Digestive and Liver Disease 48 (2016) 55–61

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Cytokeratin 18-Aspartate396 apoptotic fragment for fibrosis detection in patients with non-alcoholic fatty liver disease and chronic viral hepatitis

Chiara Rosso[a,*,1], Gian Paolo Caviglia[a,1], Maria Lorena Abate[a], Ester Vanni[a,b], Lavinia Mezzabotta[a,b], Giovanni Antonio Touscoz[b], Antonella Olivero[a], Andrea Marengo[a,b], Mario Rizzetto[a,b], Elisabetta Bugianesi[a,b], Antonina Smedile[a,b]

[a] Liver Physiopathology Lab, Department of Medical Sciences, University of Turin, Turin, Italy
[b] Division of Gastroenterology and Hepatology, Città della Salute e della Scienza University Hospital, Turin, Italy

ARTICLE INFO

Article history:
Received 3 April 2015
Accepted 19 September 2015
Available online 28 September 2015

Keywords:
Chronic liver disease
Cytokeratin 18-Asp396
Liver stiffness

ABSTRACT

Background: The combination of non-invasive markers for the detection of fibrosis in patients with chronic liver diseases is still a matter of debate.
Aims: To test the performance of cytokeratin18-Aspartate396 alone or in combination with transient elastography as a marker of fibrosis, compared to liver biopsy as gold standard.
Methods: In 259 prospectively enrolled patients with chronic liver diseases, clinical, biochemical, and histological features were assessed. Serum cytokeratin18-Aspartate396 and Fibroscan were performed within 6 months prior to liver biopsy.
Results: Cytokeratin18-Aspartate396 levels predicted both significant and advanced fibrosis in non-alcoholic fatty liver disease group, correctly identifying 83.7% and 80.8% of cases, respectively. Liver stiffness performed best in predicting severe fibrosis in patients with chronic viral infection, correctly identifying 78.7% of chronic hepatitis B and 88.6% of chronic hepatitis C subjects. The combination of cytokeratin18-Aspartate396 and liver stiffness improved their diagnostic performance for the detection of significant and advanced fibrosis in non-alcoholic fatty liver disease group, only (sensitivity = 78.3%, specificity = 90.7%; sensitivity = 91.7%, specificity = 71.6%, respectively).
Conclusion: Cytokeratin18-Aspartate396 and liver stiffness can improve the non-invasive prediction of significant and advanced fibrosis in patients with non-alcoholic fatty liver disease, while in hepatitis B and C virus infected patients their combined use had no advantage over the diagnostic accuracy of transient elastography alone.

1. Introduction

Chronic hepatitis B (CHB) and C (CHC), along with non-alcoholic fatty liver disease (NAFLD), are the major leading cause of chronic liver disease (CLD) worldwide. The prognosis and clinical management of these patients are strongly influenced by the development of fibrosis and its progression over time. A number of indirect markers are currently replacing liver biopsy for the non-invasive detection of fibrosis [1–4], but all of them have significant limitations, both intrinsic and/or related to the aetiology of liver damage. Combining two unrelated markers generally yields better results than the use of a single one, as no specific test has an advantage over the others in the prediction of the severity of liver disease. Among the different strategies, algorithms combining transient elastography and serum biomarkers are the most attractive and validated one [5,6]. A growing body of evidence suggest that apoptosis contributes to liver fibrogenesis [7–11] and plays an important role in both acute and chronic liver diseases [12]. In hepatic cells, apoptosis leads to permeabilization of the external membrane of mitochondria and activation of the caspases cascade (mainly caspase 3), resulting in cleavage of cytokeratin-18 (CK18), the principal intermediate filament protein in the liver [13]. Despite intrinsic

* Corresponding author at: Liver Physiopathology Lab, Department of Medical Science, University of Turin, Via San Massimo 24, 10126 Turin, Italy.
Tel.: +39 0116333532; fax: +39 0116333976.
E-mail addresses: crosso3@cittadellasalute.to.it, chiara.rosso84@tiscali.it (C. Rosso).
[1] These authors equally contributed to this manuscript.

http://dx.doi.org/10.1016/j.dld.2015.09.008
1590-8658/

limitations, CK18-Asp396 apoptotic fragment has been included in the AGA/AASLD/ACG guidelines for diagnosis and management of NAFLD as the sole biomarker of NASH, although recent studies have questioned its reliability. However, most non-invasive tests of fibrosis perform best at excluding severe fibrosis-cirrhosis (F3–F4), with negative predictive values (NPV) > 90%, but typically have low PPV [14]. Conversely, CK18-Asp396 has a high PPV as marker of fibrosis in NAFLD, hence their combination could be useful. Circulating CK18-Asp396 fragments are increased also in liver diseases of different aetiology and have been associated to the extent of both liver fibrosis and steatosis in CHC [15–17] and to degree of necroinflammation in CHB subjects [18]. Transient elastography (FibroScan), a non-invasive method which measures liver stiffness (LS) as a function of the extent of fibrosis [3,19,20], is currently the most used imaging tool for the indirect detection of liver fibrosis and it has been validated in several large-scale prospective studies [21–23].

In this study (1) we tested the effectiveness of CK18-Asp396 as biomarker of fibrosis in patients with CLD of different aetiologies (CHB, CHC and NAFLD) by using liver biopsy as reference standard and (2) we evaluated its diagnostic accuracy compared to and in combination with LS, since algorithms combining transient elastography and serum biomarkers are the most attractive and validated [14].

2. Materials and methods

Two hundred and seventy-seven patients with chronic hepatitis were prospectively recruited at the Division of Gastroenterology of the San Giovanni Battista Hospital in Turin from 2010 to 2013. All patients underwent a liver biopsy. Chronic hepatitis B was defined by positive serology and detectable serum HBV-DNA. Chronic hepatitis C was defined by detectable anti-HCV antibodies and serum HCV-RNA. Diagnosis of NAFLD was confirmed by liver biopsy in patients with altered liver function tests and no other known cause of liver disease. Patients with other aetiologies of chronic hepatitis, such as autoimmune hepatitis, primary biliary cirrhosis, alcoholic liver disease and hemochromatosis were excluded. Personnel involved in the study was blinded to clinical characteristics of the patients. The study protocol was conformed to the principles of the 1975 Declaration of Helsinki. The study was approved by the local Ethics Committee and all patients provided written informed consent.

2.1. CK-18 Asp396 determination

Blood samples were collected at the time of liver biopsy and stored at −80 °C for further analysis. The serum levels of CK18-Asp396 were assessed by the commercial M30-Apoptosense ELISA Kit (PEVIVA, Sweden) according to the manufacturer's instructions. Briefly, samples were placed into wells coated with mouse monoclonal antibody as a catcher. After washing, a horseradish peroxidase-conjugated antibody (M30) was used for detection. The absorption was determined with an ELISA reader at 450 nm using the cubic spline algorithm. Inter and intra-assay coefficient of variation were below 10%. The concentration of CK18-Asp396 was expressed as units per litre (U/L).

2.2. Transient elastography

Liver stiffness measurements were performed within 6 months before liver biopsy (Fibroscan, Echosens, Paris, France). An ultrasound probe connected to a vibrating system was placed on the right side of the patient's chest. The velocity of the low-frequency elastic shear wave propagating through the liver is directly related to the tissue stiffness. The success rate was calculated as the ratio of the number of the successful measurements over the total number of acquisitions as described by Caviglia et al. [5]. Liver stiffness was expressed in kilopascals (kPa) as the median value of the successful determinations. LS values with at least 10 successful measurements, success rate higher than 60% and interquartile range (IR) minor of 30%, were considered reliable and used for analysis.

2.3. Liver biopsy

Specimens were analysed by an expert pathologist. Liver fibrosis was staged according to Ishak score in viral patients [24] and to Kleiner classification [25] in NAFLD subjects. For the purpose of this study, all the biopsies were further reviewed and classified according to Metavir score [26]: F0, absence of fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.

2.4. Statistical analysis

Data are reported as mean and standard deviation (SD) for continuous normally distributed variables, as median and 95% confidence interval for the median (95% CI) for continuous non-normally distributed variables and as number and frequency (%) for categorical variables. Comparisons between groups were performed using the two-tailed Student's *t*-test for normal continuous variables and the Kruskal–Wallis non-parametric test for non-normal continuous variables. For categorical data, the Fisher exact test or the Chi-square test were used as appropriate. To assess the relationship among CK18-Asp396 and LS with biochemical parameters and fibrosis, Pearson (r) or Spearman's (r_S) correlation test were performed as appropriate. Univariate analysis and stepwise logistic regression analyses were performed in each group to assess the association of CK18-Asp396 and LS with both significant and advanced fibrosis. All the models were adjusted for age, sex, body mass index (BMI), aminotransferase levels, and steatosis in CHC and CHB groups and for age, sex, BMI, aminotransferase levels, steatosis and NAS score in NAFLD cohort. Receiver Operating Curves (ROC) analysis was used to investigate the diagnostic accuracy of CK18-Asp396 and LS (alone or in combination) for the discrimination of both $F \geq 2$ and $F \geq 3$. For this purpose, we used predictive probabilities obtained from the logistic regression models. Values of $p < 0.05$ were considered statistically significant. All the analysis were performed with SPSS software version 20.0 (IBM SPSS Statistics for Windows, Chicago, IL).

3. Results

3.1. Clinical, biochemical and histological features

A total of 259 patients were enrolled in the study out of 277 who underwent screening. Reasons for exclusion were liver biopsy length <11 mm ($n = 5$) and unreliable LS measurement ($n = 13$, 10 NAFLD and 3 CHC), according to the criteria previously described. The flowchart of patient enrolment is depicted in Fig. 1. The final cohort included 57 patients with CHB, 97 with CHC and 105 with NAFLD. Clinical, biochemical and histological characteristics of the study patients are reported in Table 1. The three groups were comparable for age. As expected, BMI was higher in NAFLD subjects. Fibrosis was absent or mild (F0/F1) in 49.2% of CHB, 47.5% of CHC and 40.9% of NAFLD subjects; significant fibrosis ($F \geq 2$) was found in 50.8% of CHB, 52.5% of CHC and 59.1% of NAFLD patients, while advanced fibrosis ($F \geq 3$) was diagnosed in 29.8%, 29.8% and 36.2% of CHB, CHC and NAFLD subjects, respectively.