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Journal of Hepatology 44 (2006) 686–693

Journal of Hepatology

www.elsevier.com/locate/jhep

Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C

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***Background/Aims*: In chronic hepatitis C, biopsy is the gold standard for assessment of liver fibrosis. Non-invasive markers have been proposed but their use is limited by diagnostic accuracy. Our aim was to increase the diagnostic performance of non-invasive markers of liver fibrosis by combining them in sequential algorithms.**

***Methods*: One hundred and ninety patients with chronic hepatitis C were evaluated for AST to platelets ratio (APRI), Forns' index and Fibrotest at the time of liver biopsy and stepwise combination algorithms were developed and validated prospectively in 100 additional patients.**

***Results*: Three algorithms were developed: (1) significant fibrosis ($F \geq 2$ by METAVIR) was identified with high diagnostic performance (>94% accuracy) using APRI as screening test, followed by Fibrotest in APRI non-classified cases and restricting liver biopsy to patients classified F0–F1 by non-invasive tests. (2) A slightly modified algorithm had similar performance when applied to hepatitis C carriers with normal ALT. (3) Identification of cirrhosis (95% accuracy) was achieved using a dedicated algorithm with different cut-off, reducing by 60–70% the liver biopsies needed.**

***Conclusions*: Stepwise combination of non-invasive markers of liver fibrosis improves the diagnostic performance in chronic hepatitis C. Need for liver biopsy is reduced by 50–70% but cannot be completely avoided.**

***Keywords*: Chronic hepatitis C; Liver fibrosis; Liver biopsy; Non-invasive markers; Stepwise combination algorithms**

1. Introduction

Chronic infection with hepatitis C virus remains a major health problem with around 200 million individuals affected worldwide [1]. The natural course of chronic hepatitis C is characterised by progressive fibrosis in the inflamed liver with structural and hemodynamic changes leading to cirrhosis, which is followed by end-stage complications [2]. Accordingly, the prognostic evaluation and clinical management of still compensated chronic hepatitis C is largely based on assessment of the type and degree of liver fibrosis and several semiquantitative scoring systems have been proposed and validated [3–6]. Liver biopsy is the gold standard for fibrosis staging in chronic hepatitis C as in many other chronic liver diseases. However, liver biopsy is invasive and complications occur in 0.6–5% of patients [7–9]. Moreover, liver biopsy is costly and requires hospitalisation of at least 6–18 h [10]. Finally, recent studies performed in chronic hepatitis C have demonstrated that inadequate liver biopsy sample size frequently leads to underestimation of fibrosis stage [11,12]. Furthermore, laparoscopic studies have shown that cirrhosis is missed by percutaneous liver biopsy in 10–30% of the cases [13–15]. For all these reasons a great

Received 17 October 2005; received in revised form 17 January 2006; accepted 19 January 2006; available online 8 February 2006
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doi:10.1016/j.jhep.2006.01.007

interest and many studies have been recently dedicated to the development of non-invasive markers as surrogates of liver biopsy. These non-invasive markers include the AST to platelet ratio index (APRI) proposed by Wai et al., the Forns' index based on age, platelets, γGT and cholesterol and more sophisticated model like Fibrotest [16–18]. Fibrotest is based on five serological parameters including bilirubin, γGT, apolipoprotein A1 (ApoA1), alfa-2-macroglobulin (A_2M) and haptoglobin [18]. These different methods have been usually applied individually in the different validation studies. All of them have limitations. APRI and Forns' index leave many patients unclassified while Fibrotest is more expensive and uses two uncommon parameters. Furthermore, the diagnostic accuracy of most methods has not exceed 80–85% [12]. There are no published studies in which these non-invasive markers of liver fibrosis have been combined in the attempt to improve diagnostic accuracy. We have here measured APRI, Forns' index and Fibrotest in a consecutive series of patients with chronic hepatitis C. Having first assessed the diagnostic accuracy of each individual method using liver biopsy as gold standard, we have then combined them with the aim of defining stepwise algorithms of higher diagnostic accuracy to be used in most common clinical scenarios, i.e. for identifying cases with significant fibrosis and those with cirrhosis among patients presenting with chronic HCV infection.

2. Patients and methods

2.1. Patients

This study included two cohorts of consecutive patients with a recent diagnosis of chronic hepatitis C who underwent percutaneous liver biopsy at the Department of Clinical and Experimental Medicine at the University of Padova. The first cohort (training set) included 190 patients seen between March 2003 and June 2004. The second cohort (validation set) consisted of 100 consecutive patients seen between July 2004 and April 2005. All patients were positive for serum HCV-RNA by polymerase chain reaction and had compensated chronic HCV infection. The exclusion criteria were any other cause of chronic liver disease, co-infection with HBV or HIV and co-morbidities that could confound the results of the non-invasive markers adopted. These included current alcohol intake (>20 g/die), haemolysis, Gilbert's syndrome. All biopsies were obtained with 16G Menghini type needle. To limit the risk of fibrosis underestimation, patients with biopsy samples shorter than 1.5 cm or containing less than seven portal tracts were excluded [19]. According to these criteria, 76 patients with chronic hepatitis C were excluded. Informed consent was obtained from all patients participating in the study, that was conducted according to the rules of the Declaration of Helsinki.

2.2. Methods

2.2.1. Histological assessment

Liver biopsies were fixed in formalin and embedded in paraffin. The slides were stained with hematoxylin–eosin, van Gieson stain for collagen, PAS after diastase digestion and Perls' Prussian blue method. The slides were evaluated by a single, independent Pathologist (M.G.) who was unaware of clinical data. Fibrosis was scored according to the METAVIR system [5]. Intra-observer agreement was assessed, using k statistics, by re-evaluating a subset of 50 randomly chosen samples: k value was higher than 0.90.

2.2.2. Non-invasive markers

All patients were evaluated for APRI, Forns' index and Fibrotest using fasting serum samples obtained at the time of liver biopsy. For this purpose platelet count, AST, ALT, γGT, cholesterol levels, haptoglobin, ApoA1, A_2M, total bilirubin were routinely determined using validated methods. All the patients were also tested for prothrombin time (international normalised ratio, INR), albumin, viral load (Amplicor™; Roche, Diagnostic Systems, Basel, Switzerland) and HCV genotype (InnoLipa; Innogenetics, Bayer, Ghent, Belgium). APRI and Forns' index were calculated using cut-off values indicated in the original reports [16,17]. Fibrotest results were kindly provided by T. Poynard, Universite Paris VI, Paris, France.

2.2.3. Statistical tools

Descriptive results were expressed as mean ± SD or number (percentage) of patients with a condition. The *t*-test or non-parametric Mann–Whitney test was used to compare quantitative data and the χ^2-test was applied for comparison of frequency data. *P*-values less than 0.05 were considered significant. The diagnostic performance of the non-invasive methods for liver fibrosis was measured as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratios (LR). Sensitivity, specificity, PPV, NPV and accuracy were expressed as percentage. The diagnostic value of the three methods was assessed by calculating the areas under the curves (AUC) and their corresponding 95% confidence intervals (CI). The diagnostic algorithms combining the non-invasive methods were developed by modelling the best algorithm for liver fibrosis in different clinical scenarios, as described in Section 3.

3. Results

The main demographic, laboratory and histological features of the two sets of patients are summarized in Table 1. The mean length of liver specimens was 1.77 ± 0.31 cm and mean complete portal tracts number was 11.03 ± 3.17. Baseline characteristics of patients were comparable for all parameters considered. Around 34 and 28% of patients in the training and in the validation set, respectively, had chronic hepatitis C with persistently normal ALT (PNALT) as defined by at least three normal values 2 months apart over 6 months [20]. The two sets had all fibrosis stages represented.

3.1. Diagnostic performance of individual non-invasive methods in the training set

The first analysis was aimed to determine the diagnostic performance of the three non-invasive methods in detecting significant fibrosis as defined by METAVIR fibrosis stage ≥F2. In this analysis, patients with elevated ALT and with PNALT were considered separately. The corresponding results are shown in Tables 2 and 3.

In patients with elevated transaminases, Fibrotest classified all cases while both APRI and Forns' index were unable to classify almost half of the patients. APRI had the best PPV with the 1.5 cut-off and the best NPV with the 0.5 cut-off. It also showed the best accuracy in patients classified by the 0.5 cut-off. Fibrotest showed slightly better AUC but the difference against Forns and APRI was