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Journal of Hepatology 50 (2009) 165–173

Journal of Hepatology

www.elsevier.com/locate/jhep

Comparison of blood tests for liver fibrosis specific or not to NAFLD☆

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***Background/Aims*: To compare blood tests of liver fibrosis specific for NAFLD: the FibroMeter NAFLD and the NAFLD fibrosis score (NFSA) with a non-specific test, APRI.**

***Methods*: Two hundred and thirty-five NAFLD patients with liver Metavir staging and blood markers from two independent centres were randomly assigned to a test ($n = 121$) or a validation population ($n = 114$).**

***Results*: The highest accuracy – 91% – for significant fibrosis was obtained with the FibroMeter whose (i) AUROC (0.943) was significantly higher than those of NFSA (0.884, $p = 0.008$) and APRI (0.866, $p < 10^{-3}$; $p = 0.309$ vs NFSA) in the whole population, and (ii) misclassification rate (9%) was significantly lower than those of NFSA (14%, $p = 0.04$) and APRI (16%, $p = 0.002$) and did not vary according to centre (14 vs 7%, $p = 0.07$), unlike those of NFSA (25 vs 9%, $p = 0.001$) and APRI (29 vs 11%, $p < 10^{-3}$). By using thresholds of 90% predictive values, liver biopsy could have been avoided in most patients: FibroMeter: 97.4% vs NFSA: 86.8% ($p < 10^{-3}$) and APRI: 80.0% ($p < 10^{-3}$). A new classification provided three reliable diagnosis intervals: F0/1, F0/1/2, F2/3/4 with 91.4% accuracy for FibroMeter, avoiding biopsy in all patients.**

***Conclusions*: FibroMeter NAFLD had high performance and provided reliable diagnosis for significant fibrosis, significantly outperforming NFSA and APRI.**

***Keywords*: Blood fibrosis markers; NAFLD; Liver biopsy; Liver fibrosis; Sensitivity; Specificity**

Received 10 April 2008; received in revised form 1 June 2008; accepted 2 July 2008; available online 7 October 2008
Assistant Editor: Silvia Fargion

☆ Paul Calès, Frédéric Oberti, Isabelle Hubert, and Françoise Lunel have mentioned potential conflict of interest due to stock ownership in a society (BioLiveScale) recently created under the auspices of University of Angers.

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Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AUROC, area under the receiver operating characteristic; BMI, body mass index; CLD, chronic liver disease; NAFLD, non-alcoholic fatty liver disease; NFSA, NAFLD fibrosis score of Angulo et al.; NPV, negative predictive value; PPV, positive predictive value.

0168-8278/$34.00
doi:10.1016/j.jhep.2008.07.035

1. Introduction

Several blood tests have been proposed to diagnose liver fibrosis [1]. Some tests are simple, like the aspartate aminotransferase to platelet ratio index (APRI) [2]. Others are more complex, constructed as algorithms (regression score) like the FibroMeter [3]. Most of them have been developed in chronic hepatitis C or in miscellaneous causes [4]. However, in a previous study, we observed that the cause of CLD was an independent predictor of fibrosis and thus it was preferable to develop specific tests for alcoholic or viral CLD to improve accuracy [3].

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized condition in several countries [5] that can lead to cirrhosis or liver cancer. Some simple variables [6–8], fibrosis blood markers [9,10] and other tests [4,11] have been evaluated in NAFLD but these studies are rare or performed in only a few patients [4] and no blood test had been specifically designed for this prevalent disease until recently. We thus designed a simple algorithm in a previous study [12]. More recently, the NAFLD fibrosis score of Angulo et al. (NFSA) has been implemented in a large cohort with excellent performance [13]. However, this test was designed for severe fibrosis whereas most tests have been designed for significant fibrosis and usually for chronic hepatitis C. Some of the latter have been validated in NAFLD [11,14].

The main aim of the present study was to implement a blood test for significant liver fibrosis specifically designed for NAFLD with high diagnostic performance. The secondary aims were to compare this test to the only other specific test, the NFSA, and to a non-specific reference test, i.e. APRI, the simplest test. Other aims were to evaluate the factors influencing this diagnostic performance, such as diagnostic targets and fibrosis stages, as well as reproducibility.

2. Patients and methods

2.1. Centres

Two tertiary centres, Angers and Rennes, provided, respectively, 73 and 162 patients, for a total of 235. The centres were independent for study design, patient recruitment, blood measurements, and liver interpretation. Due to differences in size and patient characteristics, especially fibrosis stages, between the two centres (Table 1), all patients were pooled then randomly divided into test (121 patients) and validation (114 patients) populations with stratification based on Metavir fibrosis stages.

2.2. Patients

Patients were considered as having NAFLD and prospectively included between 2001 and 2006 if they had abnormal liver blood tests or ultrasonography showing diffuse hyperechogenicity compared to that of the spleen, together with at least one of the five clinical features used in the definition of metabolic syndrome according to the Adult Treatment Panel III Working Group [12,15] detailed elsewhere [12]. In addition, liver specimen had to be compatible [16] and alcohol consumption had to be <30 g/day for the past five years according to a standard questionnaire as described elsewhere [12,17]. Patients were not included if they had another cause of CLD, complicated cirrhosis or were given putative anti-fibrotic treatment in the past 6 months. In the Angers centre, 48.8% of patients with suspected NAFLD were not included as liver biopsy had not been performed; they were characterized by less severe liver disease (data not shown). The study protocol conformed to the ethical guidelines of the current Declaration of Helsinki and was approved by a local Ethics Committee.

2.3. Methods

2.3.1. Clinical data and blood tests

Diabetes was defined as fasting glucose $\geqslant$126 mg/dL at inclusion or patient under drug treatment [18]. Fasting blood samples were taken at inclusion (date of liver biopsy $\pm$7 days). The usual blood

Table 1
Characteristics of populations.

	Whole	By centre			After randomization		
		Angers	Rennes	*p*	Test	Validation	*p*
N patients	235	73	162	–	121	114	–
Sex (% male)	74.5	64.4	79.0	0.02	70.3	78.9	0.13
Age (years)	51.1 ± 11.0	54.8 ± 11.8	49.4 ± 10.2	$<10^{-3}$	51.2 ± 12.3 12	51.0 ± 9.5	0.88
Body weight (kg)	82.9 ± 16.0	87.6 ± 21.6	80.8 ± 12.2	0.003	83.5 ± 17.9	82.2 ± 13.8	0.55
BMI (kg/m^2)	28.7 ± 4.9	30.8 ± 6.7	27.8 ± 3.5	$<10^{-3}$	29.1 ± 5.5	28.4 ± 4.2	0.28
Metavir fibrosis stage:				$< 10^{-3}$			> 0.99
F0 (%)	43.4	17.8	54.9	–	43.0	43.9	–
F1 (%)	28.9	23.3	31.5	–	28.9	29.0	–
F2 (%)	8.9	13.7	6.8	–	9.1	8.8	–
F3 (%)	8.1	23.3	1.2	–	9.3	7.9	–
F4 (%)	10.6	21.9	5.6	$<10^{-3}$	10.7	10.5	0.96
Significant fibrosis (%)	27.7	58.9	13.6	$< 10^{-3}$	28.1	27.2	0.88
Severe fibrosis (%)	18.7	45.2	6.8	$<10^{-3}$	19.0	18.4	0.91
Metavir fibrosis score	1.1 ± 1.3	2.1 ± 1.4	0.7 ± 1.0	$<10^{-3}$	1.2 ± 1.4	1.1 ± 1.3	0.88
Liver specimen size (mm)	30 ± 12	21.5 ± 10	34 ± 11	$<10^{-3}$	27 ± 12	34 ± 11	$<10^{-3}$
APRI	0.53 ± 0.54	0.77 ± 0.66	0.43 ± 0.44	$<10^{-3}$	0.59 ± 0.65	0.47 ± 0.39	0.09
NFSA	0.20 ± 0.24	0.39 ± 0.31	0.12 ± 0.16	$< 10^{-3}$	0.22 ± 0.27	0.18 ± 0.22	0.22
FibroMeter	0.28 ± 0.35	0.55 ± 0.37	0.15 ± 0.26	$< 10^{-3}$	0.31 ± 0.36	0.24 ± 0.34	0.11