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Assessment of liver fibrosis in primary biliary cholangitis: Comparison between indirect serum markers and fibrosis morphometry

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ABSTRACT

Background: The accuracy of non-invasive methods for the quantification of liver fibrosis in primary biliary cholangitis (PBC) is still debated.
Aims: To determine the histo-morphometric measurement of fibrotic tissue and to explore the possible association between indirect markers (APRI, FORNS, FIB-4, and Lok) and morphometry.
Methods: Retrospective analysis of medical data from patients with PBC, on whom needle liver biopsy was performed as part of the diagnostic assessment. One section of each biopsy stained with Sirius red was used for calculating the percentage of collagen. Quantitative measure of fibrotic tissue (fibrosis morphometry) was calculated as a percentage of collagen content by digital image analysis. Morphometry results were divided into four groups reflecting Ludwig's staging and compared with values for indirect serum markers.
Results: 50 PBC patients were enrolled (86% females, mean age 57 ± 12.30 years), 19 were Ludwig's stage I (38%), 14 stage II (28%), 12 stage III (24%), and five stage IV (10%). Morphometry results were significantly different among Ludwig stages ($p < 0.05$). No significant differences were found for indirect serum markers. A significant correlation was found between morphometry results and indirect serum markers tested ($p < 0.05$).
Conclusion: In our cohort, the histo-morphometric values of fibrotic tissue increased progressively with Ludwig's stages of PBC, while non-invasive markers did not.

1. Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic disease with a progressive course that may ultimately lead to the development of the full range of complications associated with decompensated liver cirrhosis [1]. A number of staging systems have been proposed to describe the histologic progression from the typical early lesions of chronic non-suppurative destructive cholangitis to cirrhosis [2–5]. Although each staging system has been shown to have predictive value for the development of liver-related morbidity and patient survival [3], the quest for a better staging system is still active.

Similarly to other chronic liver diseases, the progression of PBC to cirrhosis is associated with extensive fibrosis. This "biliary-type" fibrosis originates in the periportal areas, where it is associated with the typical inflammatory infiltrate of the "interface hepatitis" [6]. The formation of perilobular septa and cirrhotic nodules preserves terminal hepatic veins until the late stage of advanced cirrhosis.

Recently, a quantitative method was proposed for the analysis of liver fibrosis in hepatitis C virus (HCV) infection to reduce inter- and intra-observer variability in the measurement of histological fibrosis and to explore different degrees of fibrosis within cirrhotic livers. The collagen proportionate area (CPA) is a validated method that assesses fibrosis quantity and predicts clinical outcomes in HCV-positive cirrhotic and non-cirrhotic patients [7–10]. Moreover, Tsochatzis et al. [11] found that CPA accurately subclassifies cirrhosis, and it is the only independent predictor of clinical decompensation among the other histological systems described to date. The quantitative assessment of fibrosis would be very important in PBC patients, as they may have oesophageal

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varices in early histological stages [12], although portal venopathy or nodular regeneration have been proposed as a possible explanation. Haboubi et al. [13], in studies conducted in a renal transplant series, suggested that this mechanism in the liver can result from sinusoidal endothelial cell damage that induces Disse space fibrosis, which then leads to focal hepatocyte atrophy, followed by compensatory nodular regenerative hyperplasia. Some studies [14] have described nodular hyperplasia in the early PBC stage and suggested that this may contribute to the development of portal hypertension.

It has also been suggested that non-invasive methods for the quantification of liver fibrosis may be useful since they are not subjected to liver biopsy sampling errors and may thus reflect the fibrotic state of the whole organ. However, the results obtained in these patients by Floreani et al. [15] did not confirm those reported in previous studies on HCV-infected patients.

The aims of the present study were to quantitatively determine the amount of fibrotic tissue using morphometry and to analyse indirect markers of liver fibrosis in the detection of different histological stages of PBC. Additionally, we aimed to assess the possible association between indirect serum markers and fibrotic tissue morphometry.

2. Patients and methods

2.1. Patients

Patients with PBC referred between January 1994 and December 2008 to the Hepatology outpatient services of a single tertiary centre in Florence, Italy, were considered for the study.

The diagnosis of PBC was established in accordance with the EASL 2009 guidelines [16]. In patients with PBC, on whom needle liver biopsy was performed as part of the diagnostic assessment, a retrospective collection of medical data was performed including anti-mitochondrial antibodies (AMA), serum aminotransferases, cholic acid, alkaline phosphatase, cholesterol, triglyceride, gammaglutamyl-transpeptidase, fasting glucose, platelet count, and international normalised ratio (INR). The exclusion criteria were: hepatocellular carcinoma, co-infection with hepatitis B virus (HBV) and/or HCV and/or human immunodeficiency virus, overlap syndrome between PBC and autoimmune hepatitis (AIH), metabolic liver disease, vascular disease of the liver, average daily alcohol consumption >50 g/day, or the use of hepatotoxic drugs. The applied criteria to exclude patients with the overlap syndrome between PBC and AIH were the "Paris criteria" [17], based on simplified criteria of PBC alone and AIH alone, requiring at least two of the three accepted criteria for both AIH and PBC. These criteria have been endorsed by the European Association for the Study of the Liver [16], whereby histological evidence of moderate to severe lymphocytic piecemeal necrosis (interface hepatitis) is mandatory.

2.2. Indirect serum markers

All patients were assessed for the following surrogate markers of liver fibrosis: APRI [18], FIB-4 [19], and scores published by Forns (FORNS score) [20] and by Lok (LOK score) [21]. The scores of indirect serum markers of fibrosis were calculated with laboratory tests carried out within one month of liver biopsy. These tests were reported in the medical records of hospitalisations at the time of liver biopsy.

2.3. Liver biopsy

All patients had undergone liver biopsy performed with an ultrasound-guided technique in the right hepatic lobe with a 16G needle (BIOMOL; Hospital Service, Aprilia, Italy) under local anaesthesia. The liver samples were formalin-fixed and paraffin-embedded for histological analysis. Sections of liver tissue were stained with haematoxylin/eosin and Sirius red. Only samples with a >25 mm length and including at least 11 complete portal tracts were considered adequate for the study.

Each block was processed and analysed for collagen content. 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 quantitative measure of fibrotic tissue (morphometry) was calculated as the percentage of collagen content by digital image analysis (ImageJ [22]). This software enables, through a greyscale 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. The percentage of total and collagen area was calculated. After whole-section digital image capture, the measurement included editing to eliminate structural collagen in large portal tracts, blood vessel walls, artefacts, vascular cavities, and lymphoid aggregates. The results of the digital analysis were compared with the standard Ludwig semi-quantitative score used in the original clinical evaluation. Because of possible differences in the actual cut-off, i.e., progressive reduction of the block thickness, the paraffin blocks of all samples was re-staged according to the Ludwig score. Histological staging diagnosis was established according to Ludwig's classification [4]: in stage 1, damage to interlobular bile ducts is seen in the form of the florid duct lesion; in stage 2, the damage involves the periportal areas and ductular reaction, probably representing a compensatory reaction to bile duct loss; in stage 3, the damage is characterised as a pre-cirrhotic stage, with bridging fibrosis; and in stage 4, the main feature is cirrhosis.

2.4. Statistical analysis

All results were expressed as the mean ± standard deviation. Patients were divided into four groups according to Ludwig's classification. A numerical comparison of continuous morphometry data and APRI, FORNS, FIB-4, and LOK scores was performed using the Wilcoxon signed-rank sum test, a nonparametric test. This test is often used as an alternative to the *t-test* when the population cannot be assumed to be normally distributed. When the sample size is small and the distribution of the outcome is not known and cannot be assumed to be approximately normally distributed, nonparametric tests are recommended. In Ludwig's stages I and II, fibrosis is not mentioned. This analysis permits detection of fibrosis, however mild, in Ludwig's stages I and II through the use of morphometry and indirect serum markers, and could give a more accurate staging of fibrosis. Linear regression analysis between two variables was performed using Pearson correlation. Statistical significance was set at a value of $p < 0.05$.

3. Results

The main clinical parameters of the study population are shown in Table 1.

Overall 62 PBC patients were initially considered eligible for the study; 4 were excluded due to overlap syndrome between PBC and AIH, 2 were excluded for HCC, 2 were HCV-positive, 1 was HBV-positive, and 3 patients had metabolic syndrome. Finally 50 patients who fulfilled the histopathological requirements were enrolled and were considered for the statistical analysis; 8 (16%) were AMA-negative (AMA ≤ 1:40). In this case, the diagnosis of PBC was confirmed by biopsy. The liver biopsies included in the study were collected between 1994 and 2008 (8 in 1994, 5 in 1995, 5 in 1996, 5 in 1997, 3 in 1998, 2 in 1999, 1 in 2000, 4 in 2001, 2 in 2002, 2 in 2003, 7 in 2004, 3 in 2007, and 3 in 2008).