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Diagnostic and predictive factors of significant liver fibrosis and minimal lesions in patients with persistent unexplained elevated transaminases. A prospective multicenter study☆

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***Background/Aims*: In patients with unexplained elevated transaminases, prognosis of the liver disease and factors associated with increased risk of liver fibrosis and normal/subnormal liver are unknown. The aim of this prospective study was to identify diagnosis and clinical and biological factors associated with significant (bridging) fibrosis and minimal lesions of the liver in patients with persistent unexplained elevated ALT levels.**

***Methods*: From July 2002 through October 2004, all consecutive asymptomatic patients with unexplained chronically elevated ALT levels were included. All patients had clinical, biological, ultrasonographic examination and a liver biopsy.**

***Results*: 272 patients (60.3% males, mean age 46.4 years, BMI 26.7) were included. Pathological findings were: minimal lesions (18.7%), steatosis (26.8%), NASH (32.7%), and miscellaneous (21.7%). Significant fibrosis was found in 27.4% of cases, including 9 cases of cirrhosis. By multivariate analysis, independent predictors of significant fibrosis were tobacco use (OR 2.5, 95% CI 1.34–4.74 $p = 0.04$), BMI > 25 (2.49, 1.31–4.73 $p = 0.005$) and diabetes (4.41, 1.73–11.29 $p = 0.002$). Independent factors associated with minimal lesions were female gender (OR 3.4 95% CI 1.73–6.75 $p < 0.0001$) and BMI < 25 (3.55, 1.8–6.98, $p < 0.0001$).**

***Conclusions*: In patients with unexplained chronically elevated transaminases, significant fibrosis is statistically associated with tobacco use, BMI > 25 and diabetes, and minimal lesions are significantly associated with female gender and BMI < 25.**

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1. Introduction

Among adults, the most common abnormalities found in the absence of symptoms are an elevated alanine aminotransferase (ALT) and/or γ-glutamyltransferase (GGT) activity. Elevated levels of ALT are found in 2.8% of the overall population [1]. In approximately 10% of the cases, no cause of chronic hypertransaminasemia is found and the prognosis of this liver disease is unknown [2,3].

Only retrospective studies have evaluated pathological findings in patients with unexplained elevated transaminases [4–6]. In these studies, the most prevalent group of patients was represented by non-alcoholic fatty liver disease (NAFLD) including non-alcoholic steatohepatitis (NASH) and steatosis [4,5,7]. The second prevalent group is often drug-related liver disease. Finally, some patients have normal or near-normal liver biopsy [5]. In the setting of chronic elevated transaminases, bridging fibrosis is observed in 20–30% of patients and 1.5–6% of the patients have cirrhosis [5,7]. To our knowledge, no prospective study has evaluated histological lesions in patients with unexplained elevated transaminases. Moreover, the risk of liver fibrosis in such cases, regardless of histological lesions, is unknown, as is the need for a liver biopsy.

The aim of this prospective multicenter study was to evaluate, in patients with chronically elevated ALT, diagnosis and predictive factors associated with significant fibrosis, defined by the presence of at least some septa (bridging fibrosis) and minimal lesions of the liver.

2. Patients and methods

2.1. Inclusion criteria

From July 2002 to October 2004, consecutive patients with unexplained chronically elevated ALT (at least three tests above the upper limit of normal for at least 6 months) were prospectively included in 20 French centers. All patients were HBs Ag, HCV Ab and HIV Ab negative. None of the patients were either alcohol (<40 g per day for males and <20 g per day for females) or drug users, and they had no autoimmune or genetically induced (hemochromatosis, Wilson's disease or α1-antitrypsin deficiency) liver disease. Patients who started new drugs in the preceding 6 months were not included. No patient had abnormal thyroid stimulating hormone level, cardiac insufficiency, or neoplasia. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the Hospital of Bordeaux, France. Patients were enrolled after written informed consent was obtained.

2.2. Clinical characteristics of patients

A detailed history was obtained by the main investigator of each center to determine current medication use, previous and/or concurrent medical conditions, tobacco use and quantity of past alcohol intake. All subjects underwent a physical examination documenting height and weight. Overweight was defined as a body mass index (BMI) between 25.0 and 29.9 kg/m^2; obesity was defined as a BMI $\geqslant$ 30.0 kg/m^2 [8]. Diabetes refers to previously diagnosed diabetes.

2.3. Laboratory tests

After fasting overnight, the following laboratory tests were performed: serum glucose, aspartate aminotransferase (AST), ALT, alkaline phosphatase, GGT, triglycerides, total cholesterol, LDL and HDL cholesterol, ferritin, percent transferrin saturation and platelet count. Other biochemical tests were CPK and aldolase activities, serum protein electrophoresis, glycated hemoglobin, insulin and carbohydrate-deficient transferrin. Virological tests included HBV DNA (real-time PCR) and HCV RNA (Cobas Amplicor HCV 2.0, Roche Diagnostics). Immunological tests included anti-SLA and anti-neutrophil cytoplasmic antibodies. Since celiac disease and subclinical Addison's disease could be associated with chronic elevation of ALT level [9,10], anti-endomysium antibodies were tested and Synacthen test was performed.

The insulin resistance index was calculated according to homeostasis model assessment index (HOMA) as insulin resistance = insulin in μIU/ml × glucose in mmol/l/22.5. The metabolic syndrome was defined as the presence of at least three factors: fasting glucose $\geqslant$1.1 g/l, BMI > 30 kg/m^2, blood pressure $\geqslant$130/85 mmHg, triglycerides $\geqslant$1.7 mmol/l, HDL cholesterol $\leqslant$0.9 mmol/l, treatment for diabetes or hypertension.

2.4. Histological analysis

All patients underwent a percutaneous liver biopsy. Specimens were fixed in 10% buffered formalin and embedded in paraffin. Sections, 3-μm thick, were stained with H& E-saffron, Masson's trichrome (or picrosirius red) and Perls. All biopsies were examined by the same pathologist (BLB), who was unaware of the patient's clinical or biological evolutions. The form used for histological analysis included 18 individual histological parameters (including the degree of fibrosis) followed by 4 possible conclusions: minimal lesions (normal or near normal liver histology), bland steatosis (i.e. steatosis alone without significant inflammation or hepatocyte ballooning), NASH and miscellaneous. Patients with biopsies considered too small or too fragmented to perform the histological diagnosis and to provide a reliable conclusion were excluded.

A definite diagnosis of NASH was considered using E Brunt criteria [11] corresponding to a NAS score of 5 or more [12]. The activity of the disease was classified from grade 1 to 3 according to the Brunt system [11].

Fibrosis was evaluated using METAVIR score, from 0 normal to 4 (cirrhosis), as previously described [13]. Patients were thereafter separated into 2 groups: patients with no or mild fibrosis (F0 or F1 according to their METAVIR scores) and those with significant fibrosis (F2, F3, F4 according to their METAVIR score). In NASH cases, significant fibrosis was defined as bridging fibrosis (stade 3 in Brunt system [11]) and subdivided in F2 or F3 METAVIR equivalent, basing on the number of septa. For steatosis, a semi-quantitative evaluation was performed (% of hepatocytes). Steatosis <5% was considered as normal/subnormal.

Minimal lesions were defined by the presence of isolated or combined minor abnormalities (steatosis <5%, F1 fibrosis, mild iron overload) and in the absence of vascular and biliary lesions.

2.5. Statistical analysis

Results are expressed as means ± SEM. Comparisons of quantitative data were made using the Student's *t*-test or the non-parametric Mann–Whitney rank-sum test when data did not exhibit a normal distribution. Qualitative data were analyzed using the χ^2 test. The odds