2020

Utility of serum biomarker indices for staging of hepatic fibrosis before and after venesection in patients with hemochromatosis caused by variants in HFE

Justin Chin

Lawrie W. Powell

Louise E. Ramm

Gunter F. Hartel

John K. Olynyk
Edith Cowan University

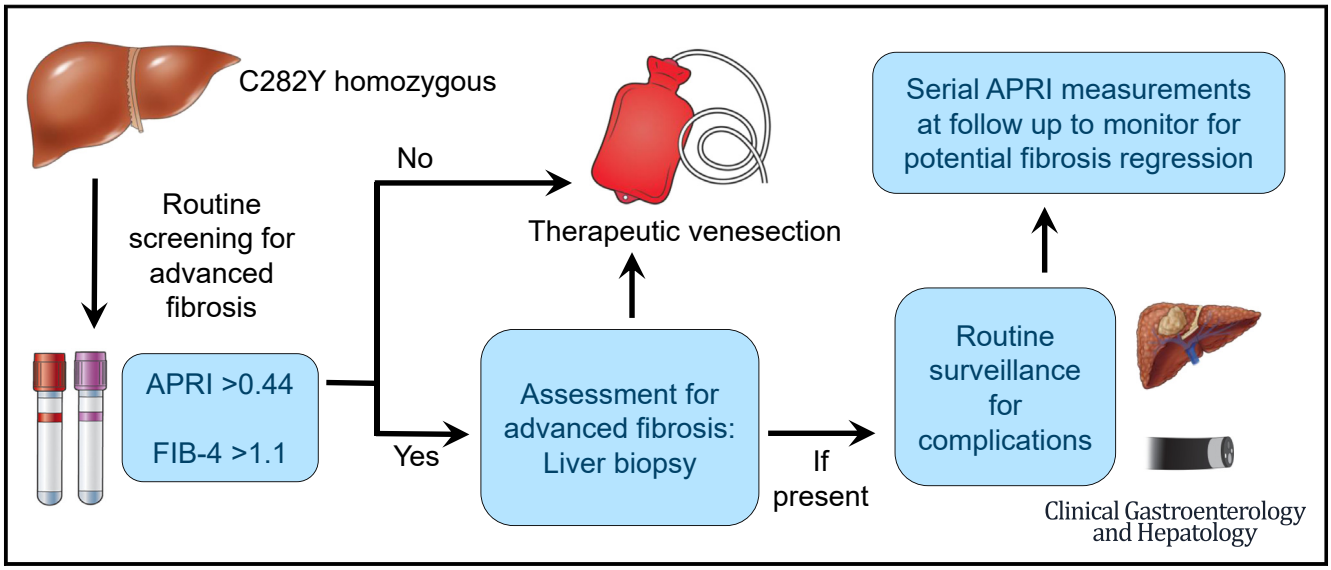
See next page for additional authors

10.1016/j.cgh.2020.07.052 Chin, J., Powell, L. W., Ramm, L. E., Hartel, G. F., Olynyk, J. K., & Ramm, G. A. (2020). Utility of serum biomarker indices for staging of hepatic fibrosis before and after venesection in patients with hemochromatosis caused by variants in HFE. *Clinical Gastroenterology and Hepatology*, Advance online publication. https://doi.org/10.1016/j.cgh.2020.07.052

Utility of Serum Biomarker Indices for Staging of Hepatic Fibrosis Before and After Venesection in Patients With Hemochromatosis Caused by Variants in *HFE*

Justin Chin,[*] Lawrie W. Powell,[‡] Louise E. Ramm,[§] Gunter F. Hartel,[‖] John K. Olynyk,[*,¶,b] and Grant A. Ramm[‡,§,b]

[*]Department of Gastroenterology & Hepatology, Fiona Stanley Fremantle Hospital Group, Murdoch, Western Australia, Australia; [‡]Faculty of Medicine, University of Queensland, Herston, Brisbane, Queensland, Australia; [§]Hepatic Fibrosis Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; [‖]Statistics Unit, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; and [¶]School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia



BACKGROUND & AIMS: **Hemochromatosis that is associated with variants in the homeostatic iron regulator gene (HFE) is characterized by intestinal absorption of iron and excessive body and hepatic iron stores; it can lead to hepatic fibrosis and cirrhosis. Fibrosis has been staged by analysis of liver biopsies, but non-invasive staging methods are available. We evaluated the ability of aspartate aminotransferase:platelet ratio index (APRI), the fibrosis-4 (FIB-4) index, and gamma-glutamyl transferase:platelet ratio (GPR) to assess hepatic fibrosis staging in subjects with HFE-associated hemochromatosis, using liver biopsy-staged fibrosis as the reference standard.**

METHODS: **We performed a retrospective, cross-sectional analysis of 181 subjects with HFE-associated hemochromatosis and hepatic fibrosis staged by biopsy analysis and available serum samples. We calculated APRI, FIB-4, and GPR at diagnosis for all 181 subjects and following venesection therapy in 64 of these subjects (7 subjects had follow-up biopsy analysis). We used area under the receiver operating characteristic curve (AUROC) analysis to assess the relationships**

[b]Authors share co-senior authorship.

Abbreviations used in this paper: APRI, aspartate aminotransferase-to-platelet ratio; AUROC, area under the receiver operator characteristic curve; CI, confidence interval; FIB-4, fibrosis-4; GPR, gamma-glutamyl transferase ratio; HFE, homeostatic iron regulator gene; HH, HFE hemochromatosis; ROC, receiver operator characteristic.

1542-3565
https://doi.org/10.1016/j.cgh.2020.07.052

between APRI score, FIB-4 score, and GPR and advanced (F3–F4) fibrosis and to select cut-off values.

RESULTS: Hepatic fibrosis stage correlated with APRI score (r = 0.54; $P < .0001$), FIB-4 score (r = 0.35; $P < .0001$), and GPR (r = 0.36, $P < .0001$). An APRI score above 0.44 identified patients with advanced fibrosis with an AUROC of 0.88, 79.4% sensitivity, 79.4% specificity, and 81% accuracy. A FIB-4 score above 1.1 identified patients with advanced fibrosis with an AUROC of 0.86, 80% sensitivity, 80.3% specificity, and 81% accuracy. A GPR above 0.27 identified patients with advanced fibrosis with an AUROC of 0.76, 67.7% sensitivity, 70.3% specificity, and 69% accuracy. APRI score was significantly more accurate than GPR ($P = .05$) in detecting advanced fibrosis; there was no difference between APRI and FIB-4. Venesection treatment was associated with significant reductions in APRI ($P < .0001$) and GPR ($P < .001$), paralleling fibrosis regression observed in available liver biopsies. Post-venesection APRI identified 87% of subjects with advanced fibrosis that decreased to levels that indicate stage F1–F2 fibrosis.

CONCLUSIONS: In a retrospective study of 181 subjects with HFE-associated hemochromatosis, we found that APRI and FIB-4 scores identified patients with advanced hepatic fibrosis with 81% accuracy. APRI scores might also be used to monitor fibrosis regression following venesection.

Keywords: HH; Disease Progression; Respond to Treatment; Blood Test.

Homeostatic iron regulator gene (HFE) hemochromatosis (HH) is a common genetic disorder of iron metabolism,[1] characterized by dysregulated hepcidin expression, resulting in increased intestinal absorption of iron and excessive total body and hepatic iron stores.[2–4] In some individuals advanced fibrosis and cirrhosis may develop, increasing mortality and morbidity.[5] Liver biopsy has been the gold standard for fibrosis staging in HH patients, because early identification of advanced hepatic fibrosis or cirrhosis is crucial in guiding appropriate clinical management. However, liver biopsies are not without risk, and the heterogeneous distribution of fibrosis development may result in an underestimation of the actual staging of fibrosis. In addition, liver biopsy does not allow for easy, dynamic, ongoing assessment of fibrosis progression.

There has been a progressive evolution of noninvasive modalities for the detection and staging of hepatic fibrosis in a variety of different chronic liver diseases. These include ultrasound and elastography-based technologies, blood test panels, and serum biomarker indices (for example, aspartate aminotransferase-to-platelet ratio index [APRI], gamma-glutamyl transferase-to-platelet ratio [GPR], fibrosis-4 [FIB-4]). These serum biomarker indices have been shown to be useful, easy to perform, and relatively inexpensive. In addition, these tests can be repeated frequently, unlike liver biopsies, to provide ongoing assessment of fibrosis progression. Such methods for assessing hepatic fibrosis have been validated in adult patients with viral hepatitis, nonalcoholic fatty liver disease, human immunodeficiency virus/hepatitis B coinfection, as well as in children with cystic fibrosis–associated liver disease (a condition with a similar heterogeneous pattern of fibrosis deposition).[6–14] However, no large studies have assessed the efficacy of these biomarkers in HH. Other studies have evaluated different models in the prediction of advanced fibrosis in HH. For example, the clinical parameters of serum ferritin >1000 μg/L, with an elevated aspartate aminotransferase level and a platelet count >200 $\times$ 10^9/L, were shown to predict cirrhosis in the majority of HH subjects.[15] However approximately 30%–64% of patients with cirrhosis do not fulfill all 3 criteria.[15,16] Serum hyaluronic acid levels >46.5 ng/mL have also been shown to have high sensitivity and specificity in identifying the presence of cirrhosis in HH patients and together with serum ferritin level >1000 μg/L obviate the need for liver biopsy in 60% of patients.[16] Although transient elastography for assessment of fibrosis has been used in viral hepatitis and nonalcoholic fatty liver disease,[6,7] its use in HH has not been clearly defined. Magnetic resonance imaging elastography has been assessed in HH, but as with all forms of elastography, cost and accessibility can be significant limiting factors.[17] Serum biomarker indices such as APRI, FIB-4, and GPR may offer a more viable alternative because they are likely to be highly cost-effective and readily available via liver function tests performed during routine blood workup at clinic visits for patients with HH.

Therefore, the aim of this study was to assess the potential of these simple, readily available, and inexpensive noninvasive serum biomarker indices (APRI, GPR, and FIB-4) to predict the stage of fibrosis and determine cutoff thresholds for the detection of advanced hepatic fibrosis in a large, well-characterized cohort of liver biopsy-validated subjects with HH before and after venesection treatment.

Methods

Patients

The study subjects were derived from a database of all HH subjects referred between 1983 and 2013 to the Royal Brisbane and Women's Hospital, Australia. Inclusion criteria were met by 181 subjects, requiring complete baseline demographics, total number of venesections, alcohol consumption, serum biochemistry, and liver biopsy histologic assessments (with formal scoring of fibrosis) of subjects to be extracted from the QIMR Berghofer Medical Research Institute HH database. The alcohol consumption of subjects in the study was recorded by using methods by the National Health and Medical Research Council of Australia, which define one standard drink as containing 10 g of alcohol (equivalent to 12.5 mL of pure alcohol). All subjects were confirmed as being C282Y homozygous on genetic testing. All subjects were routinely offered a liver biopsy as part of baseline assessment. Venesection treatment was performed weekly until a serum ferritin level <100 μg/L was achieved. Liver biopsy was also performed in 7 subjects after treatment for clinically indicated reasons. APRI, GPR, and FIB-4 data were calculated for all study subjects at the time of liver biopsy before commencing venesection. These biomarker indices were also calculated in a subgroup of 64 subjects after completion of venesection, including 7 patients who underwent a second biopsy. Exclusion criteria included age <16 years or other forms of chronic liver disease (chronic viral hepatitis, immune-mediated, metabolic liver diseases), which were assessed through standard, routine testing and clinical assessment as previously described.[18] Subject age was defined as the age when the liver biopsy was performed. All subjects were untreated at the time of study inclusion. Paraffin-embedded sections were stained with hematoxylin-eosin and Perls' Prussian blue and reviewed by liver histopathologists with expertise in HH who classified fibrosis stage according to the grading system of Scheuer: F0, no fibrosis; F1, mild fibrosis with enlarged portal tracts; F2, moderate periportal and portal-portal septa but intact architecture; F3, severe fibrosis with architectural distortion; and F4, cirrhosis with architectural distortion.[19] For the purposes of this study, subjects with hepatic fibrosis stages F3–F4 were combined and termed *advanced fibrosis*. These studies were approved by the Human Research Ethics Committees of the Royal Brisbane and Women's Hospital and the QIMR Berghofer Medical Research Institute, Brisbane, Australia, and informed written consent was obtained at the time of entry into the study.

Statistical Analysis

All data are presented as mean ± standard error of the mean unless otherwise specified. Spearman's rank correlation was used to assess associations with increasing stage of hepatic fibrosis. The Student *t* test or analysis of variance was used to analyze differences between groups. Receiver operator characteristic (ROC) curve analysis was performed to evaluate the discriminatory capacity of APRI, GPR, and FIB-4 for the diagnosis of advanced fibrosis and to establish appropriate cutoffs. In addition, dual cutoff values to demonstrate best accuracy to rule in (specificity $>90\%$) and rule out (sensitivity $>90\%$) advanced fibrosis were also determined. The method described by Hanley and McNeil[20] was used to compare performance of the ROC curves. To assess the impact of venesection on APRI, GPR, and FIB-4 we performed a Wilcoxon signed rank paired *t* test on paired patient biomarker indices post-venesection vs pre-venesection and generated Bland-Altman plots showing relative fold-change of indices with venesection for F0–F2 and F3–F4 fibrosis cohorts. To assess the potential clinical utility of post-venesection APRI, GPR, and FIB-4 in predicting fibrosis regression, logistic regression was used to model fibrosis stage (dichotomized as mild fibrosis, F1-F2 and advanced fibrosis, F3–F4) versus APRI, GPR, or FIB-4 at biopsy. A cutoff value was selected to maximize the Youden's index (sensitivity + specificity). This cutoff was applied to APRI, GPR, and FIB-4 values determined after venesection (de-ironing) to predict fibrosis stage. The effect of alcohol consumption on biomarker indices both at biopsy and after de-ironing was assessed by using analysis of variance and Tukey-

What You Need to Know

Background

Hemochromatosis that is associated with variants in the homeostatic iron regulator gene (HFE) is characterized by intestinal absorption of iron and excessive body and hepatic iron stores; it can lead to hepatic fibrosis and cirrhosis. Fibrosis has been staged by analysis of liver biopsies, but noninvasive staging methods are available.

Findings

This retrospective study of 181 subjects with HFE-associated hemochromatosis found that aminotransferase:platelet ratio index (APRI) and fibrosis-4 (FIB-4) scores identify patients with advanced hepatic fibrosis (stage F3–F4) with 81% accuracy. Post-venesection APRI identified 87% of subjects with advanced fibrosis that decreased to levels that indicate stage F1–F2 fibrosis.

Implications for patient care

APRI and FIB-4 measurements can be used to noninvasively identify patients with HFE-associated hemochromatosis who have advanced hepatic fibrosis. APRI scores might also be used to monitor fibrosis regression after venesection.

Table 1. Diagnostic Accuracy of APRI, GPR and FIB-4 Using Optimal Cutoffs in the Diagnosis of Advanced Fibrosis in HH Subjects

	AUROC (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV (%)	NPV (%)	% Accuracy	Cutoff	z value	*P* value
APRI	0.88 (0.81–0.96)	79.4 (63.2–89.7)	79.3 (72–85.1)	79.3	79.4	81	0.44		
GPR	0.76 (0.67–0.85)	67.7 (50.8–80.9)	70.3 (62.5–77.1)	69.5	68.5	81	0.27	1.96	.05
FIB-4	0.86 (0.78–0.95)	80 (60.9–91.1)	80.3 (72.6–86.3)	80.2	80.1	69	1.11	1.27	.20

NOTE. 95% confidence intervals in parentheses. *P* values derived from Hanley-McNeil comparison of z values for GPR and FIB-4 versus APRI.[22]
APRI, aspartate aminotransferase-to-platelet ratio; AUROC, area under the receiver operator characteristic curve; CI, confidence interval; FIB-4, fibrosis-4; GPR, gamma-glutamyl transferase-to-platelet ratio; HH, HFE hemochromatosis; NPV, negative predictive value; PPV, positive predictive value.

Kramer honestly significant difference. Statistical significance was assigned as $P \leq .05$. All statistical tests were conducted by using GraphPad Prism 7 (GraphPad Software, San Diego, CA) and JMP Pro (SAS Institute, Cary, NC).

Results

Baseline characteristics of all subjects are presented in Supplementary Table 1. Mean age was 42.7 ± 1.1 years for male patients and 46 ± 2.3 years for female patients. Mean alcohol consumption was 28.5 ± 2.5 g/day (19.8 ± 3.5 g/day for female patients and 31.7 ± 3.1 g/day for male patients; $P = .01$). Advanced hepatic fibrosis was identified in 34 subjects and was more prevalent in male patients. Mean APRI, GPR, and FIB-4 were significantly higher in those with advanced fibrosis versus those without (Supplementary Table 1). ROC curve analysis assessed the discriminant ability of APRI, GPR, and FIB-4 (Table 1). Comparison of the ROC curves[20] demonstrated significantly higher area under the ROC curve (AUROC) for APRI versus GPR ($P = .05$), but there was no significant difference between APRI and FIB-4 or between FIB-4 and GPR. Figure 1 shows a significant correlation between all 3 biomarkers and increasing hepatic fibrosis stage (APRI, $r = 0.54$, $P < .0001$; GPR, $r = 0.36$, $P < .0001$; FIB-4, $r = 0.35$, $P < .0001$).

Diagnostic Accuracy of Aspartate Aminotransferase-to-Platelet Ratio Index, Gamma-Glutamyl Transferase Ratio, and Fibrosis-4 for the Prediction of Advanced Fibrosis

The AUROC for APRI was 0.88 (95% confidence interval [CI], 0.81–0.96), providing an optimal threshold for detection of advanced fibrosis of 0.44 (Figure 2*A*), with sensitivity of 79.4%, specificity of 79.3%, and diagnostic accuracy of 81% (Table 1). Dual cutoff values were also identified with best accuracy to rule in advanced fibrosis, APRI ≥0.59 (specificity 90.3%), and rule out advanced fibrosis, APRI ≤0.37 (sensitivity 91.1%) (Table 2). Using the identified cutoff value of >0.44, 29 of 34 patients (85.3%) with F3–F4 fibrosis were accurately staged, whereas 21.2% of patients with F0–F2 fibrosis were staged incorrectly.

The AUROC for GPR was 0.76 (95% CI, 0.67–0.85), providing an optimal threshold for detection of advanced fibrosis of 0.27 (Figure 2*B*), with sensitivity of 67.7%, specificity of 70.3%, and diagnostic accuracy of 69% (Table 1). Dual cutoff values were also identified with best accuracy to rule in advanced fibrosis, GPR ≥0.57 (specificity 90.3%), and rule out advanced fibrosis, GPR ≤0.15 (sensitivity 91.2%) (Table 2). Using the identified cutoff value of >0.27, 23 of 34 patients (67.6%) with F3–F4 fibrosis were correctly staged, whereas 29.5% of patients with F0–F2 fibrosis were staged incorrectly.

The AUROC for FIB-4 was 0.86 (95% CI, 0.78–0.95), providing an optimal threshold for detection of advanced fibrosis of 1.11 (Figure 2*C*), with sensitivity of 80%, specificity of 80.3%, and diagnostic accuracy of 81% (Table 1). Dual cutoff values were also identified with best accuracy to rule in advanced fibrosis, FIB-4 ≥1.38 (specificity 90.6%), and rule out advanced fibrosis, FIB-4 ≤0.73 (sensitivity 96.0%) (Table 2). Using the identified cutoff value of >1.11, 20 of 25 patients (80%) with F3–F4 fibrosis were correctly staged, whereas 18.9% of patients with F0–F2 fibrosis were staged incorrectly.

Effect of Venesection on Aspartate Aminotransferase-to-Platelet Ratio Index, Gamma-Glutamyl Transferase Ratio, and Fibrosis-4 and Potential to Monitor Fibrosis Regression

After venesection therapy (when serum ferritin levels decreased to <100 μg/L), APRI, GPR, and FIB-4 were recalculated. The mean (± standard error of the mean) interval time between the initial (at biopsy) and follow-up (at de-ironing) assessments was 2.66 ± 0.3 years (range, 0.03–10.5 years). Therapeutic venesection of 64 HH subjects led to a significant reduction in their APRI ($P < .0001$) values (Figure 3*A*), including in subjects with F0, F0–F2, or F3–F4 fibrosis (Figure 4). Figure 3*B* shows APRI plotted as fold-change after venesection vs APRI measured at biopsy for F0–F2 vs F3–F4 fibrosis. GPR was also significantly reduced after venesection (Figure 3*A*,

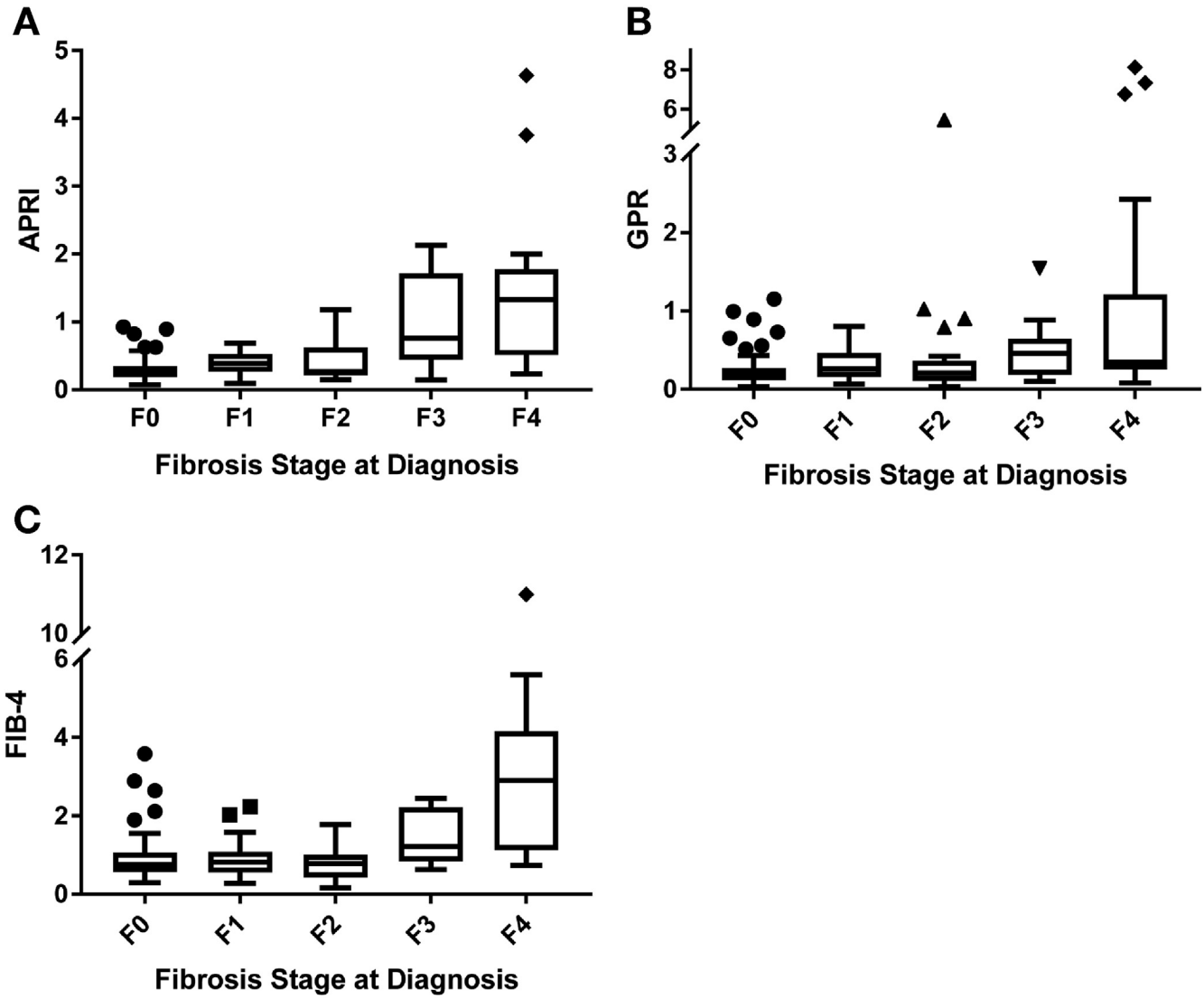


Figure 1. There was a significant correlation between increasing hepatic fibrosis stage and (*A*) APRI ($r = 0.54$, $P < .0001$), (*B*) GPR ($r = 0.36$, $P < .0001$), and (*C*) FIB-4 ($r = 0.35$, $P < .0001$). APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis 4; GPR, gamma-glutamyl transferase ratio.

$P < .001$), including in subjects with F0 or F0–F2 fibrosis, but not in subjects with F3–F4 fibrosis (Supplementary Figure 1). Figure 3*C* shows GPR plotted as fold-change after venesection vs GPR measured at biopsy for F0–F2 vs F3–F4 fibrosis. In contrast, FIB-4 demonstrated no significant changes with therapy (Figure 3*A* and *D*), including when subjects were analyzed at F0, F0–2, or F3–F4 fibrosis (not shown).

Because of the significant effect of de-ironing on APRI and GPR we assessed the potential for post-venesection APRI and GPR values to predict fibrosis regression from F3–F4 to mild fibrosis (F1–F2). Logistic regression of dichotomized fibrosis stage (F1–F2 and F3–F4) versus APRI at biopsy was highly significant ($P < .0001$), with odds ratio of 38.6 (95% CI, 6.3–235.0) per unit change in APRI for having advanced versus mild fibrosis. The AUROC was 0.83, with sensitivity of 61.8% and specificity of 95.9%, using APRI cutoff of 0.785. Applying this cutoff to the post-venesection APRI values, we found that of the 15 patients with F3–F4 fibrosis at diagnosis, APRI values decreased below the cutoff indicative of F1–F2 fibrosis in 13 subjects (87%; 95% CI, 62.1%–96.3%).

Logistic regression of F1–F2 and F3–F4 versus GPR at biopsy was significant ($P = .002$), with odds ratio of 2.1 (95% CI, 1.3–3.5) per unit change in GPR for having advanced versus mild fibrosis. The AUROC was 0.70, with sensitivity of 82.4% and specificity of 51.0%, using GPR cutoff of 0.225. Applying this cutoff to the post-venesection GPR values, we found that of the 15 patients with F3–F4 fibrosis at diagnosis, GPR values decreased below the cutoff indicative of F1–F2 fibrosis in only 6 subjects (40%; 95% CI, 19.8%–64.3%).

The logistic regression of F1–F2 and F3–F4 versus APRI at biopsy had a significantly higher AUROC versus GPR at biopsy (0.83 versus 070; difference 0.13, 95% CI, 0.06–0.22; $P = .0009$). The proportion of F3–F4 patients

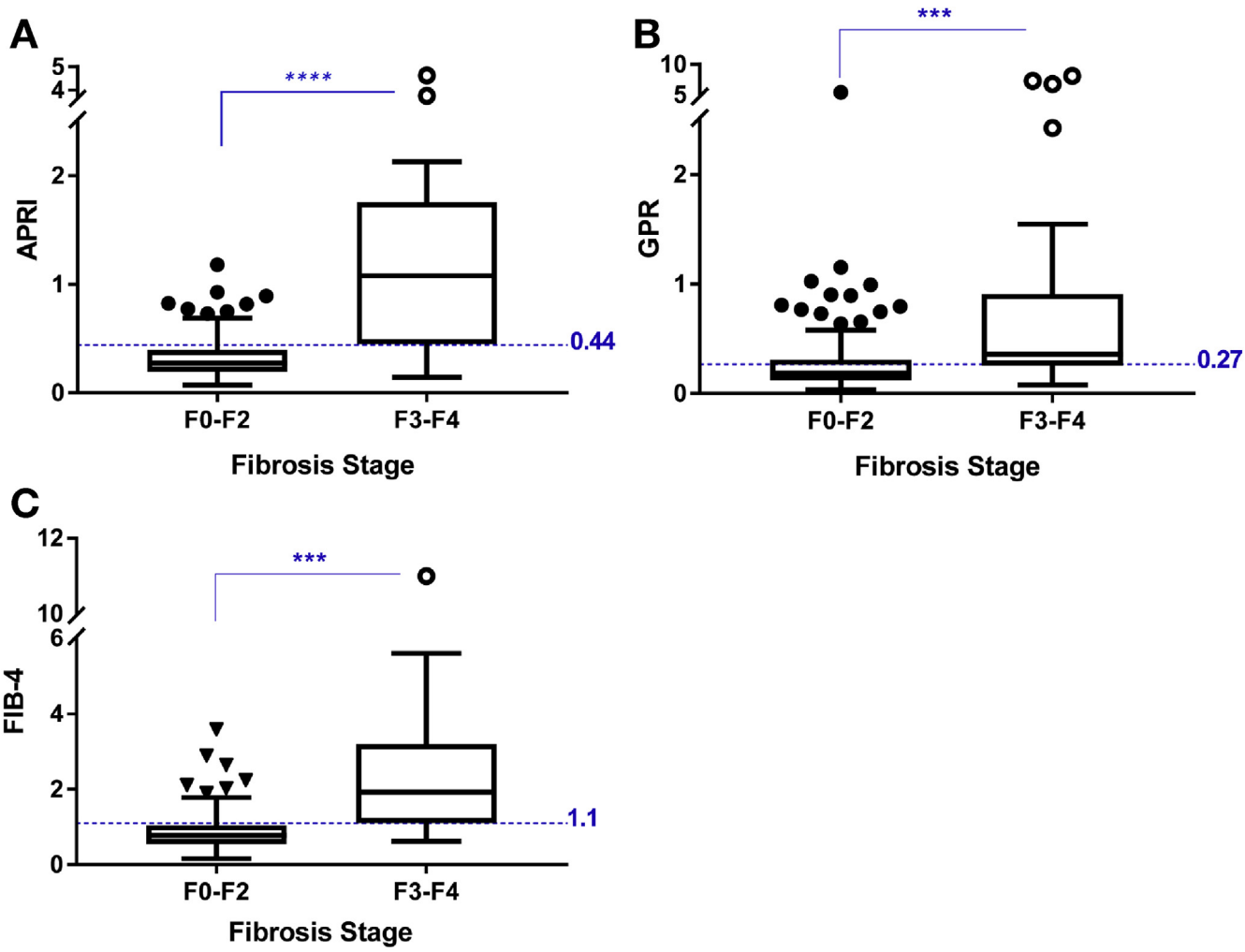


Figure 2. (*A*) APRI, (*B*) GPR, and (C) FIB-4 values for F3–F4 versus F0–F2 fibrosis with proposed cutoffs for predicting advanced fibrosis in HH patients (*dotted lines*). ***$P < .01$; ****$P < .0001$. APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis 4; GPR, gamma-glutamyl transferase ratio; HH, HFE hemochromatosis.

that decreased to F1–F2 levels was significantly higher for APRI than GPR (87% vs 40.0%; rate ratio = 2.167; 95% CI, 1.13–4.15; likelihood ratio χ^2, $P = .006$). Thus, this result suggests that APRI may be superior to GPR for the assessment of fibrosis regression after venesection therapy.

Seven subjects with F3–F4 fibrosis at diagnosis also had follow-up liver biopsies after de-ironing for clinically indicated reasons. Hepatic fibrosis regressed ≥2 F stages in 5 of 7 patients after venesection but remained unchanged in 2 patients ($P = .06$) (Supplementary Figure 2). There was a significant reduction in APRI and GPR with de-ironing in these 7 patients but no effect on FIB-4 (Supplementary Figure 2). There were no associations between pre- or post-treatment APRI, GPR, or FIB-4 values and the quantity of iron removed (not shown).

To assess the influence of alcohol, comparisons between subjects with no alcohol use, light-moderate (<30 g/day), and heavy (≥30 g/day) alcohol consumption were performed. There was no significant effect of alcohol on APRI, GPR, and FIB-4 when measured at biopsy (Supplementary Figure 3) or on the fold-change decrease in these biomarker indices after de-ironing therapy (Supplementary Figure 4). There were also no relationships observed between biomarker indices and iron indices either at biopsy or after venesection (not shown).

Discussion

This unique, liver biopsy-based study of a well-characterized cohort of HH subjects before and after treatment has demonstrated the clinical utility of APRI, GPR, and FIB-4 for the diagnosis and/or monitoring of advanced hepatic fibrosis. We found that of these markers, APRI and FIB-4 demonstrated superior diagnostic accuracy in the diagnosis of fibrosis stage. APRI and GPR values were significantly decreased after venesection treatment, including when analyzed in subjects with F0, F0–F2, or advanced (F3–F4) fibrosis. In addition, in a subset of subjects with available post-treatment liver biopsies, reductions in APRI and GPR

Table 2. Diagnostic Accuracy of APRI, GPR, and FIB-4 Using Optimal Cutoffs to Rule-in and Rule-out Advanced Fibrosis in HH Subjects

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV (%)	NPV (%)	*P* value
APRI					<.0001
≥0.59	70.6 (53.8–83.2)	90.3 (84.5–94.1)	87.9	75.4	
≤0.37	91.1 (77–97)	69.0 (61.0–75.9)	74.6	88.6	
GPR					<.0001
≥0.57	38.2 (23.9–55)	90.3 (84.5–94.2)	79.7	59.4	
≤0.15	91.2 (77–97)	31.7 (24.7–40)	57.2	78.3	
FIB-4					<.0001
≥1.38	64 (44.5–79.8)	90.6 (84.2–94.5)	87.2	71.6	
≤0.73	96 (75–98.6)	46.7 (38–55.1)	64.3	92.1	

NOTE. 95% confidence intervals are shown in parentheses.
APRI, aspartate aminotransferase-to-platelet ratio; AUROC, area under the receiver operator characteristic curve; CI, confidence interval; GPR, gamma-glutamyl transferase-to-platelet ratio; FIB-4, fibrosis-4; HH, HFE hemochromatosis; NPV, negative predictive value; PPV, positive predictive value.

values reflected fibrosis regression. Finally, we demonstrated that post-venesection APRI predicted 87% of subjects with advanced fibrosis decreased to APRI levels indicative of mild F1–F2 fibrosis. This information has important clinical implications because it extends the widespread recognition of the utility of serum biomarkers in the assessment of advanced fibrosis/cirrhosis observed in other chronic liver diseases into the management of subjects with HH.

Our data suggest that optimal cutoff values for these biomarkers for predicting advanced fibrosis in HH are lower than those observed in more aggressive conditions such as viral hepatitis B or C and alcohol-related liver disease. In previous studies in patients with hepatitis C virus or alcohol-related liver disease, an APRI cutoff threshold for advanced fibrosis of 1 was proposed with demonstrated sensitivity of 35% and specificity of 94% for the diagnosis of cirrhosis in alcohol-related liver disease.[9,21] If one were to apply an APRI threshold of 1 to our HH cohort, the sensitivity and specificity of APRI in HH would be 50% (95% CI, 34.1%–65.9%) and 99.3% (95% CI, 96.2%–99.9%), respectively. For GPR, at a threshold of 0.32 (as suggested by Lemoine et al[14] in predicting advanced fibrosis in hepatitis B virus), the sensitivity and specificity in HH would be 58.8% (95% CI, 42.2%–73.6%) and 77.2% (95% CI, 69.8%–83.3%), respectively. With regard to FIB-4, at the lower limit of 1.45 (as suggested by Vallet-Pichard et al[11] for advanced fibrosis in hepatitis C virus), the sensitivity and specificity in HH would be 64% (95% CI, 44.5%–79.8%) and 92.1% (95% CI, 86.1%–95.7%), respectively. The cutoff values we defined in HH subjects were more similar to those found in a study that evaluated the utility of APRI in subjects with cystic fibrosis–related liver disease where an APRI ≥0.462 was able to accurately identify patients with F3–F4 fibrosis.[8] This could be due to HH being a less inflammatory, more chronic condition (similar to cystic fibrosis liver disease), where fibrosis develops in subjects with lower aspartate aminotransferase levels compared with those observed in viral hepatitis.[22–25]

The method described by Beaton et al[15] was also shown to be a reliable predictor of cirrhosis in HH. However, a significant number of subjects would not fulfill all 3 criteria.[15,16] When applied to our study population, the Beaton model only successfully identified 68% of subjects with cirrhosis (15/22) and only 56% of those with F3–F4 fibrosis (19/34). In addition, 4 patients with F0–F1 and 3 patients with F2 fibrosis fulfilled the Beaton criteria for the prediction of cirrhosis, which was consistent with data from other studies.[16]

In our study, APRI, GPR, and FIB-4 demonstrated significant correlation with hepatic fibrosis stage. Of particular benefit is these biomarkers can be repeated regularly to assess potential fibrosis progression or regression. Previous studies from our group demonstrate fibrosis regression with venesection.[18,26] In a subset of this cohort, we showed that APRI and GPR were significantly decreased with venesection. Monitoring APRI after venesection could be useful in predicting fibrosis regression with APRI in 13 of 15 subjects with advanced fibrosis at diagnosis, decreasing to APRI levels indicative of mild F1–F2 fibrosis after de-ironing. Both APRI and GPR reflected biopsy-based changes in fibrosis regression after venesection, but FIB-4 did not, albeit in 7 patients where repeat liver biopsy was available. Unlike other liver diseases, HH is not typically characterized by significant necroinflammation.[22–25] Thus, improvements in fibrosis indices may be due to decreased iron-induced hepatocellular damage and, as we propose, may be reflective of improvements in fibrosis. A previous HH study, including 23 subjects with advanced fibrosis on pretreatment biopsy, demonstrated 69% of F3 and 35% of F4 subjects achieved fibrosis regression ≥2 F stages on post-treatment liver biopsy.[27] Another HH study demonstrated that fibrosis stage decreased in 73% of subjects with F3 fibrosis after treatment, and that fibrosis reduction to ≤METAVIR F2 was associated with a major reduction in long-term hepatocellular carcinoma risk.[26] Thus, APRI and potentially GPR present options for noninvasive monitoring of fibrosis regression after treatment of HH. Further prospective studies, with

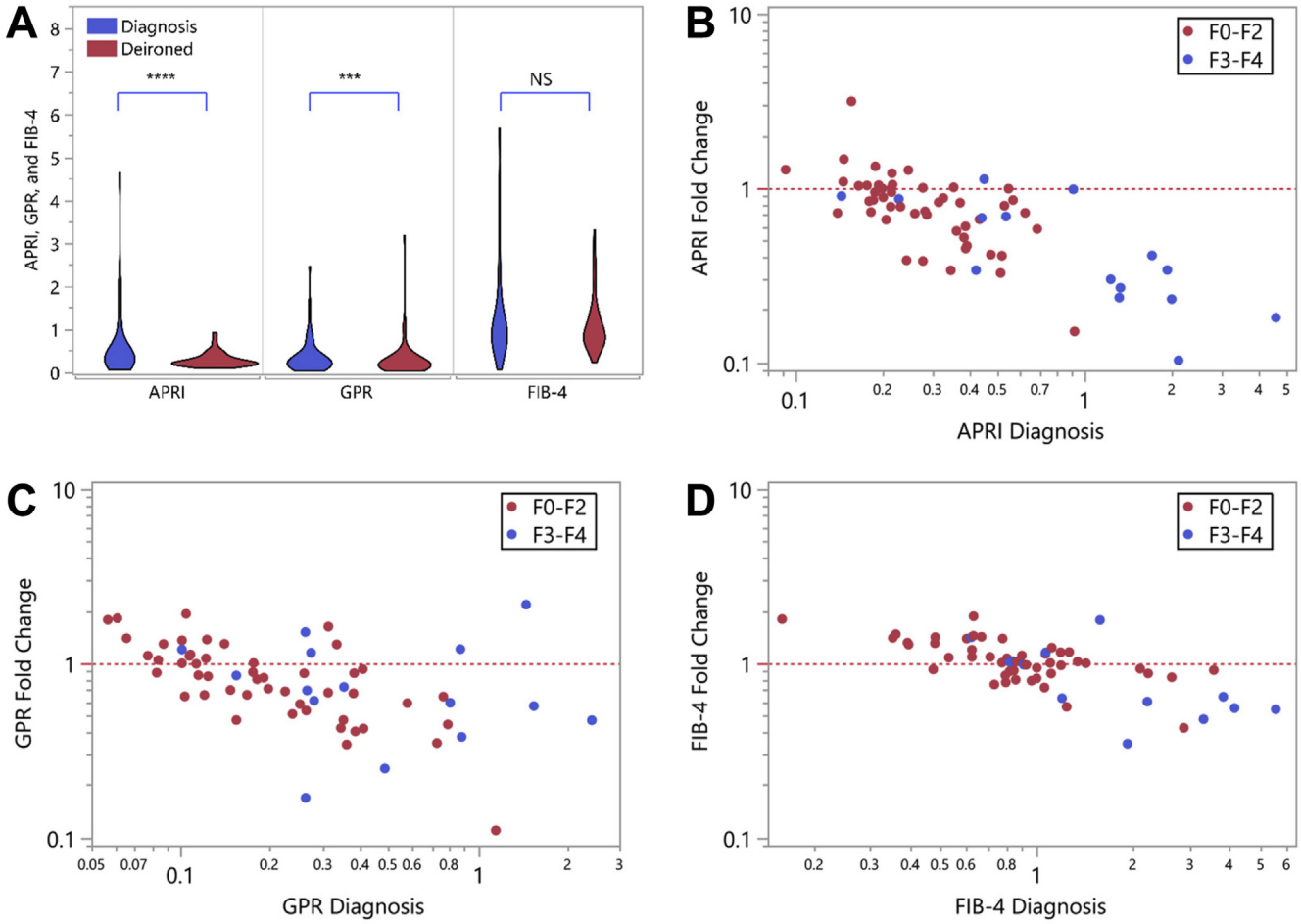


Figure 3. Effect of venesection treatment on APRI, GPR, and FIB-4 in subjects with HH (*A*) at diagnosis and after de-ironing. (*B*) APRI, (*C*) GPR, and (*D*) FIB-4 plotted as fold-change after venesection versus when measured at biopsy for F0–F2 (*red circles*) versus F3–F4 (*blue circles*) fibrosis, with line of best fit. (*A*) Wilcoxon signed rank paired *t* test on paired patient biomarker indices values after vs before venesection. ****$P < .0001$; ***$P < .001$. (*B–D*) Bland-Altman plots. APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis 4; GPR, gamma-glutamyl transferase ratio; HH, HFE hemochromatosis.

paired liver biopsies, are warranted to confirm and validate their utility in this setting.

A study by Adhoute et al[28] assessed the utility of Fibroscan and serum-based noninvasive methods of hepatic fibrosis assessment in 57 subjects with HH versus 46 controls. They found that prevalence of liver stiffness measurements at a cutoff >7.1 kPa was significantly higher in HH versus healthy controls. They also found a correlation between serum biomarkers (including APRI and FIB-4) with Fibroscan. However, their study did not include paired liver biopsies to allow for correlation of noninvasive methods with histology, and thus appropriate cutoffs for diagnosis of advanced fibrosis were not defined. Future studies could assess whether combinations of elastography and biomarkers could provide better diagnostic accuracy for advanced fibrosis, as demonstrated in other liver disease etiologies using elastography and APRI.[12,13]

We acknowledge limitations of our study including the retrospective design, which may introduce unintended bias. Also, the limited numbers of subjects with post-venesection liver biopsies require caution in interpretation of the significant decreases observed for APRI and GPR with biopsy-validated fibrosis regression. However, this study assesses the performance of 3 separate, commonly used serum biomarker indices in the diagnosis of advanced fibrosis in a large, well-characterized cohort of HH subjects with matched liver biopsies.

Conclusion

This study demonstrates the diagnostic accuracy of APRI and FIB-4 in the detection of advanced hepatic fibrosis in HH. Furthermore, APRI and GPR were significantly reduced in association with venesection therapy. We propose that APRI measurements may be clinically useful in monitoring fibrosis regression after treatment. These readily available biomarkers could be used by physicians and general practitioners to stratify subjects for management appropriate to the severity of hepatic fibrosis and guide the need for liver biopsy in HH.

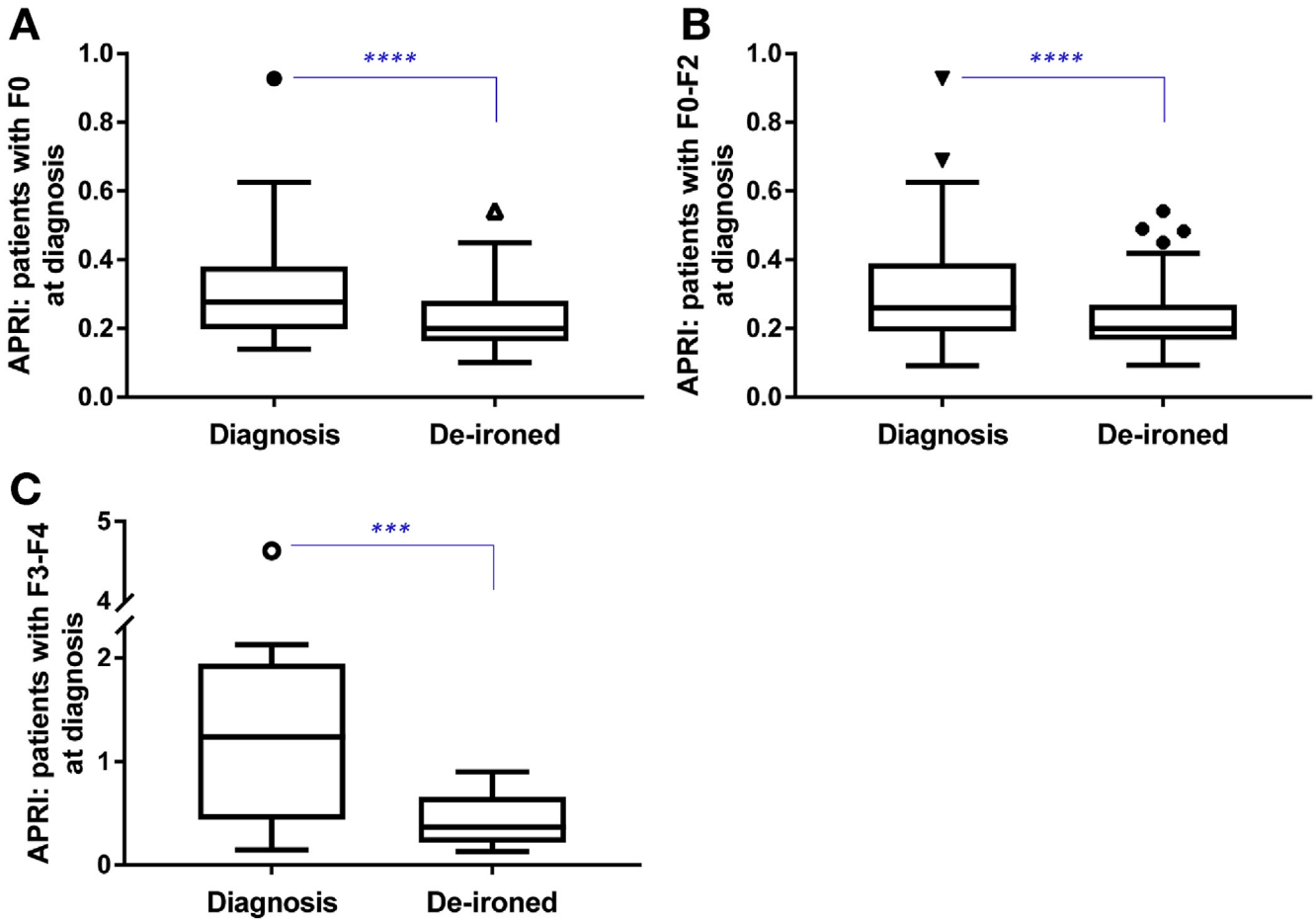


Figure 4. Changes in APRI before and after venesection in HH subjects with fibrosis stage at initial diagnosis of (*A*) F0, (*B*) F0–F2, and (*C*) F3–F4. ****$P < .0001$; ***$P < .001$. APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis 4; GPR, gamma-glutamyl transferase ratio; HH, HFE hemochromatosis.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.07.052.

References

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399–408.
2. Bridle KR, Frazer DM, Wilkins SJ, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet 2003; 361:669–673.
3. Edwards CQ, Griffen LM, Goldgar D, et al. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med 1988;318:1355–1362.
4. Olynyk JK, Luxon BA, Britton RS, et al. Hepatic iron concentration in hereditary hemochromatosis does not saturate or accurately predict phlebotomy requirements. Am J Gastroenterol 1998;93:346–350.
5. Adams PC, Speechley M, Kertesz AE. Long-term survival analysis in hereditary hemochromatosis. Gastroenterology 1991;101:368–372.
6. Cassinotto C, Boursier J, de Ledinghen V, et al. Liver stiffness in nonalcoholic fatty liver disease: a comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. Hepatology 2016;63:1817–1827.
7. Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343–350.
8. Leung DH, Khan M, Minard CG, et al. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. Hepatology 2015;62:1576–1583.
9. Lin ZH, Xin YN, Dong QJ, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology 2011;53:726–736.
10. Sterling RK, King WC, Wahed AS, et al. Evaluating noninvasive markers to identify advanced fibrosis by liver biopsy in HBV/HIV co-infected adults. Hepatology 2020;71:411–421.
11. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection—comparison with liver biopsy and fibrotest. Hepatology 2007;46:32–36.
12. Lewindon PJ, Puertolas-Lopez MV, Ramm LE, et al. Accuracy of transient elastography data combined with APRI in detection

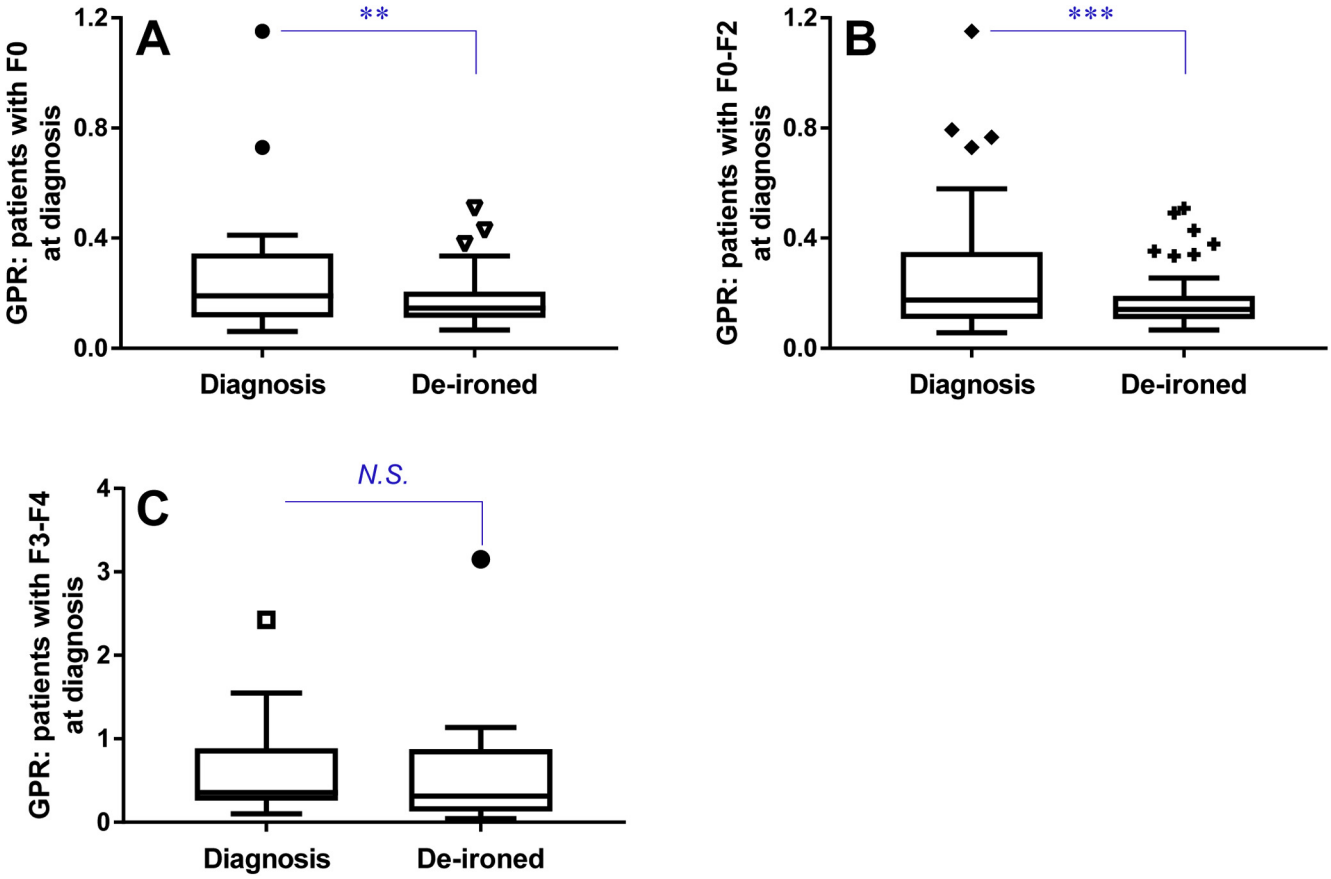
and staging of liver disease in pediatric patients with cystic fibrosis. Clin Gastroenterol Hepatol 2019;17:2561–2569.e5.
13. Calvopina DA, Noble C, Weis A, et al. Supersonic shear-wave elastography and APRI for the detection and staging of liver disease in pediatric cystic fibrosis. J Cyst Fibros 2020;19:449–454.
14. Lemoine M, Shimakawa Y, Nayagam S, et al. The gamma-glutamyl transpeptidase to platelet ratio (GPR) predicts significant liver fibrosis and cirrhosis in patients with chronic HBV infection in West Africa. Gut 2016;65:1369–1376.
15. Beaton M, Guyader D, Deugnier Y, et al. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. Hepatology 2002;36:673–678.
16. Crawford DH, Murphy TL, Ramm LE, et al. Serum hyaluronic acid with serum ferritin accurately predicts cirrhosis and reduces the need for liver biopsy in C282Y hemochromatosis. Hepatology 2009;49:418–425.
17. Olynyk JK, St Pierre TG, Britton RS, et al. Duration of hepatic iron exposure increases the risk of significant fibrosis in hereditary hemochromatosis: a new role for magnetic resonance imaging. Am J Gastroenterol 2005;100:837.
18. Powell L, Dixon J, Ramm G, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med 2006;166:294–301.
19. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991;13:372–374.
20. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983;148:839–843.
21. Moreno C, Mueller S, Szabo G. Non-invasive diagnosis and biomarkers in alcohol-related liver disease. J Hepatol 2019;70:273–283.
22. Olynyk JK, Trinder D, Ramm GA, et al. Hereditary hemochromatosis in the post-HFE era. Hepatology 2008;48:991–1001.
23. Stal P, Broome U, Scheynius A, et al. Kupffer cell iron overload induces intercellular adhesion molecule-1 expression on hepatocytes in genetic hemochromatosis. Hepatology 1995;21:1308–1316.
24. Deugnier YM, Loreal O, Turlin B, et al. Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their bioclinical correlations. Gastroenterology 1992;102:2050–2059.
25. Bridle KR, Crawford DH, Fletcher LM, et al. Evidence for a submorphological inflammatory process in the liver in haemochromatosis. J Hepatol 2003;38:426–433.
26. Bardou-Jacquet E, Morandeau E, Anderson GJ, et al. Regression of fibrosis stage with treatment reduces long-term risk of liver cancer in patients with hemochromatosis caused by mutation in HFE. Clin Gastroenterol Hepatol 2020;18:1851–1857.
27. Falize L, Guillygomarc'h A, Perrin M, et al. Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36 cases. Hepatology 2006;44:472–477.
28. Adhoute X, Foucher J, Laharie D, et al. Diagnosis of liver fibrosis using FibroScan and other noninvasive methods in patients with hemochromatosis: a prospective study. Gastroenterol Clin Biol 2008;32:180–187.

Reprint requests
Address requests for reprints to: Grant A Ramm, BSc (Hons), PhD, FAASLD, Hepatic Fibrosis Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4006, Australia. e-mail: Grant.Ramm@qimrberghofer.edu.au; fax: +61-7-3362-0191.

CRediT Authorship Contributions
Justin Chin (study design, data collection, analysis, and manuscript preparation)
Lawrie W. Powell (study design, data curation, and manuscript editing)
Louise E. Ramm (study design, data curation, and manuscript editing)
Gunter F. Hartel (data analysis and manuscript editing)
John K. Olynyk (study design, data collection, analysis, manuscript review and editing)
Grant A. Ramm (Conceptualization, study design, data collection, analysis and manuscript editing)

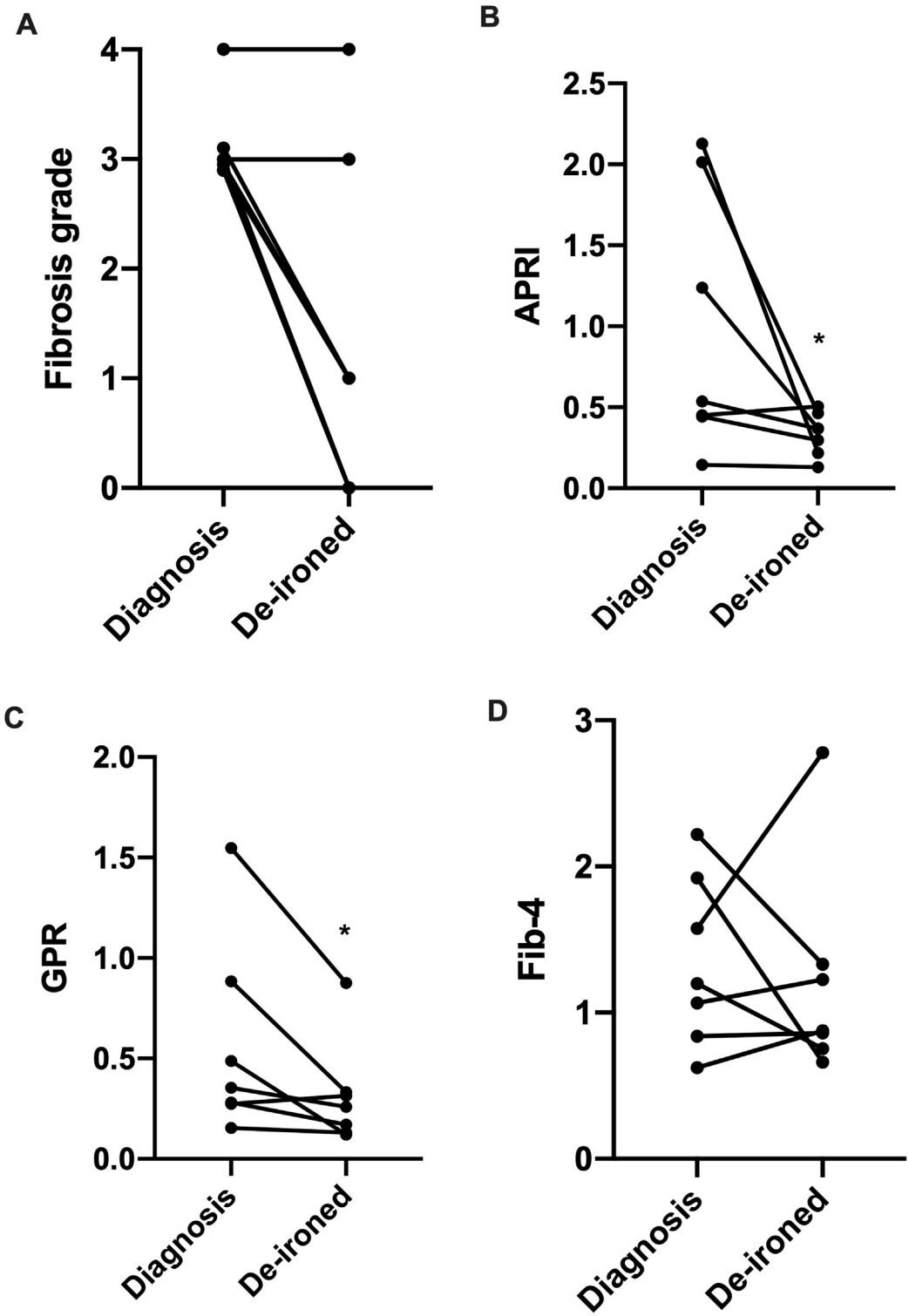
Conflicts of interest
The authors disclose no conflicts.

Funding
Supported by grants from the National Health and Medical Research Council of Australia (APP1048740; APP1061332).

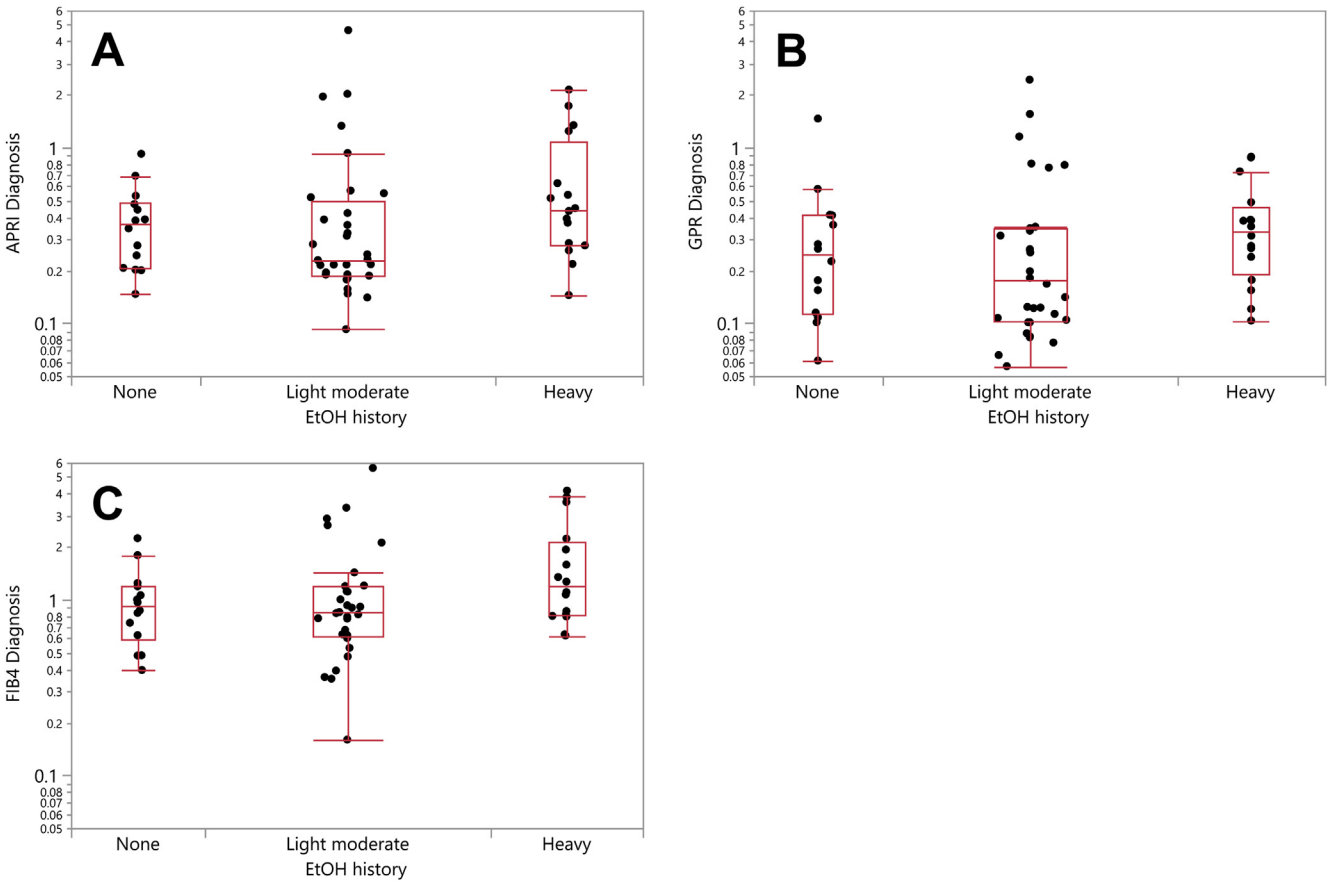


web 4C/FPO

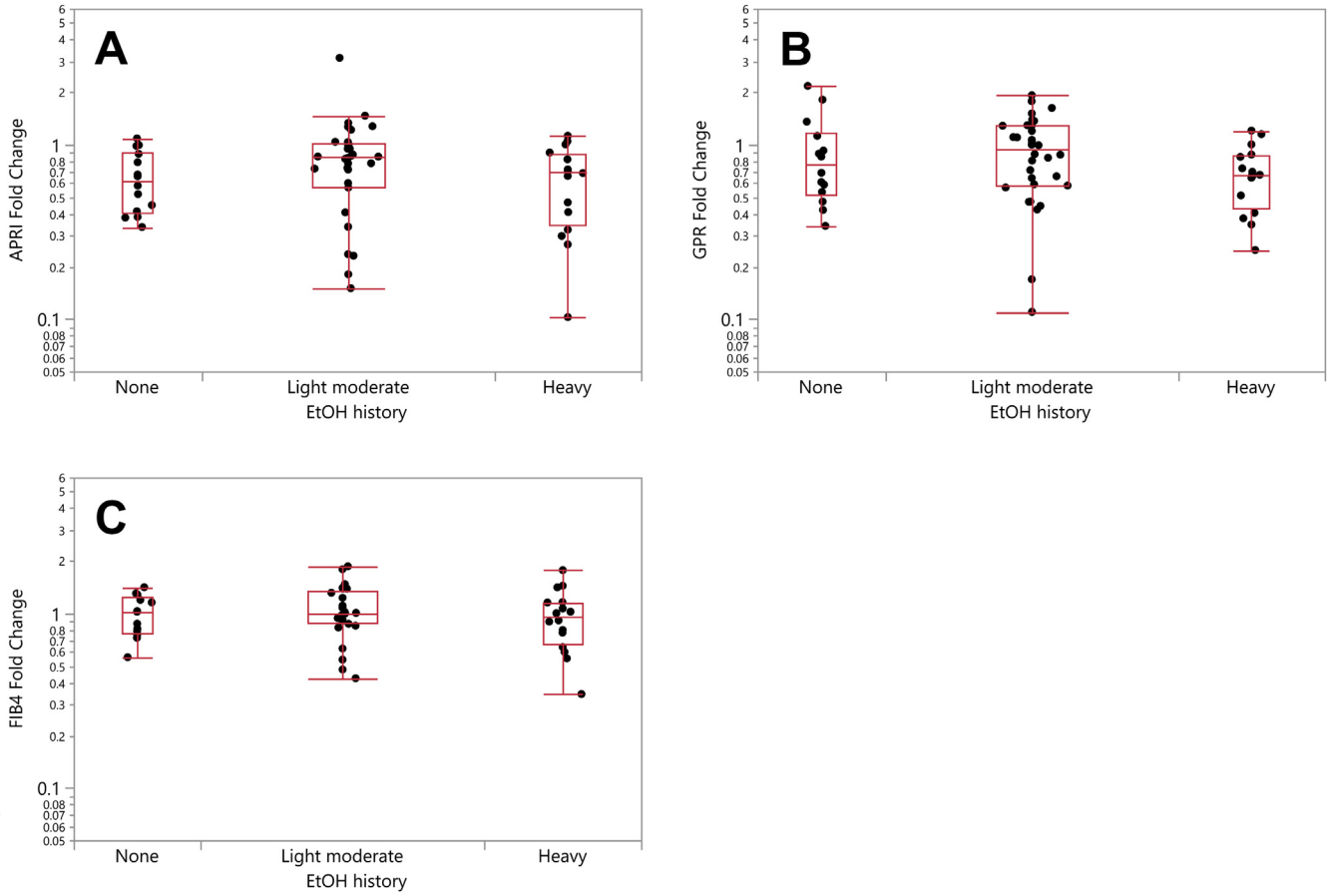
Supplementary Figure 1. Changes in GPR before and after venesection in HH subjects with fibrosis stage at initial diagnosis of (*A*) F0, (*B*) F0–F2, and (*C*) F3–F4. ***$P < .001$; **$P < .01$. APRI, aspartate aminotransferase-to-platelet ratio; GPR, gamma-glutamyl transferase-to-platelet ratio; HH, HFE hemochromatosis.



Supplementary Figure 2. Effect of venesection treatment on (*A*) hepatic fibrosis stage, (*B*) APRI, (*C*) GPR, and (*D*) FIB-4 in 7 HH subjects who had repeat liver biopsies after venesection treatment. *$P \leq .05$. APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, fibrosis-4; GPR, gamma-glutamyl transferase-to-platelet ratio; HH, HFE hemochromatosis.



Supplementary Figure 3. Effect of alcohol consumption on (*A*) APRI, (*B*) GPR, and (*C*) FIB-4 measured at biopsy. Alcohol (EtOH) consumption is reported as none, light-moderate (<30 g/day), or heavy (>30 g/day). APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, fibrosis-4; GPR, gamma-glutamyl transferase-to-platelet ratio.



Supplementary Figure 4. Effect of alcohol consumption on fold-change decrease in (*A*) APRI, (*B*) GPR, and (*C*) FIB-4 after venesection. Alcohol (EtOH) consumption is reported as none, light-moderate (<30 g/day), or heavy (>30 g/day). APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, fibrosis-4; GPR, gamma-glutamyl transferase-to-platelet ratio.

Supplementary Table 1. Baseline Characteristics of Patients With HH

Age (*y*)	
Men	42.7 ± 1.1
Women	46 ± 2.3
Gender	
Male	132 (72.9%)
Female	49 (27.1%)
Alcohol intake (*g/day*)	
Men	31.7 ± 3.1
Women	19.8 ± 3.5[a]
Fibrosis stage	
0	98 (54.1%)
1	27 (14.9%)
2	22 (12.2%)
3	12 (6.6%)
4	22 (12.2%)
Mean APRI (n = 179)	
F0–F2	0.33 ± 0.02
F3–F4	1.25 ± 0.17[b]
Mean GPR (n = 179)	
F0–F2	0.29 ± 0.04
F3–F4	1.16 ± 0.35[b]
Mean FIB-4 (n = 153)	
F0–F2	0.86 ± 0.04
F3–F4	2.52 ± 0.39[b]

NOTE. Data presented as mean ± standard error of the mean or proportions. APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4; GPR, gamma-glutamyl transferase-to-platelet ratio; HH, HFE hemochromatosis.
[a]$P < .01$.
[b]$P < .0001$.