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A panel of a mitogenic (PDGF), biochemical (albumin) and demographic (age) parameters for the non-invasive assessment of hepatic fibrosis

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ABSTRACT

Background: Several studies have investigated certain fibrosis markers that incorporate liver function tests, fragments of liver-matrix components and/or degraded products generated by hepatic stellate cells for determining the degree of hepatic fibrosis. However, the role of these molecules in the development of hepatic fibrosis is unclear. This work aimed (a) to determine whether platelet-derived growth factor (PDGF) is linked to different stages of hepatic fibrosis and (b) investigate its diagnostic performance alongside other laboratory and demographic factors in assessing liver fibrosis in chronic hepatitis C infection.

Methods: Liver-fibrosis was staged according to Fibroscan, PDGF quantified using ELISA, and liver function tests and other analytes determined by standard techniques in 239 patients with chronic hepatitis C virus infection.

Results: Patients with significant (F2-F4), advanced fibrosis (F3-F4) and cirrhotic liver disease (F4) showed significantly (P<0.0001) higher PDGF levels increase respectively compared to stage F0/1. We used this to construct the PARA-Index (**P**DGF/**a**lbumin **r**atio, **a**ge), which performed well in assessing hepatic-fibrosis stages with AUCs of 0.91, 0.87 and 0.86 for identifying F2-F4, F3-F4 and F4, respectively. Additionally, the PARA-Index correlated strongly (r=0.65, P<0.0001) with the severity of the fibrosis. An elevated PARA-index provided odds ratios of 21.0, 20.7 and 10.3 for developing F2-F4, F3-F4 and F4, respectively.

Conclusion: A panel of mitogenic (PDGF), biochemical (albumin) and demographical (age) parameters may improve liver-fibrosis staging with a high degree of accuracy in those with a hepatitis C virus infection.

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Introduction

Hepatic fibrosis is a central pathological process in progressive chronic liver disease leading to cirrhosis and hepatocellular carcinoma [1]. Knowledge of the liver fibrosis stage is essential not only for prognosis but also for treatment decisions [2]. Although the most specific and sensitive tool for liver fibrosis diagnosis and staging remains biopsy, it is an invasive and painful, and is related with high costs and clinical complications [3,4]. Advances in serological tests with blood marker panels could enable the accurate assessment of liver fibrosis and thus provide alternative to liver biopsy [1,5–12]. Indeed, several studies have been performed for investigating the clinical usefulness of fragments of the liver matrix components produced by hepatic stellate cells and other hepatic cells which include collagens and glycoproteins in addition to matrix metalloproteases and their inhibitors [11,13,14]. However, the role of molecules or factors involved in regulating fibrosis is unclear. Among factors up-regulated during fibrosis, platelet-derived growth factor (PDGF) is known to be the most effective mitogen for hepatic stellate cells in addition to collagen synthesis [15]. PDGF, mainly produced by Kupffer cells, is a dimeric protein composed of varying combinations of four polypeptide chains [16], and mRNA is markedly expressed during fibrogenesis [17,18]. We have recently proposed PDGF as a surrogate marker for identifying patients who have significant fibrosis, although their diagnostic accuracy for identifying advanced fibrosis and cirrhosis was unexplored [11]. Therefore, this work is concerned with determining the levels of PDGF in different hepatic fibrosis stages, investigating the extent to which it could affect the progression rate of liver fibrosis and estimating its diagnostic performance as a direct and complementary fibrosis marker. Hence, we hypothesized that a new scoring system incorporating PDGF and standard biochemical and demographical parameters will improve the diagnosis, not only of significant fibrosis, but also other categories of liver fibrosis. Our hypothesis was tested in patients with chronic hepatitis C virus infection in whom fibrosis is an inevitable and clinically important feature.

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Materials and methods

We recruited 239 subjects with chronic hepatitis C virus (HCV) infection from the Endemic Medicine Department, Cairo University Hospitals and the National Hepatology and Tropical Medicine Research Institute, Egypt. Informed consent was obtained from all patients who were fully informed of the diagnostic procedures and nature of their disease. The study protocol conformed to ethical guide-lines of 1975 Helsinki Declaration. Liver fibrosis staging was interpreted according to FibroScan [11,19] (Echosens, Paris, France).

Venous blood was taken into EDTA, citrate or no anticoagulant for platelet count, prothrombin time (expressed as international normalized ratio) and albumin, total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The latter were measured on an automated biochemistry analyser (A15, Biosystem, Spain), full blood count on a KX-21 Sysmex automated haematology analyser (Sysmex Corporation, Kobe, Japan), alpha-fetoprotein (AFP) by chemiluminescence (Immulite AFP kit, Diagnostic Products Corporation; Los Angeles, USA) and PDGF by ELISA (Shanghai Sunred Biological Technology Co. Ltd, Shanghai, China). All patients were tested negative for HBsAg (Dia.Pro, Milan, Italy) and positive for anti-HCV antibodies (Biomedica, Sorin, Italy). Presence of HCV-RNA was confirmed by quantitative PCR (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Pleasanton, USA).

Statistical analyses were performed using SPSS software version 15.0 (SPSS Inc., Chicago, USA) and GraphPad Prism package, version 5.0 (GraphPad Software, San Diego, USA). Continuous variables are expressed as mean with standard deviation (SD). AFP was log transformed for analysis. Correlation was evaluated by Spearman's rank method. Significant differences between groups were determined based on one-way analysis of variance (ANOVA) and Tukey's post-hoc test. $P<0.05$ was considered statistically significant. Diagnostic accuracy was assessed using area under the receiver-operating characteristic (ROC) curves. The best cut-off values for optimal prediction of significant, advanced fibrosis and cirrhosis were determined by ROC analysis. Common indicators of score performance were derived from a 2×2 contingency table.

Results

Twenty-one of the 269 patients were in F0 (8.8%), 15 were in F1 (6.3%), 22 were in F2 (9.2%), 35 were in F3 (14.6%) and 146 were in F4 (61.1%). In view of small numbers, we pooled certain groups for analysis, and in general, 84.9% (203 of 239) had significant fibrosis (F2-F4), 23.8% (57 of 239) had moderate/severe fibrosis (F2-F3) and 75.7% (181 of 239) had advanced fibrosis (F3-F4). PDGF levels were strongly linked to the severity of liver fibrosis ($P < 0.0001$) (Figure 1(a)). The median PDGF in F0-F1 was 83 ng/mL, in F2-F3 it was 112 ng/mL and in F4 it was 198 ng/mL. PDGF levels in relation to fibrosis stages are presented as box plots in Figure 1(b–d) ($P<0.0001$). Patients with F2-F4 had a 2.09-fold (109% increase) in PDGF over those in F0-F1. Patients with F3-F4 had a 1.95-fold (95% increase) in PDGF over those in F0-F2, whilst F4 patients had a 1.97-fold (97% increase) in PDGF over those in F0-F3. Based on ROC analyses, cut-off points for PDGF in assessing the risk of being in stage F2-F4 versus F0-F1 (AUC 0.85), of being in stage F3-F4 compared to F0-F2 (AUC 0.81), and of being in stage F4 compared to F0-F3 (AUC 0.79) all with very significant odds ratios (Table 1).

Having established the value of PDGF in stages of liver fibrosis, we further hypothesised that a combination of PDGF with other factors would provide a score that would have improved diagnostic accuracy. Accordingly, we measured a cross-section of routine biochemistry and haematology markers of likely interest in this setting [8–14], results of which are shown in Table 2. Of these, age, AST, ALT, the AST/ALT ratio, platelet count, APRI, albumin and INR all showed a directly linear trend with fibrosis stage. In ROC analysis, albumin was the most efficient marker for predicting F4 with an AUC of 0.72. The relationship of PDGF and albumin with the severity of liver fibrosis were further analysed, finding it a positive correlation between PDGF and severity ($r = 0.53$, $P < 0.0001$), and inverse correlation between albumin and severity ($r = -0.40$, $P < 0.0001$). Consequently, we formulated a novel index 'PDGF/albumin ratio' that performed well in predicting cirrhosis with an 0.84 AUC for identifying F4. PDGF/albumin ratio was then applied to other categories of liver fibrosis yielding AUCs of 0.86 and 0.84 for F2-F4 and F3-F4, respectively. The best cut-offs points were then selected based on ROC analysis and the diagnostic performances of PDGF/albumin ratio are shown in Table 1. This approach failed to improve the odds ratios for F0/F1 versus F2/F4, or for F0/F2 versus F3/F4, but it did improve the odds ratio for F0/F3 versus F4.

Unsurprisingly, age was linked to disease severity (Table 2). Therefore, a new index 'PARA-Index' incorporating the product of **P**DGF/**a**lbumin **r**atio and **a**ge was formulated and its diagnostic performances were summarized in Table 1. This index was accurate in predicting F2-F4, F3-F4 and F4 yielding higher AUCs of 0.91, 0.87 and 0.86, respectively, than those produced by PDGF/albumin ratio *per se* as given in Figure 2(a–c). The distribution of PARA-Index in relation to fibrosis stages is shown in Figure 2(d,f), differences statistically significant ($P<0.0001$) yielding a Spearman's rank correlation coefficient of 0.65 ($P<0.0001$). The median value for PARA-Index in F0 was 94, in F1 it was 115, in F2 it was 128, in F3 it was 155, and in F4 it was 213. The PARA-Index was then applied to a validation cohort comprising 160 patients (F0: 25; F1: 21; F2: 29; F3: 39; F4: 46) to test accuracy and

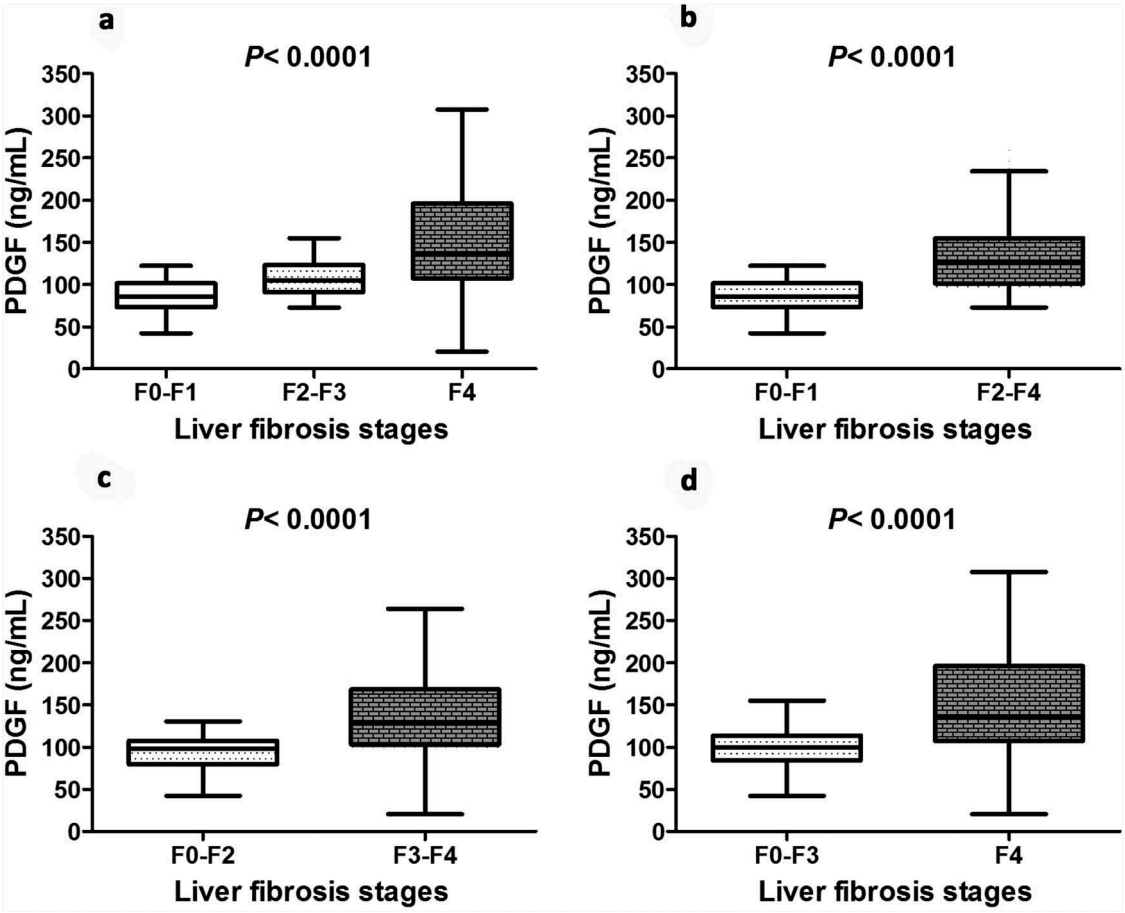

Figure 1. Box plots (median, IQR) PDGF in chronic hepatitis C virus patients (a) in different fibrosis groups; (b) for discriminating patients with significant fibrosis (F2-F4) from those without (F0-F1); (c) for discriminating patients with advanced fibrosis (F3-F4) from those without (F0-F2) and (d) for discriminating patients with cirrhosis (F4) from those without (F0-F3).

Table 1. Diagnostic performances of PDGF, PDGF/albumin ratio and PARA-Index for the prediction of the stage of fibrosis.

Variable	Cut-off	Sens.	Spec.	OR (95% CI)*
F2-F4 (n = 203) *vs.* F0-F1 (n = 36)				
PDGF	> 103	73.4	80.6	11.4 (4.7–27.6)
PDGF/Albumin	> 25	79.8	72.2	10.3 (4.6–23.0)
PARA-Index	> 134	80.8	83.3	21.0 (8.2–54.0)
F3-F4 (n = 181) *vs.* F0-F2 (n = 58)				
PDGF	> 110	67.4	79.3	7.9 (3.9–16.0)
PDGF/Albumin	> 28	69.1	75.9	7.0 (3.6–13.8)
PARA-Index	> 148	76.8	86.2	20.7 (9.1–47.1)
F4 (n = 146) *vs.* F0-F3 (n = 93)				
PDGF	> 124	61.6	86.0	9.9 (5.0–19.4)
PDGF/Albumin	> 31	70.0	83.6	12.1 (6.3–23.2)
PARA-Index	>161	72.6	79.57	10.3 (5.5–19.2)

PDGF: platelet-derived growth factor; PARA-Index: [PDGF/Albumin × age]; Sens: sensitivity; Spec: specificity; CI: confidence interval; OR: Odds ratio. *All ORs P < 0.0001

reproducibility. As a result, PARA-Index yielded AUCs of 0.89, 0.88 and 0.83 for F2–F4, F3-F4 and F4, respectively. PARA-Index >134.1 was 82% specific and 83% sensitive for F2-F4, PARA-Index >147.8 was 86% specific and 76% sensitive for F3-F4 and PARA-Index >160.9 was 78% specific and 66% sensitive for F4. The PARA-index failed to improve the odds ratios for F0-F3 versus F4, but it more than doubled the odds ratio for F0/F1 versus F2/F4, and almost tripled the odds ratio for F0/F2 versus F3/F4 (Table 1).

Discussion

There is a growing need for the development of accurate non-invasive markers for assessing the presence and the degree of liver fibrosis [1,5–12,20–22]. Hepatic fibrosis results from an imbalance between synthesis and dissolution of extracellular matrix accumulated as a consequence of activation of hepatic stellate cells by cytokines [23]. Indeed, PDGF is considered one of the most important cytokines implicated in stellate cell activation and collagen synthesis and all of their isoforms are up-regulated in the fibrotic liver and correlate with the degree of fibrosis and inflammation [16,24]. Consentient to these facts, our findings show that patients who developed F2-F4 versus F0-F1, those who F3-F4 versus F0-F2, and those with F4 versus F0-F3 had an increase in PDGF of approximately two-fold. Indeed, PDGF alone was a powerful determinant of stage with odds ratios between 7.9 and 11.4. These results may be explained by the fact that PDGF receptors are upregulated in liver fibrotic diseases which in turn directly promote the inflammatory and fibrogenic actions of hepatic stellate cells and transformation to active myofibroblastic phenotype, and so secrete a large amount of collagen, the basis of fibrosis [15,25]. Our analysis

Table 2. Clinical features of chronic hepatitis C patients in different fibrosis stages included in the present study.

Variables	F0-F1 (n = 36)	F2-F3 (n = 57)	F4 (n = 146)	P value based on Tukey's *post hoc* test		
				F0-F1 *vs.* F2-F3	F0-F1 *vs.* F4	F2-F3 *vs.* F4
Age (years)	47.2 ± 10.5	54.5 ± 7.3	55.5 ± 8.6	<0.0001	<0.0001	0.757
AST (U/L)	47 ± 22	64 ± 31	69 ± 29	0.001	0.004	0.541
ALT (U/L)	52 ± 40	58 ± 28	75 ± 44	0.005	0.542	0.006
AST/ALT	1.0 ± 0.3	1.1 ± 0.2	1.2 ± 0.1	0.637	0.082	0.001
Platelet count (10^9/L)	183 ± 83	178 ± 65	149 ± 80	0.957	0.052	0.044
APRI	0.7 ± 0.4	1.0 ± 0.4	1.3 ± 0.7	0.042	<0.0001	0.031
Albumin (g/L)	41 ± 5	39 ± .4	36 ± 4	0.429	<0.0001	<0.0001
Total bilirubin (mg/dL)	0.7 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	<0.0001	0.037	0.037
INR	1.0 ± 0.1	1.1 ± 0.2	1.2 ± 0.1	0.118	0.723	0.171
Log AFP (U/L)	0.7 ± 0.3	1.2 ± 0.4	1.0 ± 0.4	<0.0001	0.006	0.135

Data are mean ± SD. AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR: international normalized ratio; AFP: alpha fetoprotein; APRI: AST to platelets ratio index.

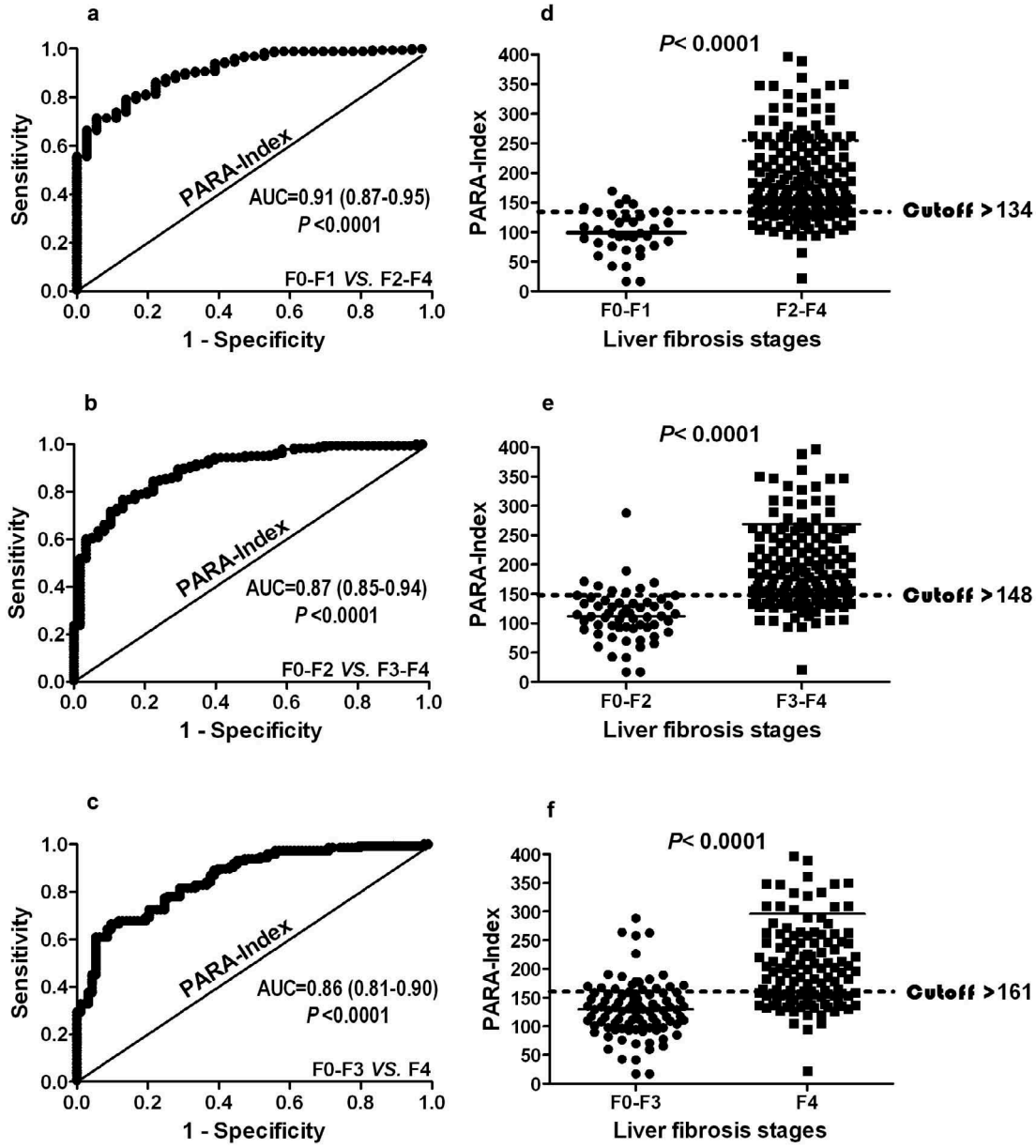

Figure 2. Diagnostic performances and distribution of PARA-Index for separating (a,b) patients who developed significant fibrosis (F2-F4) from those without (F0-F1); (c,d) patients who developed advanced fibrosis (F3-F4) from those without (F0-F2) and (e–f) patients who developed cirrhosis (F4) from those without (F0-F3). PARA-index = [PDGF/albumin × age].

found that albumin and age were also powerful predictors of stage. Levels of liver marker albumin [26] fall in patients infected with HCV as the synthetic function of the liver declines with worsening liver fibrosis [8]. Synthesis of albumin is inhibited by pro-inflammatory substances, including interleukin-6 and tumour necrosis factor-α that stimulate the fibrogenic action of hepatic stellate cells [27–29]. Thus, it is not surprising

that albumin has value in the prognosis of cirrhotic patients [30] in addition to diagnosing hepatic fibrosis [8]. In order to amplify the difference in PDGF and albumin values among patients with different fibrosis stages and to increase their aptitude, PDGF/albumin ratio was devised that showed a better AUC of 0.80 than that produced by each individually. The final step in developing our index was to enhance the efficacy of PDGF/albumin ratio to be capable of the accurate diagnosing all categories of liver fibrosis. No doubt the value of age as a marker of hepatic fibrosis seems obvious as fibrosis progression is time-dependent [31] and several studies had identified age as independent predictor of fibrosis [31–34]. Previously, Ahmed et al. [34] showed the more advanced fibrosis stages were observed in older patients, which we confirm. Therefore, we constructed a more developed index incorporating PDGF, albumin and age capable of identifying F2-F4, F3-F4 and F4. The most important issue is to know that our score has a notable advantage over that reported previously 'Fibro-mark' as it is simpler and could predict, not only significant fibrosis, but also advanced fibrosis and cirrhosis using a larger number of HCV patients. AAR and APRI are considered two of the simplest tests in a community hospital that fulfill the criteria of an ideal non-invasive test. Indeed, AAR was one of the first indirect markers for staging liver fibrosis in HCV patients. However, AAR was found to have less value than APRI [35]. The overall performance of AAR was very variable across studies, with an AUC ranging from 0.51 to 0.83. Consistent with these results, our findings showed that AAR had an AUC of 0.62. Notably, APRI is one of the most investigated non-invasive markers for assessing the degree of hepatic fibrosis [36]. In the original study, APRI enabled the correct identification of F2-F4 and F4 with an estimated AUC of 0.88 and 0.94, respectively. However, our results showed that APRI provided lower AUCs of 0.76 and 0.65 for predicting F2-F4 and F4, respectively. Our results may be supported by other studies that showed variable performance for APRI with AUCs ranging between 0.69 and 0.88 for F2-F4 and between 0.61 and 0.94 for F4 [37,38].

Further prospective studies involving a greater number of patients are warranted to validate the usefulness of the produced score in clinical practice and to validate the impact of elevated levels of PDGF on fibrosis progression, and to determine if this score is applicable in other liver disease, such as those infected with hepatitis B virus. This work represents an advance in biomedical science because it provides a three-marker model combining mitogenic (PDGF) and biochemical (albumin) together with demographical (age) parameters to improve liver fibrosis staging with a high degree of accuracy in patients infected with the hepatitis C virus.

Summary Table

What is known about this topic:

- Several studies have investigated many fibrosis markers including liver-matrix fragments or products of hepatic stellate cells for fibrosis staging.
- PDGF is an important factor in hepatic stellate cell activation, and levels are raised in hepatitis C infection.
- Liver cirrhosis causes a fall in serum albumin.

What this work adds:

- PDGF levels are linked to the severity of liver fibrosis in patients infected with hepatitis C virus with odds ratios of around 10.
- Addition of albumin and age to PDGF, in the PARA-index, improves the diagnostic accuracy of determining fibrosis stages F0-F1 versus F2-F4, and of F0-F2 versus F3-F4 with odds ratios of around 20.
- Compared to PDGF alone, the PARA-Index failed to increase the diagnostic accuracy of the determination of fibrosis stage F4 versus F0-F3.

Disclosure statement

No potential conflict of interest was reported by the authors.

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