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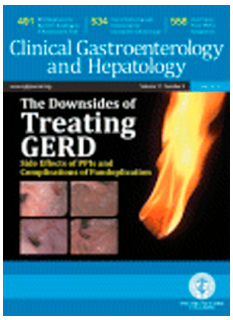

Utility of Serum Biomarker Indices for Staging of Hepatic Fibrosis Before and After Venesection in Patients With Hemochromatosis Caused by Variants in *HFE*.

Justin Chin, Lawrie W. Powell, Louise E. Ramm, Gunter F. Hartel, John K. Olynyk, Grant A. Ramm

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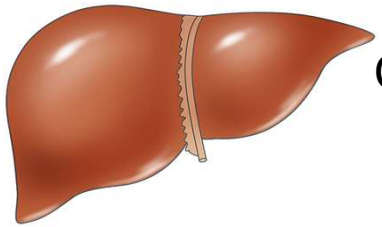
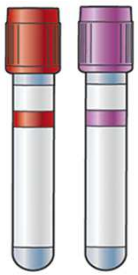


C282Y homozygous

Routine screening for advanced fibrosis



APRI >0.44

FIB-4 >1.1

No

Yes

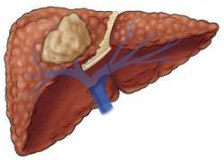
Therapeutic venesection

Assessment for advanced fibrosis: Liver biopsy

If present

Routine surveillance for complications

Serial APRI measurements at follow up to monitor for potential fibrosis regression



Clinical Gastroenterology and Hepatology

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 non-invasive 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 non-invasively identify patients with HFE-associated hemochromatosis who have advanced hepatic fibrosis. APRI scores might also be used to monitor fibrosis regression following venesection

TITLE

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

SHORT TITLE

Serum fibrosis biomarkers in hemochromatosis

AUTHORS: Justin Chin[1], Lawrie W. Powell[2], Louise E. Ramm[3], Gunter F. Hartel[4], John K. Olynyk[1,5+], Grant A. Ramm[2,3*+]

1. Department of Gastroenterology & Hepatology, Fiona Stanley Fremantle Hospital Group, Murdoch, Western Australia, Australia
2. Faculty of Medicine, The University of Queensland, Herston, Brisbane, Queensland, Australia.
3. Hepatic Fibrosis Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia
4. Statistics Unit, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia
5. School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia.

* **Corresponding author**

\+ **Equal senior authors**

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ABBREVIATIONS:

HFE Hemochromatosis, HH; AST-to-platelet ratio, APRI; GGT-to-platelet ratio, GPR; Fibrosis-4, FIB-4; liver function test, LFT; magnetic resonance imaging, MRI; area under the receiver operator characteristic, AUROC; positive predictive value, PPV; negative predictive value, NPV

CORRESPONDENCE:

Professor Grant A Ramm, BSc(Hons), PhD, FAASLD

Hepatic Fibrosis Group

QIMR Berghofer Medical Research Institute, Brisbane, Queensland

4006, Australia

Grant.Ramm@qimrberghofer.edu.au

Telephone: +61 7 3362 0222

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This study is in compliance with the Australian Code for the Responsible Conduct of Research, 2018

ABSTRACT

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 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.

KEY WORDS: HH, disease progression, respond to treatment, blood test

INTRODUCTION

HFE-hemochromatosis (HH) is a common genetic disorder of iron metabolism[1], characterised 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, since 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. Additionally, liver biopsy does not allow for easy, dynamic, ongoing assessment of fibrosis progression.

There has been a progressive evolution of non-invasive 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, AST-to-platelet ratio index [APRI], GGT-to-platelet ratio [GPR], FIB-4). These serum biomarker indices have been shown to be useful, easy to perform and relatively inexpensive. Additionally, 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, non-alcoholic fatty liver disease, HIV/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 AST level and a platelet count >200x10^9/L were shown to predict cirrhosis in the majority of HH subjects[15]. However approximately 30%-64% of patients with cirrhosis do not fulfil all three criteria[15, 16]. Serum hyaluronic acid levels of >46.5 ng/ml have also been shown to have a high sensitivity and specificity in identifying the presence of cirrhosis in HH patients, and together with a serum ferritin level >1000 µg/L obviate the need for liver biopsy in 60% of patients[16]. While transient elastography for

assessment of fibrosis has been used in viral hepatitis and non-alcoholic fatty liver disease[6, 7], its use in HH has not been clearly defined. MRI 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 as they are likely to be highly cost-effective and readily available via liver function tests performed during routine blood work up 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 non-invasive serum biomarker indices (APRI, GPR and FIB-4) to predict the stage of fibrosis and determine cut-off thresholds for the detection of advanced hepatic fibrosis in a large, well-characterised cohort of liver biopsy-validated subjects with HH, before and after venesection treatment.

PATIENTS AND 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 histological 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 using methods by the National Health and Medical Research Council of Australia, which define one standard drink as containing 10g of alcohol (equivalent to 12.5mL 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 <100µg/L was achieved. Liver biopsy was also performed in seven subjects following treatment for clinically indicated reasons. APRI, GPR and FIB-4 data were calculated for all study subjects at the time of liver biopsy, prior to commencing venesection. These biomarker indices were also calculated in a subgroup of 64 subjects following 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 was 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 and 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 to 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 ± SEM unless otherwise specified. Spearman's rank correlation was used to assess associations with increasing stage of hepatic fibrosis. Student's T-test or analysis of variance were used to analyse 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 cut-offs. In addition, dual cut-off 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 was used to compare performance of the ROC curves[20]. 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- 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 (dichotomised as 'mild fibrosis', F1-F2 and 'advanced fibrosis', F3-F4) versus APRI, GPR or FIB-4 at biopsy. A cut-off value was selected to maximize the Youden's Index (Sensitivity + Specificity). This cut-off 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 following de-ironing was assessed using ANOVA and Tukey-Kramer HSD. Statistical significance was assigned as $p \leq 0.05$. All statistical tests were conducted 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 males and 46±2.3 years for females. Mean alcohol consumption was 28.5±2.5 g/day (19.8±3.5 g/day for females and 31.7±3.1g/day for males, p=0.01). Advanced hepatic fibrosis was identified in 34 subjects and was more prevalent in males. 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 (AUROC) for APRI versus GPR (p=0.05), 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<0.0001; GPR, r=0.36, p<0.0001; FIB-4, r=0.35, p<0.0001).

Diagnostic accuracy of APRI, GPR and FIB-4 for the prediction of advanced fibrosis

APRI: The AUROC for APRI was 0.88 (95% CI, 0.81-0.96), providing an optimal threshold for detection of advanced fibrosis of 0.44 (Figure 2A), with a sensitivity of 79.4%, specificity of 79.3% and a diagnostic accuracy of 81% (Table 1). Dual cut-off 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 cut-off value of >0.44, 29/34 (85.3%) of patients with F3-F4 fibrosis were accurately staged, whilst 21.2% of patients with F0-F2 fibrosis were staged incorrectly.

GPR: 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 2B), with a sensitivity of 67.7%, specificity of 70.3% and a diagnostic accuracy of 69% (Table 1). Dual cut-off 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 cut-off value of >0.27, 23/34 (67.6%)

of patients with F3-F4 fibrosis were correctly staged whilst 29.5% of patients with F0-F2 fibrosis were staged incorrectly.

FIB-4: 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 2C), with a sensitivity of 80%, specificity of 80.3% and a diagnostic accuracy of 81% (Table 1). Dual cut-off 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 cut-off value of >1.11, 20/25 (80%) of patients with F3-F4 fibrosis were correctly staged whilst 18.9% of patients with F0-F2 fibrosis were staged incorrectly.

Effect of venesection on APRI, GPR and FIB-4 and potential to monitor fibrosis regression

Following venesection therapy (when serum ferritin levels decreased <100 μg/L), APRI, GPR and FIB-4 were recalculated. The mean (± SEM) 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 ($p<0.0001$) values (Figure 3A), including in subjects with F0, F0-F2 or F3-F4 fibrosis (Figure 4). Figure 3B 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 post-venesection (Figure 3A, $p<0.001$), including in subjects with F0 or F0-F2 fibrosis, but not in subjects with F3-F4 fibrosis (Supplementary Figure 1). Figure 3C 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 3A and 3D), including when subjects were analysed at F0, F0-2 or F3-F4 fibrosis (not shown).

Given 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). **APRI:** Logistic regression of dichotomised fibrosis stage (F1-F2 and F3-F4) versus APRI at biopsy was highly significant ($P<0.0001$) with odds ratio 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=61.8% and specificity=95.9%, using APRI cut-off = 0.785. Applying this cut-off to the post-venesection APRI values, we found that of the 15 patients with F3-F4 fibrosis at diagnosis, APRI values decreased below the cut-off indicative of F1-F2 fibrosis in 13 subjects (87%; 95% CI, 62.1%–96.3%).

GPR: Logistic regression of F1-F2 and F3-F4 versus GPR at biopsy was significant (P=0.002) with odds ratio 2.1 (95% CI, 1.3–3.5) per unit change in GPR for having advanced versus mild fibrosis. The AUROC curve was 0.70, with sensitivity=82.4% and specificity=51.0%, using GPR cut-off = 0.225. Applying this cut-off to the post-venesection GPR values, we found that of the 15 patients with F3-F4 fibrosis at diagnosis, GPR values decreased below the cut-off 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=0.0009). The proportion of F3-F4 patients 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=0.006). Thus, this result suggests that APRI may be superior to GPR for the assessment of fibrosis regression following venesection therapy.

Seven subjects with F3-F4 fibrosis at diagnosis also had follow-up liver biopsies following de-ironing for clinically indicated reasons. Hepatic fibrosis regressed ≥2 F stages in five of seven patients following venesection, but remained unchanged in 2 patients (p=0.06) (Supplementary Figure 2). There was a significant reduction in APRI and GPR with de-ironing in these seven 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 (<30g/day), and heavy (≥30g/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 following de-ironing therapy. There were also no

relationships observed between biomarker indices and iron indices either at biopsy or following venesection (not shown).

DISCUSSION

This unique, liver biopsy-based study of a well-characterized cohort of HH subjects pre- and post-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 following venesection treatment, including when analysed in subjects with F0, F0-F2 or advanced (F3-F4) fibrosis. Additionally, in a subset of subjects with available post-treatment liver biopsies, reductions in APRI and GPR 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 as 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 cut-off values for these biomarkers for predicting advanced fibrosis in HH are lower than those observed in more aggressive conditions such as viral hepatitis B (HBV) or C (HCV) and alcohol-related liver disease (ALD). In previous studies in patients with HCV or ALD, an APRI cut-off threshold for advanced fibrosis of 1 was proposed with a demonstrated sensitivity of 35% and specificity of 94% for the diagnosis of cirrhosis in ALD[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. in predicting advanced fibrosis in HBV[14]), 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 regards to FIB-4, at the lower limit of 1.45 (as suggested by Vallet-Pichard for advanced fibrosis in HCV[11]), 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 cut-off values we defined in HH subjects were more similar to those found in a study which 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 AST levels compared to that observed in viral hepatitis[22-25].

The method described by Beaton et al. was also shown to be a reliable predictor of cirrhosis in HH. However, a significant number of subjects would not fulfil all three 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). Additionally, 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 post-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 following venesection, but FIB-4 did not, albeit in 7 patients where repeat liver biopsy was available. Unlike other liver diseases, HH is not typically characterised 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 pre-treatment 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 post-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 non-invasive monitoring of fibrosis regression following treatment of HH. Further

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

A study by Adhoute et al. assessed the utility of Fibroscan and serum-based non-invasive methods of hepatic fibrosis assessment in 57 subjects with HH versus 46 controls[28]. They found that prevalence of liver stiffness measurements at a cut-off >7.1kpa were 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 non-invasive methods with histology and thus appropriate cut-offs 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 requires caution in interpretation of the significant decreases observed for APRI and GPR with biopsy-validated fibrosis regression. However, we believe this study may be the first to assess the performance of three separate, commonly utilised 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 following treatment. These readily available biomarkers could be utilized 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.

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FIGURE LEGENDS

Figure 1. There was a significant correlation between increasing hepatic fibrosis stage and (A) APRI ($r=0.54$, $p<0.0001$), (B) GPR ($r=0.36$, $p<0.0001$), and (C) FIB-4 ($r=0.35$, $p<0.0001$).

Figure 2. (A) APRI, (B) GPR and (C) FIB-4 values for F3-F4 versus F0-F2 fibrosis with proposed cut-offs for predicting advanced fibrosis in HH patients (dotted lines). ***$p<0.01$; ****$p<0.0001$.

Figure 3. Effect of venesection treatment on APRI, GPR and FIB-4 in subjects with HH, (A) at diagnosis and following 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 post- vs pre-venesection. ****$p<0.0001$, ***$p<0.001$. (B-D), Bland-Altman plots.

Figure 4. Changes in APRI pre- and post-venesection in HH subjects with fibrosis stage at initial diagnosis of (A) F0, (B) F0-F2 and (C) F3-F4. ****$p<0.0001$, ***$p<0.001$

Table 1. Diagnostic accuracy of APRI, GPR and FIB-4 using optimal cut-offs in the diagnosis of advanced fibrosis in HH subjects.

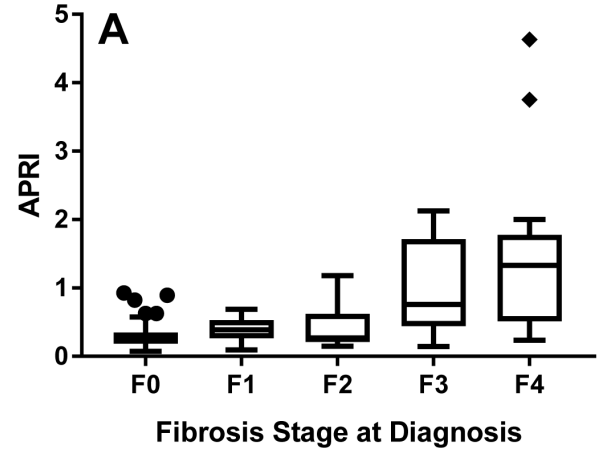
	AUROC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	% Accuracy	Cut-off	z	*p*
APRI	0.88 (0.81 – 0.96)	79.4% (63.2%-89.7%)	79.3% (72%-85.1%)	79.3%	79.4%	81%	0.44		
GPR	0.76 (0.67 – 0.85)	67.7% (50.8% - 80.9%)	70.3% (62.5-77.1%)	69.5%	68.5%	81%	0.27	1.96	0.05
FIB-4	0.86 (0.78 – 0.95)	80% (60.9% - 91.1%)	80.3% (72.6%–86.3%)	80.2%	80.1%	69%	1.11	1.27	0.20

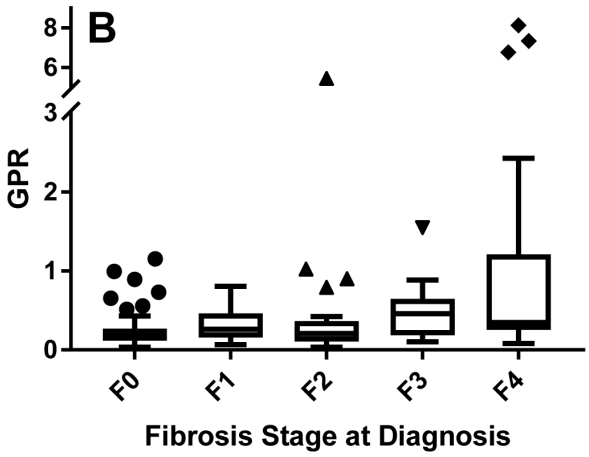
95% confidence intervals in brackets. AUROC, area under the receiver operator characteristic; APRI, AST-to-platelet ratio; GPR, GGT-to-platelet ratio; FIB-4, Fibrosis-4. p-values derived from Hanley-McNeil comparison of z-values for GPR and FIB-4 versus APRI[22].

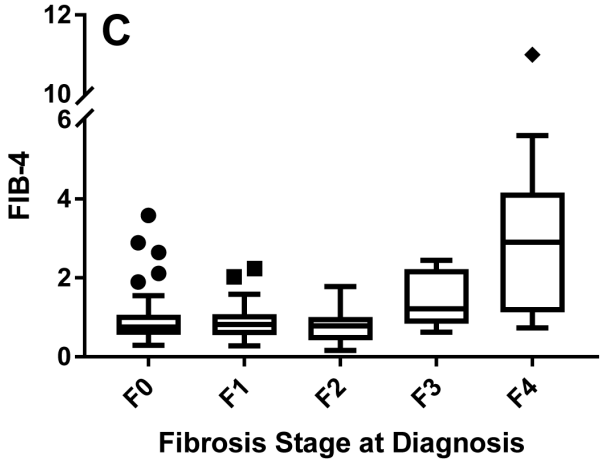
Table 2. Diagnostic accuracy of APRI, GPR and FIB-4 using optimal cut-offs to rule-in and rule-out advanced fibrosis in HH subjects.

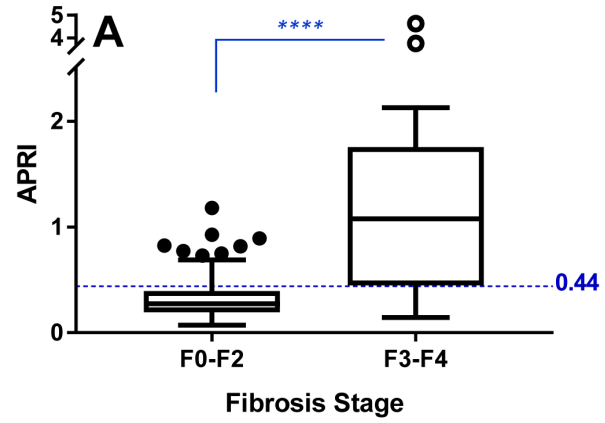
	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV**	**NPV**	***p***
APRI					
≥ 0.59	70.6% (53.8%-83.2%)	90.3% (84.5%-94.1%)	87.9%	75.4%	<0.0001
≤ 0.37	91.1% (77%-97%)	69.0% (61.0%-75.9%)	74.6%	88.6%	
GPR					
≥ 0.57	38.2% (23.9%-55%)	90.3% (84.5%-94.2%)	79.7%	59.4%	<0.0001
≤ 0.15	91.2% (77%-97%)	31.7% (24.7%-40%)	57.2%	78.3%	
FIB-4					
≥ 1.38	64% (44.5%-79.8%)	90.6% (84.2%-94.5%)	87.2%	71.6%	<0.0001
≤ 0.73	96% (75%-98.6%)	46.7% (38%-55.1%)	64.3%	92.1%	

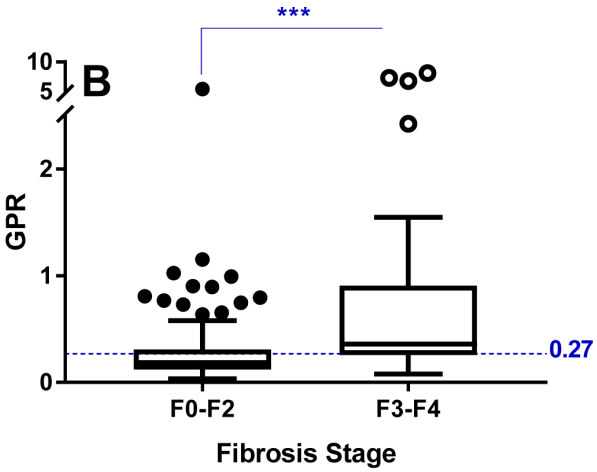
95% confidence intervals are shown in brackets. AUROC, area under the receiver operator characteristic; APRI, AST-to-platelet ratio; GPR, GGT-to-platelet ratio; FIB-4, Fibrosis-4.

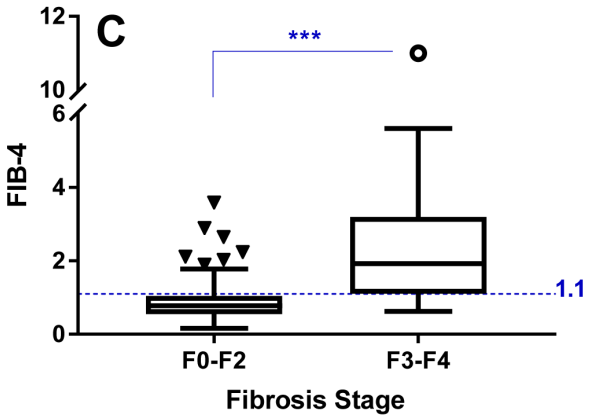

A
APRI
5
4
3
2
1
0
F0
F1
F2
F3
F4
Fibrosis Stage at Diagnosis

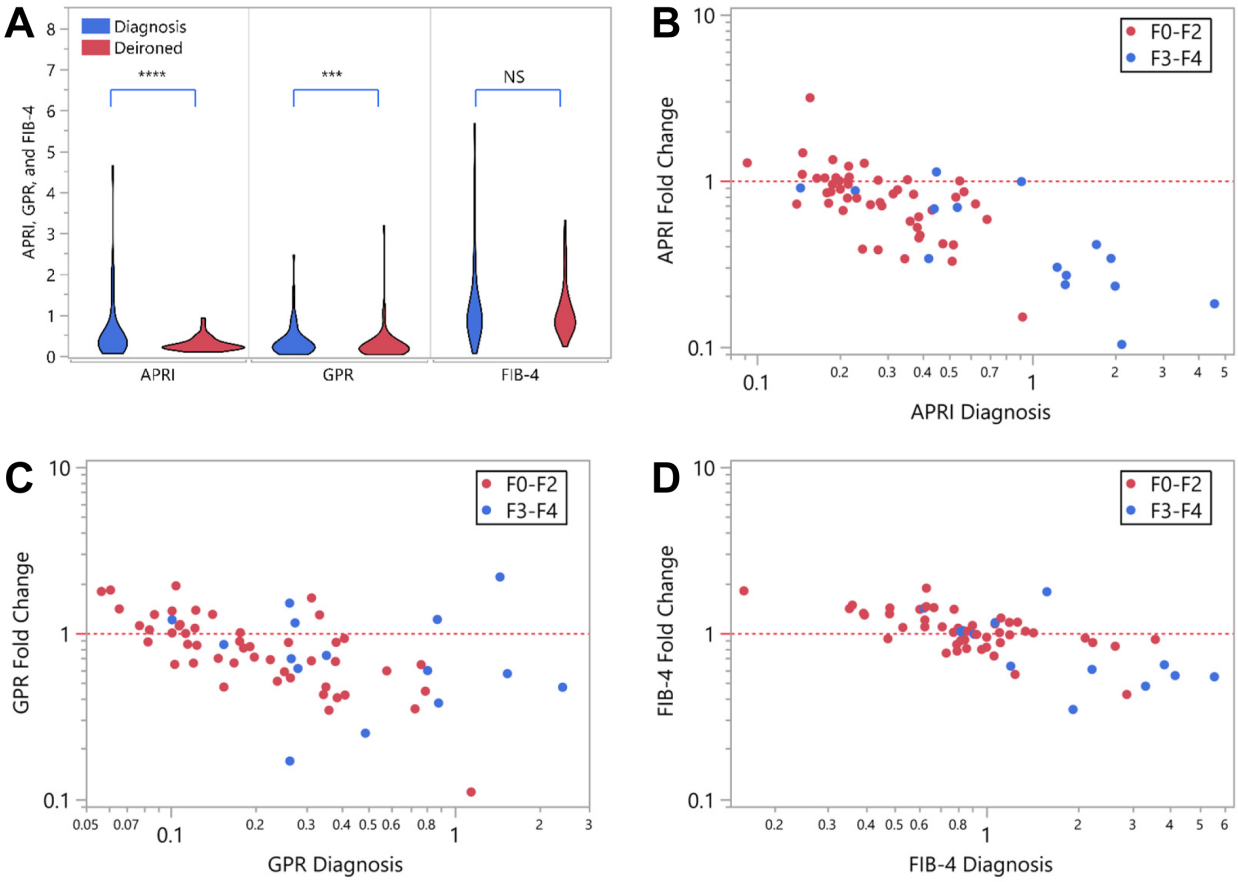

B
GPR
8
6
3
2
1
0
F0
F1
F2
F3
F4
Fibrosis Stage at Diagnosis

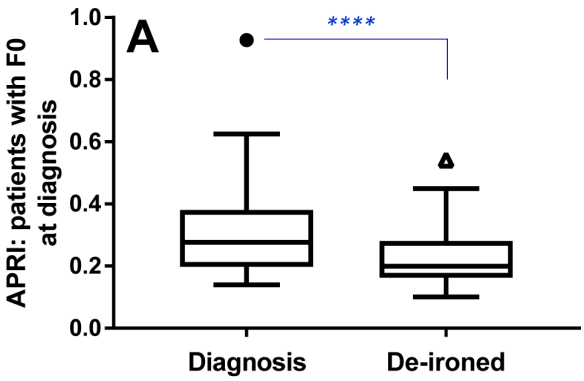

C
FIB-4
12
10
6
4
2
0
F0
F1
F2
F3
F4
Fibrosis Stage at Diagnosis

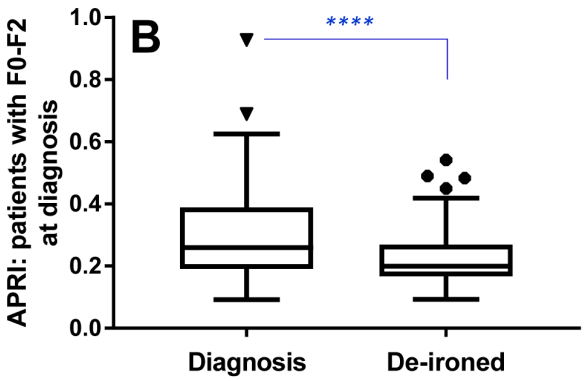

A
****
APRI
5
4
2
1
0
0.44
F0-F2
F3-F4
Fibrosis Stage

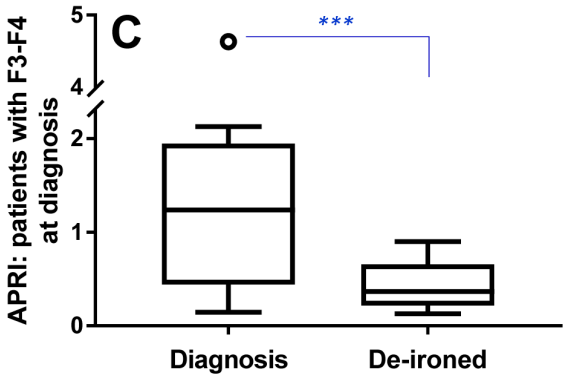

B
***
GPR
10
5
2
1
0
0.27
F0-F2
F3-F4
Fibrosis Stage


C
***
FIB-4
12
10
6
4
2
0
1.1
F0-F2
F3-F4
Fibrosis Stage


A
Diagnosis
Deironed
****
***
NS
APRI, GPR, and FIB-4
APRI
GPR
FIB-4
B
F0-F2
F3-F4
APRI Fold Change
APRI Diagnosis
C
F0-F2
F3-F4
GPR Fold Change
GPR Diagnosis
D
F0-F2
F3-F4
FIB-4 Fold Change
FIB-4 Diagnosis


A
****
APRI: patients with F0 at diagnosis
1.0
0.8
0.6
0.4
0.2
0.0
Diagnosis
De-ironed


B
****
APRI: patients with F0-F2 at diagnosis
1.0
0.8
0.6
0.4
0.2
0.0
Diagnosis
De-ironed


C
***
APRI: patients with F3-F4 at diagnosis
5
4
2
1
0
Diagnosis
De-ironed