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MANAGEMENT

Biopsy and Noninvasive Methods to Assess Progression of Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) comprises a spectrum of histopathologic features, ranging from isolated hepatic steatosis, to steatohepatitis with evidence of hepatocellular injury and fibrosis, to cirrhosis. The diagnosis and determination of NAFLD prognosis requires clinical and histopathologic assessments. Liver biopsy still is regarded as the reference for differentiating steatosis (NAFL) from nonalcoholic steatohepatitis, for staging hepatic fibrosis, and for identifying NAFLD in patients with other chronic liver disease. Standardized grading and staging histologic scoring systems, such as the NAFLD activity score and the steatosis, activity, and fibrosis score, can help guide clinical decisions and assess outcomes of clinical trials. Improved understanding of the pathophysiology of NAFLD and technologic advances have led to algorithms that can be used to assess serum biomarkers and imaging methods that are noninvasive alternatives to biopsy collection and analysis. We review the advantages and limitations of biopsy analysis and noninvasive tests as diagnostic and prognostic tools for patients with NAFLD. We also discuss techniques to improve dynamic histopathology assessment, and emerging blood and imaging biomarkers of fibrogenesis.

Keywords: Biomarkers; Hepatic Fibrosis; Histology; Imaging; Antifibrotics; Steatosis; Steatohepatitis.

Liver biopsy still is regarded as the best method for differentiating nonalcoholic fatty liver (NAFL) from nonalcoholic steatohepatitis (NASH), for staging hepatic fibrosis, and for identifying nonalcoholic fatty liver disease (NAFLD) in patients with other chronic liver disease.[1] Clinical and histologic risk assessment are important for predicting progression of NAFLD.[2] Semiquantitative categoric grading systems developed for NAFLD do not quantify the linearity of fibrosis deposition or actual matrix content. These scoring systems were derived for viral hepatitis, developed to standardize and improve observer variability, and can be used to assess the severity of chronic liver injury in clinical trials. Current noninvasive techniques for fibrosis use biochemical (serum), physical (imaging), and physiological (breath) characteristics to estimate disease severity.[3] Some of these methods initially were developed for staging in chronic hepatitis C (CHC), but continue to be refined for diagnosis of patients with NASH.[4] Clinical practice guidelines for NAFLD now include noninvasive tests for defining the presence or absence of advanced fibrosis.[5] Imaging modalities may provide an accurate assessment of steatosis compared with serum biomarkers, but a noninvasive technique to monitor progression to NASH would be of greater clinical significance.

A key challenge to refining noninvasive measures of fibrosis is to overcome the diagnostic limitations of a liver biopsy analysis. Diagnostic serum biomarker panels and imaging tools were developed in relation to a cross-sectional binary assessment of semiquantitative histopathologic scores, and do not account for the dynamic nature of fibrogenesis or variations in clinical or genetic risk factors for NASH. As we consider ways to assess disease progression, or efficacy end points for studies of antifibrotic agents, noninvasive tests cannot reliably differentiate adjacent different disease stages or quantify changes in fibrosis. New histologic methods for in situ assessment of collagen cross-links and phospholipid distribution should improve measurement of NAFLD severity and progression.

Abbreviations used in this paper: **APRI, aspartate aminotransferase to platelet ratio index; ARFI, acoustic radiation force impulse imaging; AUROC, area under the receiver operating characteristic; BARD, BMI, AST:ALT Ratio, Diabetes; BMI, body mass index; CAP, controlled attenuation parameter; CHC, chronic hepatitis C; FIB-4, fibrosis-4; ^{1}H-MRS, ^{1}H-magnetic resonance spectroscopy; K18, keratin 18; LSM, liver stiffness measurement; miRNA, microRNA; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network; SAF, steatosis, activity, and fibrosis; US, ultrasound; VCTE, vibration-controlled transient elastography.**

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Advantages and Limitations of Liver Biopsy Analysis

NAFLD encompasses several entities with different prognoses; these disorders are identified based on histologic analyses of biopsy specimens. Some histologic features have been associated with fibrosis progression and/or clinical outcome, so biopsy analyses can help predict patient outcomes. The main histologic features that indicate disease progression are summarized in Table 1. Biopsy specimens also can be used to monitor disease progression (or absence of progression). Findings from analysis of biopsy specimens collected over time are used as a primary end point in NAFLD clinical trials.

However, there is a low but real risk of morbidity for biopsy collection, and a much lower risk of death. In addition, biopsy analysis is a costly procedure that requires technical expertise; the hepatologist (or radiologist) must obtain an adequate liver sample and the pathologist must obtain the most accurate information from the biopsy sample. Based on the large number of patients with potential NAFLD, liver biopsy analysis cannot be considered as a screening procedure, but should be reserved for select patients.[6] The decision to perform a biopsy for diagnosis should be based on a patient's risk for steatohepatitis or advanced disease, discordant noninvasive NAFLD risk scores, or findings from elastography. Other chronic liver diseases should be excluded, and biopsy specimens should be collected only from patients with persistent increases in liver transaminase levels, despite lifestyle interventions, during bariatric surgery or before therapy.[5,7]

In addition to the risks of adverse events, liver biopsy analysis can produce inaccurate findings because of limitations of the biopsy procedure itself. The tissue contained in a needle biopsy sample is only a minor fraction of the liver, therefore findings from the sample might not indicate what is occurring in the entire organ.[8,9] However, features of NAFLD are distributed fairly evenly throughout zone 3 of every liver lobule, a systematic distribution of lesions that may attenuate the risk of sampling error (Figure 1*A*). Strategies to assess the quality of biopsy specimens collected for the detection of NAFLD have been derived mostly from those for other chronic liver diseases, but these appear to be appropriate. Adequacy of the biopsy sample relies on the final decision of the pathologist, but length and diameter of the biopsy core are good indicators of interpretability.[10] Although a specimen length of 25 mm is considered to be optimal, a 15-mm biopsy sample provides much information.[8,11]

The diameter of the core also should be considered. Narrow-bore needles often transect the liver lobule, making it impossible for pathologists to analyze various components or assess architectural distortion. A 16-gauge needle with an inner diameter (1.2 mm) larger than a liver lobule (0.5–1 mm) is considered adequate.[12] It also is important to consider the level of expertise of the pathologist who performs the histologic analysis.[13] Although expert liver pathologists produce more reproducible findings, general pathologists perform just as well if they are trained properly.[14]

Table 1. Histologic Patterns Associated With Disease Severity

Histologic features	Association
Fibrosis	Liver-related mortality [2,35,37] Overall mortality [2,35]
NASH	Liver-related mortality [34,37] Cardiovascular mortality [34]
Inflammation	Fibrosis[40]
Ductular reaction	Fibrosis[38]

Liver Biopsy to Determine Risk of Disease Progression

NAFLD refers to a spectrum of liver lesions ranging from simple steatosis (NAFL) to a more complex pattern that includes features of hepatocyte injury and inflammation (NASH) (for detailed review see Brunt[15] and Yeh and Brunt[16]). The risk of progression of NAFL differs from that of NASH, but histologic analysis can be used to predict disease progression for an individual. Although several algorithms, based on combinations of clinical and biological markers, have been proposed, there is no noninvasive test that firmly can identify patients with steatohepatitis or distinguish those with steatohepatitis from those with NAFL.[17,18] If there is a need to know with certainty whether or not a patient has NASH, a liver biopsy must be analyzed.

Steatosis results from an excess accumulation of triglycerides in the liver. In NAFLD, steatosis usually is macrovesicular, but it can be either a purely large droplet or a mixture of small and large droplets.[19] Microvesicular steatosis is uncommon, but it may occur in a patchy distribution in up to 10% of cases of NAFLD.[20] In adults, steatosis may be distributed in a distinct zone 3 (pericentral)-centered pattern, but abundant steatosis is panacinar (Figure 1*A*). As the disease progresses toward cirrhosis, the steatosis can become irregularly distributed or vanish. On rare occasions, the steatosis can localize to zone 1—a characteristic pattern in pediatric NAFLD.[21] A semiquantitative 4-scale grading system (from 0 to 3) is used to score the level of steatosis. It takes into account only macrovesicular and/or mediovesicular steatosis and assesses the percentage of hepatocyte decorated by steatosis vacuoles.[22]

Patients with steatosis and no additional features of liver injury often follow a benign clinical course, which is not associated with an increase in liver disease–related mortality compared with the general population of similar age and sex.[23] However, a recent meta-analysis found that simple steatosis can progress to fibrosis, but at a significantly lower rate than steatohepatitis.[24] This form of progressive steatosis may correspond histologically to that of steatosis with few inflammatory cells or clarified or ballooned hepatocytes of normal size. Together, these lesions are too mild for the lesion to be considered NASH, but they still are important to identify and follow up. Retrospective studies have shown that steatosis with mild inflammation