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Fibro-Mark: a panel of laboratory parameters for predicting significant fibrosis in chronic hepatitis C patients

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ABSTRACT

Background: Fibrosis markers are useful for the prediction of cirrhosis but clinical scores such as King's score, AST-Platelet ratio index (APRI), Biotechnology research center (BRC), Fibrosis routine test (FRT), Fibro-α score and Fibro-quotient (FibroQ) have limited accuracy for diagnosing significant fibrosis. We hypothesised that new markers (reflecting the balance between hepatic fibrogenesis and fibrolysis) together with other indirect fibrosis markers would together construct a more sensitive and specific score capable of identifying fibrosis than existing scores.
Methods: Collagen IV, hyaluronic acid, platelet-derived growth factor (PDGF) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were measured by ELISA, and AST, ALT, platelet count, albumin, total bilirubin, INR and AFP by routine methods in 148 patients with hepatitis C induced liver disease. Stepwise linear discriminant analysis and area under receiver-operating characteristic curves (AUCs) were used to create a predictive score and compare it to others.
Results: Patients with significant fibrosis (n = 100, F2–F4) showed 2.08, 2.14, 1.80 and 1.90-fold increase in collagen IV, hyaluronic acid, PDGF and TIMP-1, respectively, over patients with no or mild fibrosis (n = 48, F0/F1)(all $p < 0.01$). Significant independent predictors of F2–F4 were AFP (AUC 0.79), age (0.76), PDGF (0.74), collagen IV (0.78) and TIMP (0.75), which together formed a five-marker score 'Fibro-Mark' for predicting F2–F4. In comparison with other scores, AUC for Fibro-Mark was 0.89, BRC was 0.83, followed by FRT and King's score (both 0.82), APRI (0.80), Fibro-α (0.70) and finally Fibro Q (0.63).
Conclusions: The Fibro-Mark score provides better discrimination in hepatic-fibrosis staging in chronic hepatitis C patients than existing scores.

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Liver fibrosis; non-invasive; TIMP-1; PDGF; collagen IV; hyaluronic acid

Introduction

The disadvantages of liver biopsy, being its cost, the need for skilled pathologists, discomfort to the patient, and bleeding have stimulated identification of reliable non-invasive biomarkers [1]. Indeed, many studies searched for non-invasive fibrosis markers capable of providing accurate information about hepatic fibrosis stage in patients with chronic hepatitis C (CHC) [2]. These proposed hepatic fibrosis biomarkers can be broadly divided in two main categories: indirect markers and direct markers. The former incorporate routine clinical and laboratory data (such as liver function tests and platelet count) but do not directly reflect extracellular matrix metabolism. The latter incorporate fragments of liver matrix components involved in the molecular pathogenesis of fibrogenesis and fibrinolysis and which reflect the metabolism of hepatic extracellular-matrix [3]. These biomarkers include measures of extracellular-matrix remodelling, enzymes involved in matrix degradation, and of collagen synthesis. Individual markers are useful for the prediction of liver cirrhosis but have limited accuracy for the diagnosis of significant fibrosis [4]. Therefore, the development of more advanced scores combining both direct and indirect markers may improve the diagnostic accuracy of liver fibrosis, current defined histologically [5].

We hypothesised that a new scoring system, incorporating new and pathophysiologically relevant markers reflecting the balance between hepatic fibrogenesis and fibrolysis (that is, hyaluronic acid, platelet-derived growth factor (PDGF), tissue inhibitor of metalloproteinase-1 (TIMP-1) and soluble collagen IV) together with existing markers can construct a predictive score capable of identifying hepatic fibrosis with a high degree of accuracy. In clinical validation of this new score, we hypothesised that its performance would be superior to those of other non-invasive tests. We tested our hypotheses in patients a high risk of liver fibrosis due to chronic hepatitis C infection (CHC).

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Materials and methods

One hundred and forty-eight consecutive CHC-patients were recruited from Endemic Medicine Department, Cairo University Hospitals, Egypt. Informed consents were obtained from all participants who were fully informed concerning the diagnostic procedures involved and disease nature. The study protocol conformed to ethical guide-lines of 1975 Helsinki Declaration. Liver fibrosis staging were interpreted according to FibroScan [5] (Echosens, Paris, France) into no or mild fibrosis (F0/F1) or moderate to severe (F2–F4).

Venous blood provided serum for measurement of albumin, total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) by routine methods (A15, Biosystem, Spain). A full blood count was performed using an automated haematology analyzer (KX-21, Sysmex Corporation, Kobe, Japan). Alpha-fetoprotein (AFP) was estimated by chemiluminescence (Immulite 1000, Diagnostic Products Corporation; Los Angeles, USA). Hyaluronic acid, PDGF, TIMP-1 and collagen IV were all performed by ELISA (Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China). All patients were tested negative for HBsAg (Dia.Pro, Milan, Italy) and tested positive for anti-HCV antibodies (Biomedica, Sorin, Italy). The presence of HCV-RNA was confirmed using quantitative polymerase chain reaction assay (COBAS Ampliprep/ COBAS TaqMan, Roche Diagnostics, Pleasanton, USA).

Statistical analyses were performed on SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean (SD) or median (inter-quartile range). Correlations were sought by Spearman's rank method. The main determinant was the identification of patients with moderate to severe fibrosis (F2–F4) as this group is of great clinical interest and has been adopted as a target for most clinicians [6–8]. Statistically significant differences between groups were determined using the Student *t* test or Mann–Whitney U test. A value of $p < 0.05$ was considered statistically significant. The diagnostic accuracy was assessed by calculating area under the receiver-operating characteristic (ROC) curves (AUC) [9]. The optimum cutoff points were selected and diagnostic performances (sensitivity, specificity and accuracy) were determined. Accuracy was calculated as: [(True positive + True negative)/(Total number of tested subjects) × 100]. Likelihood of F2–F4 compared to F0–F1 is given as odds ratio with 95% confidence interval.

Results

Of 148 patients aged 52.9 (10.1), 79 (53.4%) were men. In accordance with FibroScan, patients were clustered into two groups: 48 with F0–F1 (32.4%) and 100 with F2–F4 (67.6%) (Table 1). As expected, patients with F2–F4 were older and had higher levels of AST, ALT, total bilirubin and AFP compared to F0–F1 patients (all $p < 0.01$). Collagen IV, hyaluronic acid, PDGF and TIMP-1 were all higher ($p \leq 0.01$) by a factor of 2.08-fold, 2.14-fold, 1.80-fold and 1.90-fold, respectively. ROC-derived AUCs and other data for these markers are shown in Table 2. The ROC curves giving an AUC estimating the diagnostic accuracies of the routine markers were AFP 0.79, age 0.76, AST 0.75, ALT 0.73, total bilirubin 0.67 and INR 0.65. Those indices significant in univariate analysis were entered into a multivariate regression which found that age, AFP, PDGF, collagen IV and TIMP-1 retained significance. We used further analysis to develop a score named Fibro-Mark as follows: Fibro-Mark = [0.07 × AFP] + [0.05 × Age] + [0.001 × PDGF] + [0.145 × Collagen IV] – [0.002 × TIMP-1 – 0.466].

Bivariate Spearman's rank correlation coefficient between Fibro-Mark and its component markers were determined for estimating the impact of each marker on the predictive criteria. AFP was found to have highest correlation coefficient (*r*) of 0.7 followed by collagen IV ($r = 0.65$), PDGF ($r = 0.59$), age ($r = 0.54$) and TIMP-1 ($r = 0.45$)(all $p < 0.001$). The diagnostic value of Fibro-Mark was then assessed by ROC curve showing AUC = 0.89 for identifying F2–F4 (Figure 1). Based on this, an optimal cut-off point of 3.8 enabled the correct identification of F2–F4 patients with sensitivity = 84%, specificity = 77% and efficiency = 82%. The distribution of Fibro-Mark levels in relation to fibrosis stages was

Table 1. Age and laboratory data.

Variables	No/mild fibrosis (F0–F1); $n = 48$	Significant fibrosis (F2–F4); $n = 100$	*p*-value
Age (years)	51.5 (38–55)	57.0 (52–62)	<0.001
AST	48 (34–61)	68 (53–86)	<0.001
ALT	42 (26–62)	69 (47–87)	<0.001
AST/ALT ratio	1.02 (0.95–1.22)	1.04 (0.97–1.15)	0.936
Platelet count	172 (140–223)	170 (108–223)	0.396
Albumin	40 (36–43)	40 (36–44)	0.515
Total bilirubin	13 (7–17)	15 (13–18)	0.004
INR	1.0 (0.9–1.1)	1.1 (1.0–1.2)	0.277
AFP	6.1 (3–11)	13.0 (7.8–26)	<0.001
Collagen IV	5.5 (4.5–6.8)	8.2 (6.1–9.7)	0.007
HA	74 (42–103)	105 (83–140)	0.009
PDGF	88 (71–104)	110 (95–138)	0.004
TIMP-1	70 (60–85.2)	90 (72–104)	0.01

Notes: Variables expressed as median (IQR). Reference values: aspartate aminotransferase (AST) (male < 37, female < 31 U/L); alanine aminotransferase (ALT) (male < 41, female < 31 U/L); platelet count 150–400 × 10^9/L; albumin 38–54 g/dL; total bilirubin < 17.1 µmol/L; international normalised ratio (INR) 1; alpha fetoprotein (AFP) < 10 U/L. HA = hyaluronic acid (ng/mL), PDGF = platelet-derived growth factor (ng/mL); TIMP-1: tissue inhibitor of metalloproteinase-1 (ng/mL), Unit for collagen is (µg/mL).

Table 2. Diagnostic performances and AUC for research indices for identifying patients with significant liver fibrosis (F2–F4).

	Collagen	HA	PDGF	TIMP-1
AUC	0.78	0.75	0.74	0.75
Cut-off	6.5 µg/mL	86 ng/mL	100 ng/mL	76 ng/mL
Sensitivity	70	70	70	71
Specificity	68	60	64	66
Accuracy	69	66	68	69
Odds ratio (95% CI)	4.8 (2.29–10.31)	3.35 (1.62–6.95)	4.02 (1.92–8.40)	6.88 (2.28–20.77)

Notes: AUC: area under curve. CI: confidence interval; See Table 1 for other abbreviations.

determined: the median (IQR) value for Fibro-Mark in F0–F1 and F2–F4 was 3.1 (2.5–3.7) and 4.8 (4.0–5.8), respectively ($p < 0.001$). Additionally, Fibro-Mark correlated with liver fibrosis-progression (F stage) with a Spearman's correlation coefficient of 0.63 ($p < 0.001$).

In comparing Fibro-Mark results with those of other scores, the sensitivity, specificity, efficiency and adjusted odds ratios for these non-invasive scores were calculated using cutoff values as originally described by their authors (Table 3). Fibro-Mark provided the highest AUC and odds ratio. The Fibro-Mark was then applied to a validation cohort comprising 151 patients (36 with F0–F1 and 115 with F2–F4) to test its accuracy and reproducibility. The characteristics of validation group were similar to that of the estimation group with no significant differences in any of the assessed variables. The diagnostic power of Fibro-Mark was assessed by ROC curve showing AUC = 0.84 for identifying F2–F4. A cutoff point > 3.8 provided sensitivity = 71%, specificity = 81% and efficiency = 73% for predicting F2–F4.

Discussion

We have derived a new score – Fibro-Mark – that is superior to other scores in detecting moderate to severe fibrosis. Of the new markers comprising the Fibro-Mark, collagen IV provided the best AUC for recognition of the progression of liver fibrosis. This may be explained by the fact that liver injury leads to hepatic stellate cell activation and transformation to active myofibroblastic phenotype, and secrete a large amount of collagen with inhibition of collagenase activity [10]. Previously, type IV collagen was reported as performing well in detecting F2–F4 producing AUC = 0.83 [11] compared to our finding of AUC = 0.78. One of the contributory factors to the development of hepatic fibrosis is a decrease in collagenase activity, which may be related to levels of inhibitors such as TIMP-1 [12]. We also report that TIMP-1 and hyaluronic acid have good AUCs. However, although several workers have used hyaluronic acid alone or in combination with other serum markers to differentiate different stages of hepatic fibrosis, and our univariate data support and extends their work [13–16], in our multivariate analysis it became non-significant. PDGF is considered to be one of the most important growth factors implicated in hepatic stellate cell activation and collagen synthesis and all of their isoforms are up-regulated in the fibrotic liver and correlated with the degree of fibrosis and inflammation [17,18]. Notably, therefore, we report that PDGF is linked with liver fibrosis progression producing a significant correlation coefficient and a very good AUC.

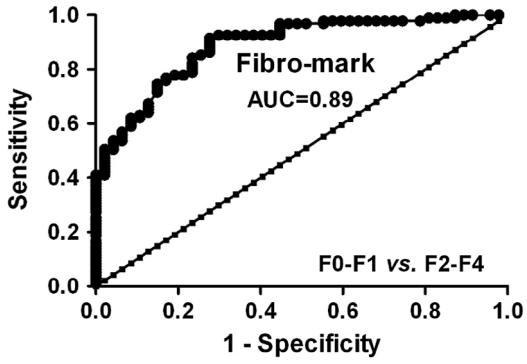

Figure 1. Area under the receiver-operating characteristic curves (AUC) for Fibro-Mark to discriminate patients with significant fibrosis from no/mild fibrosis.

We aimed to develop a more developed score using a mathematical formula to enhance the diagnostic accuracy of these non-invasive fibrosis markers. Multivariate regression modelling demonstrated that age, AFP, PDGF, collagen IV and TIMP-1 independent significant predictors. Age as marker has already been identified as a predictor of hepatic-fibrosis in several studies [19–23], but we found that AFP is the most efficient marker, which confirms and extends the work of others [24]. However, by combining age and AFP with collagen IV, TIMP-1 and PDGF, we improved the diagnostic accuracies of these fibrosis markers and create a more sophisticated score capable of discriminating patients with F2–F4 from those less several fibrosis.

In the second part of this work, we hypothesised our score would be superior to other non-invasive fibrosis scores (the Biotechnology research center (BRC) score [20], Fibrosis routine test (FRT) [21], King's score [25], AST-Platelet ratio index (APRI) [26], Fibro-α score [27] and Fibro-α score and Fibro-quotient (FibroQ) [23]) for assessing the degree of liver fibrosis. Diagnostic accuracies for these different scores were assessed based on ROC analysis while their diagnostic performances were evaluated

Table 3. Diagnostic performances and AUC of Fibro-Mark and other scores for predicting significant fibrosis.

Index (Ref)	AUC (95% CI)	Cutoff[a]	Sn	Sp	Ac	Odds ratio (95% CI)	*p*
Fibro-Mark	0.89 (0.83–0.94)	4.05	77	83	79	16.18 (6.59–39.70)	<0.001
BRC score [20]	0.83 (0.76–0.90)	7.2	97	30	76	12.86 (3.44–48.13)	<0.001
BRC score [21]	0.82 (0.75–0.89)	4.0	99	11	69	10.71 (1.21–94.60)	0.033
King's score [25]	0.82 (0.74–0.90)	12.3	94	45	79	12.25 (4.39–34.19)	<0.001
APRI [26]	0.80 (0.71–0.88	1.5	29	94	50	4.96 (1.40–17.44)	0.013
Fibro-α score [27]	0.70 (0.60–0.79)	1.28	95	19	72	3.60 (1.07–12.10)	0.038
FibroQ [23]	0.63 (0.54–0.73)	1.6	93	13	69	1.80 (0.53–6.04)	0.344

Notes: Ref: reference; AUC: area under receiver-operating characteristic curve (all scores $p < 0.001$ except FibroQ $p = 0.015$); Sn: Sensitivity, Sp: Specificity, Ac: accuracy. [a]Cut-off points were used as originally reported by their authors.

using cutoff points exactly as described in the original studies. In our population, the BRC score correctly identified patients with F2–F4 with 0.83 AUC, compared to the original validation study (0.85 AUC) but inferior to that produced in the original study (0.87 AUC) [20]. FRT gave 0.82 AUC in our sample data for predicting F2–F4, similar to those produced originally in both estimation and validation studies (0.80–0.84 AUC) [21]. King's score is another simple index comprising routinely available laboratory tests. Cross et al. [25] reported that King's score had 0.79 AUC for predicting F2–F4 which was somewhat similar to that produced in our sample data (0.82 AUC). APRI is the simplest test for predicting F2–F4, which we found to have 0.80 AUC for identifying F2–F4, the same as produced previously by Wai et al. [26] in the original study. Fibro-α is another simple non-invasive score developed by Omran et al. [27] for identifying different stages of hepatic fibrosis. Our Fibro-α produced 0.70 AUC for identifying F2–F4, similar to those previously reported by Omran et al. (0.72~0.74). However, when the original cutoff value of 1.28 was applied to our sample data so as to predict F2–F4, it was found to be unbalanced producing 95% specificity and 19% specificity compared to those produced in the original study (70% sensitivity and 60% specificity). FibroQ is another simple non-invasive score for predicting liver fibrosis stages, which in our hands produced a lower AUC (0.63) than that produced by Hsieh et al. [23] in the original study (AUC 0.78).These workers reported 79% sensitivity and 71% specificity for predicting F2–F4 at a cutoff point of 1.6. In contrast, our results showed that the originally described cutoff value was not balanced giving a higher sensitivity of 93% accompanied by a very low specificity of 13% when compared to those produced in the original study. These discrepancies may be related to differences in the prevalence of F2–F4 and to differences in the patient characteristics and the histopathological assessment.

In conclusion, we find that the Fibro-Mark shows better performance than BRC, FRT, King's score, APRI, Fibro-α score and FibroQ for identifying F2–F4 in this group of CHC patients. This work represents an advance in biomedical science because it provides a more developed score combining 'direct' and 'indirect' markers using a mathematical formula for predicting significant liver fibrosis.

Summary table

What is known about this topic:
- Disadvantages of liver biopsy have led to a drive towards identifying reliable biomarkers.
- Many studies have been dedicated to the search of non-invasive fibrosis markers.
- However, these markers are useful for most severe fibrosis, but they have limited accuracy for F2–F4.

What this work adds:
- Fibro-Mark is an accurate test for predicting F2–F4 providing an AUC of 0.89.
- Fibro-Mark showed better performance than other scores in this group of patients.

Disclosure statement

No potential conflict of interest was reported by the authors.

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