Is Liver Biopsy a Gold or an Old Standard in NAFL and NASH?

When defining the term gold standard, the most appropriate definition is "a benchmark test that is the best available under reasonable conditions."[1] When considering this definition, it becomes clear that non-invasive imaging is replacing liver biopsy as the gold standard for evaluation of fibrosis in non-alcoholic fatty liver disease (NAFLD).

Over the last 40 years, NAFLD has evolved from an unrecognised entity to a heterogeneous collection of overlapping liver diseases with a common phenotype of hepatic steatosis. Although NAFLD is quite common, affecting approximately 25% of the world's adult population, it is increasingly clear that subjects with non-alcoholic steatohepatitis (NASH), and especially those with significant fibrosis, are at the greatest risks for excess mortality and adverse clinical outcomes, as well as impairment of patient reported outcomes and significant economic burden.

Patients with NASH do not present with any obvious clinical signs until they are near liver failure. Despite the growing recognition of this important burden, there are significant challenges to accurately and non-invasively diagnose the progressive form of NAFLD. Although liver biopsy (LB) is still considered the current "gold" standard for diagnosing NASH and staging fibrosis, it is an invasive procedure with some variability in assessment of the key features of NASH.

LBs require significant expertise both to perform and to interpret the results. Two clinicians are involved in obtaining and interpreting LB which represents a huge clinical process, especially in the settings of clinical trials. Given the significant number of patients with NAFLD, the limited number of hepatologists represents a serious bottleneck, often leading to delays in biopsy reads and confirmation of results. The accuracy of LB to assess fibrosis has also been questioned due to sampling errors and intra- and inter-observer variability that may lead to over- or under-staging, with even highly skilled and trained pathologists showing inter-observer concordance rates of less than 80%.[2] The size of the biopsy specimen, which varies from 10 to 30 mm in length and from 1.2 to 2 mm in diameter, represents 1/50,000 of the total mass of the liver and is therefore subject to a significant risk of sampling error.[3]

Although LBs remain the gold standard for confirmation of liver fibrosis staging, they are costly, painful and pose risk of complications such as bleeding, damage to other organs, and potentially, although rare, death. Multiple LBs present a significant challenge in recruitment and retention of subjects in clinical trials due to above states risks and subject discomfort. Ideally, less invasive tests that are more widely available, less costly, and accurate can be confirmed and widely accepted by clinicians and regulatory bodies as the new gold standard. There are a number of such non-invasive tests such as radiographical modalities, serum markers, and non-invasive predictive algorithms undergoing investigation for identifying those with increased risk of NAFLD and NASH and for confirmation of or staging of NASH fibrosis. The ideal test to discriminate advanced liver fibrosis due to NASH would be non-invasive, widely available, affordable, accurate, and reproducible

Imaging Modalities

In the context of NAFLD, the first diagnostic challenge is to accurately show the presence of fat in the liver. Fat is thought to have its own chemical signature, which can be detected directly by magnetic resonance spectroscopy (MRS). MRS quantifies the proton density fat fraction (PDFF), a standardised measure of liver tissue.[4] However, MRS has several limitations, including: limited availability, need for expertise in protocol prescription, data collection, and the requirement for spectral analysis. Furthermore, MRS is not available on routine scanners. Therefore, now, magnetic resonance imaging (MRI) based methods have been developed using MRI-PDFF to quantify liver fat without needing spectroscopy coils, using routinely available clinical MRI scanners.[4,5]

In contrast, fibrosis has no molecular signature that can be detected by current imaging techniques, so all imaging tests for fibrosis attempt to detect fibrosis indirectly using proposed biomarkers, which include: stiffness, diffusion, perfusion, metabolites, and image texture. Testing for the presence of advanced fibrosis is a primary concern when evaluating a patient with suspected NASH. It is known that fibrosis is the only independent predictor of associated morbidity and mortality. Confirmed presence of advanced fibrosis alters clinical management and consideration for treatment, potentially in the context of clinical trials. The leading biomarker of fibrosis is liver "stiffness" (or "elasticity") and its family of related parameters.[4]

The most accurate non-invasive methods to assess the stiffness of the liver and to classify the patient into advanced versus non-advanced fibrosis include transient elastography (TE), magnetic resonance elastography (MRE), and emerging techniques such as shear wave elastography (SWE) and acoustic radial force imaging (ARFI).

TE has been clinically useful as well, and has the means to replace LB as the gold standard. TE has high accuracy when identifying patients with F3-F4 fibrosis who are at greater risk for worse clinical outcomes.[6] TE has been shown to have an area under the curve (AUROC) of 0.83 for advanced fibrosis when compared to blood tests. The most remarkable advantage of TE is that the procedure is non-invasive, without any of the complications associated with liver biopsy. In addition, its cost is one-fourth of that of liver biopsy, and it can be done in five minutes in the outpatient setting without any associated pain.

MRE has the AUROC of 0.98 in identifying patients with F3–F4 disease, so at the very least is non-inferior to liver biopsy.[7] Given its accuracy, MRE may also offer a good non-invasive tool to

monitor changes in liver fibrosis. In a placebo-controlled trial of sitagliptin in NAFLD, MRE was shown to have robust correlation coefficient between baseline and 24 weeks.[8] Longitudinal studies of contemporaneous MRE and liver biopsy are underway, and their results are eagerly awaited.

Controlled attenuation parameter (CAP) is a novel ultrasound technique that measures steatosis simultaneously with liver stiffness during vibration-controlled transient elastography. Overall, CAP is a relatively simple and inexpensive method for steatosis assessment that is reasonably accurate for the diagnosis of steatosis. When combined with other clinical assessments, it is likely to help clinicians diagnose or exclude steatosis.

Non-invasive Biomarkers in NASH

In addition to the non-invasive tests based on the imaging modalities, there is an attempt to define non-invasive biomarkers using predictive models or serum biomarkers. These non-invasive markers include those that are based on alanine aminotransferase (ALT) levels, those that include components of metabolic syndrome (MetS), measuring circulating keratin18 fragment levels, as well as tests based on soluble markers such as FibroMeter, microRNA (miRNA) panels, and lipidomic panels, etc. The NASH test combines demographic characteristics (age, sex, and BMI), serum parameters (aminotransferases and lipids), and alpha-2 macroglobulin, apolipoprotein A1, and haptoglobin.

Serum Fibrosis Markers in NASH

Serological markers for the evaluation of liver fibrosis (LF) evaluate alterations in hepatic function as well as collagen turnover.

AST/ALT ratio: The aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio helps distinguish alcoholic hepatitis from NAFLD and NASH. Using these non-invasive tests to diagnose for NASH, current studies have found that the frequency of NASH

in individuals with normal ALT (<35 U/L) was 11% whereas the frequency was 29% in those with elevated ALT (35 U/L); and if the ALT was two times the upper limit of normal (>70 U/L), predicting NASH was found to have a 50% sensitivity and 61% specificity for NASH.[9] However, another study found that individuals with NAFLD can have normal ALT levels as the disease progresses.[10]

Fibrosis-4 index: This scoring system combines age, AST, ALT, and platelet count. It has a negative predictive value of more than 90% for advanced liver fibrosis, according to experts. Results can be subject to rapid AST and ALT changes, though.

BARD score: Calculated from body mass index, the ALT/AST ratio, and the presence of diabetes, this score, reported on a 0-4 scale, is routinely used to predict liver fibrosis in NAFLD patients. Scores less than 2 have a strong negative predictive value for advanced liver fibrosis associated with NAFLD.

Enhanced liver fibrosis (ELF) test: Though not yet approved by the Food and Drug Administration and not sensitive to early-stage fibrosis, this panel is an algorithm comprised of three fibrosis markers – amino-terminal propeptide of type III procollagen, hyaluronic acid, and tissue inhibitor of metalloproteinase. It detects advanced fibrosis with high accuracy in both adult and paediatric patients.

Tailored Approach

Significant progress has been made regarding the non-invasive assessment of liver disease in patients with NAFLD. Regarding detection and grading of steatosis, MRI-PDFF is the most accurate method but appears better suited for assessment and follow-up of selected patients in clinical trials, whereas conventional ultrasound, and if no steatosis is shown, CAP, as a point of care technique, could be used as triage in large unselected populations. As for the identification of advanced fibrosis, MRE, TE, as well as FIB-4 are the most accurate and validated methods.

FIB-4 is best suited as a first-line tool in a primary healthcare setting to confidently exclude advanced fibrosis, whereas TE and MRE are more suited for referral centres. It is postulated that combinations of NITs in sequential algorithms can accurately detect advanced fibrosis while eliminating the risks associated with biopsy and reducing costs by minimising unnecessary testing.[11]

Regarding NASH, no highly sensitive and specific blood tests are available to differentiate NASH from simple steatosis. Neither imaging modality can reliably discriminate NASH from simple steatosis, although MR-based modalities are showing promise. Finally, there is increasing evidence that serum markers and liver stiffness, measured using TE, accurately identify the subgroup of patients with NAFLD at a higher risk to reach the outcome of liver-related complications and death/liver transplantation, especially when analysed together. Screening data from Phase 2 ATLAS study evaluating combinations of investigational cilofexor, firsocostat and selonsertib in advanced fibrosis due to NASH has been recently presented. This analysis demonstrates that the use of currently available NITs can accurately identify patients with advanced fibrosis due to NASH and potentially reduce the need for LB. When used in combination, the ELF test and FibroScan® (TE) accurately identified advanced fibrosis in >805 of patients (presented at The International Liver Congress 2019, Vienna).

Looking Ahead
Adopting new biomarkers in clinical trials requires substantial efforts and investment to validate the reliability of these biomarkers as surrogate endpoints. In an effort to address this challenge, developers are currently integrating exploratory markers as secondary endpoints in Phase II and Phase III studies. The field has also seen the formation of two multi-stakeholder consortia, LITMUS and NIMBLE, aimed at accelerating validation of non-invasive markers by sharing resources and patient samples.

Histological diagnosis of NASH is still required in clinical trials, however non-invasive modalities can be used more frequently to follow at-risk patients over time, as well as be instituted for screening evaluations in the absence of the morbidity that unfortunately comes with LB. MRE has emerging data to support its non-inferiority to LB in terms of accuracy in fibrosis staging and, combined with the dramatic risk profile differences, should be soon considered a superior test.

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