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Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease

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BACKGROUND & AIMS: Histologic analysis of liver biopsy specimens allows for grading and staging of nonalcoholic fatty liver disease (NAFLD). We performed a longitudinal study to investigate the long-term prognostic relevance of histologic features for patients with NAFLD. **METHODS:** We performed a retrospective analysis of 619 patients diagnosed with NAFLD from 1975 through 2005 at medical centers in the United States, Europe, and Thailand. Patients underwent laboratory and biopsy analyses, and were examined every 3–12 months after their diagnosis. Outcomes analyzed were overall mortality, liver transplantation, and liver-related events. Cumulative outcomes were compared by log-rank analysis. Cox proportional-hazards regression was used to estimate adjusted hazard ratios (HRs). Time at risk was determined from the date of liver biopsy to the date of outcome or last follow-up examination. **RESULTS:** Over a median follow-up period of 12.6 years (range, 0.3–35.1 y), 193 of the patients (33.2%) died or underwent liver transplantation. Features of liver biopsies significantly associated with death or liver transplantation included fibrosis stage 1 (HR, 1.88; 95% confidence interval [CI], 1.28–2.77), stage 2 (HR, 2.89; 95% CI, 1.93–4.33), stage 3 (HR, 3.76; 95% CI, 2.40–5.89), and stage 4 (HR, 10.9; 95% CI, 6.06–19.62) compared with stage 0, as well as age (HR, 1.07; 95% CI, 1.05–1.08), diabetes (HR, 1.61; 95% CI, 1.13–2.30), current smoking (HR, 2.62; 95% CI, 1.67–4.10), and statin use (HR, 0.32; 95% CI, 0.14–0.70). Twenty-six patients (4.2%) developed liver-related events; fibrosis stage 3 (HR, 14.2; 95% CI, 3.38–59.68) and stage 4 (HR, 51.5; 95% CI, 9.87–269.2) compared with stage 0, were associated significantly with the events. Patients with fibrosis, regardless of steatohepatitis or NAFLD activity score, had shorter survival times than patients without fibrosis. **CONCLUSIONS:** In a longitudinal study of patients with NAFLD, fibrosis stage, but no other histologic features of steatohepatitis, were associated independently with long-term overall mortality, liver transplantation, and liver-related events.

Keywords: NASH; Prognosis; PRELHIN Study; Prediction.

Nonalcoholic fatty liver disease (NAFLD) represents the most common chronic liver condition around the world. The disease encompasses a wide range of liver pathology with some patients presenting with steatosis and no additional features of liver injury whereas other patients present with nonalcoholic steatohepatitis (NASH) with or without fibrosis or cirrhosis.[1] This range of liver pathology does not necessarily imply that individuals with steatosis are at risk for NASH or advanced fibrosis or that patients with NASH inevitably will progress to cirrhosis. Nevertheless, some patients with NAFLD may develop cirrhosis and die from complications of portal hypertension, liver failure, and hepatocellular cancer (HCC)[2–5] if liver transplantation is not performed. Currently, NAFLD represents a common cause of liver transplantation, and even the second most common cause of liver transplantation in large medical centers.[6] Despite this clinical reality, the long-term prognosis of patients with NAFLD remains incompletely elucidated. When compared with the general population of the same age and sex, patients with NAFLD have a significantly higher mortality rate.[7] However, the long-term prognosis is not the same across the spectrum of the disease. Patients with steatosis and minimal or no additional features of liver injury may follow a relatively benign clinical course with overall mortality similar to the general population of similar age and sex,[8] whereas in patients with NASH the long-term mortality seems greater than in the general population of the same age and sex.[9] Unfortunately, studies on NASH confirmed by liver biopsy reported to date[9,10] have included small numbers of patients, and used a definition of NASH

Abbreviations used in this paper: **BMI, body mass index; CI, confidence interval; CRN, Clinical Research Network; HCC, hepatocellular cancer; HR, hazard ratio; IQR, interquartile range; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis.**

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that is different from currently accepted criteria,[11,12] precluding meaningful conclusions.[13,14]

Liver biopsy in patients with suspected NAFLD allows confirmation of the diagnosis, and, more importantly, grading and staging the disease. The NAFLD activity score (NAS) proposed by the NASH Clinical Research Network (CRN) is an accepted scoring system to grade 3 histologic features, namely, steatosis, hepatocellular ballooning, and lobular inflammation.[11] The NAS is composed of features of active injury that potentially are reversible in the short term, and thus are recommended for use in clinical trials. Fibrosis is not included as a component of the NAS. Although the NAS correlates with the presence or absence of NASH, the diagnosis of NASH is based not on the raw NAS but on the interpretation by the histopathologist of the presence and pattern of specific histologic abnormalities on liver biopsy.[12] Although fibrosis may be present in the absence of NASH, it is a worrisome feature on liver biopsy in NAFLD because it indicates a more advanced liver disease. In addition, portal inflammation, which is not a component of the NAS, has been associated with more severe histologic injury and fibrosis in cross-sectional studies, suggesting that portal inflammation may be indicative of a worse prognosis.[15,16] However, it remains uncertain what long-term prognostic information can be obtained from grading the different histologic features and from staging the disease in an individual patient with NAFLD. Thus, the aim of this study was to determine the long-term Prognostic RElevance of Liver Histology In NAFLD (the PRELHIN study), namely, steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning, NAS, NASH, and fibrosis stage in a large number of patients with NAFLD.

Patients and Methods

This was a longitudinal, international, multicenter cohort study. Patients were identified retrospectively by reviewing the pathology database at each participating center of subjects with the pathology diagnosis of steatosis, steatohepatitis, or fatty liver. After an extensive review of the patients' medical records including all notes from clinic visits, laboratory and imaging data, and liver biopsy reports, only patients with the diagnosis of NAFLD were included in the analysis, as described later. They were untreated, consecutive patients undergoing a biopsy who met the eligibility criteria, and were recruited from 1975 to 2005 from medical centers located in 6 different countries: United States, Denmark, Australia, Iceland, Thailand, and Scotland. The time period of 1975–2005 was chosen to include a 30-year ascertainment period, and an appropriate duration of follow-up evaluation for the last patient recruited. Follow-up evaluation was extended until the end of 2012. In more than 90% of cases included the liver biopsy was performed because of persistent increases of liver enzyme levels in patients with confirmed fatty infiltration of the liver detected on imaging studies. The liver biopsy was performed to confirm the diagnosis of NAFLD after appropriate exclusion of liver disease of other etiologies, such as alcohol-induced or drug-induced liver disease, autoimmune or viral hepatitis, and cholestatic or metabolic/genetic liver disease. These other liver diseases were excluded using specific clinical, laboratory, radiographic, and/or histologic criteria. All patients had a negative history of alcohol abuse as indicated by a weekly ethanol consumption of less than 140 g in women, and less than 210 g in men. A history of alcohol consumption was investigated specifically by interviewing the patients and in many cases also by interviewing close relatives during both the first and follow-up visits. Subjects in whom an alcohol history was not provided were excluded. Serology for viral hepatitis B and C was performed before liver biopsy or during follow-up visits in all patients and all tested negative. The study was approved by the appropriate regulatory bodies at each participating center and all patients provided consent for participation in medical research.

A total of 859 patients were identified initially (Mayo Clinic, n = 385; Denmark, n = 169; Australia, n = 119; Iceland, n = 107; Thailand, n = 46; and Scotland, n = 33). Extensive clinical and laboratory data were collected at the time the liver biopsy was performed. A complete medical history and physical examination was performed in all patients. The ethnicity (Hispanic or Latino, and not Hispanic or Latino) and race (white, Asian, Black or African American, American Indian/Alaska native, Native Hawaiian, or other Pacific Islander) of the patients were determined based on the categories proposed by the US Department of Health and Human Services Public Health Service.[17] Body mass index (BMI) was calculated using the following formula: weight (in kilograms)/height (in meters^2). Laboratory evaluation included routine liver biochemistry (alanine aminotransferase and aspartate aminotransferase, total bilirubin, albumin, alkaline phosphatase, and γ-glutamyl transpeptidase levels), complete blood count, fasting lipids, fasting glucose, serum ferritin, transferrin saturation, viral serology for hepatitis B and C infection, autoantibodies, α1 antitrypsin levels and phenotype, and ceruloplasmin levels. Data on medication use were collected from review of medical records.

Liver Histology

The liver biopsy slides of these 859 patients were shipped for central pathology reading and scoring to Dr David Kleiner at the National Cancer Institute, who was unaware of the patients' clinical and laboratory data. Particular attention was paid to the quality of the liver tissue and staining. In cases of slides with faded staining, new cuts of stored paraffin-embedded liver tissue and stains were obtained; when no stored tissue was available, the coverslip of the original slide was removed and the tissue was re-stained. Thus, all liver biopsy slides were of appropriate quality for confident grading and staging of the liver biopsy features. The grade and stage of several histologic features was based on the scoring system proposed by the NASH CRN as described in Supplementary Table 1.[11] A threshold of 5% of hepatocytes showing steatosis was required for the diagnosis of NAFLD.[11,12] To control for biopsy size, the length of the biopsy specimen was measured with a hand ruler, and the number of portal areas on one cross-section was counted. Only those liver biopsy specimens that the histopathologist deemed of appropriate size and had a sufficient number of portal tracts allowing confident grading and staging were included. Biopsy specimens showing evidence of a second histologic process also were excluded. The grade of several histologic features was scored on H&E-stained tissue. The stage of fibrosis was scored on Masson's Trichrome stained tissue. The NAS was recorded as the unweighted sum of the scores for