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

Author contributions: All authors contributed to conception, drafting, critical revision for important intellectual content and final approval of the version to be published.

Supported by FLAGS Foundation; Nuovo-Soldati Cancer Research Foundation; advanced training grant from Geneva University Hospital to NG, NIH/NIDDK R01 DK099558; and the Irma T Hirschl Trust to YH.

Conflict-of-interest statement: The authors have no conflict of interest related to the manuscript.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

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

Received: April 25, 2015
Peer-review started: April 26, 2015
First decision: June 2, 2015
Revised: June 12, 2015
Accepted: August 30, 2015
Article in press: August 31, 2015
Published online: September 28, 2015

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.
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 18\[^{th}\] to 13\[^{th}\] 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 800,000 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\[^{5}\]. 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\[^{5}\].

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. 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. 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. 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, Kupffer cells, endothelial cells, platelets, and infiltrating immune cells through a wide variety of mediators.

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. Identifying subjects at higher risk of cirrhosis nodules, type of hepatic necrosis and 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

**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 or variceal hemorrhage, 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.

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). 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. 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, prior to non-hepatic surgery, in variceal bleeding, hepatorenal syndrome or mortality prediction in alcoholic hepatitis.

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...
| 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 (25%), Cryptogenic (15%) | 100% | Bilirubin, albumin, encephalopathy, ascites and prothrombin time/INR | | [91,92] |
|         | Prognostic Index | 5-yr mortality | Biliary (9%), Alcoholic (64%), Viral (24%), Other (12%) | 100% | Albumin, INR and creatinine | | [93] |
| HCV risk score | Bell et al | 5-yr mortality | HCV | 87% | Age, platelets, sex | | [94] |
|         | HVPG       | Mortality        | Alcohol (100%) | 100% | Age, alcohol abuse and alkaline phosphatase | 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-HBV (10%) | 18% | Fibrotest (Alpha-2-macroglobulin, Haptoglobin, Apolipoprotein A1, GGT, bilirubin, ALT), FIB-4 (AST, ALT, platelets, age), APRI (AST, platelets) Only predictive in subjects with Child-Pugh score of 5 | | [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) | | [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] |
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.\[^{46,49}\]

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...
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 possibly due to a loss of hepatocyte synthesis and a decrease in growth hormone receptors[56,57], and serum levels of IGF-1 reflect liver failure and risk of HCC[58]. Consistent with these findings, IGF1 is a member of a wider set of molecular variables to initially train/define prognostic models.

| Molecular method | Risk score | Liver disease etiology | Outcomes | Sample | Proportion HCC risk | 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%) | Improved model when added with clinical data: age, gender, smoking status, alkaline phosphatase level, platelet count | [67] |
| 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 | High risk (32%) Low risk (68%) | [60] |
| Activated HSC signature | EGF | HCV | HCC recurrence and survival | Frozen hepatic tissue | 87% | 37-gene signature | High risk (33%) 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%) | [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%) | Improved model when added with clinical markers: gender, smoking status, alkaline phosphatase level, platelet count | [69] |
| CAT              | HCV | HCC risk | Blood | 100% | CAT -262*C (rs1001179) | 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 (GC, 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.
signature was recently reported for its association with HCC recurrence and death\(^{(52)}\). 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\(^{(86,87)}\) 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\(^{(54,85)}\). 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.

REFERENCES

1. Scaglione S, Kliethemres S, Cao G, Shoham D, Durazo R, Luke A, Volk ML. The Epidemiology of Cirrhosis in the United States: A Population-based Study. J Clin Gastroenterol 2015; 49: 690-696 [PMID: 25291348 DOI: 10.1097/mcg.0000000000000208]

2. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013; 58: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]

3. Friedman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol 2010; 7: 425-436 [PMID: 20585339 DOI: 10.1038/nrgastro.2010.97]

4. Schuppan D. Afidhal NH. Liver cirrhosis. Lancet 2008; 371: 838-851 [PMID: 18328931 DOI: 10.1016/s0140-6736(08)60383-9]

5. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 385: 117-171 [PMID: 25530442 DOI: 10.1016/S0140-6736(14)61682-2]

6. D’Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol 2006; 44: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]

7. Garcia-Tsao G, Groszmann RJ, Fisher RL, Conn HO, Atterbury CE, Glickman M. Portal pressure, presence of gastroesophageal varices and variceal bleeding. Hepatology 1985; 5: 419-424 [PMID: 3873388]

8. Ginés P, Quintero E, Arroyo V, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural injury and intrinsic and extrinsic factors. Natural history and prognostic factors. Hepatology 1987; 7: 122-128 [PMID: 3804191]

9. Saunders JB, Walters JR, Davies AP, Paton A. A 20-year prospective study of cirrhosis. Br Med J (Clin Res Ed) 1981; 282: 263-266 [PMID: 6779978]

10. Mancebo A, González-Díezquez ML, Cadahía V, Varela M, Pérez R, Navascués CA, Sotorrio NG, Martínez M, Rodrigo L, Rodríguez M. Annual incidence of hepatocellular carcinoma among patients with alcoholic cirrhosis and identification of risk groups. Clin Gastroenterol Hepatol 2013; 11: 95-101 [PMID: 22982095 DOI: 10.1016/j.cgh.2012.09.007]

11. Fattovich G, Strollofili T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004; 127: S35-S50 [PMID: 15508101]

12. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? Hepatology 2014; 60: 1767-1775 [PMID: 24839253 DOI: 10.1002/hep.27222]
Goossens N et al. Molecular prognostication in cirrhosis

LL. Single-centre validation of the EASL-CLIF consortium definition of acute-on-chronic liver failure and CLIF-SOFa for prediction of mortality in cirrhosis. Liver Int 2015; 35: 1516-1523 [PMID: 26026737 DOI: 10.1111/liv.12957]

29 Ahn SY, Park SY, Tak WY, Lee YR, Kang EJ, Park JG, Lee WK, Lee K, Kweon YO. Prospective validation of Baveno V definitions and criteria for failure to control bleeding in portal hypertension. Hepatology 2015; 61: 1033-1040 [PMID: 25220468 DOI: 10.1002/hep.27441]

30 Smith JM, Biggins SW, Haselby DG, Kim WR, Wedd J, Lamb K, Thompson B, Segev DL, Gustafson S, Kandaswamy R, Stock PG, Matas AJ, Samana CJ, Sleeman EF, Stewart D, Harper A, Edwards E, Snyder JJ, Kasikse BL, Israni AK, Kidney, pancreas, and liver allocation and distribution in the United States. Am J Transplant 2012; 12: 3191-3212 [PMID: 23157207 DOI: 10.1111/j.1600-6143.2012.04259.x]

31 Freeman RB, Wienssner RH, Harper A, McDiarmed SV, Lake J, Edwards E, Merion R, Wolfe R, Turcott J, Teperman L. The new liver allocation system: moving toward evidence-based transplantation policy. Liver Transpl 2002; 8: 851-858 [PMID: 12206791 DOI: 10.1013/jbt.2002.39527]

32 Malinchoch K, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intraportal portosystemic shunts. Hepatology 2000; 31: 864-871 [PMID: 10733541 DOI: 10.1015/hce.2000.5852]

33 Said A, Williams J, Holden J, Remington P, Gangnon R, Musat A, Lucey MR. Model for end stage liver disease score predicts mortality across a broad spectrum of liver disease. J Hepatol 2004; 40: 897-903 [PMID: 15158328 DOI: 10.1016/j.jhep.2004.02.010]

34 Wiensner E, Edwards F, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R. Model for end-stage liver disease (MELD) and allocation of donors. Gastroenterology 2003; 124: 91-96 [PMID: 12512033 DOI: 10.1016/j.gastro.2002.09.002]

35 Teh SH, Nagorney DM, Stevens SR, Offerd KP, Thermeau TM, Plevak DJ, Talwalkar JA, Kim WR, Kamath PS. Risk factors for mortality after surgery in patients with cirrhosis. Gastroenterology 2007; 132: 1261-1269 [PMID: 17448652 DOI: 10.1015/j.gastro.2007.01.040]

36 Reverter E, Tandon P, Augustin S, Turon F, Casu S, Bastianpillai R, Keough A, Llop E, González A, Seijo S, Berzigotti A, Ma M, Malinchoc M. Single-centre validation of the EASL-CLIF consortium network score. J Hepatol 2005; 43: 1282-1289 [PMID: 15834937 DOI: 10.1016/hep.200877]

37 Dunn W, Jamil LH, Brown LS, Wienssner RH, Kim WR, Menon KV, Malinchoc M, Kamath PS, Shah V. MELD accurately predicts mortality in patients with alcoholic hepatitis. Hepatology 2005; 41: 353-358 [PMID: 15660383 DOI: 10.1002/hep.20503]

38 Sethasine S, Jain D, Grosszmann RJ, Garcia-Tsao G. Quantitative histological-hemodynamic correlations in cirrhosis. Hepatology 2012; 55: 1146-1153 [PMID: 22109744 DOI: 10.1002/hep.24805]

39 Rastogi A, Maiwall R, Bihari C, Ahuja A, Kumar A, Singh T, Wani ZA, Sarin SK. Cirrhosis histology and Laennec staging system correlate with high portal pressure. Histopathology 2013; 62: 731-741 [PMID: 23470026 DOI: 10.1111/his.12070]

40 Kim MY, Cho MW, Baik SK, Park HI, Jeon HK, Im CK, Won CS, Kim JW, Kim HS, Kwon SO, Eom MS, Cha SH, Kim YJ, Chang SJ, Lee SS. Histological subclassification of cirrhosis using the Laennec fibrosis scoring system correlates with clinical stage and grade of portal hypertension. J Hepatol 2011; 55: 1004-1009 [PMID: 21354227 DOI: 10.1016/j.jhep.2011.02.012]

41 Kim SU, Oh HJ, Wanless IR, Lee S, Han KH, Park YN. The Laennec staging system for histological subclassification of cirrhosis is useful for stratification of prognosis in patients with liver cirrhosis. J Hepatol 2012; 57: 556-563 [PMID: 22617153 DOI: 10.1016/j.jhep.2012.04.029]

42 Calvaruso V, Burroughs AK, Standish R, Manousou P, Grillo F, Leandro G, Mainmore S, Pleguezuelo M, Xirouchakis I, Guerini GP, Patch D, Yu D, O’Beirne J, Dhillon AP. Computer-assisted image analysis of liver collagen: relationship to Ishak scoring and hepatic venous pressure gradient. Hepatology 2009; 49: 1256-1264 [PMID: 19133646 DOI: 10.1002/hep.22745]
A 7-gene signature of the recipient predicts the progression of hepatitis C.

Liver Transpl 2014; 19: e32159 [PMID: 22403631 DOI: 10.1371/journal.pone.0032159]

Nahon P, Sutton A, Rufat P, Charnaux N, Mansouri A, Moreau R, Ganne-Carrié N, Grando-Lemaire V, N’Kontchou G, Trinchet JC, Pessayre D, Beaufang M. A variant in myeloidperoxidase promoter hastens the emergence of hepatocellular carcinoma in patients with HCV-related cirrhosis. J Hepatol 2012; 56: 426-432 [PMID: 21907168 DOI: 10.1016/j.jhep.2011.08.010]

Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Barnes HL, Minasian LM, Gazzano JM, Hartline JA, Parsons JK, Bearden JD, Crawford ED, Goodman GE, Claudio J, Winquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Alabanes D, Taylor PR, Probstfield JL, Jagal Tj, Crowley J, Meyskens FL, Baker LH, Colman CA. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009; 301: 39-51 [PMID: 19066370 DOI: 10.1001/jama.2008.864]

Di Biscaglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghain MG, Morishima C, Snow KK, Dienstag JL. Prolonged therapy of advanced chronic hepatitis C with low dose peginterferon. N Engl J Med 2008; 359: 2429-2441 [PMID: 19052125 DOI: 10.1056/NEJMoa0707615]

Tan PS, Nakagawa S, Goossens N, Venkatesh A, Huang T, Ward SC, Sun X, Song WM, Koh A, Canasto-Chibucque C, Deshmukh M, Nair V, Mahajan M, Zhang B, Fiel MI, Kobayashi M, Kumada H, Yoshida Y. Clinicopathological indices to predict hepatocellular carcinoma molecular classification. Liver Int 2015; Epap ahead of print [PMID: 26058462 DOI: 10.1111/liv.12889]

Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghain MG, Morishima C, Snow KK, Dienstag JL. Prolonged therapy of advanced chronic hepatitis C with low dose peginterferon. N Engl J Med 2008; 359: 2429-2441 [PMID: 19052125 DOI: 10.1056/NEJMoa0707615]

Friedel J, Recheister M, Frick L, Böhm F, Struckmann K, Egger M, Mohch H, Heienwaldler M, Weber A. Intratumor heterogeneity in hepatocellular carcinoma. Clin Cancer Res 2015; 21: 1951-1961 [PMID: 25248380 DOI: 10.1158/1078-0432.CCR-14-0122]

Sawyers CL, van’t Veer LJ. Reliable and effective diagnostics are keys to accelerating personalized cancer medicine and transforming cancer care: a policy statement from the american association for cancer research. Clin Cancer Res 2014; 20: 4978-4981 [PMID: 25204554 DOI: 10.1158/1078-0432.CCR-14-2295]

Parkinson DR, McCormack RT, Keating SM, Gutman SI, Hamilton SR, Mansfield EA, Piper MA, Deverka P, Frueh FW, Jessup JM, McShane LM, Tunis SR, Sigman CC, Kellogg GJ. Evidence of clinical utility: an unmet need in molecular diagnostics for patients with cancer. Clin Cancer Res 2014; 20: 1428-1444 [PMID: 24634466 DOI: 10.1158/1078-0432.CCR-13-2961]

Poste G. Bring on the biomarkers. Nature 2011; 469: 156-157 [PMID: 21228852 DOI: 10.1038/469156a]

Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009; 101: 1446-1452 [PMID: 19815849 DOI: 10.1093/jnci/djp335]

April C, Klotzle B, Royce T, Wichmann-Garcia E, Boyan_wifi T, Izzo J, Cox D, Jones W, Rubio R, Holton K, Matulonis U, Quackenbush J, Fan JB. Whole-genome expression profiling...
of formalin-fixed, paraffin-embedded tissue samples. PLoS One 2009; 4: e8162 [PMID: 19997620 DOI: 10.1371/journal.pone.0008162]
80 Reis PP, Wallace L, Goswami RS, Xu W, Xuan Y, Perez-Ordonez B, Guillaneau P, Irish J, Jurisica I, Kamel-Reid S. mRNA transcript quantification in archival sections of formalin-fixed paraffin-embedded (AS-FFPE) tissue for disease classification. PLoS One 2014; 9: e86961 [PMID: 24498002 DOI: 10.1371/journal.pone.0089691]
81 Goossens N, Nakagawa S, Sun X, Hoshida Y. Cancer biomarker discovery and validation. Transl Cancer Res 2015; In press
82 Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, Fell HP, Ferree S, George SD, Grogan T, James DJ, Maysuria M, Milton JD, Oliveri P, Osborn JL, Peng T, Ratcliffe AL, Webster PJ, Davidson EH, Hood L, Dimitrov K. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 2008; 26: 317-325 [PMID: 18278033 DOI: 10.1038/nbt1385]
83 Lander ES. Cutting the Gordian helix—regulating genomic testing in the era of precision medicine. N Engl J Med 2015; 372: 1185-1186 [PMID: 25689017 DOI: 10.1056/NEJMmp1501964]
84 Welker MW, Bechstein WO, Zeuzem S, Trojan J. Recurrent hepatocellular carcinoma after liver transplantation - an emerging clinical challenge. Transpl Int 2013; 26: 109-118 [PMID: 22994652 DOI: 10.1111/tji.12277.2013.01562.x]
85 Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 2013; 10: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]
86 Plaks V, Koopman CD, Werb Z. Cancer. Circulating tumor cells. Science 2013; 341: 1186-1188 [PMID: 24301008 DOI: 10.1126/science]
87 Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med 2015; 372: 793-795 [PMID: 25635347 DOI: 10.1056/NEJMmp1505023]
88 Dufour JF. Modern hepatomany. Gastroenterology 2013; 144: 876-878 [PMID: 23528666 DOI: 10.1053/j.gastro.2013.03.015]
89 Ruf AE, Kremers WK, Chavez LL, Descalzi VI, Podesta LG, Villamil FG. Addition of serum sodium into the MELD score and long-term survival in patients with nonbiliary cirrhosis. Transpl Proc 2005; 37: 520-527 [PMID: 16201348 DOI: 10.1002 transplant.2005.50093]
90 Yuen MF, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen Jc, But DY, Chan AO, Wong BC, Mizokami M, Lai CL. Independent risk factors and predictive score for the development of hepatocellular carcinoma in patients with chronic hepatitis B. J Hepatol 2009; 50: 80-88 [PMID: 18977053 DOI: 10.1016/j.jhep.2008.07.023]
91 Wong VW, Chan SL, Mo F, Chan TC, Loong HH, Wu YY, Chan AT, Sung JJ, Yeo W, Chan HL, Mok TS. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. Clin Oncol 2010; 28: 1660-1665 [PMID: 20194845 DOI: 10.1200/jco.2009.26.26757]
92 Yoon JH, Suh YS, Jang SJ, Jang HJ, Lee SH, Ahn SH, Chan CJ, WJG
93 Ikeda M, Fujiyama S, Tanaka M, Sata M, Ide T, Yatsuhashi H, Watanabe H. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. J Gastroenterol 2005; 40: 148-156 [PMID: 15770398 DOI: 10.1007/s00535-004-1519-2]
94 Chang KC, Hung CH, Lu SN, Wang JH, Lee CM, Chen CH, Yen MF, Lin SC, Yen YH, Tsai MC, Tseng PL, Hu TH. A novel predictive score for hepatocellular carcinoma development in patients with chronic hepatitis C after sustained response to pegylated interferon and ribavirin combination therapy. J Antimicrob Chemother 2012; 67: 2766-2772 [PMID: 22899800 DOI: 10.1093/jac/dks269]
95 Chang KC, Wu YY, Hung CH, Lu SN, Lee CM, Chiu KW, Tsai MC, Tseng PL, Huang CM, Cho CL, Chen HH, Hu TH. Clinical guide risk prediction of hepatocellular carcinoma development in chronic hepatitis C patients after interferon-based therapy. Br J Cancer 2013; 109: 2481-2488 [PMID: 24084770 DOI: 10.1038/bjc.2013.564]
