Where Are We in the Search for Noninvasive Nonalcoholic Steatohepatitis Biomarkers?

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease associated with obesity and insulin resistance.1 Due to the rising prevalence of obesity and diabetes, NAFLD is presently the most common cause of liver disease in the Western world, both in adults and children. The prevalence of NAFLD in Western adults is between 20% and 30%.2 NAFLD associates with increased hepatic-related mortality.3 NAFLD ranges from the simple accumulation of triacylglycerol (TAG) in the liver (hepatic steatosis) to nonalcoholic steatohepatitis (NASH), which is characterized by steatosis, hepatocyte ballooning, scattered inflammation, fibrosis, and necrosis.4 A continuously changing hepatic environment—reflected in lipid metabolic changes and the lipotoxicity they generate, inflammatory cells and the paracrine mediators they release, oxidative stress, and insulin resistance—are thought to be critical in the progression from hepatic steatosis to NASH. Although TAG accumulation in steatosis is now understood as a beneficial, adaptive response to the increased exposure of the liver to fatty acids, NASH is a progressive disease that may ultimately progress to cirrhosis, liver failure, and hepatocellular carcinoma in a substantial proportion of patients.4 Accordingly, compared to simple hepatic steatosis, NASH has a higher liver-related mortality. The estimated prevalence of NASH in the general Western population is between 2% and 3%.5

Liver biopsy is the only widely accepted technique to diagnose NASH and establish the presence of fibrosis.6 Several systems have been proposed for the histological evaluation of NAFLD, of which the most widely used is probably the NAFLD activity score (NAS),7 which is based on the degree of steatosis, lobular inflammation, and hepatocyte ballooning, with an additional score for fibrosis. Although considered the “gold standard,” liver biopsy is an invasive, subjective, and costly procedure, associated with potential complications (risk of death of 0.01%) and prone to sampling error.6 Because of the limitations of liver biopsy and the increasing prevalence of NAFLD, identification of noninvasive NASH biomarkers may help physicians select subjects for further liver histology analysis, intensified life style counseling, treatment (i.e., vitamin E administration), as well as helping researchers select patients for clinical studies.

The amount of TAG accumulated in the liver can be assessed noninvasively by a variety of imaging techniques, including ultrasonography (US), computed tomography, magnetic resonance imaging (MRI), and proton (1H)-MRI. Compared to US and computed tomography, MRI and 1H-MRI perform better for the evaluation of hepatic TAG accumulation, and only these last two techniques show differences across steatosis grades. In a meta-analysis of the performance of US in the assessment of hepatic TAG, this technique showed a pooled area under the curve (AUC) of the receiver operator characteristic (ROC) of 0.93, but the performance of US is decreased in the morbidly obese population.8 An ideal marker would have an AUROC of 1.0 and thus a 100% sensitivity and specificity. Although imaging techniques perform as well as liver biopsy for NAFLD diagnosis, they are, however, expensive and nonspecific, because they cannot distinguish NASH from simple hepatic steatosis, or identify fibrosis.

The majority of patients with NAFLD have normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values, and the ALT/AST ratio is often greater than one in those individuals with elevated serum aminotransferases. Bilirubin and albumin values are normal in the majority of patients who have NAFLD, whereas alkaline phosphatase and gamma-glutamyl transferase (GGT) levels can be moderately elevated. A variety of scoring systems have been developed to assess NAFLD on the basis of simple laboratory test results in combination with other parameters. For instance, the fatty liver index predicts US-diagnosed NAFLD based on the combination of body

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**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; GGT, gamma-glutamyl transferase; LC, liquid chromatography; MRI, magnetic resonance imaging; MS, mass spectrometry; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TAG, triacylglycerol; TE, transient elastography; US, ultrasound.

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mass index (BMI), waist circumference, and serum TAG and GGT. SteatoTest combines age, sex, and BMI with 10 laboratory determinations (AST, ALT, bilirubin, GGT, α2-macroglobulin, apolipoprotein AI, haptoglobin, glucose, cholesterol, and TAG) to predict liver steatosis in patients with different causes of chronic liver disease (hepatitis B and C, and alcoholic and nonalcoholic liver disease), showing AUROC curves ranging from 0.72-0.86.

Different scoring systems have been developed for staging fibrosis in patients with NAFLD, based on the combination of age and BMI with simple laboratory measurements (glucose, AST, ALT, ferritin, platelet count, and albumin) or with serum cytokines (transforming growth factor-β1, platelet-derived growth factor) and components of the extracellular matrix (collagens, collagenases and their inhibitors, glycoproteins, and polysaccharides). Of these various tests, FIB-4, NAFLD Fibrosis Score (NFS), European Liver Fibrosis (ELF), and FibroTest have been validated more amply. In general, these different scoring systems are more accurate in the detection of cirrhosis than in detecting less advanced stages of fibrosis, which limits their utility in the evaluation of fibrosis in NASH.9

US-based transient elastography (TE) imaging is a technique that can be employed to measure liver stiffness by using a probe that emits a low-frequency vibration and calculating the speed of the propagating mechanical wave induced by this vibration.10 In a meta-analysis of the performance of TE in the detection of hepatic fibrosis in patients with cirrhosis, this technique showed sensitivity and specificity values close to 90%. However, the performance of TE decreases in patients with less advanced fibrosis or in obese individuals. Magnetic resonance elastography is an imaging technique related to TE that visualizes, using MRI, the tissue of patients with NAFLD who have a variety of clinicalopathological characteristics.13 More recently, we studied the serum lipidomics and amino acid profile, as a function of BMI, in about 500 biopsied individuals with normal liver, or who had been diagnosed with steatosis or NASH. This study has identified a BMI-dependent metabolic signature able to reliably distinguish NASH from steatosis, showing an AUROC of 0.85 (Barr J, Lu SC, Mato JM, unpublished data). This list of novel BMI-dependent biomarkers is made of individual molecular species belonging to different lipid categories (i.e., eicosanoids, nonesterified fatty acids, glycerophospholipids, sphingomyelins, ceramides, diacylglycerols, and TAG).

The goal of this metabolomics-based approach is to develop perfect (99%-100% sensitivity and specificity) noninvasive diagnostic tests for liver steatosis, NASH, and fibrosis by combining the tried and trusted old biomarkers with these new lipid biomarkers. These tests should also be responsive to changes in NAFLD severity due to therapeutic intervention and time. This task is not suited for every laboratory, because extreme care needs to be taken to ensure that the analytical methods used are well validated and the new specific biomarkers correctly identified. A large number of carefully selected serum samples of biopsied individuals

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of different ethnic groups (the search for biomarkers has focused almost entirely on those of European descent) with normal liver or diagnosed with various NAFLD stages are also needed, as well as powerful informatics to ensure consistent database storage and annotation of the thousands of lipid molecular species found in serum and the analysis of large quantities of experimental data.

Considering the promising results of the available studies that have searched for serum metabolic signatures of NAFLD using MS-based methods, one may envision that the development of reliable noninvasive NAFLD tests is not too far in the future. To become a common practice in the assessment of NAFLD, an MS-based diagnostic test not only needs to be accurate but also inexpensive. At present, the cost of an LC/MS metabolomics-based serum test is between US $200 and US $300, including shipment of the sample. As occurred earlier with other “omics” technologies, the price of LC/MS-based tests will decrease if it becomes widely used.

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References

9. Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. HEPATOLOGY 2006;43:S113-S120.