*, and *TLL1* in hepatitis C with or without active viral infection, and *PNPLA3*, *TM6SF2*, and *MBOAT7* in metabolic liver diseases. Transcriptome-based prognostic liver signature in diseased liver tissue has been associated with long-term HCC risk in viral and metabolic etiologies. Transcriptomic signatures of hepatic injury and specific cell type such as aggregated lymphocytes also predict HCC development. Circulating factors such as proteins and their chemical modification, nucleotides, and metabolites may serve for less-invasive assessment of short- or long-term HCC risk. These biomarkers will enable individual HCC risk-based personalized clinical management for cost-effective early HCC detection and improvement of patient survival.

Keywords

Risk prediction; cirrhosis; hepatocellular carcinoma; precision medicine

Chronic fibrotic liver disease is caused mainly by chronic infection of hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol abuse, and non-alcoholic fatty liver disease (NAFLD).¹ Presence of cirrhosis or advanced liver fibrosis is a distinct predisposing factor for liver cancer, predominantly hepatocellular carcinoma (HCC), the fastest increasing cancer type and mortality in the 1950–1959 birth cohorts, Hispanic men, and black women in the U.S.^{2–5} The substantially high risk of HCC in cirrhosis patients justifies regular screening by abdominal ultrasound with or without α -fetoprotein (AFP) for early tumor detection and application of curative treatment.⁶ Cohort studies and mathematical modeling

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Conflict of interest statement

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analyses have indicated that the HCC screening cost-effectively improves patient survival when more than 34% of cirrhosis patients are regularly screened.^{7,8} However, in a real-world clinical setting, less than 20% of HCC patients are diagnosed through the regular screening due to various patient- and provider-related factors in addition to the vast population with cirrhosis, which accounts for 2% of global mortality.^{1,9,10} Clinical intervention such as mail outreach can improve HCC screening application rate, although the improvement was only up to 50%.^{11,12} Thus, it is likely unrealistic to expect applying the recommended regular screening to the entire target patient population with the currently available medical resources.

Practice guidelines recommend biannual HCC screening uniformly applied to all patients with cirrhosis.⁶ However, it is noteworthy that individual HCC risk hugely varies across the patients. HCC incidence varies across the etiologies and clinical conditions such as liver disease severity, and even within the same clinical entity, individual HCC risk is heterogeneous across the patients for unknown reasons.^{1,10} Universally modifying screening protocol (e.g., shorter screening interval all cirrhotics) did not improve HCC detection and clinical outcome, underscoring the limitation of the current “one-size-fits-all” approach that assumes homogeneous HCC risk across the patients sharing the same clinical condition and under/over-estimates individual HCC risk.^{13,14}

Clinical and translational studies have reported HCC risk-predictive biomarkers and scores that identify cirrhosis patients at higher risk of developing HCC compared to the others.¹ Such individual HCC risk estimation may enable prioritization of a subset of patients for the regular screening and mitigate the burden on the already overwhelmed HCC screening resources. Indeed, a recent simulation-based study showed that risk-based personalized HCC screening is cost-effective even with the current low application rate by allocating the screening to those who most need it.¹⁵ Some of them have been internally or externally validated, and therefore may have potential to be translated into clinical practice. In addition to refining HCC screening, molecular HCC risk prediction may also inform management of patients at risk of HCC recurrence after treatment for first HCC tumor and guide chemopreventive intervention with specific agents. In this review, we overview several types of molecular biomarkers that may have clinical utility in HCC risk prediction and discuss their potential and limitations.

Clinical applications of molecular HCC risk stratification

Numerous molecular correlates of HCC risk have been reported in literature, and some of them have been evaluated at various levels of validation studies (Table 1). However, none of them has been translated into clinical practice to date. To be adopted in daily care of the patients, clinically meaningful utility must be demonstrated under specific clinical scenarios. Figure 1 summarizes potential application of molecular HCC risk stratification and subsequent clinical actions over the course of HCC and liver disease progression. It is worth to note that specific clinical context will determine available types of biospecimens and molecular information that can be measured using the specimens. For example, liver tissue, the major source of biomarker exploration, is available at the time of surgical treatment for HCC tumors, whereas it is less accessible before HCC development given the diminishing

utilization of liver biopsy. Body fluid-based specimens such as plasma, serum, and urine, as well as germline DNA that can be easily obtained from buccal mucosa will allow more flexible application of biomarker-based risk stratification. In addition, other sources of biospecimens such as feces and ascites may provide information to estimate HCC risk. However, several limitations have been noted for circulating biomarkers as summarized below, and paired tissue specimen will be needed to establish validity of such biomarker candidates. One potential strategy may be to obtain tissue-based reliable information at limited critical time point(s) and follow up with regular monitoring by less-invasive sampling of circulating materials. It is expected that biomolecules derived from diseased liver (measured in either tissue or circulation) reflect the molecular status of tissue microenvironment, supporting initiation and promotion of neoplastic cellular transformation. In addition, biomolecules derived from pre/neoplastic cells undergoing the process of transformation and/or expansion may also have clinical utility to predict short-term HCC risk by detecting sub-clinical neoplasia before its development into clinically detectable tumor nodule.

Germline genetic variants in viral hepatitis-related HCC

Germline genomic DNA variants, especially single nucleotide polymorphism (SNP), may represent host genetic factors that increase susceptibility to develop HCC. Single gene-based or biological hypothesis-based studies as well as unbiased survey by genome-wide association study (GWAS) have identified numerous SNPs and other polymorphisms as correlates with elevated HCC risk.^{1,16} Magnitude of their association with HCC incidence is generally modest (odds ratios [ORs] of 1.5 to 2.0 in independent validation), suggesting that their molecular functional relevance could be affected by various confounding factors. Nevertheless, robust genotyping of SNP is relatively easy even in clinical setting. Some of them have been externally validated in multiple studies and patient populations as potential candidate HCC risk biomarkers as summarized in this section (Table 1).

The *EGF*61AG variant was reported as an HCC risk allele in Eastern and Western patients with advanced liver fibrosis mostly affected with viral hepatitis (pooled OR=1.38).^{17–19} In the setting of liver transplantation, the risk allele in donors, but not in recipients, was associated with accelerated fibrosis progression in the graft livers, suggesting its functional role in liver disease progression.²⁰ *EGF* over-expression in hepatic stellate cells and macrophages was implicated in hepatocarcinogenesis in experimental studies.^{21,22}

A SNP in *IFNL3* (formally *IL28B*) gene was first discovered as a predictor of spontaneous HCV clearance and response to interferon-based antiviral therapies.^{23–26} and more recently as associated with risk of HCC development in patients with HCV genotype 1 infection who were treated with interferon-based regimen and recurrence after curative treatment of HCC (adjusted hazard ratios [HRs]=1.59–4.50).^{27–29} Variants in *IFNL3* likely mediate *IFNL3*-*IFNL4* haplotype-dependent hepatic inflammation and fibrosis.³⁰

A GWAS of Japanese HCV-infected individuals identified an HCC susceptibility-associated SNP in *MICA* gene (adjusted OR=1.36), encoding a major histocompatibility complex-related protein that activates anti-tumor immunity through natural killer cells and CD8⁺ T

cells.³¹ The SNP as well as soluble form of the protein detected in serum are associated with risk of HCV-related HCC in Japanese and Chinese patients even after viral cure, whereas opposite association was observed in Swiss patients, suggesting racial/ethnic difference in its involvement in hepatocarcinogenesis.^{32–34} Another GWAS of Japanese hepatitis C patients found an HCC risk-associated SNP in *DEPDC5* gene (adjusted OR=1.96).³⁵ Although its external validation is still lacking, the variant was also associated with fibrosis progression in European patients.³⁶

New direct-acting antivirals (DAAs) for HCV achieve a high rate of sustained virologic response (SVR) and reduce HCC incidence at comparable level with the older-generation interferon-based regimens, although the risk persists even longer than a decade.^{37,38} A retrospective GWAS identified a SNP in intron of *TLL1* gene (rs17047200) as post-SVR HCC risk locus in Japanese patients treated with interferon-based therapies (adjusted HR=1.78).³⁹ Independent validation of the HCC risk association is still lacking, but the variant was associated with presence of advanced fibrosis (F3–4) in NASH patients together with a SNP in *PNPLA3* gene (adjusted OR=2.63).⁴⁰

Chronic HBV infection has been the dominant HCC etiology in Southeast Asia and sub-Saharan Africa, although the incidence is declining.⁴¹ Universal HBV vaccination and antiviral therapies are effective as a primary and secondary HCC prevention, respectively.¹ However, the risk of HCC is not completely eliminated. A GWAS in Chinese patients showed that an intronic SNP in *KIF1B* on chromosome 1p36.22 (rs17401966) as a protective allele for HBV-related HCC, and this finding was confirmed in 5 independent Chinese cohorts (pooled OR=0.61).⁴² However, external follow-up studies from other Asian regions, including Japan, Korea, other region of China, and Thailand, could not validate the association, which may be due to population diversity.^{43,44} A more recent larger GWAS from China analyzed training cohort of 2,514 chronic HBV carriers and 6 independent validation cohorts, and identified SNPs in *STAT4* (rs7574865, adjusted OR=1.21) and *HLA-DQB1/HLA-DBA2* (rs9275319, adjusted OR=1.49).⁴⁵ A genomic DNA copy number variation (CNV)-based GWAS of Chinese HBV carriers found duplication at chromosome 15q13.3 conferring risk of HBV-related HCC with a large OR of 12.02, although its prevalence is low (2.3% in HCC cases and 0.2% in controls).⁴⁶ The CNV was positively correlated with hepatic expression of *SNORA18L5* that suppresses p53-dependent cell cycle arrest and apoptosis by attenuating ribosome biogenesis stress and inhibiting MDM2-mediated p53 ubiquitination.

Germline genetic variants in metabolic HCC

One-fourth of the global population is affected by NAFLD, and among biopsy-indicated NAFLD patients, approximately 60% are estimated to have non-alcoholic steatohepatitis (NASH) (estimated NASH prevalence in non-biopsy-indicated NAFLD patients is 7%), developing HCC annually at a rate of 5.29 per 1,000 person-years.⁴⁷ Obesity, diabetes, and metabolic syndrome are present in 51%, 23%, and 43% of NAFLD patients, respectively, suggesting that underlying molecular pathogenesis is highly heterogeneous across the patients.^{47,48} Excess alcohol intake remains a major HCC etiology and contributes to a rising HCC mortality in several regions such as northern and central Europe, whereas HCC

mortality decreases in some countries such as France accompanied with reduced alcohol use.⁴⁹ In these major metabolic liver diseases, genetic variants in *PNPLA3*, *TM6SF2* and *MBOAT7* genes have been more extensively studied compared to other genes (Table 1).

A SNP in *PNPLA3* is the first germline variant identified as a risk allele associated with NAFLD detected by proton magnetic resonance spectroscopy.⁵⁰ Subsequent studies reported its association with accelerated fibrosis progression in patients with HCV infection, alcoholic liver disease, and NAFLD/NASH.^{51–53} *PNPLA3* gene encodes patatin like phospholipase domain containing 3 (PNPLA3), which is located in lipid droplet and has hydrolase activity on triglycerides and retinyl esters in hepatocytes and hepatic stellate cells, respectively.⁵⁴ Recent paper showed that I148M substitution disrupts ubiquitylation and proteasomal degradation of PNPLA3, and results in accumulation of triglyceride by impairing mobilization of triglyceride.⁵⁵ Higher body mass index enhanced association of the SNP with risk of cirrhosis, suggesting its interaction with clinical phenotypes.⁵⁶ On the other hand, *PNPLA3* G/G genotype was significantly associated with presence of NASH among lean patients with NAFLD, suggesting that the risk allele drives disease progression irrespective of obesity.⁵⁷ Systematic reviews and meta-analyses confirmed association of the SNP with HCC risk in patients with NASH or alcohol-related cirrhosis (pooled ORs=1.40–2.20).^{58,59} This has been replicated in more recent studies of patients with alcoholic cirrhosis and HBV/HCV infection.^{60–62} Steatohepatic HCC is a histological variant often observed in patients with underlying steatohepatitis, although no association was observed with the SNP in *PNPLA3* gene.^{63–66}

Transmembrane 6 superfamily member 2A (TM6SF2) is a regulator of triglyceride metabolism in liver and small intestine and hepatic cholesterol metabolism.^{67–69} A SNP in *TM6SF2* gene was originally identified as a risk allele for presence of NAFLD, i.e., amount of fat deposition in liver.⁷⁰ Subsequent studies have reported association of the variant with alcoholic cirrhosis as well as hepatic steatosis and dyslipidemia in viral hepatitis, whereas association with fibrosis was marginal.^{71,72} NAFLD-related HCC was more frequently observed in patients carrying homozygous G allele at the locus, although the association was not statistically significant after adjusting with clinical confounding factors.⁷³ In contrast, the genetic variant in *TM6SF2* was significantly associated with HCC in alcohol-related cirrhosis (adjusted HR=1.66).⁶⁰ More studies will be needed to determine its association with HCC risk according to etiology and other factors such as patient race/ethnicity. Such diverse and distinct association of the same genes between different etiologies suggest etiology-specific involvement of the genes in HCC development presumably due to presence of etiology-specific co-factors.

A SNP in *MBOAT7* was first reported as one of risk locus for alcoholic cirrhosis.⁷¹ This variant was also modestly associate with NASH severity, including fibrosis stage, in Caucasian patients with suspected NASH (adjusted OR=1.30).⁷⁴ The variant in *MBOAT7* is independently associated with NAFLD-related HCC even in patients without advanced liver fibrosis (adjusted OR=2.10), whereas the *PNPLA3* SNP was associated with HCC in patients with severer fibrosis.⁷⁵ There was no association with HCC observed in patients with alcoholic liver disease.^{60,76}

Several more additional risk SNPs for HCC have been reported in more recent studies. A SNP in *DYSF* gene was found to be associated with NASH-related HCC in Japanese patients, and together with the SNP in *PNPLA3*, a combined polygenic score showed HCC risk association with ORs as high as 4.39 based on several cut-off values.⁷⁷ A recent GWAS identified a splicing variant in *HSD17B13* gene, encoding hydroxysteroid 17-beta dehydrogenase 13, which was associated with reduced risk of disease progression in alcoholic and non-alcoholic liver diseases, and possibly mitigates hepatic injury associated with the *PNPLA3* risk allele.⁷⁸ Although its inverse association with HCC risk did not reach statistical significance in the study, a small retrospective study suggested that its protein abundance in peri-tumoral liver may be negatively associated with HCC recurrence after curative treatment.⁷⁹

Transcriptomic dysregulation in diseased liver tissue

A transcriptome-based prognostic liver signature (PLS), consisting of 186 or 32 genes, has shown to predict long-term risk of HCC development in patients with HCV-related early-stage cirrhosis as well as *de novo* HCC recurrence after curative treatment in patients affected with HBV, HCV, alcohol abuse, or NAFLD with HRs ranging from 2.65 to 10.94 (Table 1).^{80–82} The gene signature is implemented in an FDA-approved digital transcript counting assay for clinical use.⁸¹ Because transcriptional regulation is a dynamic process that can be significantly affected by drastic change in the clinical context such as HCV cure, it will be critical to ensure robust risk determination according to the context. In an attempt of PLS-based HCC risk prediction before and after SVR, use of data normalization reference with or without active HCV infection enabled quantitative HCC risk estimation even after pharmacological cure of HCV infection.⁸³ A hepatic injury and regeneration (HIR) signature was derived from peri-tumoral tissues from HBV-related HCC patients and is predictive of late (> 2 years) recurrence after curative treatment (adjusted HR=2.2).⁸⁴ Late recurrence, defined at various cut-offs ranging from 1 to 5 years across the studies, has been empirically used to enrich *de novo* recurrence to identify HCC risk-associated molecular signatures. Genetic clonal assessment of paired initial primary and recurrent tumors may enable more precise determination of the outcome as performed in a previous study as a proof of concept.⁸⁵ Hepatic stellate cells are liver-resident fibroblasts that are activated in chronically inflamed and fibrogenic liver due to hepatitis virus infection and metabolic disorders, and contribute to creation of tissue microenvironment supporting HCC initiation and promotion.^{1,86} Expression levels of 12 cytokines captures presence of histological ectopic lymphoid-like structures (ELS) in portal areas, which is associated with increased risk of late HCC recurrence (> 2 years) after curative resection (adjusted HR=3.58).⁸⁷ Gene-expression signatures of hepatic stellate cell activation or presence were associated with HCC development or recurrence.^{88,89} Some of the transcriptome signatures encoding the information of activated stellate cells may warrant confirmation of their correlation with direct measure of portal hypertension. It is assumed that the transcriptome signatures capture molecular information from multiple cell types and tissue micro-niches, which are directly and/or indirectly involved in the process of hepatocarcinogenesis. Future studies aiming to deconvolute the signals may clarify more specific direct drivers of HCC development.

Circulating factors

Blood-based biomarkers will enable less invasive and more flexible clinical application compared to liver biopsy-based assessment in a clinical setting, although tissue acquisition will help ensure their validity and utility during the process of developing such assays.⁹⁰ Abundance of serum/plasma proteins has been associated with HCC risk in cirrhotic patients.^{91,92} Elevated levels of interleukin-6 (IL-6) and lower levels of insulin-like growth factor 1 (IGF1) in serum were associated with susceptibility to HCC development, which may reflect inflammatory response in the liver, secretion from tumor progenitor cell, and/or hepatocellular dysfunction.^{93–95} Consistent with these studies, IL-6 pathway is a part of poor prognosis-associated genes, and IGF1 is a member of the good prognosis-associated genes in the PLS.^{80,85} Some of the reported serum proteins were combined with clinical prognostic factors to develop integrative HCC risk scores.¹ Abundance of serum cytokines, including MIG, IL22, TRAIL, APRIL, VEGF, IL3, TWEAK, SCF, and IL-21 were found to be associated with post-SVR HCC development within 18 months.⁹⁶ Profiling of bisecting N-glycans on glycoprotein in serum was first reported as a biomarker to distinguish cirrhosis from non-cirrhotic chronic liver disease, which was later used to develop GlycoHCCRiskScore for HCC risk estimation (HR=2.72), although external validation is still lacking.^{97,98} Liquid chromatography-mass spectrometry (LC-MS)-based metabolomics profiling was applied to more than 1,400 serum samples, and identified two metabolites, phenylalanyl-tryptophan and glycocholate, that distinguish patients with not only large but also small (< 3cm) HCC tumors. Serum acylcarnitine level may serve as a biomarker short-term risk of NAFLD-related HCC (adjusted OR=3.68).⁶⁴

Circulating tumor cells (CTC), circulating cell-free DNA (cfDNA, which is supposed to contain circulating tumor DNA [ctDNA]), and cell-free or exosomal non-coding RNA may have a role to assess short-term HCC risk by detecting subclinical cancer cells and their products released into bloodstream. However, detection sensitivity and specificity need to be substantially improved to reliably monitor them in cirrhosis patients with no clinically recognizable tumor.^{99–101} For example, sensitivity of circulating cfDNA-based detection of somatic DNA mutations found in tissue is less than 50% even with deep sequencing of targeted genes.¹⁰² Detection of CTC and ctDNA may be biased toward specific tumor cell population prone to die, closely located to vascular system, or with other properties. In addition, site of blood sampling may affect which HCC CTC populations are preferentially captured.¹⁰³ These factors need to be taken into consideration as confounders to interpret the result. To mitigate the problem, one approach may be to combine different types of circulating biomarkers, e.g., cfDNA and protein, to complement the limitations each other.¹⁰⁴ After curative HCC resection, number and/or molecular characteristic of CTC such as mesenchymal features were associated with risk of future tumor recurrence (adjusted HRs=1.02–2.45).^{105,106} In a small case series of 10 patients with curatively treated HCC, tumor-specific *TP53* 249T mutations and aberrant methylation of *RASSF1A* and *GSTP1* in urine-derived cfDNA were detected up to 9 months prior to tumor recurrence clinically diagnosed by MRI.¹⁰⁷ *TERT* promoter mutations, a well-known early event in HCC progression, was detected in plasma DNA from HCC-free cirrhotic patients, although its capability to predict future HCC risk needs to be determined in future study.¹⁰⁸ A panel of

15 microRNA in serum were identified as a predictor of future HCC risk (adjusted HR=3.57).¹⁰⁹

Conclusions

HCC risk-predictive biomarkers will be a critical component in clinically implementing HCC early detection strategies. Individual HCC risk-based personalized or stratified HCC screening indeed improve cost-effectiveness and prolong net patient survival as suggested by a Markov model-based simulation analysis.¹¹⁰ Given the diverse risk association according to the liver disease etiology, patient population, and geographic region, future studies should focus on determining HCC risk association across distinct and well-defined patient group and clinical context to clarify the scenario of clinical application for each biomarker (Figure 1). In parallel, clinical variable-based HCC risk scores such as PAGE-B are being considered for clinical application in specific clinical contexts such as chronic HBV infection.^{1,111} Ideal HCC risk biomarkers are expected to refine the risk prediction through combination/integration with the clinical scores as well as stratification of a patient subpopulation where performance of clinical scores is limited. Newly emerging clinical entities such as non-cirrhotic NAFLD and HCV SVR should be of particular focus given their high and increasing prevalence. Unified protocols of sample acquisition, assay, and clinical follow-up will facilitate multi- and cross-institutional assessment of HCC risk biomarkers to achieve the goal more efficiently. Liver tissue biopsy will be instrumental to facilitate development of reliable non-invasive blood- or imaging-based biomarkers. Biomarker-based HCC risk stratification will also enable high-risk patient-enriched HCC chemoprevention clinical trial that requires smaller sample size and shorter follow-up time compared to the traditional all-comer design.¹ Thus, clinical translation of molecular HCC risk biomarker tests is expected to transform the care of patients with chronic fibrotic liver diseases and substantially improve the dismal HCC prognosis.

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Figure 1:
(A) HCC risk stratification in chronic liver disease patient population with clinically uncertain risk.
(B) Potential application of molecular HCC risk assessment and subsequent clinical actions over the course of HCC and liver disease progression.

Table 1.

Molecular features associated with future HCC risk.

Type of molecular information	Molecular feature	Study design	No. subjects	HCC Endpoint	Major etiology	Major race/ethnicity	Cirrhosis	Combined clinical variables	Validation	Reference
SNP	<i>EGF 61AG</i> (rs4444903, A >G)	Prospective-retrospective, cohort	816	Development	HCV	White, black	15.4%	Age, sex, smoking history, ALP, platelet	External (HBV/Asian)	17–19
	<i>IFNL3</i> (rs8099917, T >G)	Retrospective, cohort	792	Development	HCV	Asian	22.7% (F3–4)	n.a.	No (HCV/Asian)	27–29
	<i>MICA</i> (rs2596542, G >A)	Retrospective, case-control	721; 2,890 + 673; 2,596 ^{AB}	Presence	HCV	Asian	n.a.	n.a.	External(HCV, HBV/Asian)	31,112
	<i>DEPDC5</i> (rs1012068, T >G)	Retrospective, case-control	212; 765 + 710; 1,625 ^{AB}	Presence	HCV	Asian	n.a.	n.a.	External	35
	<i>TLL1</i> (rs17047200, A >T)	Retrospective, case-control	123; 333 + 130; 356 ^{AB}	Development	HCV after SVR	Asian	24.6% + 20.1% ^A (F3–4)	Age, albumin, AFP after SVR	External	39
	<i>Ip36.22</i> (rs17401966, A >G)	Retrospective, case-control	355; 360 + 1,962; 1,430 ^{AB}	Presence	HBV	Asian	n.a.	n.a.	External	42
	<i>STAT4</i> (rs7574865, G >T)	Retrospective, case-control	1,161; 2,514 + 4,319; 4,966 ^{AB}	Presence	HBV	Asian	n.a.	n.a.	External	45
	<i>HLA-DQB1/HLA-DBA2</i> (rs9275319, A >G)	Retrospective, case-control	1,161; 2,514 + 4,319; 4,966 ^{AB}	Presence	HBV	Asian	n.a.	n.a.	External (HBV/Asian)	45,113
	<i>PNPPLA3</i> 1148M (rs738409, C >G)	Prospective, cohort	532	Development	Alcohol, HCV	White	100%	Age, sex, BMI	External (NAFLD, alcohol/White)	114–116
	<i>TM6SF2</i> E167K (rs58542926, C >T)	Retrospective, case-control	751; 1,165 ^B	Development	Alcohol	White	100%	n.a.	External (alcohol/White)	60,117
Tissue transcriptome	<i>MBOAT7</i> (rs641738, C >T)	Retrospective, case-control	132; 633 ^B	Presence	NAFLD	White	27.5% (F3–4)	n.a.	No	75
	<i>MPO</i> –463AG (rs2333227, A >G)	Prospective-retrospective, cohort	205	Development	HCV	White	100%	n.a.		118
	<i>HFE</i> C282Y (rs1800562, G >A)	Prospective, cohort	301	Development	Alcohol, HCV	White	100%	n.a.	No	119
	186/32-gene signature	Prospective-retrospective, cohort	82 + 225/216/145/263A	Development, recurrence	HCV, HBV, Alcohol, NASH	Asian + n.a./white/white/Asian ^A	52.4% + n.a./100%/100%/43% ^A	AFP, vascular invasion, bilirubin, platelet, Child-Pugh class, AJCC stage	External	80–82
	HIR gene signature	Injured/regenerative livers + retrospective, cohort	72 + 96; 228 ^A	Recurrence (>2 yr)	HBV	Asian	50% + 62.5%; 92.5% ^A	n.a.	External	84
Circulating factor	ELS signature	Retrospective, cohort	82	Recurrence (>2 yr)	HCV	Asian	52.4%	n.a.	No	87
	Activated HSC gene signature	Primary cells + retrospective, cohort	247+ 226; 72 ^A	Recurrence	HBV	Asian	90.70%	n.a.	External	88
	HSC signature	Primary mouse cells + retrospective, cohort	Mouse + 216/82	Recurrence	HCV, HBV	White/Asian	100%/100% ^A	Bilirubin, platelet	No	89
	IL-6	Prospective-retrospective, cohort	330	Development	HCV	Asian	n.a.	n.a.	External (mixed/Asian, white)	94,120,121
	IGF 1	Prospective, cohort	104	Development	HCV	White	100.0%	n.a.	External (HBV/Asian)	93,122
	GlycoHCCriskScore	Prospective-retrospective, case-control	125	Development	HCV	n.a.	100.0%	n.a.	No	97
	Circulating microRNA signature	Prospective, cohort	373	Development	HBV	Asian	34.6%	AFP	No	109

Validation: “Internal”, validation in patients from the same institution(s); “External”, validation in patients from independent institution(s).

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Biomarkers evaluated in prospective patient series and/or externally validated in > 100 patients are included

SNP, single nucleotide polymorphism; HSC, hepatic stellate cell; HIR, hepatic injury and regeneration; ELS, ectopic lymphoid-like structures; IGF, insulin-like growth factor; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; HCV, hepatitis C virus; SVR, sustained virologic response; NASH, non-alcoholic steatohepatitis; HBV, hepatitis B virus; BMI, body mass index; AFP, α -fetoprotein ; ALP, alkaline phosphatase.

A Training + validation.

B Case:control.

C Major etiology/major race(s) in external validation cohort(s) additionally analyzed to the etiology/race in the original study.

„Prospective-retrospective”, indicates retrospective analysis of prospectively collected cohort in the past.