# Comprehensive comparative analysis of standard validated, genetic, and novel biomarkers to enhance prognostic risk-stratification in patients with hepatitis C cirrhosis

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## TITLE

Comprehensive comparative analysis of standard validated, genetic and novel biomarkers to enhance prognostic risk-stratification in patients with hepatitis C cirrhosis.

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## ABSTRACT

Objective:

Risk-stratifying patients with 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 horizon.

Design:

1196 HCV cirrhosis patients from the UK were recruited into a prospective study. Genetic-risk-score, collagen (e.g. PROC3), comorbidity (e.g. CirCom) and validated biomarkers from routine data (e.g. ALBI-FIB4 index) were measured at enrolment. Participants were linked to UK hospital-admission, cancer and mortality registries. Primary endpoints were: (i) Liver-Related Outcome (LRO), for compensated cirrhosis patients; and (ii) All-cause mortality, for decompensated cirrhosis. The discriminative ability of all biomarkers were quantified individually and also by the fraction of new prognostic information provided.

Results:

At enrolment, 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. 75 deaths in decompensated subgroup and 98 LROs in the compensated subgroup were reported. The discriminative ability of ALBI-FIB4 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).

Conclusion:

Inexpensive biomarkers such as the ALBI-FIB4 index predict medium-term cirrhosis prognosis moderately well, and outperform collagen, genetic and co-morbidity biomarkers. Improvement of performance was greatest when a validated test was combined with comorbidity or collagen biomarker.

KEYWORDS:

Cirrhosis; Hepatitis C; Liver function tests; Viral hepatitis; Liver fibrosis; Liver.

**STUDY HIGHLIGHTS**

WHAT IS KNOWN:

- Patients with cirrhosis and cured hepatitis C 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. ALBI-FIB4 index) are effective at discriminating between HCV cirrhosis patients with good versus poor prognosis.
- Collagen biomarkers (i.e. Nordic Pro-C6, PROC3 and C4M2) are outperformed by routine biomarkers
- Genetic risks scores are outperformed by routine biomarkers.

## 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 mortality rates that are five times greater than the general population. [2] Yet prognosis is highly variable – i.e. some patients live complication-free for more than twenty years, whilst 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 cirrhosis patients in terms of their future prognosis. This includes APRI, FIB4, MELD, ALBI and Child Pugh score. At present, it is not clear how suitable these biomarkers/models are for risk stratifying cirrhosis patients 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. 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 if these enhanced biomarkers are able to improve risk stratification, beyond what is possible with routine biomarkers.

To address these questions, we analysed data from the STOP-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; FIB4; CTP; MELD; MELD_Na; ALBI; ALBI-FIB4) 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, co-morbidity and genetic risk polymorphisms) both in isolation, and when added to existing validated biomarkers.

## METHODS:

STOP-HCV CIRRHOSIS PROSPECTIVE STUDY

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 one of 31 participating UK liver clinics for care/management of HCV infection between Jan 2015 and July 2016; and (ii) had been diagnosed with liver cirrhosis at the time of attendance (definition provided in Appendix A). 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, 1255 participants were recruited from 31 liver clinics covering all geographical areas of the UK excluding Northern Ireland; the estimated participant response rate was 75%.

DATA COLLECTION AT ENROLMENT:

Participants completed the AUDIT questionnaire and donated a 25ml blood sample at enrolment. 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, 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); (vi) medications participants were taking on the day of enrolment.

The date of sustained viral response (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 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 to 1st April 2017, 1st April 2018, and 1 April 2019, respectively. All linked data were analysed 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 STOPHCV cirrhosis participants from England and Wales, where record linkage to national data registries held by NHS Digital was performed.

Participants were bifurcated into two groups, according to whether they had or had not experienced a Liver Related Outcome (LRO) prior to enrolment. In other words, those patients without a prior LRO were assigned to the compensated cirrhosis group, whereas patients with a prior LRO were assigned to the decompensated cirrhosis group (See Figure 1).

A LRO was defined as: decompensation (i.e. ascites, bleeding varices, hepatic encephalopathy); hepatocellular carcinoma (HCC); or a liver-related death. Information from patient medical records and national registries were used to ascertain if each patient had presented with any of these conditions. The full definition for a LRO is provided in Table S1.

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

For the compensated cirrhosis group, the primary outcome event of interest was the first occurrence of a LRO. This outcome mirrors what patient are most interested in knowing – i.e. 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 two disease stages.

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

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 seven such validated biomarkers. These were: i) Aspartate-aminotransferase to Platelet Ratio Index (APRI); ii) FIB-4; iii) Model for End-stage Liver Disease (MELD); iv) MELD with sodium correction (MELD_Na); v) ALBI; vi) ALBI-FIB4; and vii) Child Pugh Turcotte (CPT). All biomarkers were calculated according to standard formulae, using the most recent laboratory test carried out prior to enrolment (but not more than 12 months previously). Further details are provided in Appendix B.

ENHANCED BIOMARKERS:

Enhanced biomarkers refer to prognostic factors that are not routinely available/measured during routine clinical practice, but that have been indicated in prior studies to have prognostic value. We determined the performance of the following enhanced biomarkers: (i) CirCom co-morbidity score;

(ii) AUDIT; (iii) Nordic biomarkers (PROC3, PROC6 and C4M2); iv) the Huang et al's "seven-gene" Genetic risk score (GRS) [13]; and (v) Innes-Buch GRS. [14]

AUDIT score was determined from the questionnaire completed at the date of study enrolment. CirCom is a comorbidity score developed by Jepson et al, specifically for patients with liver cirrhosis.[8] Hospital admission records in the five years prior to study enrolment 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 enrolment. Two GRSs were assessed to gauge the utility of currently discovered genetic polymorphisms for risk stratification. The first GRS was Huang et al's "Seven-gene Cirrhosis Risk Score", developed in 2007 to stratify patients with chronic HCV according to their risk of liver cirrhosis. [13] The second, was a GRS recently developing by Innes and Buch et al; .it comprises 9 polymorphisms (e.g. in *PNPLA3*; *HSD17B13*; *TM6SF2*; *MARC1*) associated with risk of progression to alcohol-related liver cirrhosis among individuals with high alcohol intake in the UK Biobank resource.[14] Further details are provided in appendix B.

## STATISTICAL ANALYSIS:

### SURVIVAL ANALYSIS FRAMEWORK

All analyses were underpinned by survival analysis methods, with follow-up beginning at the date of study enrolment 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 BIOMARKERS 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 ($<16^{th}$ percentile), intermediate low ($16\text{-}50^{th}$ percentile), intermediate high ($50\text{-}84^{th}$ percentile) and high ($>84^{th}$ percentile) biomarker values. We also generated a P-value to indicate if these differences were statistically significant. We used Stata's "mi test" command to do this after fitting a univariate Cox model with biomarker category (i.e. 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 four groups. Second, we determined each biomarker's discriminative ability quantitatively, using two 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), whilst 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 twenty imputations for each missing data point using either predictive mean matching (for: bilirubin, albumin, sodium, creatinine, platelet count, PROC3, PROC6, C4M2; AST, ALT); linear regression models (for: age, and GRSs). Imputation was performed separately for the compensated and decompensated subgroups. All imputations 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 datasets. Similarly, Kaplan Meier curves are based on the average estimate across the 20 imputation datasets 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 one 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 to 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 modelled as continuous variables, using Royston's multivariate fractional polynomial procedure to identify the optimal functional relationship with the outcome (i.e. whether linear or non-linear).[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 HCV cirrhosis patients with a clear and individualised 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 1196 patients with liver cirrhosis living in England or Wales. Of these, three quarters (75.8%, n=907) had compensated cirrhosis at enrolment, and the remainder (24.2%, n=289) had decompensated cirrhosis. 361 (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; See Figure 1.

### CHARACTERISTICS OF FINAL SAMPLE AT ENROLMENT:

Table 1 indicates that participants in the final sample were mainly middle-aged (mean age was 56.1 to 57.4 years) male (69% to 73% of male gender) and white (>80% were of white ethnicity). 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 six 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 enrolment, 24.1% and 37.7% of compensated and decompensated participants had achieved SVR, respectively. This increased rapidly post-enrolment to 66.5% and 68.5% in the compensated and decompensated subgroup, respectively (Table S2).

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

### PRIMARY OUTCOME EVENTS

Table 2 shows that patients with compensated cirrhosis were followed-up for 1,995 person-years (2.2 years per patient, on average). Over this time, 98 patients experienced an LRO, equating to a crude rate of 4.91 per 100 person years (95% CI: 4.03-5.99). Half of the LROs occurred following SVR achievement (49.0%) (Table S3).

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% CI: 5.78-9.09). Of these 75 deaths, 47 (62.7%) occurred following SVR achievement.

BIOMARKER PERFORMANCE:

INDIVIDUAL BIOMARKER PERFORMANCE:

Most biomarkers were significantly associated with both: (i) a LRO in patients with compensated cirrhosis, and (ii) all-cause mortality in patients with decompensated disease (see Figure S1-S5; Tables S4-S5). Yet, their discriminative ability varied widely -see Figure 2 & Figure S5.

The biomarker with the best discriminative ability was the ALBI-FIB4 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-FIB4 values. The MELD score, without sodium correction, was the weakest validated biomarker for both analyses; i.e. the C-index was 0.61 for the compensated cirrhosis analysis, and 0.64 for the decompensated cirrhosis analysis (See 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).

Figure 2 shows that validated biomarkers were generally superior to enhanced biomarkers at risk-stratifying cirrhosis patients; for example, the best performing validated biomarker (i.e. ALBI-FIB4) had considerably better discriminative ability than the best performing enhanced biomarker (i.e. PROC6).

With the exception of MELD and CPT, biomarkers generally performed better in the compensated cirrhosis analysis, versus the decompensated cirrhosis analysis. This is illustrated in Figure 4.

Finally, there were no appreciable differences between the individual biomarker performance observed in our base-case analysis (using multiple imputation), compared to the complete-case analysis restricting to participants with complete data for each biomarker. This is shown in Figures S6-S9.

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, genetic risk scores added relatively little additional prognostic information (<10% in general)- see Figure 5 and Figure S10.

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%; Figure S11).

## 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 one cirrhosis patient 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-centred"/"precision medicine" approach to managing cirrhosis patients will ultimately be built. Currently, most hepatologists have recourse to a variety of validated biomarkers/models, including FIB-4, ALBI, MELD, etc. Yet there is no consensus around which of these validated biomarkers are optimal when the goal is to risk stratify cirrhosis patients according to medium term prognosis of approximately five years. In this study we quantified the ability of 14 biomarkers to separate cirrhosis patients with cured hepatitis C according to their prognosis over a 3-4 year time horizon. We found that validated biomarkers, derived from inexpensive routine laboratory measures, are able to discriminate medium-term prognosis moderately well, with C-indexes mostly exceeding 0.65. The best of these biomarkers was the ALBI-FIB4 index [22] with a C-index of 0.72 and 0.70 in the compensated and decompensated disease analysis, respectively. Using ALBI-FIB4, we show that it is possible to categorise 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 individualised manner; i.e. by using existing biomarkers that most clinicians already have access to.

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 genetic risk scores[14], Nordic biomarkers[7], and comorbidity scores[8]. Thus far however, these biomarkers either lack external validation (the acid test of performance), or have not been compared like-for-like with existing alternatives. In the present 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-FIB4, 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 our knowledge, only two 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 1457 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 five-year survival status. All biomarkers were found to be competent at predicting survival in this study, however Fibrotest and Fibroscan performed the best with AUROC values of 0.80 and 0.82, respectively. By contrast the AUROCs for APRI and FIB4 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, for predicting HCV related liver disease progression in patients with previous non-response 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 prior analyses. Firstly, the majority of individuals in the Fontana et al and Vergniol et al studies were non-cirrhotic (80%). Secondly, both studies were conducted in the pre-DAA era before HCV cure became the norm and not the exception. Thirdly, the Vergniol et al study 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 enrolment, whereas our analysis would probably have been more clinically relevant if we had followed patients up specifically from SVR achievement. Unfortunately, we were not be able to perform a viable analysis following patients up from the point of SVR achievement in this cohort. There were two main reasons for this: 1) A sizeable number of patients without biomarker data sufficiently close to the date of SVR achievement would have had to be excluded; 2) patients who had already achieved SVR at study enrolment would also have had to be exclude because the discrimination statistics that were 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 the vast majority of participants at the enrolment time point when it was not part of standard of care. We also did not have data on alpha fetoprotein which is a relevant biomarker for HCC risk. Third, our definition of severe liver related outcome combines liver failure and liver cancer, which are two biologically distinct endpoints. Thus, the predictors for one 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 – i.e. 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 prior to 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 non-liver mortality on liver transplantation in this analysis. This may have affected our results.

Our study has a number of important strengths. Firstly, we recruited participants prospectively from a representative set of UK clinics. Secondly, we leveraged outcome data held in robust national health registries, as well as 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, co-morbidity, 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 cirrhosis patients 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-FIB4, offer reasonable discriminative power over 3-4 year time frame.

FIGURE LEGENDS:

Figure 1: Liver Related Outcome defined as prior ascites, bleeding varices, hepatic encephalopathy or hepatocellular carcinoma.

Figure 2: 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)

Figure 3: Survival curves are based on the Kaplan Meier estimate

Figure 4: No legend

Figure 5: 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 (i.e. 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).


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DATA AVAILABILITY STATEMENT:

The STOP HCV consortium welcomes collaboration with interested parties. Anonymised samples and clinical data held on the study database are accessible upon 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.

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**Table 1: Description of final sample, according to compensated and decompensated cirrhosis at enrolment.**

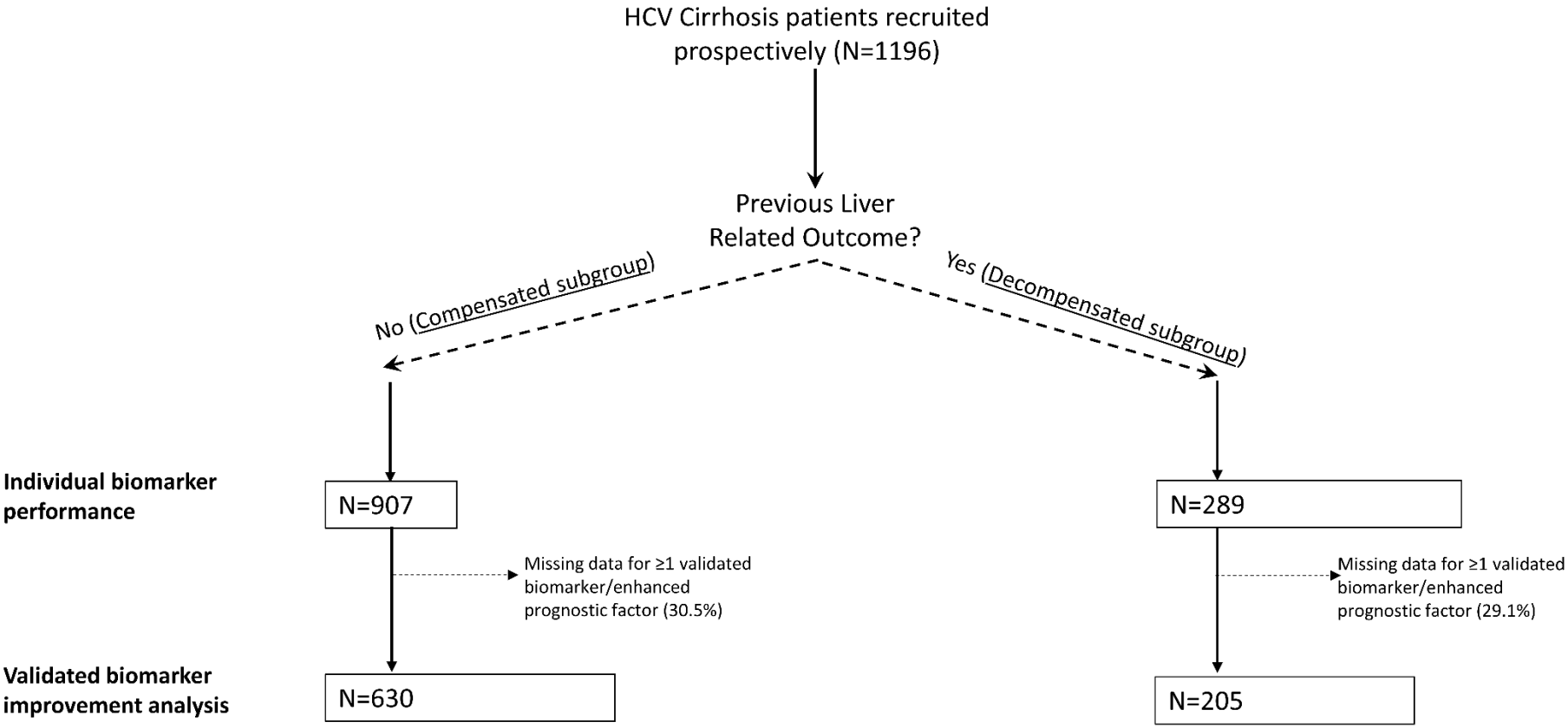
| Characteristic | | Compensated cirrhosis (N=907) | | Decompensated cirrhosis (N=289) | |
|---|---|---|---|---|---|
| | | mean/proportion | number with missing data (%) | mean/proportion | number with missing data (%) |
| Socio-demographics | Age, years | 56.1 | 6 (0.7%) | 57.4 | 4 (1.4%) |
| | % male gender | 72.9% | 0 (0.0%) | 68.5% | 0 (0.0%) |
| | % White ethnicity | 80.4% | 0 (0.0%) | 84.1% | 0 (0.0%) |
| Clinical factors | % SVR achievement | 24.1% | 0 (0.0%) | 37.7% | 0 (0.0%) |
| | % On-treatment | 29.5% | 0 (0.0%) | 25.3% | 0 (0.0%) |
| | % Encephalopathy | 0.0% | 0 (0.0%) | 15.9% | 0 (0.0%) |
| | % with ascites | 0.0% | 0 (0.0%) | 43.3% | 0 (0.0%) |
| | % genotype 3 (past or current) | 35.1% | 61 (6.7%) | 46.8% | 20 (6.9%) |
| | % Type 2 diabetes | 17.9% | 42 (4.6%) | 20.6% | 8 (2.8%) |
| Routine liver blood tests | Platelet count ($10^9$/L) | 151.2 | 48 (5.3%) | 105.8 | 18 (6.2) |
| | Albumin (g/L) | 40.9 | 33 (3.6%) | 37.1 | 14 (4.8%) |
| | Bilirubin (umol/L) | 15.3 | 33 (3.6%) | 23.1 | 14 (4.8%) |
| | Sodium (mmol/L) | 139.4 | 34 (3.7%) | 138.1 | 14 (4.8%) |
| | ALT (U/L) | 68.2 | 61 (6.7%) | 48.9 | 21 (7.2%) |
| | AST (U/L) | 71.0 | 56 (6.2%) | 61.3 | 23 (8.0%) |
| | Creatinine (umol/L) | 74.0 | 35 (3.9%) | 77.7 | 13 (4.4%) |
| | Internationalised Normal Ratio | 1.29 | 0 (0.0%) | 1.4 | 0 (0.0%) |
| Health behaviours/ liver disease risk factors | % History of heavy alcohol use | 37.9% | 61 (6.7%) | 54.5% | 14 (4.8%) |
| | % History of IVDU | 49.9% | 57 (6.3%) | 48.4% | 16 (5.5%) |
| | % Current smoker | 45.3% | 75 (8.3%) | 43.1% | 20 (6.9%) |
| | BMI | 27.9 | 174 (19.2) | 28.0 | 51 (17.6%) |
| | % Obese or with Type 2 diabetes | 41.3% | 180 (19.8%) | 42.9% | 51 (17.6%) |
| Validated biomarkers | FIB-4 | 6.0 | 106 (11.7%) | 6.8 | 45 (15.6%) |
| | APRI | 2.3 | 87 (9.6%) | 2.1 | 37 (12.8%) |
| | MELD | 9.8 | 33 (3.6%) | 11.6 | 14 (4.8%) |
| | MELD_Na | 10.1 | 33 (3.6%) | 12.3 | 14 (4.8%) |
| | ALBI | -2.8 | 34 (3.7%) | -2.3 | 14 (4.8%) |
| | ALBI-FIB4 | -2.7 | 108 (11.9%) | -2.0 | 45 (15.6%) |
| | CTP | 5.7 | 27 (3.0%) | 6.7 | 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 Genetic Risk score | 0.63 | 98 (10.8%) | 0.60 | 29 (10.0%) |
| | Innes-Buch Genetic Risk Score | 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 enrolment) - this includes data on SVR achievement.

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

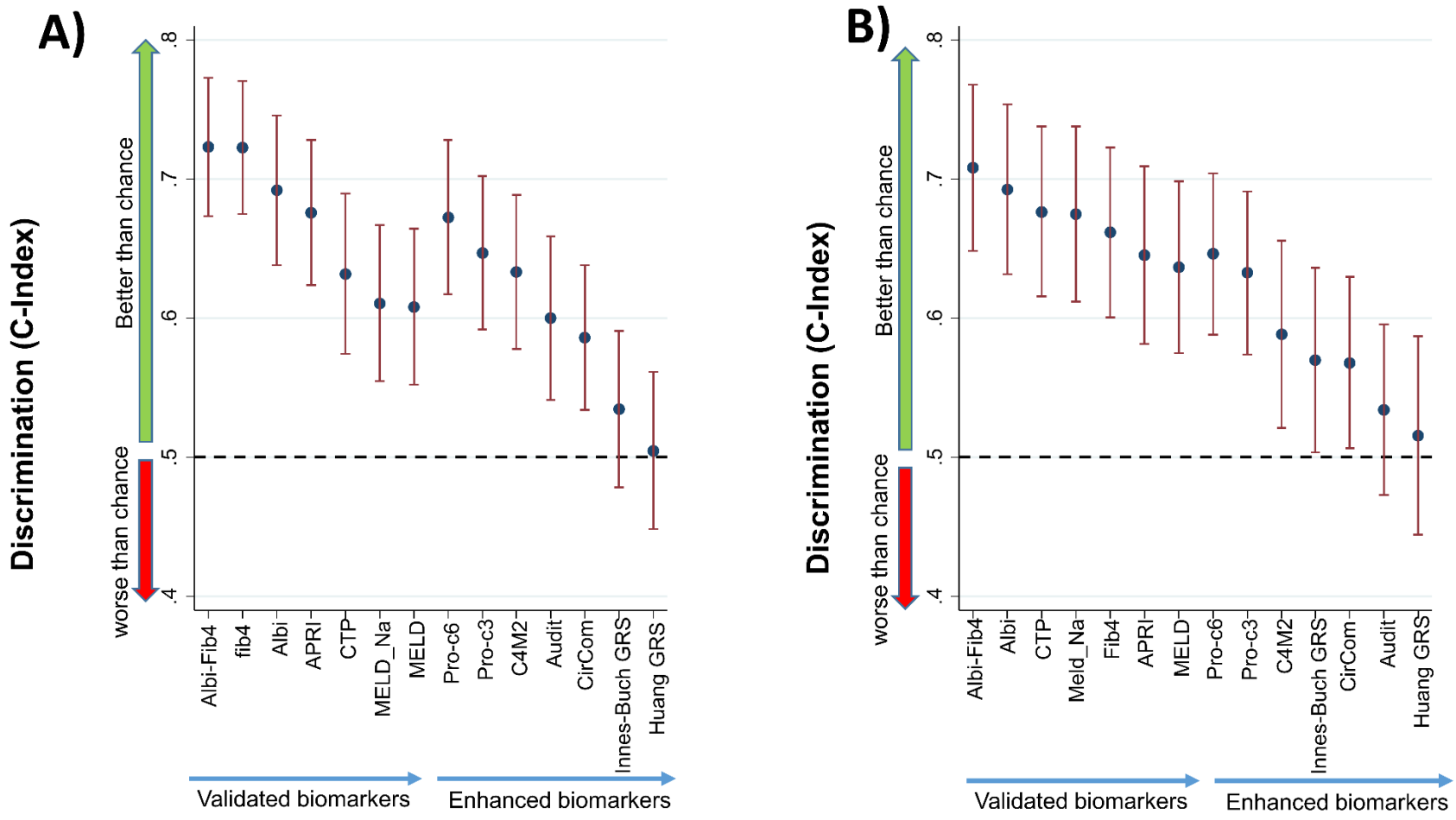
| **Subgroup** | **Outcome event** | **Total persons** | **Person Years (PYs) Fu** | | | **Outcome** | |
|---|---|---|---|---|---|---|---|
| | | | Total | Mean per patient | Median per patient | # events | Crude rate, per 100 PYs (95% CI) |
| Compensated cirrhosis | Liver Related Outcome | 907 | 1995 | 2.2 | 2.3 | 98 | 4.91 (4.03-5.99) |
| Decompensated cirrhosis | All-cause mortality | 289 | 1034 | 3.6 | 4.1 | 75 | 7.25 (5.78-9.09) |

# Fig.1 Derivation of study cohort



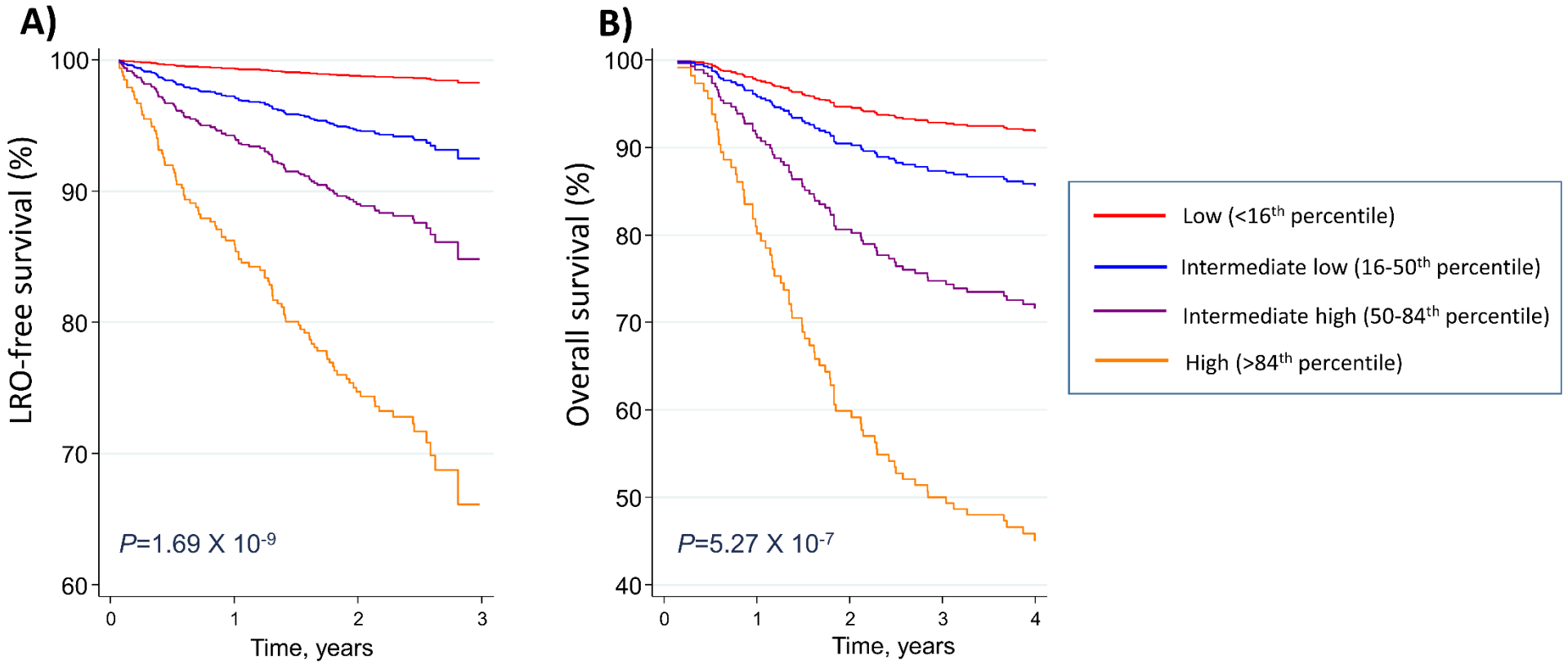

Liver Related Outcome defined as prior ascites, bleeding varices, hepatic encephalopathy or hepatocellular carcinoma

Fig.2 Biomarker discrimination for predicting: **A)** Liver Related Outcome in patients with compensated cirrhosis; and **B)** All-cause mortality in patients with decompensated cirrhosis.



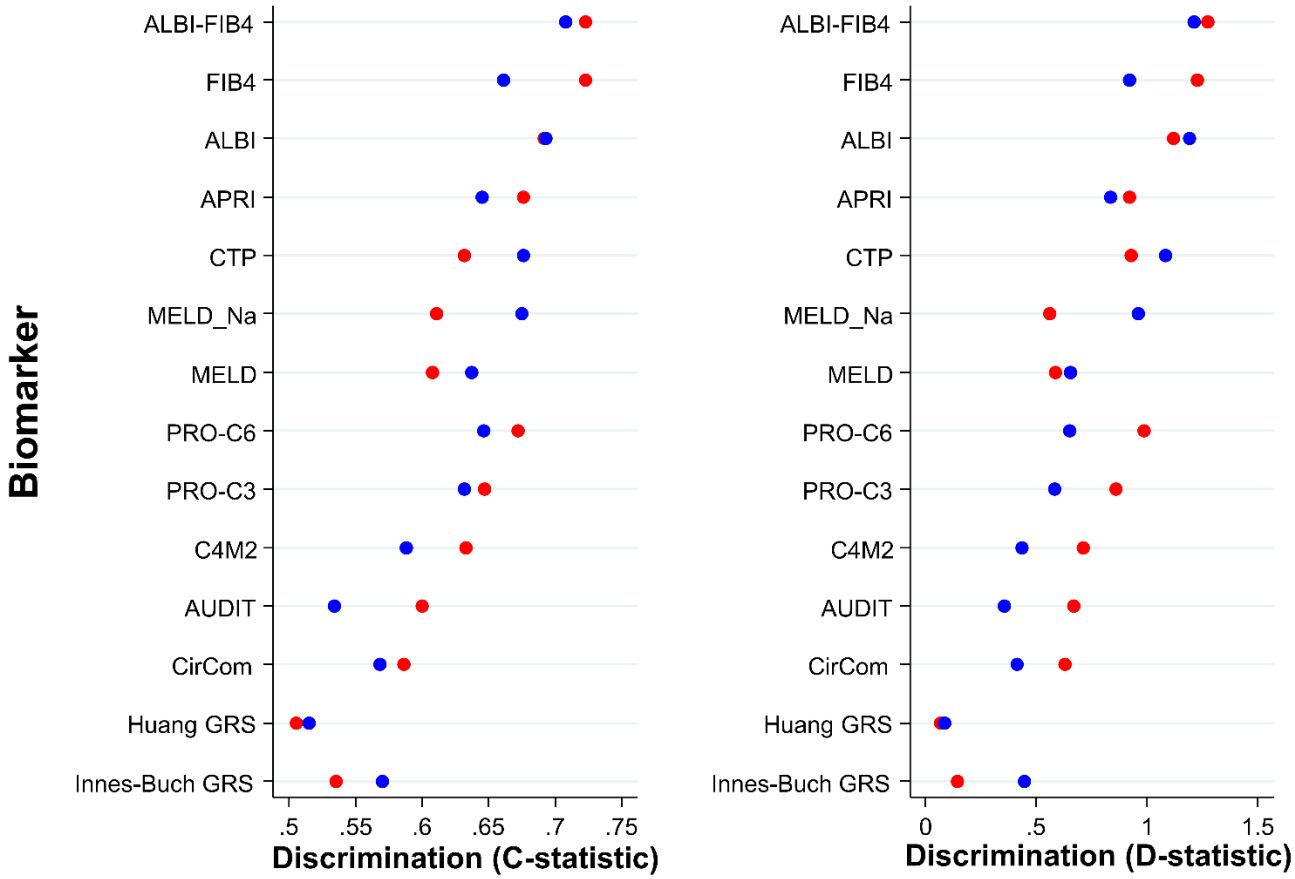

Validated and enhanced biomarkers are ordered from left to right in order of descending C-index values. Higher C-index values indicate better discrimination (and vice versa)

Figure 3. **A)** Liver Related Outcome (LRO)-free survival in patients with compensated cirrhosis; and **B)** overall survival in patients with decompensated cirrhosis, according to low, intermediate-low, intermediate-high and high ALBI-FIB4 values.




Survival curves are based on Kaplan Meier estimate.

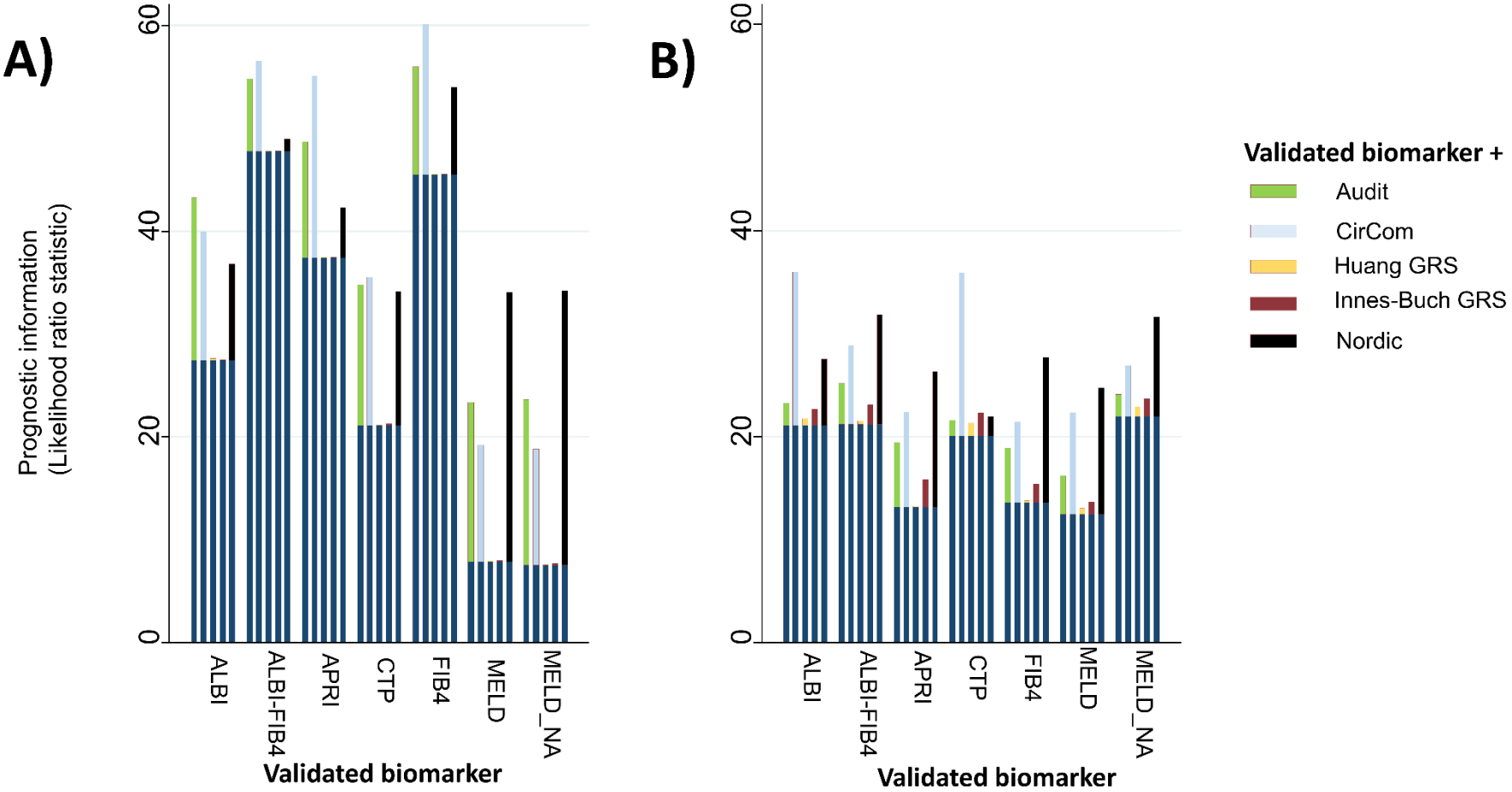
Figure.4 Biomarker discrimination for predicting: **A)** Liver Related Outcome in patients with compensated cirrhosis; and **B)** All-cause mortality in patients with decompensated cirrhosis.




**A)** Liver Related Outcome (patients with compensated cirrhosis)

**B)** All-cause mortality (patients with decompensated cirrhosis)

Fig.5 New prognostic information gained by adding an enhanced biomarker to a validated biomarker, when predicting **A)** Liver Related Outcome 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 (i.e. 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).