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Improving Noninvasive Methods of Assessing Liver Fibrosis Using an Ordinal Regression Index

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Abstract

Background & Aims—Liver fibrosis is a significant concern for patients with hepatitis C virus (HCV)/HIV co-infection. Fibrosis staging by biopsy is accurate but costly and invasive. Several fibrosis prediction models utilizing non-invasive biomarkers have been developed but are suboptimal in co-infected patients. We compared results from different staging models and ordinal regression with biopsy data.

Methods—Data from the Adult AIDS Clinical Trials Group protocol A5178 were used to evaluate 5 models of fibrosis staging; areas under receiver-operator characteristic curves (AUROC) were assessed. Individual covariates were assessed with univariable regression then entered into an ordinal logistic regression model from which a stage-wise index was developed.

Results—Data from 173 patients were evaluated; 85% were on anti-retroviral therapy, 31.2% had severe fibrosis (F3/F4) and 14% had cirrhosis (F4). Differences in CD4+ cell and platelets counts and international normalized ratio values were observed between those with and without F3/F4. Among existing models, the FIB-4 index performed best, with 88% specificity for F4 and >86% negative predictive values for F3/F4, although AUROC values were low (0.56±0.03 for F3/F4). Using patients' demographic, clinical, and laboratory data, the ordinal regression model outperformed others, with AUROC = 0.85 (SE 0.03) for predicting stage F3/F4 and 0.89 (SE 0.05) for stage 3 alone.

Conclusions—Current noninvasive methods of fibrosis assessment have poor discriminatory capacity in HCV/HIV co-infected patients. Ordinal regression analysis outperformed other non-

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invasive fibrosis prediction models. Longitudinal studies with paired biopsies will assist in refining the ordinal regression index.

Keywords

Noninvasive fibrosis assessment; HCV/HIV coinfection; Applied ordinal regression

Introduction

In patients with viral hepatitis C (HCV), the gold standard for assessing the degree of liver injury is histologic evaluation of tissue obtained at liver biopsy. Not all patients with chronic hepatitis C experience the fibrosis progression associated with infection, but it is not possible to predict who those patients might be. Because current treatments for HCV (pegylated interferon plus ribavirin) can have significant adverse effects, ascertaining fibrosis progression via biopsy is critical. Generally, severe fibrosis (METAVIR stage F3 or greater) is an absolute indication for treatment; these patients must be watched for progression to cirrhosis. However, biopsy is a relatively invasive and costly procedure. In an effort to assess degree of fibrosis and/or inflammatory activity in the liver without biopsy, some investigators have developed algorithms that utilize serologic biomarkers of fibrosis/inflammation. A wide range of tests is available, including commercial fibrosis tests and other tests of plasma/serum, and transient elastography that assesses stiffness of liver tissue. Some non-commercial indices include the AST/ALT ratio (AAR) (1;2), the Age-Platelet Index (API)(3), the AST-Platelet Ratio Index (APRI) (4), the Bonacini index (summation of ordinal scores for categories of platelet count, ALT/AST ratio, and INR) (5), the FIB-4 [(age * AST)/(platelet count × (ALT)^{1/2})] (6), the Forns index (a regression model based upon age, GGT, cholesterol, and platelet count (7)), Hepascore (modeled on bilirubin, GGT, HA, A2M, age, and gender) (8), and the Serum HA, AST, Albumin (SHASTA) index (a regression model of HA, AST, and albumin levels) (9).

However, studies have demonstrated that current noninvasive tests have limited ability for reliable and valid discrimination of fibrosis stage. They are predictive in general terms of no or minimal fibrosis (METAVIR stage F0 – F1 or Ishak score F0 – F2), or significant fibrosis (METAVIR F3 – F4 or Ishak F4 – F6).(10–13;13)

In patients with paired biopsies, a two-stage change is considered important (i.e., a jump from METAVIR F0 to F2, or F1 to F3). A one-stage change may indicate true progression, but could also be due to sampling error. Either way, knowing that a patient is truly progressing certainly has important implications for treatment decisions. Techniques are needed to improve the discriminatory capacity of these tests. Ordinal regression, or the proportional odds model, predicts the probability that an outcome such as fibrosis stage lies between a pair of cutpoints, $\alpha-1$ and α .(14) This model is appropriate for ordered outcomes, such as fibrosis stage where severity increases by stage, but only when the effects of each factor are the same for each outcome category on the log scale.(15)

Our study aimed to assess demographic, clinical, and laboratory traits as predictors of stage of fibrosis in HIV/HCV coinfecting patients via ordinal logistic regression and compare results to pre-existing non-invasive methods of fibrosis assessment.

METHODS

ACTG 5178 study design

This study consists of a secondary data analysis from a current Adult AIDS Clinical Trials Group (AACTG) protocol, A5178 (“SLAM-C”). This is an ongoing openlabel, controlled,

dual-arm crossover treatment trial to test the long-term effectiveness of pegylated interferon alfa-2a plus ribavirin (peg-IFN alfa-2a + r) in slowing progression of liver disease in HCV/HIV coinfecting patients without a 2-log reduction in HCV viral load after 12 weeks of treatment. Inclusion/exclusion information may be found in Supporting Documentation.

Data Collection and Transformation

Patient data from the first 218 patients at study entry (screening and/or baseline) were utilized, out of a total study accrual of 330. Extracted data include demographics such as age, gender, and race/ethnicity; laboratory values at screening (up to 6 weeks prior to study entry) and at the time of study entry ("baseline"); fibrosis score from previous biopsy, method of scoring, and days prior to baseline that biopsy was taken; clinical data such as HIV and HCV viral loads and CD4+ counts; and antiretroviral therapy (regimen/duration). Continuous variables were examined visually via histograms or box-and-whisker plots and assessed for normality via the Shapiro-Wilk test. Differences between laboratory and clinical values at screening and baseline were assessed for both correlation and quantitative agreement.

Because biopsies conducted prior to study entry were scored locally with various staging schema, scores were all converted to METAVIR. Previously validated conversion factors have been published (16;17). Binary variables were created for mild/moderate fibrosis (F1/F2), severe fibrosis (F3/F4), and cirrhosis (F4), as defined by the National Institutes of Health Consensus Panel on management of hepatitis C.(18)

Finally, variables were created for each previously defined method of non-invasive liver fibrosis assessment for which variables were available in this dataset via formulae described by the respective authors. These included the AAR, API, APRI, Bonacini index, and FIB-4. For each such predictor, categorical or binary variables were generated for previously defined cutpoints.

Statistical methods

Detailed methods are available in Supplemental Documentation.

Associations between previously defined indices and liver fibrosis stage—The predictive ability of each previously defined and validated index described above was assessed for the outcomes of METAVIR stage, significant fibrosis, and cirrhosis via receiver-operator characteristic curve (ROC) analysis. (19) The area under the ROC (AUROC) was used to determine the predictive capability of the index according to cutpoints predefined in the literature. The optimal cutpoints for bridging fibrosis/cirrhosis specific to this dataset was determined for each index and ROC curves were generated for ordinal categories of the index. For continuous indices, the optimal number of categories was determined. The cutpoint that maximized the sum of sensitivity and specificity was determined as the cutoff above which bridging fibrosis or cirrhosis would be best identified by the model.

Associations between individual potential predictors and liver fibrosis stage—Associations between each individual potential predictor variable and the outcomes of METAVIR stage, significant fibrosis, and cirrhosis were assessed. Predictor variables were entered into univariable ordinal regression models with fibrosis stage as the ordinal outcome. Predictor variables with two-tailed p-values ≤ 0.15 were entered into multivariable ordinal regression models. Manual backward stepwise elimination was used to generate the best-fitting multivariable, ordinal model.

Sensitivity analyses were conducted to assess the effect of time prior to entry of biopsy scoring on outcomes—subjects with biopsies occurring > 1 year prior to entry were removed from analysis, and results were compared.

RESULTS

Descriptive statistics

A total of 218 patients entered Study Step 1. Of these, 8 were missing laboratory values and were eliminated from further analysis. Demographic, clinical, and laboratory characteristics of the remaining 210 patients are detailed in Tables 1a – 1b, stratified with fibrosis by METAVIR stages F1/F2 (n = 113) versus F3/F4 (n = 60). The mean age was 47 years, most patients were Caucasian and African-American, and 80% were male. Approximately 20% of patients had elevated ALT and/or AST. Significant differences were noted in CD4+ count and in platelet count between the two fibrosis strata. Eighty-five percent of patients were on antiretroviral therapy of any type and the most common ART combination was NRTI plus PI (36.7%). Patients on ART had been treated for an average of 3 years by study entry.

Of the 210 patients, there were 168 with an actual fibrosis score and an additional 5 with a diagnosis of cirrhosis (though no score listed), totalling 173 patients with either fibrosis score or biopsy result. Of these, 157 had a described biopsy scoring method (38% used Ishak/modified HAI). Scores were converted to METAVIR stages F1 – F4 for analysis, as described in Methods. Initially, 31.2% were categorized as having significant fibrosis (Figure 1). However, including the additional five patients with cirrhosis without fibrosis score as METAVIR F4 yielded a total of 29 F4 patients. Thus, of those with laboratory scores for analysis, 35% had significant fibrosis. The majority of liver biopsies were performed within 1 year of study entry (median number of days between biopsy and study enrollment = 38.5, interquartile range 20 – 169).

Previously defined non-invasive indices for fibrosis stage assessment

Indices of fibrosis stage previously defined in the literature, and for which component data were available, were generated as described in Methods. Table 2 summarizes the areas under the ROC curve, sensitivities, specificities, and positive/negative predictive values (PPV/NPV) for each variable and each predefined cutpoint for the outcomes relevant to that index, including presence/absence of cirrhosis and significant fibrosis, and mild fibrosis. The FIB-4 had the highest AUROC for prediction of both significant fibrosis and cirrhosis.

Previously defined serologic indices analyzed continuously with new cutpoints for fibrosis stage prediction

Continuous parametric ROC curves for prediction of significant fibrosis and cirrhosis were generated for each previously defined index as described in Methods. Each index was assessed either by the number of categories scored, in the case of ordinally scored outcomes, or via the optimal number of categories as determined by the Stata program. Tables 3a and 3b summarize the results for classification of significant fibrosis and cirrhosis for each index.

Ordinal and multinomial regression models

Univariable ordinal logistic regression was performed as described in Methods. METAVIR score was regressed on all potential predictor variables for 173 subjects with the necessary laboratory results and histology. Some continuous variables were also categorized or dichotomized, such as CD4+ T cell count, HIV viral load, ALT, and AST. (Complete results of ordinal regression are available from the author on request.) Table 4 shows variables with p-values ≤ 0.15 from univariable ordinal regression. Variables not meeting the proportional

odds assumption were entered into multinomial logistic models to determine how the effect of the predictors varied. After manual backward stepwise regression, significant predictors of METAVIR fibrosis stage included elevated bilirubin (binary), INR, the African-American race interaction term (a coefficient multiplied by 1 if METAVIR stage = F2 or by 0 if otherwise, allowing the slope for METAVIR stage 2 to vary from stage 1), the log-transformed CD4+ interaction term (a coefficient multiplied by 1 if the outcome was METAVIR stage 3 and by 0 if otherwise, allowing the slope to vary for F3), and platelets^{1/2} (Table 5).

From the above ordinal logistic regression model, a new index describing the log-odds of METAVIR score 1, 2, 3, or 4, was developed: Ordinal regression index (ORI) = $(0.05 * \text{age}) - (0.84 * \text{African-American race}) - (1.29 * \text{bilirubin} > \text{ULN}) - (0.34 * \text{platelets}^{1/2} / \text{mL}) + (3.90 * \text{INR}) + (1.21 * \text{African-American race interaction term (1 if METAVIR F2, otherwise 0)}) + (0.30 * \text{CD4+ 1 if METAVIR F3, otherwise 0}) - (0.09 * \log \text{CD4+ cells/mm}^3)$ with latent variable thresholds of -0.093 , 2.17 , and 3.35 . Scores from this index were normally distributed with a mean score of 6.06 ± 1.49 (median, 5.99 ; range, $2.49 - 9.81$). The index was higher among those with significant fibrosis ($p < 0.001$) and varied significantly by METAVIR stage ($p < 0.001$).

ROC curves were generated with these scores for prediction of significant fibrosis and for cirrhosis. The AUROC for significant fibrosis was 0.85 (SE 0.03) and for cirrhosis was 0.70 (SE 0.05). The AUROC for prediction of stage F3 (significant fibrosis without cirrhosis) was 0.89 (SE 0.06). A comparison plot of each index for prediction of METAVIR stages F3/F4 is shown in Figure 3. With a cutoff ≥ 4 , 84% of patients were correctly classified as having F3/F4 fibrosis or not. Sensitivity was 63% and specificity was 89%, with 80% of patients correctly classified.

DISCUSSION

The primary aims of this study were to assess the performance of previously published non-invasive methods for liver fibrosis assessment, and to improve on discriminatory capacity using easily attainable serologic and demographic markers of liver fibrosis stage. Despite the recent influx of studies analyzing noninvasive markers of liver fibrosis in the literature, none have adequately achieved these aims. Algorithms to date have focused on dichotomous outcomes (ie, cirrhotic or noncirrhotic) and have unacceptably high rates of misclassification, precluding routine clinical use. If the ultimate goal of noninvasive fibrosis stage prediction is to avert the need for biopsy, both correct classification and changes over time are critical, highlighting the need for stagewise discrimination.

Methods of fibrosis classification that have been developed and validated by others were assessed in our current study, both via existing cutoffs for significant fibrosis/cirrhosis, and by cutoffs that were optimal for our patient population. The FIB-4 index, developed by Sterling *et al.* to predict significant fibrosis in HCV/HIV coinfecting patients, performed the best of those analyzed.⁽⁶⁾ For classifying the absence of significant fibrosis with a score ≤ 1.45 , the AUROC was 0.71 with an odds ratio of 7.72 . Similar results were obtained for predicting the absence of cirrhosis. However, classification for presence of bridging fibrosis and cirrhosis were not as strong. When the FIB-4 was log-transformed and analyzed on a continuous basis to develop new cutpoints, better results were obtained—nearly 66% were correctly classified with advanced fibrosis. The results from Sterling *et al.* suggest that 87% of patients with FIB-4 values either < 1.45 or > 3.25 (indicative of absence or presence of advanced fibrosis, respectively) would be correctly classified.⁽⁶⁾ Although the FIB-4 index was created with a subset of patients and validated on an independent subset, our assessment highlights the importance of index validation in diverse patient populations.

A comparison of patient characteristics in the current study compared to those in other studies of coinfecting subjects to date (6;9;20–23) reveals some potentially relevant differences that could affect model performance. For example, an analysis from Myers *et al.* utilized data from patients seen in a liver clinic in France rather than patients from a study population or defined cohort.(21) Patients in their analysis were younger than the ACTG 5178 population, were less likely to have significant fibrosis, were predominantly Caucasian, and had relatively low CD4+ T cell counts. Our population was ethnically diverse, with nearly 1/3 of patients having stages F3/F4. CD4+ T cell counts in our population were well-preserved. Similarly, Kelleher *et al.* studied an inner-city patient population from Baltimore, MD, which was 94% African-American.(23) Patients in this study were also known to have past histories of alcohol and drug abuse, had lower CD4+ T cell counts than the ACTG 5178 patients, and had a significant proportion (37%) with no fibrosis. A study by Macias *et al.* also had younger, Caucasian patients, of whom 45% had no or minimal fibrosis.(22) Finally, Nuñez *et al.* studied patients with similar characteristics to the ACTG 5178 population, but the sample size was small, limiting conclusions.(20) Although these study differences may not limit intra-study noninvasive liver fibrosis classification, population differences may affect inter-study comparisons. Such discrepancies suggest that any index or algorithm developed in a given cohort needs to be validated in a population of diverse patients to determine which patient characteristics may lead to misclassification with a given algorithm. In our analysis, age, race, and CD4+ T cell count were extremely important in fibrosis classification, but the level of importance for each some variables may differ by fibrosis stage. Therefore, indices developed for a given patient population, or tested in a discrete population, may yield results that would be quite different in another population with different fibrosis stage distribution.

Accurate assessment of each stage of fibrosis is important in determining treatment requirements, as patients may or may not progress over time. A patient with F1 who remains at F1 over a number of years may not necessarily be a treatment candidate; however, the shift to F2 or F3 would indicate that treatment is needed. Therefore, we developed an ordinal regression model, the Ordinal Regression Index (ORI), and tested its stagewise discriminatory capabilities via AUROC. This model demonstrates what is known about liver fibrosis severity in those with HCV; older age increases likelihood of significant fibrosis; African-Americans are less likely to have significant fibrosis/cirrhosis than Caucasians; and the lower the platelet count, the more likely one is to have significant fibrosis/cirrhosis. Thus, the log odds of METAVIR scores 1, 2, 3, or 4 increase and decrease as would be expected given the individual predictors. The AUROC for the ORI was 0.85 for significant fibrosis (F3/F4), statistically significantly greater than the other indices tested ($p < 0.0001$), with 77% of patients correctly classified. Although the ORI significantly improved prediction of significant fibrosis and METAVIR F3, it was not able to improve upon assessment of cirrhosis compared to prior models. This is likely due to the relatively small numbers of patients with cirrhosis in this population.

This study has several limitations that should be acknowledged. First and most important is the local attainment and reading of liver biopsies. Translation from the more sensitive Ishak fibrosis stage to METAVIR stage could bias our results toward lower fibrosis stages, as Ishak stages 1 and 2 are translated to METAVIR 1. Furthermore, agreement between pathologists may vary according to training and method of biopsy. The timing of biopsy varied among patients as well, ranging from baseline to up to two years prior to baseline. However, excluding patients with biopsy results from greater than one year prior to baseline did not affect results, so it is unlikely that bias or error was introduced by this inconsistency. Potential future work with this cohort will utilize staging from a single pathologist at predetermined timepoints during the study. A final biopsy limitation is the lack of data on specimen size or number of portal areas. It has been demonstrated that smaller biopsy

specimens may underestimate significant fibrosis.(24) A recent study confirmed that number of portal tracts sampled significantly correlated with proportion of patients with severe fibrosis, although biopsy length was not a factor.(25). Thus, it is possible that information on biopsy size and/or number of portal areas would have increased the classification capacity of our model. Indeed, it may be argued that biopsy itself is a tarnished standard, given the potential for sampling variability and misclassification. The most valuable method of liver fibrosis assessment will be the one that offers the most information regarding current liver damage, prognosis of future morbidity/mortality, or changes upon treatment.

Second, there were laboratory results that were not collected for this cohort that have been utilized in other serologically based fibrosis prediction algorithms. These include HA, GGT, A2M, and amino-terminal propeptide of type III collagen. Inclusion of these markers may have improved the classification capacity of some of the techniques analyzed. However, because these factors are not routinely collected in clinical care, they may not prove useful in a practical setting regardless of utility in a study setting. Another potential laboratory limitation is the lack of data on unconjugated bilirubin. It is known that certain protease inhibitors, including atazanavir and indinavir, may increase levels of total bilirubin, which was a predictor in our model. A sensitivity analysis determined that patients on ART containing these compounds did have increased odds of elevated bilirubin (OR 13.6, $p<0.001$). However, as neither ART regimen nor PI use were predictors in univariable analysis, we chose to retain total bilirubin in the model. Future studies should assess unconjugated bilirubin if possible.

Third, there are characteristics of a study population that may differentiate it from a “real-world” population. Patients in clinical trials tend to be more adherent to treatment, more motivated to receive health care, are less likely to be currently abusing alcohol or injecting drugs, etc. In this particular study, patients were a mix of prior HCV treatment non-responders and subjects naïve to treatment, and may have had additional unknown virologic or immunologic differences from others with HCV/HIV coinfection which render them less sensitive to interferon therapy. Thus, results from this study should be interpreted with caution in a non-study setting, and should be validated if possible in HCV/HIV coinfecting patients in community settings.

Fourth, the sample size for patients with cirrhosis was less than for other fibrosis stages. This could have affected classification accuracy in METAVIR stage F4. It is possible that validation with a larger, independent set of patients would improve results. As more patients are enrolled into the study, we anticipate that sample size will be sufficiently increased and that re-analysis may be useful. Similarly, there were no patients with zero fibrosis (stage F0) due to study inclusion criteria. This may have affected model results and future analyses should attempt to incorporate the full fibrosis spectrum.

In conclusion, this study attempted to address gaps in the literature that preclude the use of noninvasive, serologic markers of fibrosis in clinical care. It is clear that further work is necessary for noninvasive markers to be useful in a practice setting. Furthermore, this study demonstrates the difficulty of applying previously developed algorithms to a diverse, HCV/HIV coinfecting population—a cohort for which these markers were not created, and one that offers a more diverse set of challenges.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

A2M	α_2 macroglobulin
AACTG	Adult AIDS Clinical Trials Group
AAR	(AST)/ (ALT) ratio
ALT	alanine aminotransferase
ANOVA	analysis of variance
API	age-platelet index (a summation of ordinal scores for category of age and platelet count)
APRI	AST/platelet ratio index, expressed as $[(\text{AST}/\text{ULN}) \times 100] / [\text{platelet count}, 10^9/\text{L}]$
ART	antiretroviral therapy
AST	aspartate aminotransferase
AUROC	area under the ROC curve
CD4+	CD4-positive T-lymphocytes
ELISA	enzyme-linked immunosorbent assay
FIB-4	$\text{age} * (\text{AST}/(\text{platelet count} \times (\text{ALT})^{1/2}))$
GGT	γ -glutamyltranspeptidase
HA	hyaluronic acid
HAI	histological activity index
HCV	hepatitis C virus
HIV	human immunodeficiency virus-1
Hepascore	a model of bilirubin, GGT, HA, A2M, age, and gender
INR	international normalized ratio
Peg-IFN + r	pegylated interferon alfa-2a plus ribavirin
ROC curve	receiver-operator characteristic curve
SHASTA	a regression model built upon HA, AST, and albumin levels
SLAM-C	Suppressive Long-Term Antiviral Management of Hepatitis C Virus and HIV-1 Coinfected Subjects
ULN	upper limit of normal

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Figure 1. METAVIR fibrosis stage for subjects with laboratory data and with reported fibrosis scores (n = 173)

Figure 2.
Distribution of individuals by FIB-4 category as described by Vallet-Pichard *et al.*

Figure 3.

Comparison of Receiver-Operator Characteristic Curves for Significant Fibrosis (METAVIR F3/F4).

This plot compares AUROC for each non-invasive fibrosis index assessed. Curves were generated on a continuous basis from each index.

Abbreviations:

AUROC: Area(s) under the receiver-operator characteristic (ROC) curve

SE: standard error

ORI: ordinal regression index

APRI: aspartate aminotransferase (AST)/platelet ratio index

API: age-platelet index

AAR: AST/alanine aminotransferase (ALT) ratio

Table 1

a. Patient demographic and clinical characteristics				
	METAVIR 1/2 (n = 113)	METAVIR 3/4 (n = 60)	p-value for difference	Total* (N = 210)
Age, years				
Mean ± sd	46.92 ± 6.78	47.45 ± 6.46	0.63	46.99 ± 6.65
Median	48	48		48
Range	28 – 67	29 – 58		28 – 67
Race/ethnicity: n (%)			0.14	
Caucasian	51 (45.54)	34 (56.67)		100 (47.85)
African-American	44 (39.29)	16 (26.67)		78 (37.32)
Hispanic	15 (13.39)	6 (10.00)		23 (11.00)
Asian-Pacific Islander		1 (1.67)		1 (0.48)
Native American	1 (0.89)			2 (0.96)
Other	1 (0.89)	3 (5.00)		5 (2.39)
Missing				1 (0.48)
Gender: n (%)			0.55	
Male	91 (80.53)	46 (76.67)		168 (80.0)
Female	22 (19.47)	14 (23.33)		42 (20.0)
^aBaseline CD4+, cells/mm³			<0.0001	
Mean ± sd	629.8 ± 333.9	445.6 ± 191.4		578.0 ± 304.3
Median	565	400		503
Range	160 – 2162	135 – 891		135 – 2162
^aCD4+ ≤ 350 cells/mm³			0.01	
n (%)	20 (17.7)	21 (35.0)		47 (22.38)
^bDetectable HIV viral load at baseline			0.43	
n (%)	63 (55.75)	35 (58.33)		118 (56.19)
^bBaseline HIV viral load for those detectable, copies/mL			0.58	
Mean ± sd	7292.5 ± 16513.9	3668.7 ± 7737.0		5418.6 ± 13056.9
Median	93	60		99.5
Range	50 – 75529	50 – 33997		50 – 75529
HBV surface antigen positive	(112 tested)			
n (%)	1 (0.89)	1 (1.67)	N/A	2 (0.96)
HCV viral titer, log₁₀ IU/mL	(n = 97)	(n = 49)		(n = 180)
Mean ± sd	6.58 ± 0.55	6.42 ± 0.67	0.13	6.55 ± 0.62
Median	6.61	6.49		6.60

a. Patient demographic and clinical characteristics				
	METAVIR 1/2 (n = 113)	METAVIR 3/4 (n = 60)	p-value for difference	Total* (N = 210)
Range	5.02 – 7.73	4.14 – 8.03		4.14 – 8.03

b. Patient laboratory characteristics				
	METAVIR 1/2 (n = 113)	METAVIR 3/4 (n = 60)	p-value for difference	Total* (N = 210)
^a Baseline ALT, U/L				
Mean ± sd	72.35 ± 50.86	72.63 ± 50.45	0.75	71.79 ± 49.20
Median	62	59.5		61.5
Range	13 – 320	24 – 307		13 – 320
^a ALT ≥ 2x ULN				
n (%)	24 (21.24)	15 (25.0)	0.57	49 (23.33)
AST, U/L	(n = 100)	(n = 51)		(n = 185)
Mean ± sd	62.32 ± 44.93	61.61 ± 27.87	0.40	60.27 ± 38.22
Median	52	50		49
Range	17 – 264	25 – 149		17 – 264
AST ≥ 2x ULN				
n (%)	19 (19.0)	12 (23.53)	0.51	39 (21.08)
Total bilirubin, mg/dL	(n = 100)	(n = 51)		(n = 185)
Mean ± sd	0.99 ± 0.90	0.94 ± 0.73	0.71	0.92 ± 0.79
Median	0.7	0.7		0.7
Range	0.1 – 5.4	0.1 – 3.6		0.1 – 5.4
Bilirubin ≥ ULN				
n (%)	31 (27.43)	16 (26.67)	0.91	55 (26.19)
^b Platelet count, ×10 ⁹ /L				
Mean ± sd	221.2 ± 63.24	165.8 ± 57.43	< 0.0001	204.6 ± 67.90
Median	220	156		204
Range	48 – 395	82 – 294		48 – 431
INR				
Mean ± sd	1.03 ± 0.08	1.07 ± 0.10	0.003	1.04 ± 0.09
Median	1	1.08		1.01
Range	0.8 – 1.3	0.9 – 1.4		0.8 – 1.4
Albumin, g/dL	(n = 99)	(n = 51)		(n = 184)
Mean ± sd	4.18 ± 0.37	4.07 ± 0.37	0.08	4.17 ± 0.37
Median	4.2	4.1		4.2
Range	3.2 – 5.4	3.3 – 5.1		3.2 – 5.4

* All patients with lab results included in total N unless otherwise noted; of 210 patients, 173 had fibrosis scores

^a Screening values used where baseline values were missing, n = 26

^b Screening values used where baseline values were missing, n = 23

* All patients with lab results included in total N unless otherwise noted

^a Screening values used where baseline values were missing (n = 26)

^b Screening values used where baseline values were missing (n = 27)

Table 2

a. The AST/ALT Ratio			
Cut-points	Measure	Presence of cirrhosis (n = 29)	Presence of significant fibrosis (n = 60)
> 1	Sensitivity	0.58	0.47
n = 59	Specificity	0.72	0.73
	PPV	0.27	0.47
	NPV	0.90	0.73
	Odds Ratio (95% CI)	3.46 (1.35—9.02)	2.40 (1.12—5.16)
	p-value	0.003	0.013
	AUROC±SE (95% CI)	0.65±0.05 (0.57—0.72)	0.60±0.04 (0.52—0.68)

b. The Age/Platelet Index			
Cut-points	Measure	Presence of cirrhosis (n = 29)	Presence of significant fibrosis (n = 60)
≥ 6	Sensitivity	0.48	0.40
n = 40	Specificity	0.85	0.89
	PPV	0.37	0.67
	NPV	0.90	0.74
	Odds Ratio (95% CI)	5.44 (2.12—13.77)	5.61 (2.38—13.54)
	p-value	< 0.0001	< 0.0001
	AUROC±SE (95% CI)	0.67±0.05 (0.60—0.73)	0.65±0.03 (0.57—0.72)

c. The AST/Platelet Ratio Index		
Cut-points	Measure	Presence of cirrhosis (n = 29)
>2	Sensitivity	0.12
n = 12	Specificity	0.94
	PPV	0.27
	NPV	0.86
	Odds Ratio (95% CI)	2.23 (0.35—10.2, Fisher's exact test)
	p-value	0.38
	AUROC±SE (95% CI)	0.53±0.03 (0.46—0.59)
		Presence of significant fibrosis (n = 60)
>1.5	Sensitivity	0.20
n = 26	Specificity	0.87
	PPV	0.43
	NPV	0.68
	Odds Ratio (95% CI)	1.63 (0.58—4.40)
	p-value	0.29
	AUROC±SE (95% CI)	.53±0.03 (0.47—0.60)
		Absence of cirrhosis (n = 164)

c. The AST/Platelet Ratio Index		
Cut-points	Measure	Presence of cirrhosis (n = 29)
≤ 1	Sensitivity	0.74
n = 132	Specificity	0.46
	PPV	0.88
	NPV	0.24
	Odds Ratio (95% CI)	2.41 (0.93—6.16)
	p-value	0.04
	AUROC±SE (95% CI)	0.60±0.05 (0.49—0.70)
		Absence of significant fibrosis (n = 113)
≤ 0.5	Sensitivity	0.43
n = 70	Specificity	0.83
	PPV	0.85
	NPV	0.39
	Odds Ratio (95% CI)	3.56 (1.44—9.64)
	p-value	0.003
	AUROC±SE (95% CI)	0.63±0.04 (0.55—0.70)

d. The Bonacini Index			
Cut-points	Measure	Presence of cirrhosis (n = 29)	Presence of significant fibrosis (n = 60)
≥ 8	Sensitivity	0.07	0.07
n = 5	Specificity	0.98	0.99
	PPV	0.40	0.80
	NPV	0.86	0.67
	Odds Ratio (95% CI)	3.97 (0.31—36.0)	8.0 (0.76—397.3)
	p-value	0.16 (Fisher's exact test)	0.05 (Fisher's exact test)
	AUROC±SE (95% CI)	0.53±0.02 (0.45—0.59)	0.53±0.02 (0.45—0.61)
≥ 7	Sensitivity	0.34	0.07
n = 26	Specificity	0.91	0.99
	PPV	0.40	0.80
	NPV	0.89	0.67
	Odds Ratio (95% CI)	5.23 (1.81—14.5)	5.99 (2.15—18.1)
	p-value	0.0002	0.0001
	AUROC±SE (95% CI)	0.63±0.05 (0.55—0.69)	0.61±0.03 (0.53—0.69)
		Absence of cirrhosis (n = 126)	Absence of significant fibrosis (n = 113)
< 3	Sensitivity	0.17	0.17
n = 31	Specificity	0.97	0.89
	PPV	0.97	0.77
	NPV	0.17	0.33
	Odds Ratio (95% CI)	5.76 (0.87—244)	1.62 (0.57—5.23)
	p-value	0.08 (Fisher's exact test)	0.37 (Fisher's exact test)
	AUROC±SE (95% CI)	0.57±0.22 (0.50—0.64)	0.56±0.03 (0.48—0.63)

d. The Bonacini Index			
Cut-points	Measure	Presence of cirrhosis (n = 29)	Presence of significant fibrosis (n = 60)
≤ 3	Sensitivity	0.37	0.37
n = 67	Specificity	0.93	0.83
	PPV	0.97	0.83
	NPV	0.21	0.38
	Odds Ratio (95% CI)	8.0 (1.9—72.0)	2.93 (1.25—7.45)
	p-value	0.001 (Fisher's exact test)	0.008 (Fisher's exact test)
	AUROC±SE (95% CI)	0.65±0.03 (0.58—0.72)	0.60±0.03 (0.52—0.67)

e. The FIB-4 Index				
Cut-points	Measure	Presence of cirrhosis (n = 29)	Presence of significant fibrosis (n = 60)	
≥ 3.25	Sensitivity	0.27	0.52	
n = 25	Specificity	0.88	0.70	
	PPV	0.29	0.24	
	NPV	0.87	0.89	
	Odds Ratio (95% CI)	2.77 (0.85 – 8.20)	2.49 (0.91 – 6.79)	
	p-value	0.06 (Fisher's exact test)	0.055	
	AUROC±SE (95% CI)	0.58±0.05 (0.49—0.65)	0.56±0.03 (0.48—0.64)	
		Absence of cirrhosis (n = 126)	Absence of significant fibrosis (n = 113)	
≤ 1.45	Sensitivity	0.54	0.58	
n = 88	Specificity	0.88	0.85	
	PPV	0.96	0.90	
	NPV	0.26	0.47	
	Odds Ratio (95% CI)	8.93 (2.50—47.9)	7.72 (3.01—22.1)	
	p-value	0.0001	<0.0001	
	AUROC±SE (95% CI)	0.71±0.04 (0.64—0.78)	0.71±0.04 (0.64—0.79)	
		Mild fibrosis (METAVIR F1) (n = 56)		
≤ 0.7	Sensitivity	0.10		
n = 12	Specificity	0.97		
	PPV	0.63		
	NPV	0.68		
	Odds Ratio (95% CI)	3.51 (0.65 – 23.4)		
	p-value	0.12 (Fisher's exact test)		
	AUROC±SE (95% CI)	0.53 ± 0.02 (0.45 – 0.62)		
		Mild fibrosis (METAVIR F1) (n = 56)	Absence of cirrhosis (n = 126)	Absence of significant fibrosis (n = 113)
>0.7, ≤ 1.45	Sensitivity	0.63	0.46	0.50
n = 76	Specificity	0.73	0.88	0.85
	PPV	0.55	0.96	0.88

e. The FIB-4 Index				
Cut-points	Measure	Presence of cirrhosis (n = 29)	Presence of significant fibrosis (n = 60)	
	NPV	0.79	0.23	0.43
	Odds Ratio (95% CI)	4.63 (2.13 – 10.1)	6.41 (1.80 – 34.5)	5.68 (2.22 – 16.3)
	p-value	< 0.0001	0.001	< 0.0001
	AUROC $\pm$ SE (95% CI)	0.68 $\pm$ 0.04 (0.59 – 0.75)	0.67 $\pm$ 0.04 (0.60 – 0.75)	0.68 $\pm$ 0.04 (0.59 – 0.75)
			Absence of cirrhosis (n = 126)	Absence of significant fibrosis (n = 113)
>1.45, $\leq$ 2.4	Sensitivity		0.26	0.50
n = 53	Specificity		0.65	0.85
	PPV		0.81	0.88
	NPV		0.14	0.43
	Odds Ratio (95% CI)		0.67 (0.26 – 1.86)	5.68 (2.22 – 16.3)
	p-value		0.38	< 0.0001
	AUROC $\pm$ SE (95% CI)		0.46 $\pm$ 0.05 (0.38 – 0.53)	0.42 $\pm$ 0.04 (0.34 – 0.51)

Table 3

a: New cutpoints to predict significant fibrosis for prior indices						
Index	Cutpoint	Sensitivity	Specificity	% Correctly Classified	AUROC $\pm$ SE	95% CI
AAR	≥ 0.89	54.90%	60.00%	58.28%	0.58 ± 0.05	$0.50 - 0.66$
API	≥ 5	55.00%	79.65%	71.10%	0.70 ± 0.04	$0.62 - 0.79$
Log APRI	≥ -0.80	96.08%	39.00%	58.28%	0.70 ± 0.04	$0.62 - 0.77$
Bonacini	≥ 5	66.67%	65.49%	65.90%	0.70 ± 0.04	$0.61 - 0.78$
Log FIB-4	≥ 0.36	82.35%	57.00%	65.56%	0.73 ± 0.04	$0.64 - 0.81$

b: New cutpoints to predict cirrhosis for prior indices						
Index	Cutpoint	Sensitivity	Specificity	% Correctly Classified	AUROC $\pm$ SE	95% CI
AAR	≥ 0.89	65.4%	60.0%	60.8%	0.65 ± 0.05	$0.56 - 0.71$
API	≥ 7	44.8%	91.5%	84.5%	0.72 ± 0.05	$0.65 - 0.78$
Log APRI	≥ -0.34	100%	37.2%	46.8%	0.71 ± 0.04	$0.63 - 0.77$
Bonacini	≥ 5	75.9%	60.4%	62.3%	0.74 ± 0.05	$0.67 - 0.80$
Log FIB-4	≥ 0.36	88.5%	52.4%	57.9%	0.75 ± 0.05	$0.68 - 0.81$

Table 4

Predictor variables with p-values ≤ 0.15 in univariable ordinal regression

Predictor variable	N	Odds ratio (SE)	95% CI	Z score	p-value	Pseudo-R ²	Brant test p-value
Age, years (continuous)	173	1.04 (0.02)	0.99 – 1.08	1.76	0.08	0.007	0.25
Age, years (categorical (4))	173	1.26 (0.16)	0.98 – 1.62	1.78	0.07	0.007	0.54
Log CD4+ cells/mm ³ (continuous)*	173	0.48 (0.13)	0.28 – 0.83	-2.65	0.008	0.016	0.02
CD4+ cells/mm ³ (categorical (4))*	173	0.79 (0.10)	0.62 – 1.01	-1.85	0.06	0.008	0.03
Log ₁₀ HCV IU/mL (continuous)	146	0.67 (0.18)	0.40 – 1.14	-1.48	0.14	0.006	0.65
INR (continuous)**	173	1446.14 (2409)	55.2 – 37878	4.37	<0.0001	0.04	0.85
Platelets ^{1/2} /mL (continuous)	173	0.70 (0.05)	0.62 – 0.80	-5.42	<0.0001	0.07	0.25
Bilirubin > ULN (binary)*	151	0.43 (0.18)	0.19 – 0.99	-1.99	0.05	0.01	0.02
Albumin gm/dL (continuous)	150	0.39 (0.16)	0.18 – 0.88	-2.28	0.02	0.01	0.47

* Proportional odds assumption violated

** Brant test negative but INR distribution significantly non-normal so ran multinomial logistic

Abbreviations for tables 6 and 7 (from top, left to right):

SE, standard error

CI, confidence interval

HCV, hepatitis C virus

IU/mL, international units per milliliter

INR, international normalized ratio for prothrombin time

ULN, upper limits of normal

gm/dL, grams per deciliter

Table 5

Multivariable ordinal regression model with interactions

Parameter	Df	Odds Ratio	SE	Wald 95% CI	Chi-square statistic	p-value
Intercept1	1	-0.09	3.38	-6.71 – 6.52		
Intercept2	1	2.17	3.39	- 4.47 – 8.80		
Intercept3	1	3.35	3.39	-3.30 – 10.01		
Age, years	1	1.05	0.03	1.00 – 1.10	3.4	0.065
Bilirubin, mg/dL > ULN	1	0.28	0.13	0.11 – 0.68	7.76	0.0053
Platelets ¹⁰ ⁹ / mL	1	0.71	0.06	0.60 – 0.84	15.83	<0.0001
INR	1	49.36	97.16	1.04 – 2338.79	3.92	0.0476
African-American race	1	0.43	0.21	0.17 – 1.12	3	0.0831
African-American race interaction term	1	3.35	1.92	1.09 – 10.31	4.45	0.0348
Log CD4+ cells/mm ³	1	1.35	0.10	1.16 – 1.57	0.07	0.7919
Log CD4 interaction term	1	0.91	0.31	0.47 – 1.79	15.62	<0.0001

Abbreviations:

DF, degrees of freedom

SE, standard error

CI, confidence interval

ULN, upper limit of normal

mg/dL, milligrams per deciliter

INR, international normalized ratio for prothrombin time

Table 6

Cutpoints for prediction of significant fibrosis/cirrhosis by the ORI

Index	Cutpoint	Sensitivity	Specificity	% Correctly Classified	AUROC $\pm$ SE	95% CI
Significant fibrosis	≥ 6.66	66.7%	82.0%	76.8%	0.83 ± 0.03	$0.77 - 0.89$
Cirrhosis	≥ 5.83	80.8%	50.7%	55.3%	0.69 ± 0.05	$0.62 - 0.76$