Comprehensive Comparative Analysis of Standard Validated, Genetic, and Novel Biomarkers to Enhance Prognostic Risk-Stratification in Patients With Hepatitis C Virus Cirrhosis

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INTRODUCTION: **Risk-stratifying patients with hepatitis C virus (HCV) cirrhosis according to medium-term prognosis will inform clinical decision-making. It is unclear which biomarkers/models are optimal for this purpose. We quantified the discriminative ability of 14 diverse biomarkers for prognosis prediction over a 4-year time.**

METHODS: **We recruited 1196 patients with HCV cirrhosis from the United Kingdom for a prospective study. Genetic risk score, collagen (e.g., PROC3), comorbidity (e.g., CirCom), and validated biomarkers from routine data were measured at enrollment. Participants were linked to UK hospital admission, cancer, and mortality registries. Primary endpoints were (i) liver-related outcomes for patients with compensated cirrhosis and (ii) all-cause mortality for decompensated cirrhosis. The discriminative ability of all biomarkers was quantified individually and also by the fraction of new prognostic information provided.**

RESULTS: **At enrollment, 289 (24%) and 907 (76%) had decompensated and compensated cirrhosis, respectively. Participants were followed for 3–4 years on average, with >70% of the follow-up time occurring post-HCV cure. Seventy-five deaths in the decompensated subgroup and 98 liver-related outcomes in the compensated subgroup were reported. The discriminative ability of the albumin-bilirubin-fibrosis-4 index (C-index: 0.71–0.72) was superior to collagen biomarkers (C-index = 0.58–0.67), genetic risk scores (C-index = 0.50–0.57), and comorbidity markers (0.53–0.60). Validated biomarkers showed the greatest prognostic improvement when combined with a comorbidity or a collagen biomarker (generally >30% of new prognostic information added).**

DISCUSSION: **Inexpensive biomarkers such as the albumin-bilirubin-fibrosis-4 index predict medium-term cirrhosis prognosis moderately well and outperform collagen, genetic, and comorbidity biomarkers. Improvement of performance was greatest when a validated test was combined with comorbidity or collagen biomarker.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A765 and http://links.lww.com/CTG/A766.

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INTRODUCTION

Liver cirrhosis is a major milestone in the natural history of chronic liver disease. It heralds a step change in the risk of multiple adverse health outcomes, such as bleeding varices, ascites, hepatic encephalopathy, hepatocellular carcinoma, and premature death (1). Patients with liver cirrhosis exhibit all-cause

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mortality rates that are 5 times greater than the general population (2). Yet, prognosis is highly variable; some patients live complication-free for more than 20 years, whereas others die shortly after diagnosis (1,3).

The ability to risk-stratify patients with cirrhosis is important and can inform clinical decision-making at multiple levels. A variety of biomarkers/models are currently available to clinicians that may be useful for risk-stratifying patients with cirrhosis in terms of their future prognosis. This includes aspartate aminotransferase-to-platelet ratio (APRI), fibrosis-4 (FIB-4), model for end-stage liver disease (MELD), albumin-bilirubin (ALBI), and Child-Pugh score. At present, it is not clear how suitable these biomarkers/models are for risk-stratifying patients with cirrhosis over a medium-term time horizon nor if some are superior to others; very few head-to-head comparisons have been performed up until now (4,5).

Previous research indicates that several additional prognostic factors/biomarkers, not routinely available to clinicians, may be useful for stratifying patients according to their risk of liver-related outcomes (LROs). This includes the Alcohol Use Disorder Identification Test (AUDIT) (6), Nordic biomarkers (7), CirCom comorbidity score (8), and genetic polymorphisms such as rs738409 (in PNPLA3), rs58542926 (in TM6SF2), and rs72613567 (in HSD17B13) (9–12). It is not known whether these enhanced biomarkers are able to improve risk-stratification, beyond what is possible with routine biomarkers.

To address these questions, we analyzed data from the STOP-hepatitis C virus (HCV) cirrhosis study, a prospective cohort of patients with HCV-related liver cirrhosis recruited from UK liver clinics. The main objective of this study was to evaluate the performance of validated biomarkers (APRI, FIB-4, computer tomography perfusion, MELD, MELD-sodium, ALB, and ALBI-FIB-4) for prognostic risk-stratification in a cohort of HCV-related cirrhosis followed for 4 years on average. A secondary objective was to explore the prognostic performance of factors that examine a wider breadth of information (serum markers of fibrogenesis, alcohol intake, comorbidity, and genetic risk polymorphisms) both in isolation and when added to existing validated biomarkers.

METHODS

Participants

The STOP-HCV cirrhosis study is a prospective longitudinal cohort study, comprising patients with HCV-related liver cirrhosis. Individuals were invited to participate in this study if they were (i) in attendance at 1 of 31 participating UK liver clinics for care/management of HCV infection between January 2015 and July 2016 and (ii) had been diagnosed with liver cirrhosis at the time of attendance (definition provided in Appendix A, Supplementary Digital Content 1, http://links.lww.com/CTG/A765 and see Supplemental Material, Supplementary Digital Content 2, http://links.lww.com/CTG/A766). Exclusion criteria for this prospective study were (i) actively waiting for a liver transplant, or (ii) had an isolated portal vein thrombosis, or (iii) unable to provide informed written consent.

In total, 1,255 participants were recruited from 31 liver clinics covering all geographical areas of the United Kingdom, excluding Northern Ireland. The estimated participant response rate was 75%.

Data collection at enrollment

Participants completed the AUDIT questionnaire and donated a 25-mL blood sample at enrollment. The blood sample was used to measure Nordic biomarkers and to generate host genotyping information using the Affymetrix UK Biobank Array.

Routine clinical information was extracted through medical chart review. This captured information on (i) detailed liver disease outcomes (i.e., instances of hepatic decompensation, hepatocellular carcinoma [HCC], and liver transplantations); (ii) achievement of sustained viral response (SVR) through antiviral therapy; (iii) routine liver blood tests; (iv) screening interventions including recent ultrasound and endoscopy examinations; (v) comorbid health conditions including heart failure, angina; diabetes, kidney disease, and also history of heavy alcohol use; and (vi) medications participants were taking on the day of enrollment.

The date of SVR achievement was defined as the date the treatment course leading to SVR was completed.

Linkage to NHS digital data

For study participants in England and Wales, we linked individual-level information acquired from the STOP-HCV cirrhosis study to individual-level information held on national registries in England and Wales. Of note, this included the admitted care hospital admission database, cancer registrations, and the mortality register held by National Health Service (NHS) Digital. Approval for this linkage was given by NHS Digital's Data Access Request Service. All participants consented to, and were successfully traced, for this linkage. At the time of analysis, cancer registrations, in-patient hospital admission, and mortality records were complete through April 1, 2017, April 1, 2018, and April 1 2019, respectively. All linked data were analyzed within the University of Glasgow's Safe Haven facility, using Stata version 12.

Study population and primary outcome events

The present analysis was confined specifically to STOP-HCV cirrhosis participants from England and Wales, where record linkage to national data registries held by NHS Digital was performed.

Participants were bifurcated into 2 groups, according to whether they had or had not experienced an LRO before enrollment. In other words, those patients without a previous LRO were assigned to the compensated cirrhosis group, whereas patients with a previous LRO were assigned to the decompensated cirrhosis group (Figure 1).

An LRO was defined as decompensation (i.e., ascites, bleeding varices, and hepatic encephalopathy), HCC, or a liver-related death. Information from patient medical records and national registries was used to ascertain whether each patient had presented with any of these conditions. The full definition for an LRO is provided in Table S1, Supplementary Digital Content 2, http://links.lww.com/CTG/A766.

We hereafter refer to patients without a previous LRO, as having compensated cirrhosis, and those with a previous LRO as having decompensated cirrhosis.

For the compensated cirrhosis group, the primary outcome event of interest was the first occurrence of an LRO. This outcome mirrors what patients are most interested in knowing: their risk of developing any serious morbidity event. For patients with decompensated cirrhosis, the primary outcome of interest was overall survival (or conversely, death from any cause). These outcome events align with the prediction/risk-stratification priorities for clinicians and patients at these 2 disease stages.

We also collected information on SVR achievement occurring after enrollment through medical notes. Again, date of SVR was defined as the date the treatment course leading to SVR was completed.

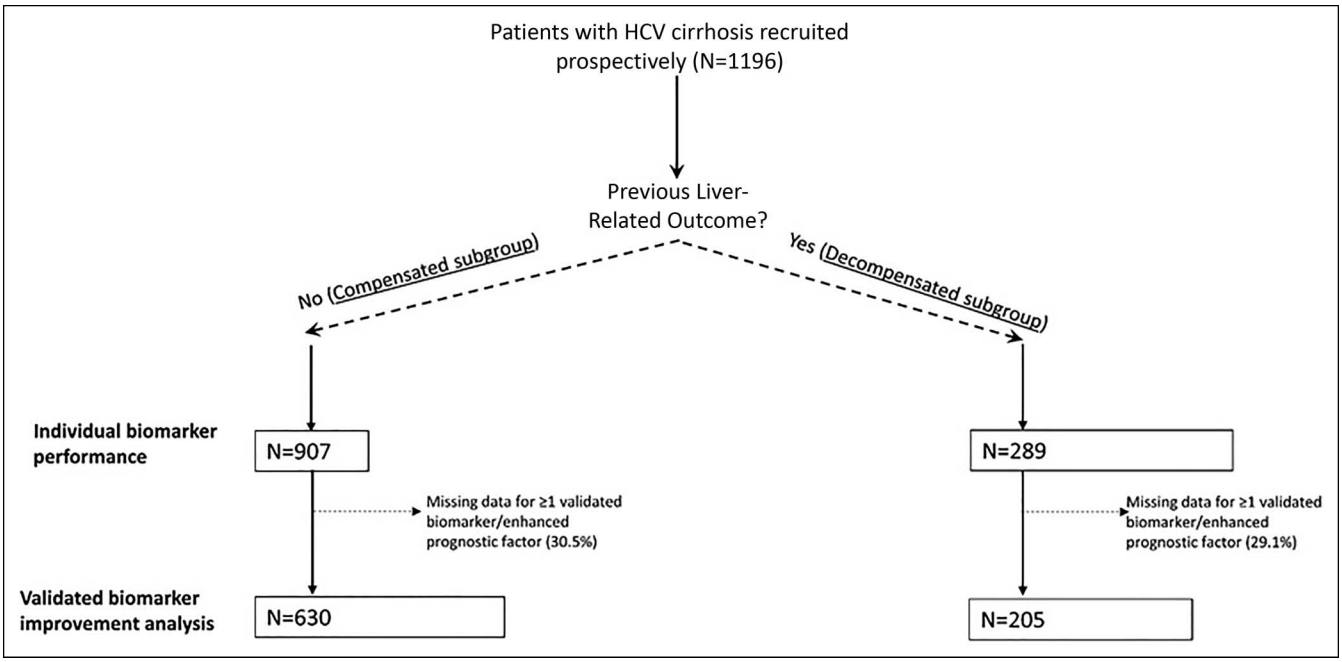

Figure 1. Derivation of the study cohort. Liver-related outcomes defined as previous ascites, bleeding varices, hepatic encephalopathy, or hepatocellular carcinoma.

Validated biomarkers

Validated biomarkers refer to those that can be calculated from tests available in routine clinical practice and that have previously been shown to confer prognostic accuracy/benefit. We assessed the risk-stratification ability of 7 such validated biomarkers. These were APRI, FIB-4, MELD, MELD-Na, ALBI, ALBI-FIB-4, and Child-Pugh-Turcotte. All biomarkers were calculated according to standard formula, using the most recent laboratory test performed before enrollment (but not more than 12 months previously). Further details are provided in Appendix B, Supplementary Digital Content 1, http://links.lww.com/CTG/A765 (see supplemental material, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

Enhanced biomarkers

Enhanced biomarkers refer to prognostic factors that are not routinely available/measured during routine clinical practice, but that have been indicated in previous studies to have prognostic value. We determined the performance of the following enhanced biomarkers: CirCom comorbidity score, AUDIT, Nordic biomarkers (PROC3, PROC6, and C4M2), the Huang et al.'s (13) "7-gene" genetic risk score (GRS), and Innes-Buch GRS (14).

AUDIT score was determined from the questionnaire completed at the date of study enrollment. CirCom is a comorbidity score developed by Jepson et al. (8) specifically for patients with liver cirrhosis. Hospital admission records in the 5 years before study enrollment were used to ascertain comorbidities for each patient and apply the CirCom algorithm. Nordic biomarkers and genetic polymorphisms were measured using the participant blood sample donated at enrollment. Two GRSs were assessed to gauge the utility of currently discovered genetic polymorphisms for risk-stratification. The first GRS was Huang et al.'s (13) "7-gene Cirrhosis Risk Score," developed in 2007 to stratify patients with chronic HCV according to their risk of liver cirrhosis. The second was a GRS recently developing by Innes and Buch et al. (14) which comprises 9 polymorphisms (e.g., in *PNPLA3*; *HSD17B13*; *TM6SF2*; and *MARC1*) associated with risk of progression to alcohol-related liver cirrhosis among individuals with high alcohol intake in the UK Biobank resource. Further details are provided in Appendix B, Supplementary Digital Content 1, http://links.lww.com/CTG/A765 (see supplemental data, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

STATISTICAL ANALYSIS

Survival analysis framework

All analyses were underpinned by survival analysis methods, with follow-up beginning at the date of study enrollment and ending at the date of outcome or registry completion. Specifically, we right-censored follow-up at April 2018 for the LRO analysis and April 2019 for all-cause mortality analysis. These dates reflect the completion dates of the relevant registries at the time of analysis.

Biomarker performance

Individual biomarker performance. Discrimination refers to the degree to which a score/biomarker can distinguish individuals who develop the outcome of interest from those who do not. First, we assessed the discrimination of each biomarker visually. As recommended by Royston et al. (15), we did this by plotting cumulative incidence for participants with low (<16th percentile), intermediate-low (16th–50th percentile), intermediate-high (50th–84th percentile), and high (>84th percentile) biomarker values. We also generated a *P* value to indicate whether these differences were statistically significant. We used Stata's "mi test" command to do this after fitting a univariate Cox model with biomarker category (low, intermediate-low, intermediate-high, and high) as the only independent variable. For this *P* value, the null hypothesis is that the risk of the outcome is equal in all 4 groups. Second, we determined each biomarker's discriminative ability quantitatively, using 2 independent metrics: Harrell's C-index and Royston-Sauerbrei D-statistic (16,17). Higher values

for Harrell's C-index indicate better discrimination; a value of 0.5 indicates zero discrimination (i.e., no better than chance), whereas a value of 1.0 indicates perfect discrimination. Similarly, higher values for Royston's D-statistic indicate greater discriminative ability. All biomarkers were handled as continuous variables when calculating these discrimination statistics.

All the above analyses were performed after multiple imputation procedure to replace missing data with plausible imputed values. We generated 20 imputations for each missing data point using either predictive mean matching (bilirubin, albumin, sodium, creatinine, platelet count, PROC3, PROC6, C4M2, aspartate aminotransferase, and alanine aminotransferase) or linear regression models (age and GRSs). Imputation was performed separately for the compensated and decompensated subgroups. All imputation models included the Nelson-Aalen estimate of the baseline cumulative hazard and the outcome variable as covariates. We used Rubin's rules to combine C-index and D-statistic estimates across imputation data sets. Similarly, Kaplan-Meier curves are based on the average estimate across the 20 imputation data sets created.

Improving performance of validated biomarkers. We assessed the degree to which validated biomarkers are improved by adding information on enhanced biomarkers. Thus, we fitted 1 model for each validated/enhanced biomarker combination. The amount of prognostic information provided by each combination model was quantified using the likelihood ratio statistic and compared with the likelihood ratio statistic for the validated biomarker only model. We also calculated Harrell's Adequacy Index, defined as: 1-(LR_{SB}/LR_{SB+EB}), where LR_{SB+EB} is the likelihood ratio statistic for the validated biomarker + enhanced biomarker model, and LR_{SB} is the likelihood ratio statistic for the validated biomarker model only (17). In this way, the adequacy index reflects the fraction of new prognostic information provided by each enhanced prognostic factor over and above the validated biomarker. All biomarkers were modeled as continuous variables, using Royston's multivariate fractional polynomial procedure to identify the optimal functional relationship with the outcome (linear or nonlinear) (18).

Of note, this analysis was only carried for participants with complete data for all validated biomarkers and enhanced biomarkers (n = 835). We did not use multiple imputation here because it is incompatible with the calculation of likelihood ratio statistics and also because there is no clear consensus on how to combine multiple imputation with Royston's multivariate fractional polynomial procedure.

Patient and public involvement

Patients with liver cirrhosis experience significant uncertain-future anxiety, driven by the prospect of developing liver cancer and dying prematurely (19–21). The STOP-HCV cirrhosis study aims to allay these concerns by providing patients with HCV cirrhosis with a clear and individualized picture of their likely prognosis. Patients were not directly involved in the design of this study. However, there has been patient representation on the STOP-HCV project steering group; thus, some patient oversight was/is present indirectly. There are no plans to disseminate the findings generated from this cohort to the study participants themselves.

RESULTS

Derivation of final sample size

The final sample comprised 1,196 patients with liver cirrhosis living in England or Wales. Of these, 75.8% (n = 907) had compensated cirrhosis at enrollment, and the remainder (24.2%, n = 289) had decompensated cirrhosis. Three hundred sixty-one (30.2%) individuals were missing data for ≥1 biomarkers; thus, our complete case analysis, used in the biomarker improvement analysis, was based on data for 835 participants (Figure 1).

Characteristics of final sample at enrollment

Participants in the final sample were mainly middle-aged (mean age was 56.1–57.4 years), male (69%–73% of male sex), and white (>80% were of white ethnicity; Table 1). About half had acquired their HCV infection through intravenous drug use, and more than two-fifths had a history of heavy alcohol use (defined as consuming >50 units/week for a sustained period of at least 6 months). Also, about two-fifths of participants had metabolic syndrome-related risk factors for liver disease, *viz* obesity and/or type 2 diabetes.

Follow-up data

Achievement of sustained viral response. At enrollment, 24.1% and 37.7% of compensated and decompensated participants had achieved SVR, respectively. This increased rapidly after enrollment to 66.5% and 68.5% in the compensated and decompensated subgroups, respectively (see Supplemental Table S2, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

Overall, more than 70% of the overall person-years of follow-up time occurred at the post-SVR stage (71% in the compensated subgroup and 76% in the decompensated subgroup).

Primary outcome events. Patients with compensated cirrhosis were followed up for 1,995 person-years (2.2 years per patient, on average; Table 2). Over this time, 98 patients experienced an LRO, equating to a crude rate of 4.91 per 100 person-years (95% confidence interval [CI]: 4.03–5.99). Half of the LROs occurred after SVR achievement (49.0%) (see Supplemental Table S3, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

Patients with decompensated cirrhosis were followed up for 1,034 person-years (3.6 years per patient, on average). Over this time, 75 patients died, equating to a crude mortality rate of 7.25 per 100 person-years (95% confidence interval: 5.78–9.09). Of these 75 deaths, 47 (62.7%) occurred after SVR achievement.

Biomarker performance

Individual biomarker performance. Most biomarkers were significantly associated with both an LRO in patients with compensated cirrhosis and all-cause mortality in patients with decompensated disease (see Supplemental Figure S1–S5 and Tables S4–S5, Supplementary Digital Content 2, http://links.lww.com/CTG/A766). Yet, their discriminative ability varied widely (Figure 2; see Supplemental Figure S5, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

The biomarker with the best discriminative ability was the ALBI-FIB-4 index. This had a C-index of 0.72 for differentiating LRO risk in patients with compensated cirrhosis and 0.70 for differentiating all-cause mortality risk in patients with decompensated cirrhosis. Accordingly, Figure 3 highlights the distinct risk profiles apparent for participants with low, intermediate-low, intermediate-high, and high ALBI-FIB-4 values. The MELD score, without sodium correction, was the weakest validated biomarker for both analyses; the C-index was 0.61 for the

Table 1. Description of final sample, according to compensated and decompensated cirrhosis at enrollment

Characteristic	Compensated cirrhosis (N = 907)		Decompensated cirrhosis (N = 289)	
	Mean/proportion	Number with missing data (%)	Mean/proportion	Number with missing data (%)
Sociodemographics				
Age, yr	56.1	6 (0.7)	57.4	4 (1.4)
% Male sex	72.9%	0 (0.0)	68.50%	0 (0.0)
% White ethnicity	80.4%	0 (0.0)	84.10%	0 (0.0)
Clinical factors				
% SVR achievement	24.1%	0 (0.0)	37.50%	0 (0.0)
On-treatment	29.5%	0 (0.0)	25.30%	0 (0.0)
% Encephalopathy	0.0%	0 (0.0)	15.90%	0 (0.0)
% With ascites	0.0%	0 (0.0)	43.30%	0 (0.0)
% Genotype 3 (past or current)	35.1%	61 (6.7)	46.80%	20 (6.9)
% Type 2 diabetes	17.9%	42 (4.6)	20.60%	8 (2.8)
Routine liver blood tests				
Platelet count (10^9/L)	151.2	48 (5.3)	105.80%	18 (6.2)
Albumin (g/L)	40.9	33 (3.6)	37.10%	14 (4.8)
Bilirubin (μmol/L)	15.3	33 (3.6)	23.10%	14 (4.8)
Sodium (mmol/L)	139.4	34 (3.7)	138.10%	14 (4.8)
ALT (U/L)	68.2	61 (6.7)	48.90%	21 (7.2)
AST (U/L)	71.0	56 (6.2)	61.30%	23 (8.0)
Creatinine (≥μmol/L)	74.0	35 (3.9)	77.70%	13 (4.4)
INR	1.29	0 (0.0)	1.40%	0 (0.0)
Health behaviors/liver disease risk factors				
% History of heavy alcohol use	37.9%	61 (6.7)	54.50%	14 (4.8)
% History of IVDU	49.9%	57 (6.3)	48.40%	16 (5.5)
% Current smoker	45.3%	75 (8.3)	43.10%	20 (6.9)
BMI	27.9	174 (19.2)	28.00%	51 (17.6)
% Obese or with type 2 diabetes	41.3%	180 (19.8)	42.90%	51 (17.6)
Validated biomarkers				
FIB-4	6.0	106 (11.7)	6.80%	45 (15.6)
APRI	2.3	87 (9.6)	2.10%	37 (12.8)
MELD	9.8	33 (3.6)	11.60%	14 (4.8)
MELD-Na	10.1	33 (3.6%)	12.30%	14 (4.8)
ALBI	−2.8	34 (3.7%)	−2.30%	14 (4.8)
ALBI-FIB-4	−2.7	108 (11.9%)	−2.00%	45 (15.6)
CTP	5.7	27 (3.0%)	6.70%	6 (2.1)
Enhanced prognostic factors				
PRO-C3	19.9	49 (5.4%)	21.8	16 (5.5)
PRO-C6	10.3	49 (5.4%)	14.2	16 (5.5)
C4M2	33.5	49 (5.4%)	37.9	16 (5.5)
CirCom	0.37	0 (0.0%)	0.76	0 (0.0)
AUDIT score	3.3	93 (10.3%)	3.2	25 (8.7)
Huang et al. GRS	0.63	98 (10.8%)	0.60	29 (10.0)
Innes-Buch GRS	0.46	109 (12.0%)	0.47	31 (10.7)

N.B validated biomarkers refer to those that can be calculated from tests available in routine clinical practice and that have previously been shown to confer prognostic accuracy/benefit.
All values in the table relate specifically to the baseline time point (i.e. study enrollment)—this includes data on SVR achievement.
ALBI, albumin-bilirubin; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio; AST, aspartate aminotransferase; AUDIT, Alcohol Use Disorder Identification Test; BMI, body mass index; CTP, Child-Turcotte-Pugh score; FIB-4, fibrosis-4; GRS, genetic risk score; INR, internationalized normal ratio; IVDU, intravenous drug users; MELD, model for end-stage liver disease; MELD-Na, model for end-stage liver disease-sodium; SVR, sustained viral response.

Table 2. Description of follow-up data and outcome events observed for patients with compensated and decompensated cirrhosis at enrollment

			Person-years (PYs) Fu			Outcome	
Subgroup	Outcome event	Total persons	Total	Mean per patient	Median per patient	# Events	Crude rate, per 100 PYs (95% CI)
Compensated cirrhosis	Liver-related outcome	907	1995	2.2	2.3	98	4.91 (4.03–5.99)
Decompensated cirrhosis	All-cause mortality	289	1,034	3.6	4.1	75	7.25 (5.78–9.09)

CI, confidence interval.

compensated cirrhosis analysis and 0.64 for the decompensated cirrhosis analysis (Figure 2).

There was also wide variability in the discriminative performance of enhanced biomarkers. For example, ranging from Huang et al. GRS (C-stat for differentiating LRO risk in compensated cirrhosis:0.51) to PROC6 (C-stat for differentiating LRO risk in compensated cirrhosis: 0.66).

Validated biomarkers were generally superior to enhanced biomarkers at risk-stratifying patients with cirrhosis (Figure 2). The best performing validated biomarker (i.e. ALBI-FIB-4) had considerably better discriminative ability than the best-performing enhanced biomarker (i.e., PROC6).

Except for MELD and Child-Pugh-Turcotte, biomarkers generally performed better in the compensated cirrhosis analysis vs the decompensated cirrhosis analysis (Figure 4).

Finally, there were no appreciable differences between the individual biomarker performance observed in our base-case analysis (using multiple imputation), compared with the complete-case analysis restricting to participants with complete data for each biomarker (see Supplemental Figures S6–S9, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

Improving performance of validated biomarkers. The fraction of new prognostic information provided by adding enhanced biomarkers to validated biomarkers was greatest in relation to adding CirCom, Audit, and Nordic biomarkers (generally >30% of new information added by these biomarkers). Conversely, GRSs added relatively little additional prognostic information (<10% in general; Figure 5 and Supplemental Figure S10, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

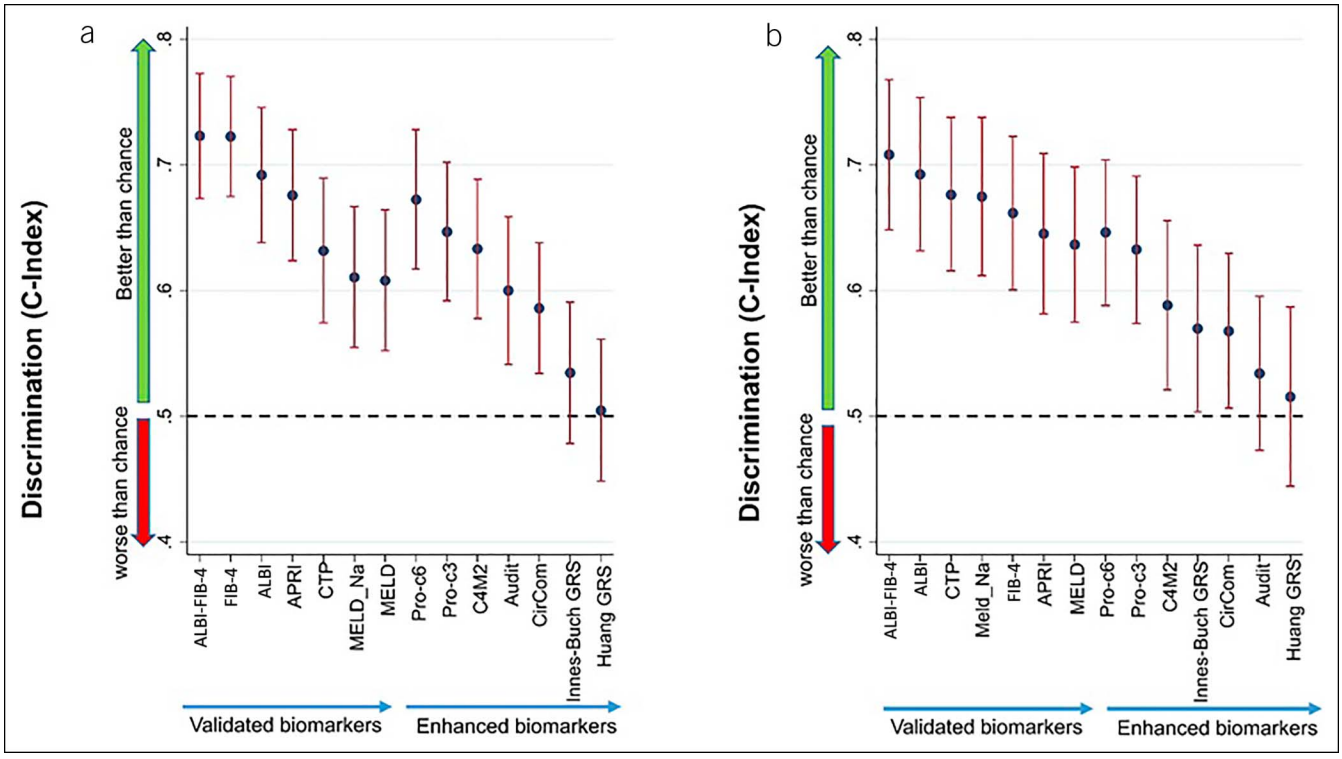

Figure 2. Biomarker discrimination for predicting (**a**) liver-related outcomes in patients with compensated cirrhosis and (**b**) all-cause mortality in patients with decompensated cirrhosis. Validated and enhanced biomarkers are ordered from left to right in order of descending C-index values. Higher C-index values indicate better discrimination (and vice versa). ALBI, albumin-bilirubin; APRI, aspartate aminotransferase-to-platelet ratio; AUDIT, Alcohol Use Disorder Identification Test; CTP, Child-Turcotte-Pugh score; FIB-4, fibrosis-4; GRS, genetic risk score; MELD, model for end-stage liver disease; MELD-Na, model for end-stage liver disease-sodium.

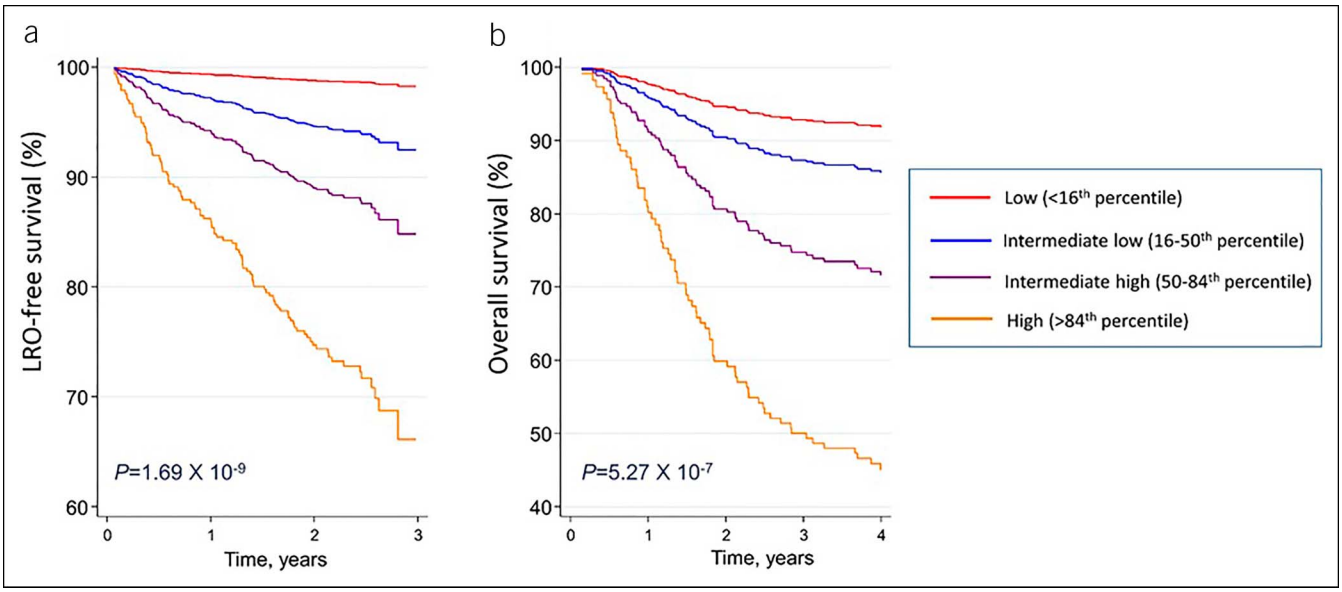

Figure 3. (**a**) Liver-related outcome–free survival in patients with compensated cirrhosis. (**b**) Overall survival in patients with decompensated cirrhosis, according to low, intermediate-low, intermediate-high, and high albumin-bilirubin-fibrosis-4 values. Survival curves are based on the Kaplan-Meier estimate. LRO, liver-related outcome.

In a *post hoc* analysis, we also assessed how much new prognostic information is provided by adding Nordic biomarkers to validated biomarker + CirCom models. Against this higher benchmark, the fraction of new prognostic information provided by Nordic biomarkers still remained considerable (generally >10%; see Supplemental Figure S11, Supplementary Digital Content 2, http://links.lww.com/CTG/A766).

DISCUSSION

Liver cirrhosis is a gateway to a variety of major sequelae including decompensation, hepatocellular carcinoma, and premature mortality (1). However, the likelihood of developing these complications can be highly variable from 1 patient with cirrhosis to the next (1,2). The ability to differentiate higher risk patients from lower risk patients *ex ante* over a relevant time frame is the cornerstone on which any risk-centered/precision medicine approach to managing patients with cirrhosis will ultimately be built. Currently, most hepatologists have recourse to a variety of validated biomarkers/models, including FIB-4, ALBI, and MELD. Yet, there is no consensus around which of these validated biomarkers are optimal when the goal is to risk-stratify patients with cirrhosis according to medium-term prognosis of approximately 5 years.

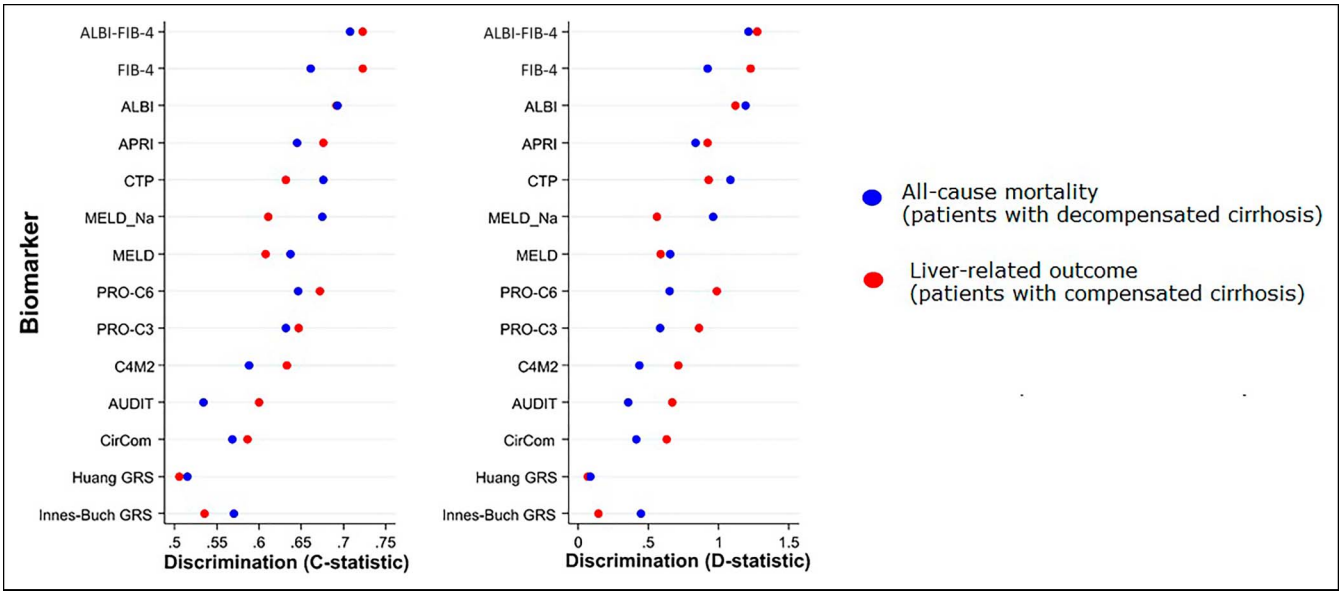

Figure 4. Biomarker discrimination for predicting liver-related outcomes in patients with compensated cirrhosis and all-cause mortality in patients with decompensated cirrhosis. ALBI, albumin-bilirubin; APRI, aspartate aminotransferase-to-platelet ratio; AUDIT, Alcohol Use Disorder Identification Test; CTP, Child-Turcotte-Pugh score; FIB-4, fibrosis-4; GRS, genetic risk score; MELD, model for end-stage liver disease; MELD-Na, model for end-stage liver disease-sodium.

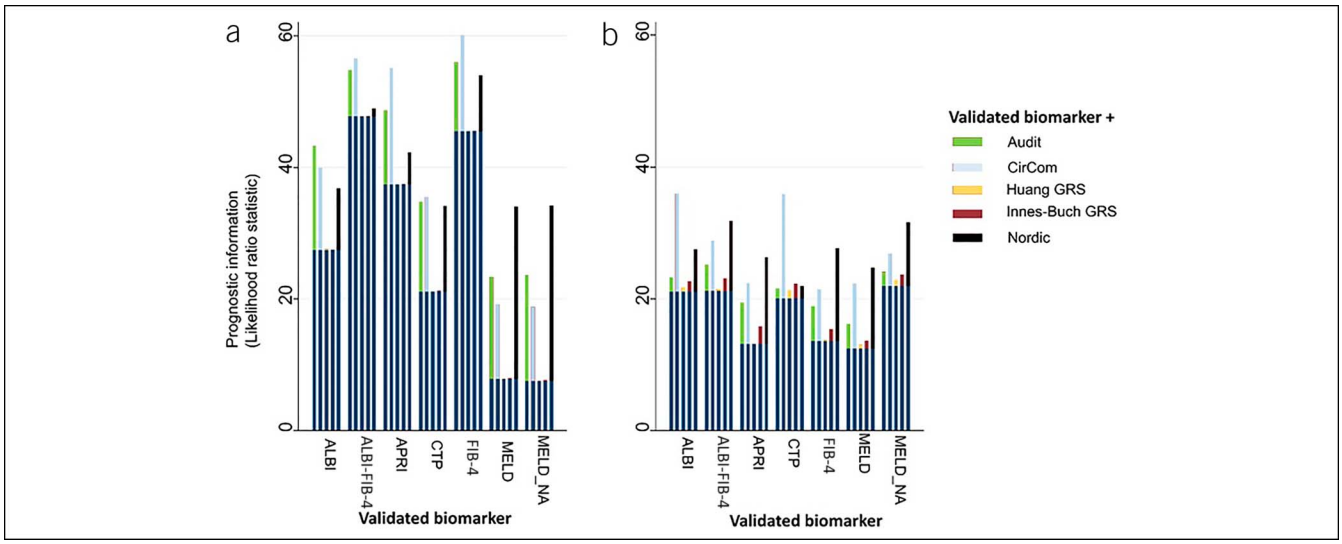

Figure 5. New prognostic information gained by adding an enhanced biomarker to a validated biomarker, when predicting (**a**) liver-related outcomes in patients with compensated cirrhosis and (**b**) all-cause mortality in patients with decompensated cirrhosis. The y axis indicates the amount of prognostic information provided by each model. Specifically, it is the difference between the likelihood ratio statistic of the validated biomarker model and the likelihood ratio statistic of the null model (a Cox model with no covariates). The additional portion of each bar indicates the increase in this quantity when the validated biomarker model is replaced with a validated biomarker + enhanced biomarker model (i.e., a model including the validated and enhanced biomarker as covariates). ALBI, albumin-bilirubin; APRI, aspartate aminotransferase-to-platelet ratio; AUDIT, Alcohol Use Disorder Identification Test; CTP, Child-Turcotte-Pugh Score; FIB-4, fibrosis-4; GRS, genetic risk score; MELD, model for end-stage liver disease; MELD-Na, model for end-stage liver disease-sodium.

In this study, we quantified the ability of 14 biomarkers to separate patients with cirrhosis with cured HCV according to their prognosis over a 3- to 4-year time horizon. We found that validated biomarkers, derived from inexpensive routine laboratory measures, can discriminate medium-term prognosis moderately well, with C-indexes mostly exceeding 0.65. The best of these biomarkers was the ALBI-FIB-4 index (22) with a C-index of 0.72 and 0.70 in the compensated and decompensated disease analysis, respectively. Using ALBI-FIB-4, we show that it is possible to categorize patients *ex ante* into groups with clearly distinct risk profiles (Figure 3). This has important clinical implications because it highlights the latent potential to manage HCV cirrhosis in a more individualized manner by using existing biomarkers to which most clinicians already have access.

Recent studies have proposed a number of more innovative biomarkers that are not currently routinely available to/collected by hepatologists, but arguably should be. This includes GRSs (14), Nordic biomarkers (7), and comorbidity scores (8). Thus far, these biomarkers either lack external validation (the acid test of performance) or have not been compared like-for-like with existing alternatives. In this study, we have tried to tackle these gaps in the evidence base. In general, we found that as single variables, these enhanced biomarkers performed no better than existing validated biomarkers such as ALBI-FIB-4 and, in most cases, performed considerably worse. We also examined the degree to which enhanced biomarkers could augment the performance of validated biomarkers when considered in combination. In general, our results indicate that adding Nordic biomarkers or CirCom to a validated biomarker led to the greatest improvements in model performance. Consequently, these biomarker combinations may be worth considering in future studies. Naturally, however, any decision to bring a new prognostic test into clinical practice must trade-off incremental prognostic benefit against opportunity cost (both in terms of economic value and ease of implementation). We believe that the analyses outlined in this study provide a useful framework for assessing the incremental benefit aspect of this trade-off.

To the best of our knowledge, only 2 studies have quantified the performance of multiple and diverse competing biomarkers for predicting liver disease complications over a longer-term time horizon in chronic HCV (4,5). This includes an analysis of 1,457 patients with chronic HCV by Vergniol et al. (4), where the authors compared the ability of APRI, liver biopsy, FibroTest, FibroScan, and FIB-4 to discriminate patients in terms of their 5-year survival status. All biomarkers were found to be competent at predicting survival in this study; however, FibroTest and FibroScan performed the best with area under the receiver operating characteristic values of 0.80 and 0.82, respectively. By contrast, the area under the receiver operating characteristic values for APRI and FIB-4 were 0.75 and 0.66, respectively. Similarly, a study by Fontana et al. assessed performance of hyaluronic acid, TIMP-1, and YKL-40, as well as other biomarkers, in predicting HCV-related liver disease progression in patients with previous nonresponse to pegylated interferon and ribavirin (5). They found that baseline hyaluronic acid and platelet counts were best at predicting disease progression, but area under the curve values were relatively modest at $\leq$0.663. Our current study has some important distinctions to these previous analyses. First, most individuals in the studies by Fontana et al. and Vergniol et al. were noncirrhotic (80%). Second, both studies were conducted in the pre-direct acting antiviral era before HCV cure became the norm and not the exception. Third, the study by Vergniol et al. was only able to investigate survival as an outcome and did not consider episodes of cirrhosis-related morbidity as we did in this study. Thus, it is our view that the present analysis fills an important gap in the literature. Nevertheless, much more research is still needed in this area.

The main limitation of this study is that patients were followed up from study enrollment, whereas our analysis would probably have been more clinically relevant if we had followed patients up

specifically from SVR achievement. Unfortunately, we were not able to perform a viable analysis after patients up from the point of SVR achievement in this cohort. There were 2 main reasons for this. First, a sizeable number of patients without biomarker data sufficiently close to the date of SVR achievement would have had to be excluded. Second, patients who had already achieved SVR at study enrollment would have had to be exclude because the discrimination statistics central to our analysis (i.e., the C-index) are not compatible with delayed entry survival data. Nevertheless, in our current analysis, the majority of both follow-up time and outcome events take place at the post-SVR stage, and in this sense, our cohort is more reflective of post-SVR liver disease than pre-SVR liver disease. However, our findings should be replicated in a cohort where all patients are followed up from the point of SVR. A second limitation is that some important biomarkers were not included in this study. In particular, the enhanced liver fibrosis test by Siemens was not available for this study. FibroScan was also not considered because these data were missing for most participants at the enrollment time point when it was not part of standard of care. We also did not have data on α-fetoprotein, which is a relevant biomarker for HCC risk. Third, our definition of severe LROs combines liver failure and liver cancer, which are 2 biologically distinct endpoints. Thus, the predictors for 1 event may not be the same as the predictors for the other event (and vice versa). However, our current approach is rooted in what many patients want to know regarding their future prognosis—that is their risk of developing any type of severe liver morbidity. A fourth limitation is that we did not consider whether change/trend in biomarker values before baseline can provide prognostic information over and above its absolute value at baseline; this would be worthy of further research. Finally, the GRSs examined in this study did not perform very well, either individually or when added to a validated biomarker. However, it is important to point out that these scores were developed to predict a different outcome from those considered in this study. Finally, we did not take account of competing risk events such as nonliver mortality on liver transplantation in this analysis. This may have affected our results.

Our study has 3 important strengths. First, we recruited participants prospectively from a representative set of UK clinics. Second, we leveraged outcome data held in robust national health registries and information from medical records. A third key strength is the sizeable breadth of biomarker data considered, capturing information on liver enzymes, synthetic liver function, platelet count, fibrosis markers, genetics, comorbidity, and alcohol consumption. Despite its limitations, therefore, this study is unique and represents an important contribution to the current literature.

In conclusion, this study has quantified the ability of 14 different biomarkers for stratifying patients with cirrhosis with cured HCV according to their medium-term prognosis. We show that there is a wide performance spectrum, but also highlight that inexpensive routine biomarkers, particularly ALBI-FIB-4, offer reasonable discriminative power over 3- to 4-year time frame.

CONFLICTS OF INTEREST

Guarantor of the article: Indra Neil Guha, PhD.

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Data availability statement: The STOP HCV consortium welcomes collaboration with interested parties. Anonymized samples and clinical data held on the study database are accessible on successful application to the HCVRUK Tissue Data Access Committee (TDAC). However, we cannot share linked NHS Digital data or any data derived from linked NHS digital data. HCVRUK operate a cost-recovery system in relation to providing data and/or biological samples to researchers. More information on the STOP consortium can be found at our web site [http://www.stop-hcv.ox.ac.uk]. Contact Dr Neil Guha (neil.guha@nottingham.ac.uk) or Prof Will Irving (will.irving@nottingham.ac.uk) regarding prospective TDAC applications for data/samples from this cohort.

Study Highlights

WHAT IS KNOWN

- ✓ Patients with cirrhosis and cured hepatitis C virus remain at higher risk of liver-related morbidity and mortality.
- ✓ Risk-stratification biomarkers are urgently needed to inform long-term follow-up.

WHAT IS NEW HERE

- ✓ Validated biomarkers (e.g., albumin-bilirubin-fibrosis-4 index) are effective at discriminating between patients with hepatitis C virus cirrhosis with good vs poor prognosis.
- ✓ Collagen biomarkers (i.e., Nordic Pro-C6, PROC3, and C4M2) are outperformed by routine biomarkers.
- ✓ Genetic risk scores are outperformed by routine biomarkers.

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