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TOPIC HIGHLIGHT

2015 Advances in Cirrhosis

Molecular prognostic prediction in liver cirrhosis

Nicolas Goossens, Shigeki Nakagawa, Yujin Hoshida

Nicolas Goossens, Shigeki Nakagawa, Yujin Hoshida, Division of Liver Diseases, Department of Medicine, Liver Cancer Program, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

Nicolas Goossens, Division of Gastroenterology and Hepatology, Geneva University Hospital, 1211 Geneva, Switzerland

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Correspondence to: Yujin Hoshida, MD, PhD, Division of Liver Diseases, Department of Medicine, Liver Cancer Program, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1470 Madison Ave, New York, NY 10029, United States. yujin.hoshida@mssm.edu
Telephone: +1-212-8248862
Fax: +1-646-5379576

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Abstract

The natural history of cirrhosis varies and therefore prognostic prediction is critical given the sizable patient population. A variety of clinical prognostic indicators have been developed and enable patient risk stratification although their performance is somewhat limited especially within relatively earlier stage of disease. Molecular prognostic indicators are expected to refine the prediction, and potentially link a subset of patients with molecular targeted interventions that counteract poor prognosis. Here we overview clinical and molecular prognostic indicators in the literature, and discuss critical issues to successfully define, evaluate, and deploy prognostic indicators as clinical scores or tests. The use of liver biopsy has been diminishing due to sampling variability on fibrosis assessment and emergence of imaging- or lab test-based fibrosis assessment methods. However, recent rapid developments of genomics technologies and selective molecular targeted agents has highlighted the need for biopsy tissue specimen to explore and establish molecular information-guided personalized/stratified clinical care, and eventually achieve "precision medicine".

Key words: Cirrhosis; Gene expression; Prognosis; Hepatocellular carcinoma; Biomarker

Core tip: Molecular-based prediction of prognosis in liver cirrhosis is coming of age with the emergence of clinically applicable genomic assays, which are expected to further refine clinical indicator-based prognostication. Such biomarkers could also guide individualized molecular targeted therapeutic and/or preventive interventions to improve patient prognosis in the near future.

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INTRODUCTION

The prevalence of cirrhosis has been estimated at 0.3% in the United States and in Western Europe[1,2], and 1% to 2% globally[3,4]. Major etiologies of liver disease leading to cirrhosis are hepatitis C virus (HCV), hepatitis B virus (HBV), non-alcoholic fatty liver disease (NAFLD), and alcohol-related liver disease (ALD). Cirrhosis is an increasing cause of morbidity and mortality worldwide. According to the most recent assessment of global burden of disease, cirrhosis was estimated to cause over 1.2 million deaths globally in 2013, or 2% of total deaths, an increase of 47% compared to 1990[5]. In addition, when ranked for global years of life lost, the rank of cirrhosis rose from 18th to 13th between 1990 and 2013. Cirrhosis is also strongly associated to hepatocellular carcinoma (HCC) development, the most common cause of primary liver cancer, which was estimated to claim an additional 800000 deaths worldwide in 2013[5].

Although cirrhosis has a clear case definition, its prognosis ranges widely from a one-year mortality of 1% per year in well-compensated cirrhotics without signs of portal hypertension to up to 57% 1-year mortality in decompensated cirrhotics with a gastrointestinal bleed, which require intensive and costly medical care[6]. The high mortality of advanced cirrhosis and high global prevalence of cirrhosis have highlighted the necessity to further refine our capacity to predict prognosis. This has led to numerous attempts to identify clinical prognostic indicators that could help the clinician in guiding decision-making and allotting limited resources, such as liver transplantation, to cirrhotics who need them most. Molecular prognostic markers have been explored, although few are successfully validated and incorporated into clinical practice.

In this review, we overview the natural history of cirrhosis in the context of prognosis prediction, identify key clinical and molecular prognostic predictors in cirrhotic subjects, discuss potential applications, challenges in the development, and conclude by discussing future perspectives of molecular prognostic biomarkers.

NATURAL HISTORY AND PATHOGENESIS OF CIRRHOSIS

Clinically, cirrhosis gradually progresses towards more advanced stages associated with increased morbidity and mortality. In the initial, asymptomatic, compensated cirrhosis stage, portal pressure is under the threshold to develop esophageal and other varices and hepatic venous pressure gradient (HVPG) is generally below 10 mmHg[6,7]. As the liver disease progresses, portal pressure increases, protein synthetic function is reduced resulting in the development of ascites, portal hypertensive hemorrhage, hepatic encephalopathy and/or jaundice. The occurrence of any of these complications signals the transition to a decompensated phase, generally initially indicated by the development of ascites[6,8,9]. The rate of progression from a compensated to a decompensated stage has been estimated to be approximately 5%-7% per year and survival decreases sharply from a median survival of over 12 years in compensated disease to approximately 2 years in decompensated cirrhosis[6]. Further progression of liver disease and increase of portal pressure and HVPG above 16-20 mmHg often leads to severe complications of cirrhosis such as refractory ascites, bacterial infection, recurrent variceal hemorrhage, hepatorenal syndrome and, without therapy, invariably death. An approach to standardize the clinical classification of cirrhosis severity has suggested four clinical stages, from stage 1 which encompasses cirrhotic patients with no ascites and no esophageal varices and a very low mortality to stage 4 characterized by gastrointestinal bleeding and a high mortality of over 50% at 1 year[6].

Cirrhosis is also a major risk factor for HCC development. The risk of developing HCC in cirrhosis depends largely on the underlying condition, reaching 5-year cumulative risks of 17%-30% in HCV cirrhosis, 21% in hemochromatosis, 8%-12% in alcoholic cirrhosis but only 4% in biliary cirrhosis[10-12]. Importantly, HCC can also occur on the background of non-cirrhotic liver, especially in the context of chronic HBV infection and, increasingly recognized, non-cirrhotic NAFLD[13]. Prediction of HCC risk in liver disease remains an ongoing challenge requiring improvement in current stratification of HCC risk across multiple etiologies of liver disease.

Cirrhosis is the end-stage manifestation of hepatic fibrosis, as characterized histologically by the formation of regeneration parenchymal nodules, separated by fibrotic septa and associated with major distortion in vascular architectural[14,15]. Fibrosis is a ubiquitous pathological process, resulting from cellular and molecular responses triggered by an injury, ultimately leading to parenchymal scarring and organ dysfunction[16]. Fibrogenesis accounts for substantial morbidity and mortality as it can affect virtually any organ system including cardiac, hepatic, renal, pancreatic and pulmonary organ systems. Fibrosis stage was reported to be associated with step-wise increase of annual HCC incidence in HCV-infected individuals[17]. The histological alterations leading to hepatic fibrosis and cirrhosis result in architectural vascular alterations such as angiogenesis, vascular occlusion leading to parenchymal extinction, major

microvascular changes and formation of intrahepatic shunts. Increased resistance to portal blood flow and splanchnic vasodilatation mediated through increased NO and reduced response to vasoconstrictors are major factors leading to portal hypertension and ensuing complications such as ascites and variceal bleeding.

Chronic damage to hepatocytes or biliary epithelium leads to a release of inflammatory and fibrotic mediators such as reactive oxygen species, cell death signals, hedgehog ligands and nucleotides[15]. A complex series of mechanisms centering on the hepatic stellate cell, mediated through intracellular inflammasome activation, the nuclear receptor family, such as farsenoid-X-receptor, peroxisome proliferator-activated receptors and others, and other transcriptional events contribute to stellate cell activation. Autophagy was recently identified to play a role in providing energy for the activation of hepatic stellate cells and the autophagic response has also been linked to endoplasmic stress and the unfolded protein response[18,19]. Interestingly, dietary fat composition and an altered microbiome has been linked to increased fibrogenic potential in animal models, possibly mediated by pathogen-associated molecular signaling such as activation of toll-like receptors[20]. The activated hepatic stellate cell promotes liver scarring through proliferation, contractility, fibrogenesis, matrix degradation and inflammatory signaling. A number of inflammatory and immune cell interactions perpetuate the activation or inhibition of stellate cell activation including hepatocytes, liver progenitor cells, Kuppfer cells, endothelial cells, platelets, and infiltrating immune cells through a wide variety of mediators[15].

It is important to note that until recently, fibrosis and cirrhosis were deemed irreversible however this perception has been evolving with reports of fibrosis and cirrhosis regression after control of the underlying hepatic insult, such as treatment of chronic hepatitis C or B[21,22]. Identifying subjects at higher risk of progressive disease and HCC risk despite correction of the underlying etiology of liver disease is becoming an important unmet need in the era of highly efficacious therapies for HCV[23].

Considering the high prevalence of cirrhosis, more than 630000 adults in the United States alone according to recent population-based estimations[1], the population that requires monitoring and screening in compliance with clinical guidelines is huge and likely unmanageable. For instance, despite guidelines recommending HCC surveillance in cirrhotics[24], most patients at risk of HCC in the United States do not receive recommended regular surveillance. Only 12% of cirrhotic HCV patients had routine annual surveillance in one United States Veterans Affairs series and only 2% of HCV patients who developed HCC had previous appropriate screening in another series[25,26]. Additionally, in a population-based United States study, less than 20% of patients with cirrhosis who developed HCC received regular surveillance[27]. With the emergence of non-invasive tools to diagnose cirrhosis such as elastography, the burden of regular monitoring and HCC surveillance is increasing and overtaxing currently available medical resources. Thus, prognostic indicators for cirrhosis are urgently needed to enable effective clinical management of the patients[23].

CLINICAL PROGNOSTIC SYSTEMS

A number of non-invasive and invasive clinical markers and systems have been proposed and some of them are clinically well established to assess prognosis in liver disease, in particular, cirrhosis (Table 1). Although a number of risk scores have been developed for acute conditions in cirrhotic subjects, such as acute-on-chronic liver failure[28] or variceal hemorrhage[29], we do not consider these scores in this review as molecular stratification of prognosis in these acute conditions probably still has limited value. Cirrhosis severity is clinically manifested as impaired normal liver function, and readily available clinical symptom and laboratory variable-based prognostic systems have been used to prognosticate cirrhotic patients to guide indication of interventional therapies such as transection for esophageal varices and/or allocation of medical resources such as donor livers for transplantation. One of the earlier attempts to develop an objective measure, the Child-Turcotte-Pugh (CTP) score, adopted in the US in 1998 for liver transplantation allocation was later replaced by the model for end-stage liver disease (MELD) in 2002 due to less objectivity of the clinical symptom variables in the CTP score and insufficient validation of prognostication on the transplantation waiting list[30,31]. The MELD score, consisting of bilirubin, creatinine, and INR, was initially developed as a prognostic tool in cirrhotic patients undergoing a transjugular intrahepatic portosystemic shunt (TIPS)[32]. It has since been adopted by many liver transplant programs in the world due to accurate prognostication of death in a broad spectrum of liver disease with improved prognostic capacity when compared to CTP[33,34]. Outside of liver transplant allocation, the use of the MELD score has been broadened in cirrhotic subjects to assessment of risk prior to TIPS placement[32], prior to non-hepatic surgery[35], in variceal bleeding[36] hepatorenal syndrome[37] or mortality prediction in alcoholic hepatitis[38].

Historically, liver histology has been established as the gold standard of disease staging and one of the prime indicators of prognosis in liver disease. Based on morphological assessment of fibrosis/cirrhosis, including thickness of fibrotic septa, number and size of cirrhosis nodules, type of hepatic necrosis and cellular infiltrates, several classification systems to subclassify cirrhosis have been established to attempt

Table 1 Clinical prognostic system in cirrhotic patients

Outcome	Risk score	Outcome assessed	Etiology of liver disease	Proportion of cirrhotics	Variables	Note	Ref.
Death	MELD	3-mo mortality	Multiple	100%	Creatinine, bilirubin and INR	Used by UNOS for liver allocation	[34]
	MELD-Na	3 and 6-mo mortality	HCV (25%) Chronic cholestasis (23%) Autoimmune hepatitis (14%) Alcohol (13%) Cryptogenic (12%) Other (13%)	100%	Components of MELD score and serum sodium		[90]
	CTP	Mortality	Alcohol (53%) Hepatitis (23%) Cryptogenic (15%) Biliary (9%)	100%	Bilirubin, albumin, encephalopathy, ascites and prothrombin time/INR		[91,92]
	Prognostic Index	5-yr mortality	Alcohol (64%) Viral (24%) Other (12%)	100%	Albumin, INR and creatinine		[93]
	HCV risk score	5-yr mortality	HCV	87%	Age, platelets, sex		[94]
	Bell *et al*	Mortality	Alcohol (100%)	100%	Age, alcohol abuse and alkaline phosphatase		[95]
	HVPG	Mortality	Alcohol (44%) HCV (36%) HBV (9%) Other (11%)	100%	HVPG		[96]
	Liver stiffness measurement	Composite outcome: death, liver transplantation, variceal bleeding and ascites	Alcohol (51%) HCV (20%) NASH (8%) HBV (3%) Other (18%)	100%	Liver stiffness measurement		[97]
	Non-invasive assessment of fibrosis: FibroTest, FIB-4, APRI	Overall survival	HCV (90%) HCV-HIV (10%)	18%	Fibrotest (Alpha-2-macroglobulin, Haptoglobin, Apolipoprotein A1, GGT, bilirubin, ALT) FIB-4 (AST, ALT, platelets, age) APRI (AST, platelets)		[98]
	FIB-4	Survival in cirrhotic Child-Pugh class A subjects with HCC	HCV (70%) HBV (16%) Other (14%)	100%	FIB-4 (AST, ALT, platelets, age)	Only predictive in subjects with Child-Pugh score of 5	[99]
	Collagen proportionate area	Liver decompensation	Alcohol (38%) HCV (28%) HBV (9%) NASH (9%) Other (17%)	100%	Measuring collagen proportionate area on liver histology		[47]
HCC	ADRESS-HCC	1-yr HCC risk	HCV (46%) Alcohol (18%) NASH (18%) HBV (3%) Other (15%)	100%	Age, diabetes, race, etiology of cirrhosis, sex, and severity of liver dysfunction (Child-Pugh score)		[100]
	Velazquez *et al*	4-yr HCC risk	Alcohol (59%) HCV (29%) HBV (7.5%) Other (3%)	100%	Age, anti-HCV positive, prothrombin time and platelet count		[101]
	UM regression model	3 and 5-yr HCC risk	HCV (47%) Cryptogenic (19%) Alcohol (15%) Other (19%)	100%	AFP and gender	A machine-learning algorithm was also derived using 23 variables	[51]
	GAG-HCC	5 and 10-yr HCC risk	HBV	15%	Age, gender, HBV DNA, core promoter mutations, cirrhosis		[102]
	CU-HCC	5-yr HCC risk	HBV	38%	Age, albumin, bilirubin, HBV DNA, and cirrhosis		[103]

LSM-HCC	3 and 5-yr HCC risk	HBV	31%	Liver stiffness, age, albumin, HBV DNA	[104]
REACH-B	3, 5 and 10-yr HCC risk	HBV	0% discovery cohort, 18% validation cohort	Sex, age, ALT, HBeAg status, and serum HBV DNA level	[105]
Risk index	Incidence of HCC	HCV after SVR	10%	Age, AST, platelet count	[106]
score$_{HCC}$	Incidence of HCC	HCV after SVR	30%	Age, AFP level, low platelets and advanced fibrosis	[107]
Chang *et al*	5-yr HCC risk	HCV after therapy	45% fibrosis stage 3-4	Age, male sex, AFP level, low platelet, advanced fibrosis, HCV genotype 1b and non SVR	[108]
El-Serag *et al*	Incidence of HCC	HCV	100%	AFP, ALT, platelets, interaction terms, and age	[50]
HALT-C model	5-yr HCC risk	HCV	41%	Age, race, Alkaline phosphatase, esophageal varices, ever smoked, and platelet count	[109]
REVEAL-HCV	5-yr HCC risk	HCV	4%	Age, ALT, AST/ALT ratio, HCV RNA, cirrhosis and HCV genotype	[110]
Liver stiffness measurement	5-yr HCC risk	HBV	50%	Liver stiffness measurement	[111]
FIB-4	Incidence of HCC	HBV	10%	FIB-4 (AST, ALT, platelets, age)	[112]

HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease-sodium.

to correlate these findings with clinical endpoints and HVPG as a surrogate marker[39-42]. Quantification of fibrotic collagen tissue can be performed with digital image analysis with staining of collagen[43] which have been validated against HVPG measurements and clinical outcomes (for instance fibrosis progression and clinical decompensation), mostly in the setting of HCV recurrence after liver transplantation[44,45]. Recently, an automated assessment method combining quantification of histopathological architectural features on unstained histological slides has been developed to allow more accurate assessment of fibrosis[46]. A recent report suggested that collagen proportionate area may perform better than other histological measures to predict risk of decompensation in cirrhotic subjects although this will require further validation in larger patient cohorts[47]. Although liver biopsy-based histological assessment provides more deterministic evidence of cirrhosis, and HVPG could complement the suboptimal robustness of histological cirrhosis assessment affected by sampling variability in liver biopsy, these methods are relatively invasive especially in patients with more advanced cirrhosis with impaired blood coagulation. Multiple clinical-based scoring systems have also been proposed to predict outcomes in subjects with cirrhosis in a wide range of etiologies (Table 1). Liver stiffness measurement by transient elastography or MR-elastography, is another non-invasive, imaging-based techniques mainly developed as a diagnostic tool to assess liver fibrosis severity. With a cutoff of 21.1 kPa in elastography, one report found that it accurately predicted portal hypertension related complications in subjects with chronic liver disease (65% cirrhotic) and that it was significantly correlated to HVPG, an indicator of portal pressure and prognosis[48,49].

Another important clinical goal in caring for patients with liver disease is prediction of risk of HCC. Numerous clinical scores, especially in HBV and HCV-related liver disease, have been developed to assess for HCC risk in cirrhotic subjects (Table 1). However, no universal risk score encompassing all types of liver disease etiologies has emerged in clinical use. The incorporation of clinical and/or molecular risk scores in HCC screening strategies could potentially boost efficacy and uptake of HCC screening in high-risk populations, while significantly reducing costs, as we discuss below.

GENOME-BASED MOLECULAR PROGNOSTIC SYSTEMS

The clinical variable-based prognostic systems have yielded reasonably good capability in discriminating subsets of patients with either severe cirrhosis or milder fibrosis. However, patients in the middle of the spectrum, *i.e.*, clinically asymptomatic, early-stage cirrhosis, still account for a sizable population requiring regular clinical follow-up such as biannual HCC surveillance, as evidenced by the extremely low application rate (17%) in the United States[27] In addition, it is more challenging to make prognostic prediction within this subset of patients, even with sophisticated machine-learning approaches based on

Table 2 Molecular prognostic systems in cirrhotic patients

Molecular method	Risk score	Liver disease etiology	Outcomes	Sample	Proportion cirrhosis	Molecular marker	Risk groups and proportion of subjects	Note	Ref.
Gene expression	186-gene signature	HCV	Overall death, Progression to advanced cirrhosis, HCC	FFPE liver needle biopsy	100%	186-gene signature	Poor (25%) Intermediate (47%) Good prognosis (28%)		[52,53]
	HIR gene signature 65-gene signature	HBV (89%)	223-gene sig: late HCC recurrence, 65-gene sig: early HCC recurrence	Frozen hepatic tissue	78%	223 (HIR) & 65-gene signature	HIR signature: High risk (32%) Low risk (68%)		[60]
	Activated HSC signature	HBV (92%)	HCC recurrence and survival	Frozen hepatic tissue	87%	37-gene signature	High risk (53%) Low risk (47%)		[62]
SNP	*EGF*	HCV	6-yr HCC risk	Blood	39%	*EGF* 61*G (rs4444903)	When combined with clinical markers: High risk (14%) Intermediate risk (29%) Low risk (57%)	Improved model when added clinical data: age, gender, smoking status, alkaline phosphatase level, platelet count	[67]
	Cirrhosis risk score	HCV	Fibrosis progression after liver transplantation	Blood	41% progressed to at least F3 fibrosis	7-SNP signature	High risk (44%) Intermediate risk (29%) Low risk (24%)		[64]
	PNPLA3	Alcohol (52%) HCV (48%)	6-yr HCC risk	Blood	100%	*PNPLA3* 444*G (rs738409)	When combined with clinical markers (alcoholic cirrhosis): High risk (25%) Intermediate risk (55%) Low risk (20%)		[113]
	MPO	HCV	HCC risk	Blood	100%	*MPO* -463*G (rs2333227)	High risk (GG, 51%) Intermediate risk (AG, 35%) Low risk (AA, 14%)		[69]
	CAT	HCV	HCC risk	Blood	100%	*CAT* -262*C (rs1001179)	High risk (CC, 68%) Intermediate risk (CT, 28%) Low risk (TT, 4%)	Not yet implemented in risk score	[69]
	HFE	Alcohol (54%) HCV (46%)	HCC risk	Blood	100%	*HFE* C282Y (rs1800562)	In alcoholic cirrhosis: High risk (GA, 8%) Low risk (GG, 92%)	Not predictive in HCV cirrhosis in this study	[114]

FFPE: Formalin fixed paraffin embedded; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HIR: Hepatic injury and regeneration; HSC: Hepatic stellate cell; SNP: Single nucleotide polymorphism.

clinical variables, because most of the values are within normal reference range[50,51]. Genome-wide molecular profiling is an approach to overcome the issue of a limited number of clinical variables by using a much wider set of molecular variables to initially train/define prognostic models.

Genome-wide profiles of RNA expression and DNA variant, *i.e.*, single nucleotide polymorphism (SNP), have been studied to define molecular prognostic indicators (Table 2). A 186-gene expression signature, derived from non-tumoral liver tissues of subjects undergoing hepatic resection for HCC, has proven prognostic not only for HCC recurrence but also for liver disease progression, HCC development and overall survival in subjects with early-stage HCV cirrhosis[52-54]. The signature was present in the liver of rodent models of fibrosis/cirrhosis-driven HCC, and the poor prognosis pattern of the signature was reversed in association with the HCC chemopreventive effect of an FDA-approved EGFR inhibitor, erlotinib[55], which is now being tested in a phase 1 trial with the gene signature as a companion biomarker (ClinicalTrials.gov, NCT02273362). Insulin-like growth factor one (IGF-1) has been shown to reflect hepatocellular dysfunction possibly due to a loss of hepatocyte synthesis and a decrease in growth hormone receptors[56], and serum levels of IGF-1 reflect liver failure and risk of HCC[57,58]. Consistent with these findings, IGF1 is a member of the good prognosis-correlated genes in the 186-gene prognostic liver signature[52,54]. Similarly, liver tissue-derived transcriptome signatures have been associated with multicentric HCC development and late recurrence after curative HCC treatment attributable to *de novo* HCC development[59-61]. A gene signature of hepatic injury and regeneration was associated with late HCC recurrence[60] and a hepatic stellate cell gene

signature was recently reported for its association with HCC recurrence and death[62]. Several germline SNPs were reported to be associated with increased HCC risk and other liver disease-related outcomes (Table 2). A 7-gene SNP assay named cirrhosis risk score was associated with risk of developing cirrhosis in HCV-infected individuals[63] and fibrosis progression after liver transplantation for HCV-related cirrhosis[64]. Numerous other germline SNPs have been reported as HCC risk variants in HCV cirrhosis, although very few of them are replicated in independent patient series/cohorts[65]. The *EGF* 61*G allele was associated with HCC risk in a prospective cohort of patients with HCV-related advanced fibrosis (39% cirrhotic)[66,67]. Despite diverse allele frequency across patient populations, association between the *EGF* genotype and HCC risk remains significant and independent of patient race[68]. A SNP in an antioxidant enzymes, *MPO* was associated with HCC risk in a prospective study in HCV-cirrhotic subjects[69].

POTENTIAL APPLICATIONS OF MOLECULAR PROGNOSTIC PREDICTION IN CIRRHOSIS

One of the goals of molecular prediction of prognosis in cirrhotic subjects is to predict risk of major liver-related endpoints such as HCC development, liver disease progression, liver transplantation, or death beyond clinically available prognostic indicators. Besides merely predicting prognosis, molecular prognostic predictions linked to specific molecular deregulation could be used to guide therapeutic and/or preventive intervention with molecular targeted therapies. The value of molecular prognostic biomarkers especially in the setting of HCC chemoprevention cannot be overemphasized. Cancer chemoprevention trials have been regarded as highly resource-intensive, requiring the enrollment of thousands of patients, a follow-up time approaching decade(s), and rarely yielding positive results[70,71]. HCC risk biomarker-based clinical trial enrichment will drastically lower the bar to conduct cancer chemoprevention trials by substantially reducing required sample size and the duration of follow-up comparable to oncology trials enrolling advanced-stage cancer patients[23]. In patients with HCC, another application of molecular analysis is the subclassification of HCC into distinct molecular subtypes linked to different clinical and pathological characteristics[72,73], although intratumoral molecular heterogeneity within a tumor nodule or between nodules in a patient remains a challenge that must be resolved before applying the molecular classification to therapeutic decision-making, especially for selective molecular targeted agents[74].

Clinical deployment of molecular prognostic biomarkers is still a challenging task due to many hurdles as evidenced by the extremely low rate of successful clinical translation (0.1%) of biomarkers[6,75-77]. Study design/setting, from which analyzed biospecimens are derived, is a key issue to ascertain robust prognostic association of molecular biomarkers, and can be graded to inform reliability of the finding[78]. Although prospective assessment is ideal to establish clinical utility of biomarkers, requirement for financial and medical resources as well as observation time is the major limiting factor in establishing prognostic biomarkers. An alternative approach to overcome this challenge was proposed, namely "prospective-retrospective" design, where archived samples from previously completed prospective trials are retrospectively analyzed[78]. Capability to analyze archived real-world formalin-fixed, paraffin-embedded (FFPE) tissue specimens will greatly enhance applicability of this approach[52-54,79-81]. Although many modern biomarkers are developed using a variety of technologies, a key factor for implementation in clinical practice is the choice of assay technology for clinical laboratory use[82]. Reproducibility and robustness of the measurement, complexity of the assay, and cost are the major determinants of the assay selection. Historically, immunohistochemistry, including fluorescent *in-situ* hybridization, and quantitative PCR-based nucleic acid assays have been the dominant technologies employed to deploy molecular biomarkers. However, subjectivity in the quantification and experimental artifact in the process of target amplification, for example, are the major limitation to provide reliable results. Recently developed technologies such as digital transcript counting without target amplification[80,83] are expected to overcome the issue by providing more objective and robust readout. Genome-wide sequencing of germline DNA variants has posed ethical issues regarding incidental findings[84]. Regulatory oversight, which hugely varies across countries/regions, is another key factor affecting clinical translation and implementation of biomarkers whilst inclusion in clinical practice guidelines will support wider use and reimbursement from insurance companies.

CONCLUSION

As was the case for clinical prognostic indicators such as CTP and MELD scores, it is expected that molecular prognostic indicators are evaluated in more specific and additional clinical contexts/scenarios to address specific unmet need in patient management. For example, post-transplantation disease progression is a topic understudied by molecular biomarkers, which will greatly help decision on limited donor organ allocation[64,85]. In addition, there is a trend towards non-invasive biomarker assessment based on emergence of highly sensitive genomic assay technologies, *e.g.*, single cell profiling, analysis of RNA, DNA, or circulating cells derived from body fluid-derived specimens such as whole blood, plasma,

serum, ascites, and urine[86,87]. Although promising, tissue specimens are still needed to establish validity of such strategy (so-called liquid biopsy)[86]. Depending on clinical utility and requirement for robust and reliable readout, acquisition of liver biopsy could still be justifiable.

In conclusion, ever-evolving genomics technologies has enabled to identify a variety of molecular prognostic indicators in cirrhosis, which have great potential to refine clinical care of the patients as well as guide development of new therapeutic and/or preventive approaches to realize "precision medicine"[88] and enable a modern alternative to the ancient Babylonian practice of hepatomancy, the reading of omens from the liver of sacrificed animals[89]. Liver tissue acquisition by biopsy will keep playing the key role in the process.

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