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ORIGINAL RESEARCH

The Utility of Retinol-Binding Protein 4 in Predicting Liver Fibrosis in Chronic Hepatitis C Patients in Response to Direct-Acting Antivirals

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Background: Hepatic fibrosis grading is crucial for chronic hepatitis C (CHC) patients in monitoring liver disease progression and antiviral treatment indication. Retinol-binding protein 4 (RBP4), an adipokine secreted by adipocytes and hepatocytes, has variable levels in health and disease.

Purpose: To comparatively evaluate RBP4 serum levels in predicting liver fibrosis in CHC versus fibroscan, noninvasive fibrosis, and inflammatory indices.

Patients and Methods: Cohort study included 50 naive non-obese CHC patients and 20 age-, sex- and body mass index-matched healthy subjects. Fibroscan, RBP4, and noninvasive fibrosis as APRI, CDS, FIB-4, GUCI, Lok index indices based on serological markers, and inflammatory indices as platelet to lymphocyte ratio (PLR) and liver regeneration markers as; alpha-fetoprotein (AFP) and APRI, were evaluated in response to direct-acting antivirals (DAAs).

Results: RBP4 was significantly lower in patients than in controls (P=0.0001) and progressively decreased with the increase in fibrosis grade (F0-F=41.42±3.08), (F2=39.32±1.43), (F3-F4= 35.31±0.5), (P=0.0001). Liver function, stiffness, and RBP4 significantly improved after treatment (P=0.0001). RBP4 negatively correlated with viral load (r=−0.78, p=0.0001), fibroscan fibrosis grade (r=−0.52, p=0.0001), AFP (r=−0.63, p=0.0001), and positively correlated with platelet (r=0.424, p=0.0001), and white cell count (r=0.298, p=0.002). RBP4 at a cutoff value <40.55 ng/mL might predict significant fibrosis (90.48% sensitivity, 62.5% specificity, AUROC=0.811, 95% CI=67.5–90.0) and at a cutoff value <35.9 ng/mL could predict advanced fibrosis (100% sensitivity, 100% specificity, AUROC =1.0, 95% CI=0.929–1).

Conclusion: RBP4 showed excellent accuracy, sensitivity, specificity, PPV, and NPV. RBP4 has a superior diagnostic performance in predicting advanced fibrosis grads in CHC patients and hence can replace expensive invasive procedures.

Keywords: APRI, FIB-4, GUCI, noninvasive fibrosis indices, platelet lymphocyte ratio, transient elastography fibroscan

Introduction

Precise staging and grading of hepatic fibrosis/cirrhosis are of critical concern in chronic hepatitis C (CHC) patients not only for monitoring the progression of liver disease but also for antiviral treatment indication.[1]

Retinol-binding protein 4 (RBP4), an adipokine primarily secreted by adipocytes and hepatocytes, is the sole transporter of retinol from liver stores to peripheral tissues, so its serum estimate reflects serum retinol concentration and the

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increase in retinoic acid genesis is related to the progressive decrease in serum RBP4 levels and hepatic vitamin A storage exhaustion and hence the liver functional status, both of which accelerate liver fibrosis giving the chance for tumor development.[2]

Liver necrosis or active regeneration due to ongoing inflammation and/or fibrosis with an altered hepatocyte–hepatocyte interaction and loss of normal architecture produces elevation of alpha-fetoprotein (AFP).[3]

The benefit of extended sustained virological response (SVR) in reaction to the antiviral drug is to block the fibrogenic progression, thus escaping the progressive normal tissue destruction or the replacement of hepatic parenchyma, changing micro-environment from pro-inflammatory to anti-inflammatory restorative state, and redirect the synthesis of inflammatory molecules to a regenerative state.[4]

Little known about the effect of current direct-acting antiviral drugs (DAAs) on RBP4. This study aimed to estimate serum RBP4 as a screening tool for hepatocellular regeneration in response to Sofosbuvir-based treatment regimen in genotype 4, naïve CHC Egyptian patients, and relate the finding to the result of transient elastography (TE) – Fibroscan and non-invasive liver fibrosis marker testing in liver fibrosis grading and diagnosis of early cirrhosis.

Materials and Methods

The current study is a prospective case-control study, involving 50 naïve CHC non-obese patients, who were a candidate for antiviral therapy and monitored at the outpatient clinics of Tropical Medicine and Gastroenterology department – Qena University Hospital, from January 2018 to December 2018. 20 healthy subjects, matched by age, sex, and BMI, form a control group.

The inclusion criteria were treatment naïve patients, age older than 18 years with HCV RNA positivity. The study was conducted after approval by the Qena faculty of medicine – South Valley University institutional ethics committee review board, according to the Declaration of Helsinki and its subsequent amendments, and after obtaining written informed consent from all subjects.

The virological assessment of HCV-RNA level determined three times: at the baseline (before treatment), at the end of treatment (week 12), and 12-week post-treatment, using the Cobas TaqMan HCV assay V.2.0 (Roche Diagnostics – Mannheim, Germany). The lower limit of detection (15 IU/mL). Sustained virological response (SVR12) is defined as an undetectable HCV-RNA at 12 weeks after completion of therapy. The HCV genotypes were identified by direct sequencing of the untranslated regions using RT-PCR-based assay (Ampli Sens 61 HCV-genotype-FRT PCR kit).

Treatment Regimen

Based on European Association for Study of Liver (EASL) guidelines, for treatment of HCV, patients were classified into two groups: easy-to-treat group, including (naive patients with a serum bilirubin level ≤1.2 mg/dl, albumin ≥3.5 gm/dl, INR level ≤1.2 and the platelet count ≥ 150×10^9/L, their regimen was sofosbuvir 400 mg and daclatasvir 60 mg once daily for 12 weeks), and difficult-to-treat group (total bilirubin ≥1.2 mg/dl, albumin level ≤3.5 gm/dl, INR ≥ 1.2 and the platelet count < 150×10^9/L) treated by sofosbuvir 400 mg and daclatasvir 60 mg and dose-weighted ribavirin (800–1200 mg) daily for 12 weeks.[5]

Exclusion Criteria

Pregnancy, BMI > 25, acute exacerbation of viral hepatitis, or other forms of liver diseases such as concomitant hepatitis-B and HIV infection, schistosomal, autoimmune or alcoholic hepatitis, diabetes, drug addiction or alcohol abuse, hepatocellular carcinoma (HCC), evidence of decompensated liver cirrhosis, cardiac cirrhosis or primary biliary cirrhosis or hereditary liver condition, presence of cancer or hepatoma, patients received recent platelet transfusion and associated kidney disease.

All the patients subjected to history taking, including (age, sex, previous operation, disease duration, past history, and family history), and physical examination, abdominal ultrasonography to assess the presence of liver cirrhosis; portal vein diameter, splenic size, the existence of ascites and evaluation of cirrhosis clinical-grade using Child–Pugh score.[6]

Blood Sampling

10-mL fasting venous blood sample, collected under aseptic conditions and divided into 3 tubes; an EDTA tube for a complete blood count, a citrate tube for prothrombin time, and a plain tube for liver functions and ELISA techniques. Serum samples were obtained by centrifugation of the clotted blood at 3000 × g for 10 min at room temp and allocated and kept in sterile containers at – 80°C till the time of RBP4 assay.

Complete Blood Count

Using cell dyne-1800 (Abbott Diagnostics – Santa Clara, CA, USA). Platelet count (PLT) and the neutrophil and lymphocyte counts used to calculate the platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) to reflect the patient's inflammation and immune response status.

Liver Function Assessment

Alanine (ALT) and aspartate (AST) aminotransferases, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transferase, albumin, total bilirubin, glucose, urea, and creatinine, using Cobas c311 (Roche Diagnostics, Mannheim, Germany). prothrombin time (PT) using STA Compact Max® Coagulation System (Stago-USA).

Hepatitis B (HBsAg) and HCV Antibody Testing

By an enzyme immunoassay (EIA)-Cobas e411 (Roche Diagnostics, Mannheim, Germany).

Alpha-Fetoprotein (AFP)

According to the manufacturer's instructions, by an automated immunoassay system – Tosoh Bioscience (ST AIA-PACK™ AFP Cat. No. 0025252) (Tosoh Corporation Minato-Ku,- Tokyo, Japan). The adult reference interval for serum AFP was <7 ng/mL and the coefficient of variation was ≤5% across the linear range (1–1210 ng/mL) of measurement and the minimal detectable concentration (MDC) was 1.0 ng/mL. The normal level was defined as 0–10 ng/mL.

Retinol-Binding Protein 4 (RBP4)

According to the manufacturer's directions, using ELISA (Kit Cat. No: E0929h-EIAab® Science Co. Ltd. Wuhan – China). The MDC was 0.051 ng/mL and the detection range was 0.156–10 ng/mL.

Liver Stiffness Measurement

Using Transient Elastography (TE) Fibroscan (Echosens, FS-502 touch device, Paris, France). The results expressed in kilopascals (kPa), with normal values, range from 2.5 to 7.0 kPa. Patients were classified into subgroups based on the optimal cutoff value of liver stiffness ranges 2 to 7 kPa = F0-F1 (no liver scarring – mild liver scarring), 8 to 9 kPa = F2 (moderate liver scarring), 9 to 14 kPa = F3 (severe liver scarring), 14 kPa or higher = F4 (advanced liver scarring or cirrhosis).[7] The calculated computed scores and indices according to published or patented formulas APRI, Cirrhosis discriminate score (CDS), FIB-4, GUCI, and Lok index.[8–12]

Statistical Analyses

All calculations performed using the Statistical Package for Social Sciences (SPSS) version 20 software for Windows (IBM Corporation, Armonk, NY, USA). The data tested for normality using the Kolmogorov–Smirnov test and for homogeneity variances before further statistical analysis. Symmetrically distributed continuous variables presented as mean and standard deviation (mean, SD). The skewed continuous variables presented as median and range. Categorical variables presented as number (NO.) and percentage (%). Quantitative data were analyzed using the Mann–Whitney *U*-Test and Kruskal–Wallis test and the χ^2 probability test for the categorical data. Spearman correlation coefficients used to study the correlation between different parametric variables. Receiver operating characteristic (ROC) curves constructed to determine the optimal cutoff value to each biomarker. The accuracy and the diagnostic performance of noninvasive fibrosis tests compared with the area under the receiver operating curves (AUROC) and their corresponding 95% confidence intervals (CI) and the probability of a true positive (sensitivity) and a true negative (specificity), positive predictive value (PPV), negative predictive value (NPV), calculated. The diagnostic accuracy and AUROC calculated based on TE Fibroscan performance as a reference. All calculation was tow-tailed, and P-value <0.5 considered significant.

Results

Demographic data: 50 CHC patients (28 males and 22 females), with a mean age of (55.66 ± 10.49 years), BMI (22.27 ± 2.10 kg/m^2). 45 patients received (SOF/DAC) therapy and five patients received (SOF/DAC/RBV) therapy. The HCV RNA median viral load was (929,350.00 IU/mL) with a mean of (1,449,308.18 ± 1,114,712.26), 58% (≥800,000 IU/mL) and 42% (<800,000 IU/mL). All patients achieved both rapid virological response and SVR. Twenty healthy subjects selected as a control group with a mean age of (54.60 ± 3.56 years), (11 males and 9 females) and BMI of (22.74 ± 2.13 kg/m^2).

Laboratory investigation and TE fibroscan findings in CHC patients before and after treatment compared to controls: all parameters showed significant improvement after

therapy with a reduction of liver stiffness as assessed by TE fibroscan and improvement of liver synthetic function and reduction of liver enzymes (Table 1).

Laboratory investigation and noninvasive fibrosis indices in CHC patients concerning Fibroscan fibrosis grade (Table 2).

Correlations in CHC patients before therapy: RBP4 showed a positive association with the platelets, WBC, neutrophil, lymphocyte counts, and a negative link with fibrosis grade, viral load,

AFP, the five non-invasive fibrosis indices (Table 3).

To differentiate no or mild fibrosis (F0–F1) from significant and advanced liver fibrosis (F2–F3–F4): the diagnostic performance of the Fib-4 score is the most superior followed by GUCI, APRI, and LOK index with an excellent performance, followed by the good performance of CDC, RBP4, and PLR. (Table 4 and Figure 1).

To differentiate significant liver fibrosis (F0 - F1 - F2) versus advanced liver fibrosis (F3 - F4): the diagnostic performance of RBP4 is the most superior; likewise, fibroscan, with an outstanding performance at a cutoff value <35.9 ng/mL, with 100% sensitivity, 100% specificity, 100% PPV, 100% NPV, AUROC =1.0, 95% CI=0.929–1), followed by Fib-4 score, GUCI, and APRI showed excellent performance, and very good performance for LOK index and CDC, and good performance for PLR (Table 4 and Figure 2).

Discussion

The liver performs a critical role in regulating and suppressing inflammation by controlling both local and systemic inflammatory reactions. In CHC, the inflammatory response is linked to the progression of extra-hepatic and

Table 1 A Laboratory Investigation and Fibroscan Findings in CHC Patients and Healthy Control

Parameter	Pre-Treatment	After Treatment	Control	*P*-value[¥]
Fibroscan KPa*	8.8(3.2–13.6)	7.7(2.8–11.83)	–	**0.0017**
Fibroscan** F0–1 F2 F3–4	No(%) 8(16%) 26(52%) 16(32%)	No(%) 34(68%) 1(2%) 15(30%)	–	**0.00001**
Total protein (g/dl)	7.2(6–8.3)	7.4(6.4–8.3)	8(6.9–8.5)	**0.00001**
Albumin (g/dl)	3.8(2.8–5)	4(3.2–5)	4.1(3.8–4.4)	**0.0015**
PT-INR	1.02(1–1.8)	1(1–1.5)	1(1–1.05)	**0.0224**
Total bilirubin (mg/dl)	0.9(0.46–1.72)	0.7(0.2–1.25)	0.56(0.25–0.75)	**0.00001**
AST (U/L)	46.5(16–291)	34(10–109)	14(10–30)	**0.00001**
ALT (U/L)	45(10–228)	30(10–58)	13.5(8–32)	**0.00001**
GGT (U/L)	34.5(15–58)	26.5(11–51)	13.5(9–31)	**0.00001**
ALP (U/L)	119.5(46,191)	103(40–160)	49.5 (36–62)	**0.00001**
LDH (U/L)	175.5(110–250)	153(97–190)	116(100–155)	**0.00001**
AFP (ng/mL)	8.5(3.7–23)	5(2–15)	1.8(1.30–3.30)	**0.00001**
RBP4 (mg/l)	38.4(34.4–47.1)	45.5(40.4–51.3)	55.57(47.50–62.30)	**0.00001**
APRI	0.59(0.18–5.18)	0.35(0.11–1.22)	0.1(0.07–0.26)	**0.00001**
CDS	5(1–9)	4(2–8)	2.5(1–4)	**0.00001**
Fib-4 score	2.13(0.28–8.17)	1.35(0.05–4.2)	0.65(0.44–1.19)	**0.00001**
GUCI	0.62(0.18–6.22)	0.35(0.11–1.4)	0.11(0.07–0.26)	**0.00001**
Lok index	0.4(0.1–1)	0.27(0.06–0.96)	0.16(0.06–0.29)	**0.00001**
Platelets (10^9/L)	182.5(60–415)	350.5(121–411)	332(236–411)	**0.00001**
WBCs (10^9/L)	5600(3200–11,020)	6850(4500–14,070)	5460(4250–10,900)	**0.005**
[#]Neutrophil count	2742(961–7163)	3660(1440–10,975)	3537(2233–7725)	**0.0097**
[#]Lymphocyte count	2130(735–5220)	2567(1140–4800)	1703(796–3509)	**0.0002**
[#]Monocyte count	321(105–746)	333(84–846)	254(87–763)	0.2208
NLR	1.27(0.44–5.33)	1.35(0.5–4.70)	2.14(0.64–4.76)	**0.0012**
PLR	81.04(26.89–204.44)	85.59(38.65–310.75)	198.88(70.96–474.87)	**0.00001**

Notes: [¥]Kruskal–Wallis test; *Man Mann–Whitney *U*-Test; **Chi-Square; n (%): number (percentage); [#]Absolute. Bold:*P* < 0.05

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate amino-transferase; AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GGT, gamma-glutamyl transpeptidase; GUCI, Göteborg University Cirrhosis Index; INR, international normalized ratio; LDH, kPa: kilopascal; lactate dehydrogenase; PT-NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; RBP4, Retinol-binding protein 4; WBCs, white blood cell.

Table 2 The Laboratory Investigation and Noninvasive Fibrosis Indices in CHC Patients Concerning Fibroscan Fibrosis Grades

Mean ±SD	F0-F1 (n=8)	F2 (n=26)	F3-F4 (n=16)	*P*-value
Age (years)	52.25±17.24	55.77±17.24	57.19±8.12	0.874
BMI (kg/m^2)	22.80±1.72	23.53±1.05	23.07±1.45	0.635
Fibroscan (kPa)	4.68±1.20	8.66±0.21	12.19±1.12	**< 0.00001**
Viral load	1,511,700±1,196,509	1,400,648.34±1,154,236.60	1,400,648.31±1,154,236.60	0.951
Albumin (g/dl)	4.2±0.5	3.89±0.39	3.66±0.43	**0.036**
PT-INR	1.05±0.07	1.04±0.07	1.17±0.2	**0.004**
AST (U/L)	30.5±12.38	47.19±20.38	101.12±84.31	**0.003**
ALT (U/L)	42.87±27.05	47.19±30.5	87.75±66.79	0.124
ALP (U/L)	77.12±26.42	116.96±38.87	116.06±49.7	0.060
GGT (U/L)	27.62±13.04	30.27±11.40	37.87±11.70	0.096
LDH (U/L)	174±37.44	164.08±35.34	171.29±30.96	0.549
AFP (ng/mL)	7.88±1.77	7.98±2.46	9.71±3.63	0.132
RBP4 (mg/l)	41.24±3.08	39.32±1.43	35.31±0.50	**< 0.00001**
APRI	0.25±0.6	0.61±0.38	1.80±1.26	**< 0.00001**
Fib-4 score	0.79±0.34	2.01±0.65	4.60±1.92	**< 0.00001**
CDS	2.88±1.64	4.77±0.86	6.75±1.44	**< 0.00001**
Lok index	0.19±0.13	0.39±0.12	0.65±0.20	**< 0.00001**
GUCI	0.26±0.07	0.63±0.38	2.06±1.41	**< 0.00001**
Platelets (10^9/L)	297±86	205±57	136±52	**< 0.00001**
WBCs (10^9/L)	6240±1550	6040±1770	5440±2130	0.511
NLR	1.35±0.58	1.32±0.66	2.01±1.45	0.079
PLR	132.99±61.38	88.94±29.50	81.97±40.95	**0.012**

Notes: Bold:*P* < 0.05.

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate amino-transferase; AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GGT, gamma-glutamyl transpeptidase; GUCI, Göteborg University Cirrhosis Index; INR, international normalized ratio; LDH, kPa, kilopascal; lactate dehydrogenase; PT-NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; RBP4, Retinol-binding protein 4; WBCs, white blood cells.

Table 3 Spearman Correlation Analysis Between Viral Load, AFP, RBP4, and PLR, and Noninvasive Fibrosis Indices; Fib-4, APRI, CDS, Lok Index, and GUCI

	Viral Load		AFP		RBP4		PLR	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Age (years)	−0.041	0.6942	−0.035	0.733	−0.016	0.871	−0.1045	0.301
FibroScan (kPa)	0.312	**0.0015**	0.337	**0.001**	−0.5249	**0.0001**	−0.323	**0.001**
Viral load	-	-	0.639	**0.0001**	−0.781	**0.0001**	−0.0037	0.713
AFP (ng/mL)	0.639	**0.0001**	-	-	−0.632	**0.0001**	0.0395	0.696
RBP4 (mg/l)	−0.586	**0.00001**	−0.632	**0.00001**	-	-	0.154	0.126
APRI	0.192	0.0553	0.360	**0.0002**	−0.546	**0.0001**	−0.307	**0.002**
Fib-4 score	0.187	0.0630	0.277	**0.005**	−0.538	**0.0001**	−0.450	**0.001**
CDS	0.124	0.218	0.130	0.197	−0.405	**0.0001**	−0.520	**0.0001**
Lok index	0.133	0.1861	0.223	**0.025**	−0.481	**0.0001**	−0.518	**0.0001**
GUCI	0.186	0.0642	0.364	**0.0002**	−0.543	**0.0001**	−0.269	**0.003**

Notes: r: Spearman correlation coefficient; Bold: *P*<0.05.

Abbreviations: AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GUCI, Göteborg University Cirrhosis Index; PLR, platelet to lymphocyte ratio; RBP4, Retinol-binding protein 4.

hepatic injuries as fibrosis, cirrhosis, and hepatocellular carcinoma. The degree of liver fibrosis is the best indicator of disease progression.[13]

All CHC patients were genotype 4; this was in line with the inconsistent finding of others,[14,15] and all treated patients achieved (SVR12).

Table 4 The Performance of Fibrosis Serum Markers in Detecting Fibrosis Stage

No or Mild Liver Fibrosis (F0-F1) Versus Significant and Advanced Liver Fibrosis (F2-F3-F4)											
	AUC	**Cutoff**	**Sensitivity**	**95% CI**	**Specificity**	**+LR**	**-LR**	**PPV**	**NPV**	**Accuracy**	***P* -value**
FibroScan (kPa)	1	> 6.4	100	0.929–1	100	–	0	100	100	100	**<0.001**
FIB-4 score	0.985	> 1.17	95.24	0.902–1	100		0.048	100	80	97.62	**<0.001**
GUCI	0.948	> 0.37	90.48	0.846–0.991	100		0.095	100	66.7	95.24	**<0.001**
APRI	0.946	> 0.35	90.48	0.843–0.990	100		0.095	100	66.7	95.24	**<0.001**
LOK INDEX	0.921	> 0.18	100	0.809–0.978	75	4	0	95.5	100	87.5	**<0.001**
CDS	0.893	> 3	97.62	0.773–0.963	75	3.9	0.032	95.3	85.7	86.31	**<0.001**
RBP4 (mg/l)	0.811	≤ 40.6	90.48	0.675–0.908	62.5	2.41	0.15	92.7	55.6	76.49	**0.004**
PLR	0.738	≤115.8	85.71	0.594–0.852	62.5	2.29	0.23	92.3	45.5	74.11	**0.042**
AFP (ng/mL)	0.577	> 6	83.33	0.409–0.697	37.5	1.33	0.44	87.5	30	60.415	0.604
Mild & moderate (F0-F1-F2) versus advanced liver fibrosis and cirrhosis (F3-F4)											
	AUC	**Cutoff**	**Sensitivity**	**95% CI**	**Specificity**	**+LR**	**-LR**	**PPV**	**NPV**	**Accuracy**	***P*- value**
FibroScan (kPa)	1.000	> 8.9	100	0.929–1	100	–	0	100	100	100	**<0.001**
RBP4 (mg/l)	1.000	≤ 35.9	100	0.929–1	100	–	0	100	100	100	**<0.001**
FIB-4 score	0.940	> 2.31	93.75	0.835–0.988	88.24	7.97	0.071	78.9	96.8	90.995	**<0.001**
GUCI	0.935	> 1.27	75	0.827–0.985	97.06	25.5	0.26	92.3	89.2	86.03	**<0.001**
APRI	0.923	> 0.59	100	0.811–0.979	76.47	4.25	0	66.7	100	88.235	**<0.001**
LOK INDEX	0.893	> 0.55	81.25	0.774–0.963	85.29	5.52	0.22	72.2	90.6	83.27	**<0.001**
CDS	0.882	> 5	75	0.760–0.956	94.12	12.75	0.27	85.7	88.9	84.56	**<0.001**
PLR	0.650	≤ 77.36	62.50	0.502–0.779	70.59	2.13	0.53	50.0	80.0	66.55	**<0.001**
AFP (ng/mL)	0.638	> 7	100	0.490–0.769	35.29	1.55	0	42.1	100	67.645	0.067

Note: Bold: $P<0.05$.

Abbreviations: AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; AUC, area under the curve; CDS, cirrhosis discriminant score; CI, confidence intervals; Fib-4, fibrosis index based on the four factors; GUCI, Göteborg University Cirrhosis Index; NPV, NPV, negative predictive value; LR, likelihood ratio; PLR, platelet-to-lymphocyte ratio; PPV, positive predictive value; RBP4, Retinol-binding protein 4.

CHC patients had liver dysfunction with a significant increase in hepatic enzyme levels reflecting the extent of liver cell cytolysis, and the hepatic synthetic function worsens with progression of fibrosis and hepatocyte loss as revealed by a decreased in serum albumin, RBP4, and platelet count and increase of PT-INR, this was in agreement with other reports.[16,17] Serum ALT values were slightly greater in the marked fibrosis-cirrhosis patient group.

CHC patients showed a significant decrease in the mean platelet count related to disease severity and the hepatic fibrosis grade, which improved after therapy. This was in line with other authors.[14,18–21] Moreover, platelet count correlated positively with RBP4 and negatively with the fibrosis grade and the five non-invasive fibrosis scores. This certified that platelets add to the inflammatory reaction after the liver injury that contributes to both liver fibrogenesis and regeneration.[22]

TE fibroscan is a reliable, objective technique to measure hepatic tissue elasticity,[23] which is correlated with the stages of liver fibrosis in CHC.[24] Yet, several limitations could affect its clinical usefulness. The cost can bar its widespread availability, and it has a decreased accuracy in specific patient groups (obese or patients with ascites or hepatitis flare) that may limit its use in assessing the degree of liver fibrosis in CHC patients.[25]

Fibroscan was useful for monitoring fibrosis progression in untreated CHC cases and showed significant fibrosis regression in patients achieving SVR after therapy; this was in-line with others.[1,26,27]

Several non-invasive blood test indices developed to predict hepatic fibrosis to overcome liver biopsy limitations. In this study, we evaluated the performance of five indices in predicting liver fibrosis grade, and we found that with the increasing hepatic fibrosis severity, as estimated by fibroscan; the values of non-invasive indices increased significantly together with the rise of AFP and the decrease of albumin, RBP4, PT-INR, platelet count, and PLR. This implies that all can objectively evaluate the various stages of liver fibrosis in CHC patients. This was in agreement with many authors.[28–32]

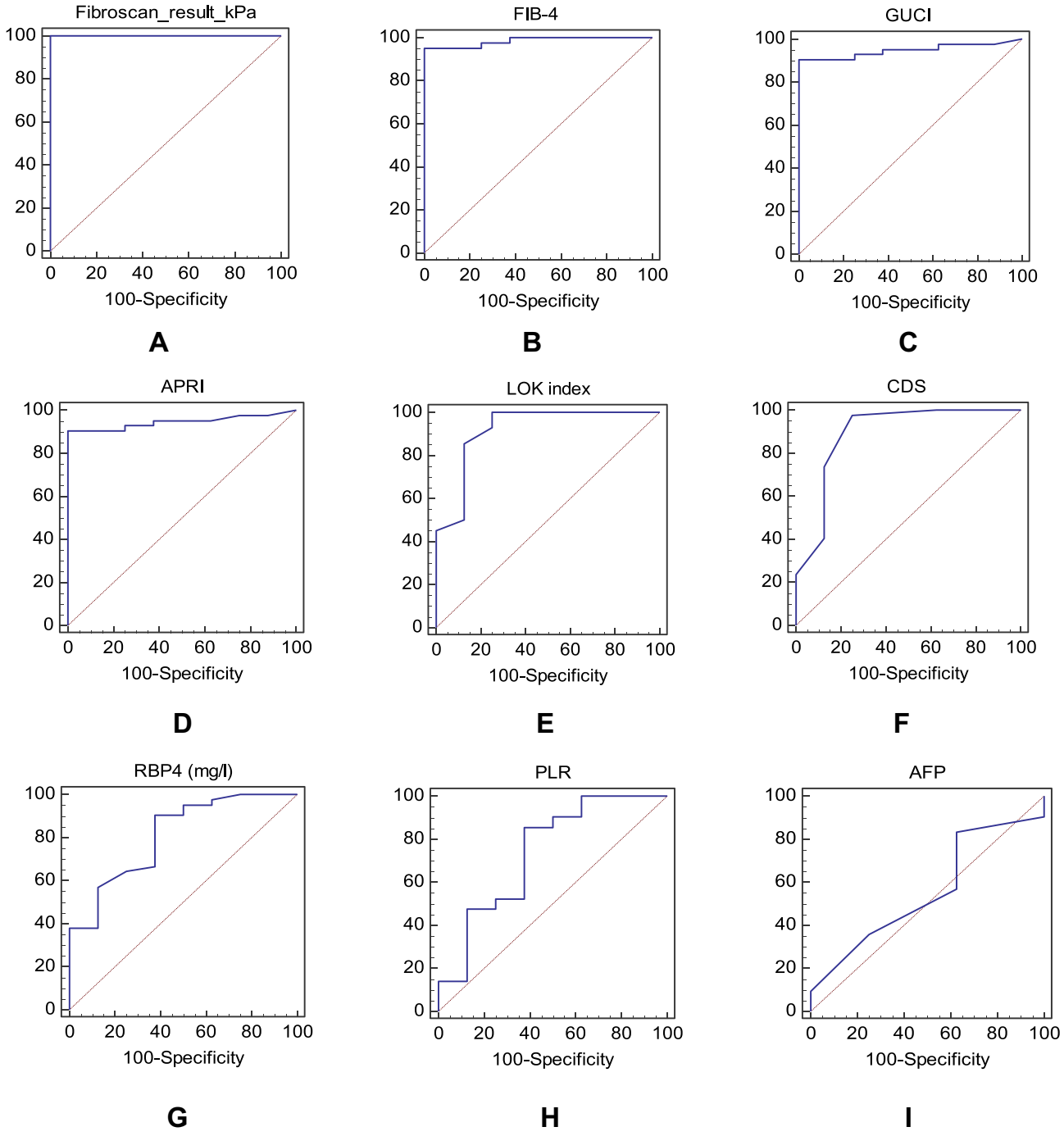

Figure 1 The area under the receiver operating characteristic (ROC) curves of RBP4, FIB-4, GUCI, APRI, LOK index, CDS, PLR, and AFP for the diagnosis of advanced liver fibrosis using Fibroscan as the reference.

The viral load correlated positively with the hepatic fibrosis severity and AFP, whereas RBP4 correlated positively with the platelet count, and negatively with fibrosis stage, viral load, AFP, and the five non-invasive fibrosis indices (APRI, CDS, Fib-4, GUCI, Lok index). PLR correlated negatively with fibrosis stage and the five non-invasive fibrosis indices. This was in line with the finding of other authors.[29–35]

RBP4 is the most superior, likewise fibroscan, with an outstanding performance in predicting significant and advanced fibrosis, followed by Fib-4 score, GUCI, APRI, LOK index, CDC, and PLR. This is in line with other authors.[8–12]

In this study, 68% of CHC patients had higher AFP levels, in contrast to former studies that observed higher

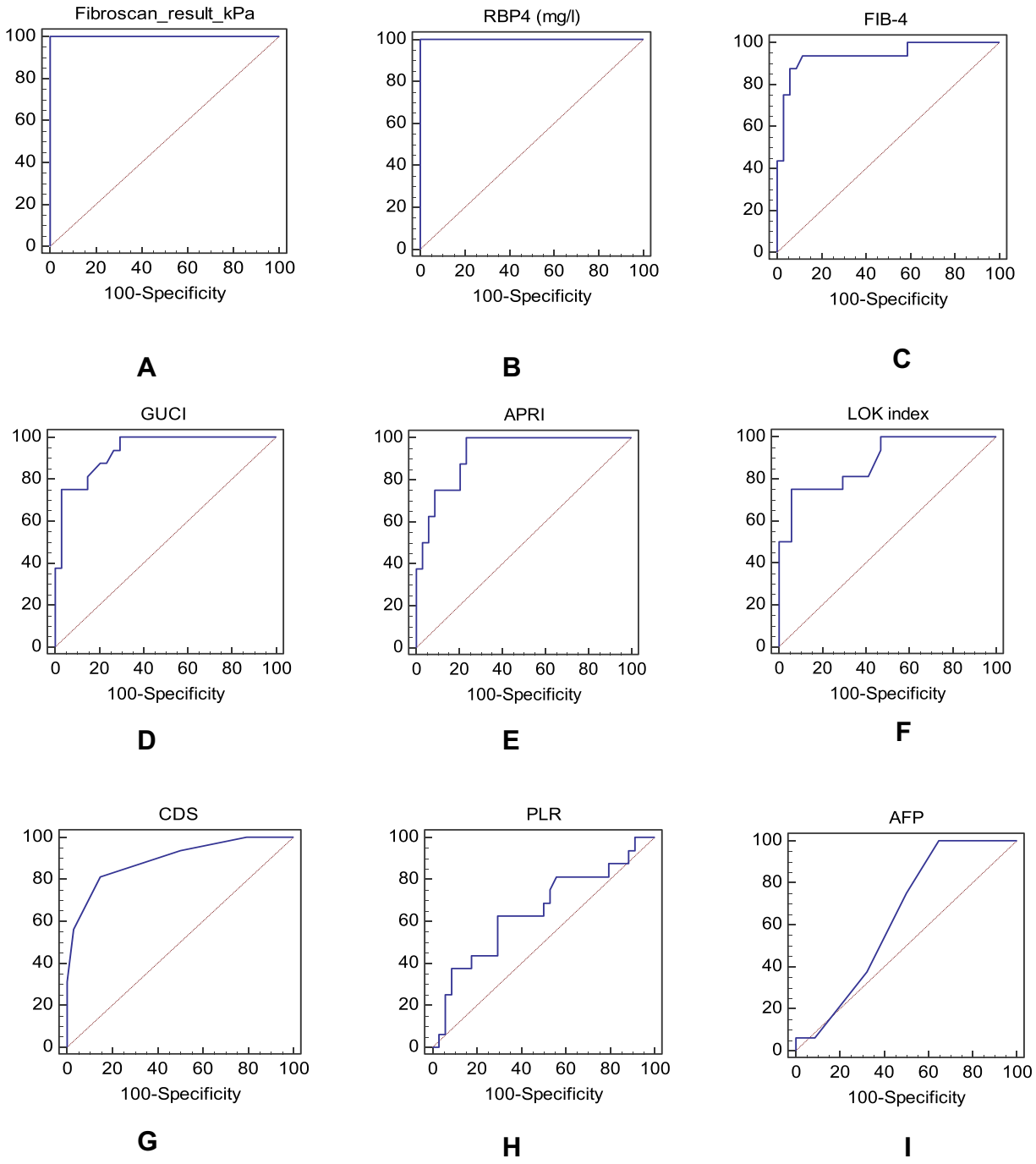

Figure 2 The area under the receiver operating characteristic (ROC) curves of RBP4, FIB-4, GUCI, APRI, LOK index, CDS, PLR, and AFP for the diagnosis of advanced liver fibrosis using Fibroscan as the reference.

Abbreviations: AFP, alpha-fetoprotein; APRI, aspartate transaminase-to-platelet ratio index; CDS, cirrhosis discriminant score; Fib-4, fibrosis index based on the four factors; GUCI, Göteborg University Cirrhosis Index; PLR, platelet-to-lymphocyte ratio; RBP4, retinol-binding protein 4.

serum AFP levels with a prevalence ranging from 15% to 58% of patients with CHC and 11% to 47% of patients with cirrhosis, and they settled that AFP levels increase during regeneration of liver cell, especially after massive hepatic necrosis and this correlates with raised ALT.[17,36,37]

CHC patients had a higher serum AFP, related to the loss of normal hepatic architecture arrangements and altered hepatocyte–hepatocyte interaction that correlated to fibrosis and cirrhosis extent, with a significant drop after DAAs and SVR achievement, reflecting the

improvement in disease activity and the amelioration of both inflammatory-necrosis and hepatocellular injury process. This was in agreement with other authors.[16,38–41]

AFP showed a positive correlation with fibrosis stage, viral load, and four non-invasive fibrosis indices (APRI, Fib-4, GUCI, and Lok index) and had a negative correlation with WBC, neutrophil, lymphocyte count, and RBP4. In contrast, Tai and his coworkers found that AFP levels correlated positively with the age, ALT elevation, fibrosis grade, inflammation score, and negatively with platelet count. However, in our study, it has poor discrimination power to differentiate the stage of liver fibrosis.[42]

In this study, the CHC patient had a significant reduction in the mean RBP4 that improved after therapy. This was in agreement with many authors.[43–47] The inverse association between RBP4 and disease severity could be explained by the fact that retinoic acid is a suppressor of type I collagen expression by hepatic stellate cells and hence lower RBP4 levels were involved in activating stellate cells to overexpress and deposit type I collagen in the liver.[48]

In CHC patients, RBP4 is a valuable serological marker to assess disease severity, inflammatory activity, and fibrosis grade evaluation, which could be valued as a sign of DAA success. For the diagnosis of significant fibrosis, the performances of fibroscan and serum biomarkers from routine blood tests were comparable. So in resource-limited countries, non-invasive tests are recommended for hepatic fibrosis assessment instead of invasive or expensive methods. To increase the diagnostic accuracy, fibroscan and the non-invasive fibrosis index combination (either stepwise or sequential) proposed through using an algorithm starting with a screening test using non-invasive fibrosis indices and restricting fibroscan or liver biopsy in patients classified as (F0–F1) by non-invasive serological tests.[27,49–51]

This study had some limitations: the small number of patients and the exclusion of patients with concomitant liver disease, thus potentially limiting the generality of our results to other populations.

Conclusion

Serum RBP4 rises after successful HCV eradication considered a marker of inflammation regression and DAA treatment success. RBP4 showed excellent accuracy, sensitivity, specificity, PPV, and NPV. Likewise, fibroscan has a superior diagnostic performance in predicting advanced fibrosis grads in CHC patients and hence can replace expensive invasive procedures.

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Disclosure

The authors have declared no potential conflicts of interest in this work.

References

1. Castera L. Non-invasive assessment of liver fibrosis in chronic hepatitis C. *Hepatol Int*. 2011;5:625–634. doi:10.1007/s12072-010-9240-0
2. Petta S, Camma C, Di Marco V, et al. Retinol-binding protein 4: a new marker of virus-induced steatosis in patients infected with hepatitis c virus genotype 1. *Hepatology*. 2008;48:28–37. doi:10.1002/hep.v48:1
3. Chen CH, Lin ST, Kuo CL, et al. Clinical significance of elevated alpha-fetoprotein (AFP) chronic hepatitis C without hepatocellular carcinoma. *Hepatogastroenterology*. 2008;55:1423–1427.
4. Tacke F, Trautwein C. Mechanisms of liver fibrosis resolution. *J Hepatol*. 2015;63:1038–1039. doi:10.1016/j.jhep.2015.03.039
5. European Association for Study of Liver. EASL recommendations on treatment of hepatitis C 2015. *J Hepatol*. 2015;63:199–236. doi:10.1016/j.jhep.2015.03.025
6. Peng Y, Qi X, Guo X. Child-pugh versus MELD score for the assessment of prognosis in liver cirrhosis: a systematic review and meta-analysis of observational studies. *Medicine (Baltimore)*. 2016;95(8):e2877. doi:10.1097/MD.0000000000002877
7. Shiha G, Seif S, Maher M, et al. Comparison between Transient Elastography (FibroScan) and liver biopsy for the diagnosis of hepatic fibrosis in chronic hepatitis C patients. *MJVH*. 2016;2(1):17–25.
8. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38(2):518–526. doi:10.1053/jhep.2003.50346
9. Bonacini M, Hadi G, Govindarajan S, et al. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol*. 1997;92(8):1302–1304.
10. 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 fibro test. *Hepatology*. 2007;46:32–36. doi:10.1002/(ISSN)1527-3350
11. Islam S, Antonsson L, Westin J, et al. Cirrhosis in hepatitis C virus-infected patients can be excluded using an index of standard biochemical serum markers. *Scand J Gastroenterol*. 2005;40:867–872. doi:10.1080/00365520510015674
12. Lok AS, Ghany MG, Goodman ZD, et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology*. 2005;42:282–292. doi:10.1002/hep.20772
13. Zampino R, Marrone A, Restivo L, et al. Chronic HCV infection and inflammation: clinical impact on hepatic and extra-hepatic manifestations. *World J Hepatol*. 2013;5(10):528–540. doi:10.4254/wjh.v5.i10.528
14. Azzazi MO, Mohamed MA, Mousa MM, et al. Multicentre study of hepatitis C virus status in Egyptian patients with B-cell non-Hodgkin's lymphoma with assessment of patients' immunological state. *Egypt J Haematol*. 2017;42:19–30. doi:10.4103/1110-1067.206435
15. Miller FD, Abu-Raddad LJ. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. *Proc Natl Acad Sci USA*. 2009;107:14757–14762. doi:10.1073/pnas.1008877107
16. Allam A, Gabr S, Ajarem J, et al. Bcl-2 and p53 expression in hepatic tissues of Egyptian patients with chronic hepatitis C. *J Pak Med Assoc*. 2015;65(11):1186–1192.

17. Goldstein NS, Blue DE, Hankin R, et al. Serum alpha-fetoprotein levels in patients with chronic hepatitis C. Relationship with serum alanine aminotransferase values, histologic activity index, and hepatocyte MIB-1 scores. *Am J Clin Pathol*. 1999;111:811–816. doi:10.1093/ajcp/111.6.811
18. Alsebaey A, Elhelbawy M, Waked I. Platelets-to-lymphocyte ratio is a good predictor of liver fibrosis and insulin resistance in hepatitis C virus-related liver disease. *Eur J Gastroenterol Hepatol*. 2018;30(2):207–211. doi:10.1097/MEG.0000000000001013
19. He Q, He Q, Qin X, et al. The relationship between inflammatory marker levels and hepatitis C virus severity. *Gastroenterol Res Pract*. 2016;2016:2978479. doi:10.1155/2016/2978479
20. Meng X, Wei G, Chang Q, et al. The platelet-to-lymphocyte ratio, superior to the neutrophil-to-lymphocyte ratio, correlates with hepatitis C virus infection. *Int J Infect Dis*. 2016;45:72–77. doi:10.1016/j.ijid.2016.02.025
21. Miyaki E, Imamura M, Hiraga N, et al. Daclatasvir and asunaprevir treatment improves liver function parameters and reduces liver fibrosis markers in chronic hepatitis C patients. *Hepatol Res*. 2016;46:758–764. doi:10.1111/hepr.v46.8
22. Ripoche J. Blood platelets and inflammation: their relationship with liver and digestive diseases. *Clin Res Hepatol Gastroenterol*. 2011;35:353–357. doi:10.1016/j.clinre.2011.02.012
23. Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology*. 2005;41:48–54. doi:10.1002/hep.20506
24. Castera L, Pinzani M, Bosch J. Noninvasive evaluation of portal hypertension using transient elastography. *J Hepatol*. 2012;56:696–703. doi:10.1016/j.jhep.2011.07.005
25. Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient Elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology*. 2008;134:960–974. doi:10.1053/j.gastro.2008.01.034
26. 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. doi:10.1053/j.gastro.2004.11.018
27. Ogawa E, Furusyo N, Toyoda K, et al. The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon-alpha-2b and ribavirin. *Antiviral Res*. 2009;83:127–134. doi:10.1016/j.antiviral.2009.04.002
28. Snyder N, Gajula L, Xiao SY, et al. APRI: an easy and validated predictor of hepatic fibrosis in chronic hepatitis C. *J Clin Gastroenterol*. 2006;40:535–542. doi:10.1097/00004836-200607000-00013
29. Li SM, Li GX, Fu DM, et al. Liver fibrosis evaluation by ARFI and APRI in chronic hepatitis C. *World J Gastroenterol*. 2014;20:9528–9533. doi:10.3748/wjg.v20.i28.9528
30. Amorim TG, Staub GJ, Lazzarotto C, et al. Validation and comparison of simple, noninvasive models for the prediction of liver fibrosis in chronic hepatitis C. *Ann Hepatol*. 2012;11:855–861. doi:10.1016/S1665-2681(19)31410-3
31. Khan DA, Fatima-Tuz-Zuhra FA, Mubarak A. Evaluation of diagnostic accuracy of APRI for prediction of fibrosis in hepatitis C patients. *J Ayub Med Coll Abbottabad*. 2008;20:122–126.
32. Baranova A, Lal P, Birerdinc A, et al. Noninvasive markers for hepatic fibrosis. *BMC Gastroenterol*. 2011;11:91. doi:10.1186/1471-230X-11-91
33. Derbala M, Elbadri ME, Amer AM, et al. Aspartate transaminase to platelet ratio index in hepatitis C virus and Schistosomiasis coinfection. *World J Gastroenterol*. 2015;21(46):13132–13139. doi:10.3748/wjg.v21.i46.13132
34. Adler M, Gulbis B, Moreno C, et al. The predictive value of FIB-4 versus FibroTest, APRI. Fibro Index and Forns index to noninvasively estimate fibrosis in hepatitis C and non-hepatitis C liver diseases. *Hepatology*. 2008;47(2):762–763. doi:10.1002/hep.22085
35. Fouad SA, Esmat S, Omran D, et al. Noninvasive assessment of hepatic fibrosis in Egyptian patients with chronic hepatitis C virus infection. *World J Gastroenterol*. 2012;18(23):2988–2994. doi:10.3748/wjg.v18.i23.2988
36. Bayati N, Silverman AL, Gordon SC. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. *Am J Gastroenterol*. 1998;93:2452–2456. doi:10.1111/ajg.1998.93.issue-12
37. Chu CW, Hwang SJ, Luo JC, et al. Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J Clin Gastroenterol*. 2001;2:240–244. doi:10.1097/00004836-200103000-00014
38. Di Bisceglie AM, Sterling RK, Chung RT, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol*. 2005;43(3):434–441. doi:10.1016/j.jhep.2005.03.019
39. Nguyen K, Jimenez M, Moghadam N, et al. Decrease of alpha-fetoprotein in patients with cirrhosis treated with direct-acting antivirals. *J Clin Transl Hepatol*. 2017;5(1):43–49. doi:10.14218/JCTH.2016.00057
40. Tachi Y, Hirai T, Ishizu Y, et al. α-fetoprotein levels after interferon therapy predict regression of liver fibrosis in patients with sustained virological response. *J Gastroenterol Hepatol*. 2016;31:1001–1008. doi:10.1111/jgh.13245
41. Takayama K, Furusyo N, Ogawa E, et al. Direct-acting antiviral-based triple therapy on alpha-fetoprotein level in chronic hepatitis C patients. *World J Gastroenterol*. 2015;21:4696–4706. doi:10.3748/wjg.v21.i15.4696
42. Tai WC, Hu TH, Wang JH, et al. Clinical implications of alpha-fetoprotein in chronic hepatitis C. *J Formos Med Assoc*. 2009;108(3):210–218. doi:10.1016/S0929-6646(09)60054-1
43. Huang JF, Dai CY, Yu ML, et al. Serum retinol binding protein 4 is inversely correlated with disease severity of chronic hepatitis C. *J Hepatol*. 2009;50(3):471–478. doi:10.1016/j.jhep.2008.10.023
44. Kataria Y, Deaton RJ, Enk E, et al. Retinoid and carotenoid status in serum and liver among patients at high-risk for liver cancer. *BMC Gastroenterol*. 2016;16:30. doi:10.1186/s12876-016-0432-5
45. Kwon JH, Park ST, Kim GD, et al. The value of serum retinol-binding protein 4 levels for determining disease severity in patients with chronic liver disease. *Korean J Hepatol*. 2008;15:59–69. doi:10.3350/kjhep.2009.15.1.59
46. Peres WA, Chaves GV, JCS G, et al. Vitamin A deficiency in patients with hepatitis C virus-related chronic liver disease. *Br J Nutr*. 2011;106(11):1724–1731. doi:10.1017/S0007114511002145
47. Wang L, Attard FA, Tankersley LR, et al. Effect of retinoic acid on the enhancing effect of acetaldehyde on mouse type I collagen expression. *Arch Biochem Biophys*. 2000;376(1):191–198. doi:10.1006/abbi.2000.1723
48. Gruys E, Toussaint MJM, Niewold TA, et al. Acute phase reaction and acute phase proteins. *J Zhejiang Univ Sci B*. 2005;6(11):1045–1056. doi:10.1631/jzus.2005.B1045
49. Boursier J, Vergniol J, Sawadogo A, et al. The combination of a blood test and fibroscan improves the non-invasive diagnosis of liver fibrosis. *Liver Int*. 2009;29:1507–1515. doi:10.1111/liv.2009.29.issue-10
50. Poynard T, Ingiliz P, Elkrief L, et al. Concordance in a world without a gold standard: a new non-invasive methodology for improving accuracy of fibrosis markers. *PLoS One*. 2008;3:e3857. doi:10.1371/journal.pone.0003857
51. Sebastiani G, Vario A, Guido M, et al. Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. *J Hepatol*. 2006;44(4):686–693. doi:10.1016/j.jhep.2006.01.007