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Non-invasive assessment of liver fibrosis in patients with HBV-related chronic liver disease undergoing antiviral treatment: A preliminary study

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ABSTRACT

In chronic hepatitis B (CHB) patients, fibrosis assessment during antiviral treatment is a key step in the clinical management. Aim of this study was to evaluate the performance of elastography in assessing fibrosis stage in CHB before and after two years of nucleoside/nucleotide analogues (NUC) treatment in comparison with indirect serum markers. CHB diagnosis was made according to standard criteria. A clinical and virological evaluation was performed at baseline and again at 3, 6, 9, 12 18, and 24 months during treatment. Fibrosis was evaluated by liver biopsy, elastography and indirect serum markers. Of 75 patients, 50 had CHB, HBeAg negative and were deemed eligible for this study. Of these, 22 underwent liver biopsy. Mean histomorphometric values of fibrotic tissue differed significantly in the stage < S3 vs. stage ≥S3: 2.01 ± 2.62% vs. 12.85 ± 7.31% (p=0.03), respectively. At 18 and 24 months, stiffness values were statistically reduced from those previously observed (**P**=0.03 and **P** < 0.001). At 24 months the values of APRI, FIB-4 and LOK were not different from baseline values, while the value of FORNS score at 24 months was the only one statistically reduced. In two patients with fibrosis stage S3 and S6, respectively, fibrosis regressed to stage S2 and S5. In conclusion, the results of the present study show that liver histology, stiffness and FORNS score improve significantly during a long-term follow-up of HBV patients successfully treated with NUC. These results strongly suggest that the non-invasive evaluation of liver fibrosis represents a key step in the management and treatment of chronic HBV hepatitis.

1. Introduction

Chronic liver disease represents a major public health problem worldwide due to its morbidity, mortality, and associated economic costs (Minino et al., 2007). The introduction of an effective vaccine against the hepatitis B virus (HBV) has reduced the prevalence of hepatitis, as well as its health and economic impact in industrialised countries. In Europe, the WHO estimates that 13.3 million people are HBV infected and Italy falls among the countries with low endemicity (positivity for HBsAg < 2%) (Schweitzer et al., 2015; Stasi et al., 2015).

According to the latest recommendations, antiviral treatment for CHB needs to be considered in the presence of HBV-DNA > 2000 IU/**ml**, elevated ALT, and/or moderate liver fibrosis (Ishak ≥2). In HBeAg positive patients, the primary therapeutic goal is to achieve a stable seroconversion HBeAg/anti-HBe. Patients with immune tolerance or high levels of viremia (HBV DNA > 2×10^7 IU/**ml**) do not require treatment in the absence of hepatocellular damage, although they should still be monitored. Tenofovir, entecavir and peginterferon alfa-2a are the preferred first-line treatments for both HBeAg-positive and HBeAg-negative CHB infected patients (Carosi et al., 2011).

Some studies suggest that the complete long-term suppression of HBV replication by nucleosides/nucleotides results in a long-term improved outcome that significantly reduces the risk of developing liver cirrhosis, hepatocellular insufficiency, and, hepatocellular carcinoma (Tan and Sun, 2013). Moreover, longitudinal histopathological evaluation has demonstrated a regression of liver tissue fibrosis during

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entecavir/tenofovir therapy (Papachrysos et al., 2015).

To date, few studies have evaluated the longitudinal changes of liver fibrosis in CHB positive patients with transient elastography (TE). The aim of the current study was therefore to evaluate whether TE and indirect serum markers could represent a valuable clinical resource for monitoring tissue fibrosis during and after antiviral therapy.

2. Materials and methods

Patients with HBV referred between January 2010 and December 2015 to the Hepatology outpatient services of the Azienda Ospedaliero Universitaria Careggi (AOUC), Florence, Italy, were considered for the study.

The treatment of HBV patients was established in accordance with the Italian recommandations (Carosi and Rizzetto, 2008).

The study was clearly explained to the patients, and their written informed consent was obtained. An information form on the study design and on the treatment of clinical data collected during the same protocol was released to each patient. There was no restriction regarding current treatments for other diseases except for those therapies/diseases listed in the exclusion criteria.

Inclusion criteria were as follows: patients naïve to antiviral treatment with nucleoside/nucleotide analogues, aged between 18 and 70 years, HBsAg positive, HBV DNA > 2000 IU/**ml**, HBeAg negative, anti-HBe positive, with liver fibrosis assessed by liver biopsy and/or by FibroScan; patients naïve to treatment presenting clinical and biochemical diagnosis of HBV related cirrhosis (biopsy was not performed for these patients). Exclusion criteria were as follows: ALT > 5 x ULN, HBeAg positive patients, BMI > 30, coinfections (HIV, HCV, HDV), pregnancy, connective tissue diseases, psychiatric illnesses compromising compliance with therapy, presence of ascites at ultrasound, hepatocellular carcinoma (HCC), alcohol or drug related liver disease, treatment with corticosteroids and/or interferon alpha in the six months prior to enrolment in the study, resistance to antiviral treatment or the presence of side effects requiring an association or replacement with another drug.

2.1. Non-invasive assessment

2.1.1. Indirect serum markers

All patients were assessed with the following surrogate markers of liver fibrosis: APRI (Wai et al., 2003), FIB-4 (Vallet-Pichard et al., 2007), Forns score (Forns et al., 2002), Lok score (Lok et al., 2005). The above scores were calculated using biochemical tests carried out within one month before liver biopsy. The same tests were repeated and the scores calculated 24 months following the initiation of treatment.

The entire cohort of patients evaluated by Ishak score, together with the cirrhotic patients on the basis of clinical and ultrasound evaluation, was evaluated with HUI score (HUI et al., 2005), a non-invasive biomarker validated for HBV to distinguish between significant and non significant fibrosis.

2.1.2. Transient elastography

Liver stiffness was measured using FibroScan® (Echosens, Paris, France), according to the manufacturer instructions. In all patients, TE was performed after an overnight fasting (Arena et al., 2013). Procedures with 10 successful acquisitions, expressed in kilopascal (kPa), with a success rate of at least 60% and an interquartile range (IQR) lower than 30% of the median value, were considered reliable. Liver stiffness was measured at commencement, and again at 3, 6, 9, 12 18, and 24 months during treatment.

2.2. Invasive assessment

2.2.1. Liver biopsy

On the same study day, patients underwent a measurement of liver stiffness by TE. Ultrasound-guided percutaneus liver biopsy was then performed on the right lobe of the liver with a 16-gauge semiautomatic modified Menghini needle system (BIOMOL; Hospital Service, Aprilia, Italy) under local anaesthesia. Liver specimens were formalin-fixed and paraffin-embedded for histological evaluation. Sections of liver tissue were stained with haematoxylin, eosin and Sirius red. These were then examined by an experienced pathologist who was unaware of the liver stiffness results. All liver specimens had a length > 25 mm and included at least 11 complete portal tracts, reflecting adequate standards (Guido et al., 2004). The presence of necro-inflammatory activity (grading) and fibrosis (staging) was established according to the method proposed by Ishak (Ishak et al., 1995). Each unit participating in the study, if external to AOUC, provided three unstained paraffin sections. At the end of the study, histopathological examination of all samples (pre- and post-treatment) was repeated by a pathologist. In the event of disagreement between the two pathologists, a review of the scoring by collegial observation was scheduled.

2.2.2. Morphometry

Only the sections of each biopsy stained with Sirius red were used for calculating the percentage of collagen, which was performed by one author (C.S.). The percentage of collagen content was calculated by digital image analysis (Documentation – RSB Home Page). This software enables, through a grey scale slider, to select the total tissue area of liver biopsy. Subsequently, red, green, and blue (RGB) light channels were used to select the collagen area. Before the measurement of liver fibrosis structural collagen in large portal tracts, blood vessel walls, artefacts, vascular cavities, and lymphoid aggregates were eliminated. The results of the digital analysis were compared with the standard Ishak score (Ishak et al., 1995).

2.3. Statistical analysis

All results are expressed as mean ± standard deviation. After checking similar variances within the groups using Levine's test for equality of variances, the numerical comparison of continuous data was performed using the Student's *t*-test for unpaired and for paired samples with Bonferroni correction. Statistical significance was set at **P** < 0.05.

To evaluate factors associated with hepatic fibrosis, patients were divided in 2 groups: patients with non significant (< 6 kPa) or significant fibrosis (≥6 kPa) along the EASL-ALEH guidelines (2015). Univariate analysis explored each variable in the data set, including red blood cells, white blood cells, platelets, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha-fetoprotein, gamma-glutamyltransferase (GGT), ferritin, sideremia, creatinine, glucose, cholesterol, INR, bilirubin, albumin and all non invasive serum markers.

Logistic regression models were used for multivariate analysis to identify the most significant correlation among the variables. All variables in univariate analyses with P < 0.05 were introduced into the multivariate analysis.

3. Results

The study evaluated 75 patients (48 males and 27 females, mean age 48.45 ± 13.98). Of these, 50 CHB, HBeAg negative patients were deemed eligible. The mean stiffness value of these patients was 10.52 ± 6.05 kPa. The biochemical parameters of the entire study population are shown in Table 1. Baseline values of APRI, FORNS, FIB-4 and LOK were 0.91 ± 1.65, 4.5 ± 1.93, 0.73 ± 0.68, 0.29 ± 0.17, respectively. In these patients, when univariate analysis was conducted to evaluate