Non-alcoholic steatohepatitis
A multifactorial, frequent, paucisymptomatic liver disease with a fibrotic outcome

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RÉSUMÉ
Objectif — Etudier les caractéristiques morphologiques, cliniques et biologiques de la stéatohépatite non alcoolique, comprendre sa pathogénie pour prévenir la cirrhose.


Résultats — Vingt et une femmes et 10 hommes étaient inclus (âge moyen = 54 ans). Dix-neuf (61,3 %) étaient asymptomatiques. Le taux sérique de l’activité de l’alanine aminotransférase, principal signe révélateur, était corrélé au degré de stéatose (P = 0,008). Hypertension, surcharge pondérale, troubles glucidiques, dyslipidémies étaient observés chez respectivement 10 (32,2 %), 24 (77,4 %), 16 (51,6 %) et 18 (58,1 %) malades. Parmi les treize malades (41,9 %) ayant un taux élevé d’auto-anticorps sériques, aucun n’avait de cirrhose ; 18 (58,1 %) présentaient une surcharge ferrique. Une mutation du gène de l’hémochromatose (HFE) était détectée chez 14 malades (56 %) sur 25. Cinq (16,1 %) cirrhoses étaient observées. La concentration hépatique en fer n’était corrélée ni à l’extension de la fibrose, ni à la mutation.

Conclusion — La stéatohépatite non alcoolique est révélée par une élévation de l’activité de l’alanine aminotransférase. Les auto-anticorps sériques, la surcharge ferrique et des mutations du gène HFE sont détectés avec une forte prévalence. Le diagnostic de cette pathologie est nécessaire en raison de l’association possible avec une cirrhose.

SUMMARY
Background — The aim of this study was to evaluate the morphological, clinical and biochemical characteristics of non alcoholic steatohepatitis to understand its pathogenesis.

Patients and methods — From January 1993 to June 2000, 44 patients were selected on histological criteria. Alcohol intake, blood pressure, weight, glycaemia, lipid, immune, iron profiles hemochro-matosis (HFE) gene mutations were analyzed. Patients were re-examined thereafter or in June 2000.

Results — Twenty one women and 10 men were included (mean age = 54). Nineteen patients were asymptomatic (61.3%). Patients often presented with an increase in alanine aminotransferase. This was correlated with steatosis (P = 0.008). Hypertension, excess weight, abnormal serum glucose levels and dyslipidaemia were respectively observed in 10 (32.2 %), 24 (77.4 %), 16 (51.6 %) and 18 (58.1 %) patients. Thirteen of these patients (41.9 %) presented abnormal autoantibodies titers without autoimmune hepatitis; 18 (58.1 %) presented an iron overload. A mutation of the HFE gene was detected in 14 of 25 patients (51.6 %). Liver iron concentrations were not correlated to the extent of fibrosis extension or with mutations.

Conclusion — Increased alanine aminotransferase levels usually revealed non alcoholic steatohepatitis. A high prevalence of autoantibodies, iron overload and mutation of the HFE gene were detected. Non alcoholic steatohepatitis should be diagnosed because it can be associated with cirrhosis.

Non-alcoholic steatohepatitis (NASH), an entity described in 1980 by Ludwig et al. [1], is an acquired liver disease generally related to a metabolic cause. The histological definition of NASH has evolved over time. Currently, minimal histological criteria required for diagnosis are the presence of steatosis and intralobular necrotic inflammatory reactions [2]. Mallory bodies are not now considered necessary for diagnosis [3]. There is not however general agreement on one single histological description [4]. Liver damage is exactly the same as observed after chronic alcohol abuse [5]. The diagnosis of NASH thus requires careful examination of both the clinical signs and anatomic findings.

The first description of NASH was made in obese diabetic women with high blood pressure and dyslipidaemia treated with various drugs [1, 5-10]. NASH can however occur in several other clinical situations [11-13]. The diagnosis of NASH, rarely proposed by clinicians, is generally made when the liver biopsy reveals unexplained anomalies. Once alcoholic abuse is out ruled, the diagnosis of NASH raises two questions: what is the cause, and what is the prognosis? Most patients have a rather benign disease that does not progress [6-8]. Others may develop fibrosis or even cirrhosis [1, 2, 5, 8, 10, 12, 14-16]. The purpose of this work was to explore the morphological, clinical, and biochemical signs of steatohepatitis and better define the causes in order to establish optimal treatment and avoid progression to cirrhosis.
Patients and methods

Patients

Forty-four patients attending the outpatient clinic of the Gastroenterology and Hepatology Unit of the Saint-Etienne University Hospital from January 1993 to June 2000 were selected on the basis of the histological criteria of steatohepatitis defined by Lee [1]. Minimal inclusion criteria were steatosis and associated intralobular necrotic inflammatory reactions, with or without Mallory bodies. Patients with diseases with a known liver tropism and progression to cirrhosis were excluded. The total number of patients with the histological diagnosis of steatosis in the pathology department’s records for this same period was also noted.

Methods

CLINICAL STUDY

Clinical data recorded at the time of the liver biopsy were: gender, age, height, weight, medical history, drug use, and presence of hepatomegaly, splenomegaly, ascites, esophageal varicose veins, or associated extra-hepatic disease.

Liver biopsy was performed on informed patients and/or family members or friends by several consulting physicians. Patients were excluded if their daily alcohol intake was > 30 g in men and > 20 g in women. Body mass index (BMI), defined as weight/height², was calculated. Patients were considered overweight if their BMI was over 18.5-23.5 in men and 19.5-24.5 in women. Obesity was defined as BMI > 29.5 for men and women.

The following laboratory tests were obtained for all patients: serum ALAT, ASAT, alkaline phosphatase, gamma-GT, bilirubin, iron, transferrin, total iron binding capacity, transferrin saturation, ferritin, cholesteryl, triglycerides, glucose, electrolytes, blood cell count, platelets, differential cell count, coagulation factors, protein electrophoresis, hepatitis B and hepatitis C virus serology, ceruloplasmin, α1-antitrypsin, autoimmunity antibodies (anti-liver kidney microsome I (LKM), antimitochondria, anti-nuclear, anti-smooth muscle).

GENETIC STUDY

Polymerase chain reaction (PCR) was performed on DNA extracted from whole blood to search for the hemochromatosis gene (HFE) mutations C282Y and H63D.

HISTOLOGY

Liver biopsy specimens were available for 43 patients; a surgical biopsy was obtained in one. Specimens were fixed in 10% formol, embedded in paraffin, and stained with hematoxylin-eosin, Perls stain, and sirius red. At least twelve different sections were studied. The following data were recorded: type of steatosis (micronuclear or macrovacuolar), semi-quantitative assessment of the extent of steatosis (10 - 20% of the hepatocytes involved = 1+; 20 - 50% = 2+; > 50% = 3+), intensity and type of hepaticocyte necrosis, presence of Mallory bodies, megamitochondria, glycogen clusters, PAS-positive hyalin bodies, intensity of lobular inflammatory reaction, type of inflammatory infiltrate, and extent of fibrosis. The grade and stage of NASH was established according to the criteria described by Lee [2]. Grade was defined by the intensity of the necrotic inflammatory reaction: A0 = no steatohepatitis no hepatocellular abnormalities; A1 = minimum steatohepatitis associated with rare hepaticocyte lesions and a discrete intralobular inflammatory infiltrate; A2 = mild steatohepatitis associated with focal hepaticocyte lesions and a mild intralobular inflammatory infiltrate; A3 = moderate steatohepatitis associated with hepatocellular lesions and moderate intralobular inflammatory infiltrate; A4 = severe steatohepatitis with numerous hepaticocyte lesions associated with marked intralobular inflammatory infiltrate. The stage of fibrosis was assessed as: F0 = no periporal or centrallobular fibrosis; F1 = periporal fibrous septa with bridging and/or mild peri-sinusoidal fibrosis; F2 = periporal fibrosis with bridging and peri-sinusoidal fibrosis; F3 = numerous interportal or porta-centrallobular fibrous bridges with parenchymatous architectural anomalies without cirrhosis; F4 cirrhosis.

The presence and extent of iron overload was assessed with the histology iron score [17]. Iron concentration in liver tissue was measured with the same technique for all biopsy specimens with a threshold set at 36 µmol/g dry weight. Negative and positive control specimens were also scored.

FOLLOW-UP

The clinical features and laboratory tests listed above were checked at each follow-up visit. Follow-up liver biopsies were not obtained.

STATISTICAL ANALYSIS

Statistical analysis was performed with Spearman correlation, ANOVA, and Student t test as appropriate, taking P < 0.05 as the threshold of significance.

Results

Forty-four patients with steatohepatitis were selected on the basis of the histological criteria. There were 25 women and 19 men, mean age 51.5 years (range 25-77). Thirteen patients were excluded: excessive alcohol intake (> 30 g/day for men, > 20 g/day for women) in 8 patients and liver or systemic disease with known risk of fibrosis in 5 (chronic hepatitis C, rheumatoid arthritis, systemic sclerosis). Thirty-one patients were included in the final analysis: 21 women and 10 men, mean age 54 years (range 25-77 years). Twenty-seven of the 31 patients were reviewed in June 2000. Three patients had died and one was lost to follow-up. During this same period, the pathology department records showed 313 patients with the diagnosis of steatosis and an identified alcoholic or viral liver disease.

Clinical findings and laboratory results

Nineteen patients (61.3%) had clinical signs. Among the 12 others (38.7%), six (19.3%) complained of fatigue and six (19.3%) of abdominal pain or heart-burn. The inaugural sign in one patient was digestive track bleeding due to rupture of esophageal varices. Hepatomegaly was noted in six patients (19.3%).

Liver tests were abnormal in 28 patients (90.3%): serum ALAT (mean = 90 IU/l, range 36-249 IU/l) and serum ASAT (mean = 143 IU/l, range 60-709 IU/l) were elevated in 23 patients (74.2 %). The ASAT/ALAT ratio was less than 1 in 92.3% of the patients. Elevated alkaline phosphatase was noted in ten patients (32.3%) and elevated gamma-GT in ten (32.3%). Hepatocyte and Kupffer cell iron overload was moderate, detected in 18 of the histology specimens (58.1%). Mean iron score was 3.4 (range 1-15). Iron concentration in liver tissue was 36 µmol/g dry weight in 9 specimens. There was a statistically significant correlation between the histological iron score and intrahepatic iron level (P = 0.01). Intrahepatic iron was elevated alone in three patients. There was no correlation between intrahepatic iron concentration or histological iron score and serumaminotransferase levels (P > 0.05). Likewise, 12 of the 19 patients with iron overload had an elevated serum ferritin (mean = 898.35 µg/l, range 205.1-2826 µg/l). Transferrin saturation was elevated in four patients (mean 63.2%, range: 49-85%).

Metabolism results

Ten (32.2%) patients were taking treatments for high blood pressure (mean systolic pressure 131 mmHg, range 120-150 mmHg). Three (9.7%) were taking long-term corticosteroid therapy and four others (12.89%) were on hormone treatments (oral contraception and hormone replacement therapy for meno-
pause). One woman (3.2%) was taking both corticosteroids and hormone therapy. Twenty-four patients (77.4%) were overweight (mean BMI = 30.1 kg/m², range 24.6-38.1 kg/m²). Among these patients, eleven (35.5%) were considered obese (BMI > 29.5 kg/m²). Glucose control was perturbed in 16 patients (51.6%) mean serum glucose 1.39 g/l, range 0.8-3.83 g/l; ten had non-insulin-dependent diabetes mellitus, two had insulin-dependent diabetes mellitus, and 4 exhibited glucose intolerance. Eighteen patients (58.1%) had dyslipidemia (mean serum cholesterol 8.9 mmol/l, range 5.25-22.2 mmol/l; mean serum triglycerides 2.3 mmol/l, range 0.8-16 mmol/l): hypercholesterolemia was noted in four patient (12.9%), hypertriglycereidemia in five (16.1%), and mixed dyslipidemia in 9 (29%).

Sera from 13 patients (41.9%) were positive for at least one autoantibody, with a significant titer in ten patients (32.2%). Antinuclear antibodies were found alone in ten patients (32.2%) (mean titer 1/248, range: 1/10-1/1280). Anti-smooth muscle antibodies were associated with other autoantibodies (anti-nuclear, anti-Sjögren A and B, antinative deoxyribonucleic acid) in one patient (3.2%) and with anti-gastric parietal cell antibodies in one other (3.2%). These three latter types of antibodies were detected fortuitously in a patient with no apparent autoimmune disease.

Genetic results

Search for C282Y and H63D mutations was performed in 25 patients. Six patients declined genetic screening. Fourteen patients (56%) had an HFE gene mutation: eleven heterozygous H63D mutation, one heterozygous C282Y mutation, and one heterozygous composite C282Y/H63D mutation. No mutation was noted in eleven patients (35.5%). None of the patients were homozygous for the C282Y mutation. HFE gene mutation was associated with histological iron overload in 57% of the cases. Hepatic iron overload was not correlated with HFE gene mutation: mean iron concentration in liver tissue in patients with the HFE mutation was 27.9 (range 3-84) compared with 26.6 (range 7-57) in those without the HFE mutation.

Histology results

Severe steatosis (3+) was noted in 17 patients (54.8%), moderate steatosis (2+) in six (54.8%) and mild steatosis (1+) in eight (25.8%). ALAT level and the extent of steatosis were significantly correlated between grades 1+ and 2+ (P = 0.04), between grades 1+ and 3+ (P = 0.01), but not between grades 2+ and 3+. Hepatocyte necrosis was noted minimal in four (12.9%), mild in 27 (77.4%), and moderate in three (12.9%). The inflammatory infiltration was generally intralobular and composed of polymorphonuclear cells, lymphocytes and macrophages in variable proportions. Mallory bodies were scarce, observed in eight patients (26.8%). Histology grading was A1 in four patients (12.9%), A2 in 24 (77.4%) and A3 in three (9.7%). Two patients had no sign of fibrosis (F0), 24 had mild fibrosis (F1) and five had cirrhosis (F4). All patients with cirrhosis (F4) were over 50 years of age; the percentages of patients with F4 for BMI > 28.8, diabetes, hyperglycieridemia, and ALAT > 2N were 80%, 80%, 40% and 20% respectively.

Among the patients with no fibrosis or mild fibrosis (FO-F1), 65% were over 50 years of age; the percentages for BMI > 28.8 diabetes or glucose intolerance, hyperglycieridemia, and ALAT > 2N were 42%, 28.5%, 50% and 73%, respectively.

Discussion

Our findings demonstrate that non-alcoholic steatohepatitis (NASH) is a paucisymptomatic disease often disclosed by elevated ALAT levels. Other frequently observed metabolic disorders include iron overload, HFE gene mutation, and elevated autoantibody titers. NASH is a potentially severe disease due to the risk of cirrhosis.

Most of the published series report that the majority of the patients are asymptomatic [5, 6, 12, 18]. Most patients consult with moderately elevated aminotransferase levels. ALAT level exhibits a statistical relationship with the extent of steatosis. One recent study found steatosis as the most frequent finding in asymptomatic patients with liver disease caused by various conditions and disclosed by moderately elevated aminotransferase levels, particularly ALAT [19]. Consequently, the elevated level leading to most of the NASH diagnoses would probably be caused by steatosis. Steatosis is a common finding in alcoholic liver disease as it is in NASH. The ASAT/ALAT ratio is however greater than 1 in alcoholic liver disease and less than 1 in non-alcoholic steatohepatitis. The elevated ALAT levels observed in NASH, which appear to be a consequence of steatosis, might be caused by a different mechanism than the cell damage observed in alcoholic disease which leads to elevated ALT levels; hence the mechanism might involve steatosis necrosis. The ASAT/ALAT ratio is a useful marker for the differential diagnosis which is often difficult to establish between non-alcoholic and alcoholic steatohepatitis. It would also be interesting to study the course of NASH because other authors [20] report that inversion of the ASAT/ALAT ratio (> 1) is predictive of progression towards cirrhosis. In our series, two of five patients with cirrhosis had an ASAT/ALAT ratio > 1.

Our patients were predominantly overweight females with metabolic disorders (poorly controlled glucose, dyslipidemia), and often hypertension. Most authors have also found this association between metabolic disorders and NASH and a predominance of patients aged over 50 years at diagnosis [1, 5-7, 18]. Eight of our patients with NASH were taking corticosteroids and/or oral contraception. Such patients have been excluded from certain series [18, 21], distinguishing artificially primary NASH with an unknown cause and secondary NASH with a recognized cause, specifically drugs. For us, this distinction is not logical because the histological lesions are identical, probably resulting from very similar mechanisms triggered by different causes.

We found iron overload in nearly two-thirds of our patients. This overload was moderate, detected by histology examination or iron assay in liver tissue. The cause of iron overload in NASH could be related to the HFE gene mutations often observed. In our region (Rhône-Alpes) HFE gene mutation is more frequent than in the general population (56% versus 19%) (personal data). We did not however find a significant correlation between HFE gene mutation and iron overload. The H63D mutation predominated, a finding known to be insufficient to explain iron overload [22, 23]. On the contrary, George et al. [24] reported a high frequency of C282Y mutation associated with iron overload in their series of NASH patients. The ethnic background of our patients [25] and their small number might explain this discrepancy. Two of our five patients with cirrhosis had iron overload. Cirrhosis, with portal hypertension, sometimes observed in patients with NASH might explain part of the iron overload [26] since it is known that this condition causes hepatocyte and Kupffer cell iron overload [26]. Inversely, the iron overload observed in NASH patients does not appear to favor progression to fibrosis. Unlike George et al. [24], we did not find any correlation between the extent of fibrosis and intensity of iron overload.
Similar findings were also reported in a recent American study [27]. Among our patients, all those aged 60 years and over had iron overload, men and women alike. This would suggest that iron overload is related to the duration of NASH. Cell damage would not appear to solely explain iron overload since there was no statistical correlation between intrahepatic iron concentration and ALT level. Comparing these two parameters may not however be sufficient to rule out prolonged hepatocyte necrosis as the cause of iron overload. Several clinical features and morphological findings are similar in NASH and dysmetabolic hepatosiderosis [28]. These two liver diseases are both associated with diverse metabolic disorders, iron overload and HFE gene mutations. NASH and dysmetabolic hepatosiderosis might be different expressions of metabolic liver diseases.

One particularly intriguing finding was that several patients with NASH has a high level of autoantibodies without histological proof of autoimmune liver disease. The antibody titers observed in our patients were higher than those found in the general population of the same age. One third of our patients had antinuclear antibodies. Although Ludwig et al. [13] did mention autoantibodies in their review of the literature, no work has been specifically devoted to this aspect of NASH. The autoimmune mechanism involved in the pathogenesis of NASH remains obscure. One of the questions raised is to determine whether the elevated autoantibody titers affect the course of NASH. Our study provides little data on this point, but it was noted that the five patients with NASH that had progressed to cirrhosis were negative for autoantibodies. Clinicians should be aware that the diagnosis may be NASH in patients with an elevated serum ALAT and different metabolic diseases who are found to be positive for autoantibodies, making it unnecessary to proceed with a complete autoimmune work-up.

Much like other teams, we found that NASH is a severe disease with a possible cirrhotic outcome [1, 5-7, 15, 16]. The proportion of our patients with cirrhosis is similar to that reported by others [6]. We identified two distinct populations: patients with minimal fibrosis (F0-F1) and patients with cirrhosis (F4). This distinction draws attention to the progressive nature of NASH. Our study had two biases. The first one was due to recruitment of patients with two clinical situations: unexplained elevation of liver enzymes and obesity. Another cause of liver injury with alcoholic hyaline? Dig. Dis. Sci 1982;27:265-8.

In conclusion, non-alcoholic steatohepatitis is a multifactorial metabolic liver disease. Iron overload, HFE mutation of the HFE gene, and immune system anomalies are frequent in patients with non-alcoholic steatohepatitis but do not have a direct effect on disease outcome. Careful search for the metabolic causes of non-alcoholic steatohepatitis is crucial because correction, followed by improved liver tests, might prevent progression to cirrhosis.

**REFERENCES**


