Diagnostic Modalities for Non-alcoholic Fatty Liver Disease (NAFLD), Non-alcoholic Steatohepatitis (NASH) and Associated Fibrosis

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Abstract (275/275)

NAFLD is a spectrum comprised of isolated steatosis, NASH, advanced fibrosis, and cirrhosis. The majority of NAFLD subjects do not have NASH and don’t carry a significant risk for adverse outcomes (cirrhosis and mortality). Globally, the prevalence of NAFLD is approximately 25%. In Asia, a gradient of high prevalence rates to low rates are noted from urban to rural areas. Given the prevalence of NAFLD, the clinical and economic burden of NAFLD and NASH can be substantial. With increasing recognition as an important liver disease, the diagnosis of NASH still requires a liver biopsy which is suboptimal. Although liver biopsy is the most accurate modality to diagnose and stage the severity of NASH, it suffers from being invasive, costly, associated with potential complications, and plagued with interobserver variability of individual pathologic features. A number of non-invasive modalities to diagnose NASH and stage liver fibrosis are being developed. These include predictive models (NAFLD fibrosis score) and serum biomarkers such as Enhanced Liver Fibrosis, (ELF). Other tests are based on radiologic techniques such as transient or MR elastography (MRE) which are used to estimate liver stiffness as a potential surrogate of hepatic fibrosis. Although a dynamic field of research, most of these diagnostic modalities have AUROC between 0.76 to 0.90% with MRE having the best predictive performance. In summary, developing accurate, safe and easily accessible non-invasive modalities to accurately diagnose and monitor NASH and associated fibrosis is of utmost importance in clinical practice and clinical research. These tests are not only important to risk stratify subjects at the greatest risk for progressive liver disease but to serve as appropriate surrogate endpoints for therapeutic clinical trials of NASH.

Key words: predictive models, noninvasive, biomarkers, imaging
Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, worldwide. [1] Based on a recent meta-analysis, 25% of the general adult population in the world are potentially affected by NAFLD. [1] Although NAFLD was initially reported to be more prevalent among Hispanics, it is now increasingly reported from all regions of the world. [1]. Furthermore, the prevalence of NAFLD in children is also high and estimated to be around 10% [2,3,4].

Data on the incidence of NAFLD are quite sparse. Of the available data, the estimated NAFLD incidence is reported to be between 28 to 52/1000 person-years. [1]. However, these incidence rates are probably underestimations, given the rising incidence of obesity and diabetes, two of the primary associated risk factors for NAFLD.

The long-term outcome of the spectrum of NAFLD has been described in observational studies. In fact, data suggest that most patients with NAFLD die primarily of cardiovascular complications [4-6]. Nevertheless, there are subgroups of NAFLD patients, primarily those with histologic NASH or significant hepatic fibrosis, who are at risk for developing advanced liver disease, hepatocellular carcinoma (HCC), excess liver-mortality, or become candidates for liver transplantation (1, 7-12). In fact, more recent studies have suggested that stage of fibrosis, independent of any other histologic feature, predicts mortality in NAFLD.

In addition to the clinical burden, NASH places significant burden on patient reported outcomes (PROs) and the economy [13-18]. A recent Decision Analytic Markov Model estimated tremendous economic burden of NAFLD and NASH to the US and European economies. [19] These data provide strong evidence that the progressive form of NAFLD or NASH, especially those with significant fibrosis pose tremendous clinical, PRO, and economic burden to individuals and the society. [14-20]
The Initial Steps in the Diagnosis of NAFLD:

Prior to the initiation of exhaustive diagnostic tests for NAFLD, it is important for health care providers to exclude other etiologies for steatosis and coexisting common causes of chronic liver disease. Specifically, excessive alcohol consumption and other exogenous factors (ex, steatogenic medications) must be excluded. [1,3,21] Furthermore, in patients with persistently high serum ferritin, and increased iron saturation, especially in the context of homozygote or heterozygote C282Y HFE mutation, iron overload must be considered. Although low titer autoimmune markers can be frequently seen in patients with NAFLD, autoimmune liver disease should be ruled out in patients with high serum titers of autoantibodies in association with other features such as high serum globulins. [1, 3, 21]

Once other causes of steatosis have been ruled out, NAFLD should be considered. In this context, presence of commonly associated comorbidities such as obesity, dyslipidemia, insulin resistance or diabetes, hypothyroidism, polycystic ovary syndrome, and sleep apnea should be determined. [3, 21]

Role of Histopathology in the Clinical Research and Management of Patients with NAFLD

The liver biopsy is the definitive technique for the diagnosis and classification of NAFLD in which the role of histopathology is to establish a diagnosis, characterize the liver lesions, and correlate the lesions with potential clinical outcomes in the context of the natural history of the disease. (7, 23-30) Historically, the terminology and concepts of the histopathologic features of NAFLD were derived from alcoholic liver disease. Thus, alcoholic fatty liver (AFL) is analogous to simple steatosis (NAFL), alcoholic hepatitis to nonalcoholic steatohepatitis (NASH), and
alcoholic cirrhosis to the cirrhotic stage of NAFLD. [23] Although there may be differences in
degree, the features are sufficiently similar to preclude an etiologic diagnosis based on histology
alone. Therefore, the characteristic histopathologic features which are investigated when
diagnosing AFL or NAFL include: 1). Fat – hepatocellular triglyceride accumulation, 2).
Hepatocellular injury in the centrilobular location which is most severe in the acinar zone, 3).
Cytoskeletal damage shown as hepatocellular ballooning with or without Mallory-Denk bodies,
4). Parenchymal inflammation where lymphocytes and macrophages predominate, though
neutrophils may be present in severe cases, and 5). Perisinusoidal fibrosis seen as collagen
deposition in the space of Disse. (Figure 1)

As an aid to help characterize these lesions and allow for statistical analysis in clinical trials, the
pathologists of the National Institute of Health NASH Committee (NIH NASH CRN) devised a
grading system called the NAFLD Activity Score (NAS). [29] (Table 1) After studying the inter-
and intra-observer variability of a variety of histologic features, the features with the greatest
reproducibility (severity of steatosis, hepatocellular ballooning, and lobular inflammation) were
chosen to formulate the NAS score. The NAS system then assigns a numerical grade to each
feature such that the severity of steatosis is graded from zero to three (0 to 3), hepatocellular
ballooning is graded from zero to two (0 to 2), and lobular inflammation is graded from zero to
three (0 to 3). The NAS score is the unweighted sum of these three numbers with a range from
zero to eight (0 to 8). Improvement in histologic severity is accompanied by a decrease in the
NAS. [29] More recently, a refinement of this scoring system, called the SAF Score, has been
proposed. Although on the face of it SAF appears to be a very similar system, the SAF Score
separates degree of steatosis (S) from grade of necroinflammatory activity (A) and so may
provide greater granularity in terms of disease activity and help distinguish steatosis from
NASH. [30]
However, the histologic feature with the greatest reproducibility is fibrosis, a feature which is not part of the NAS score because fibrosis is considered a sign of the stage of disease rather than a grade of injury. Fibrosis staging is, therefore, scored separately. Accordingly, in the NASH CRN system fibrosis stage 0 = no fibrosis; stage 1 = centrilobular pericellular fibrosis (or periportal fibrosis in children); stage 2 = centrilobular and periportal fibrosis; stage 3 = bridging fibrosis; and stage 4 = cirrhosis. [8,22,30] (Figure 1)

As a result of this histologic work done on NAFLD, the natural history of the various lesions associated with NAFLD are gradually being elucidated. For example, fatty liver, alone or with some lobular inflammation but without evidence of cytoskeletal damage (ballooning or Mallory-Denk bodies) or fibrosis, has long been considered non-progressive liver disease; however, recent follow-up studies, have found that some patients do, in fact, eventually develop fibrosis and even cirrhosis. [30] Another example is NASH with ballooning ± Mallory-Denk bodies, which has long been thought to be the progressive form of NAFLD; however, recent long-term follow-up studies have found that the single histologic feature that predicted mortality was not NASH but fibrosis in the liver biopsy. [8,22,30] Furthermore, studies of NAFLD patients with paired biopsies found that spontaneous regression of fibrosis may be an important feature in NASH, both in the long-term and short-term. [22,25]

With these findings in mind, a collaborative effort with NIH NASH CRN was undertaken where two scoring systems were developed to grade the level of liver injury associated with NAFLD (NAS) and stage the level of disease associated with NAFLD- fibrosis score. As such, the use of histology in diagnosing NAFLD has allowed a more in-depth understanding of the natural history of the various NAFLD histologic lesions, but more information is still needed on the mechanisms of fibrosis progression and development of cirrhosis in patients with NAFLD.
The Role of Radiologic Modalities for Diagnosing, Staging and Monitoring NASH

Although, liver biopsy is the gold standard to diagnose NASH and assess the stage of fibrosis in patients with NAFLD, its many limitations (cost, sampling error, complications leading to morbidity, and though rare, death) prohibit its routine use. [11, 25, 26] However, when a clinician sees a patient for the first time with suspected NAFLD, he/she would like to know the following: 1) Whether the patient has NAFLD, 2) Whether the patient is likely to have underlying NASH, 3) Whether the patient has any fibrosis, 4) Whether the patient has any advanced fibrosis? As a result, there is an urgent need for an accurate non-invasive diagnostic modality for the diagnosis and staging of NAFLD and NASH. [31]

In the context of NAFLD, the first diagnostic challenge is to accurately show the presence of fat in the liver. Currently, 20%-33% is considered a reliable level at which steatosis is detected by conventional means; however, the presence of fat greater than 5% to 10% is considered abnormal. [22, 31-36]

Fat is thought to have its own chemical signature which can be detected directly by magnetic resonance spectroscopy (MRS). When performed properly, MRS quantifies the proton density fat fraction (PDFF), a standardized measure of liver tissue [TG]. [34] However, the limitations of MRS include: restricted availability, need for expertise in protocol prescription, data collection and spectral analysis is required. Furthermore, MRS is not available on routine scanners. Therefore, now, MR Imaging based methods have been developed using MRI-PDFF to quantify liver fat without needing spectroscopy coils using routinely available clinical MRI scanners (33-40). MRI- Proton density fat fraction (PDFF) addresses confounding factors and is not affected by scanner field strength, patient factors (age, sex, BMI, etiology of liver disease), and concomitant liver abnormalities such as iron overload or necroinflammation. [34,41-43]
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. However, the leading biomarker is liver “stiffness” (or “elasticity”) and its family of related parameters. [34] The rationale for using “stiffness” or “elasticity” is that the collagen deposition associated with fibrosis imparts parenchymal rigidity which on imaging tests is considered as assessing “stiffness” or “elastography”. (34-43)

The most accurate noninvasive methods to assess the stiffness of the liver and to dichotomize the patient into advanced versus non-advanced fibrosis include transient elastography (TE), magnetic resonance elastography (MRE), and emerging techniques such as shear wave elastography and acoustic radial force imaging [38,44-46] (Figure 2)

Transient elastography has been shown to have an AUC of 0.83 for advanced fibrosis when compared to blood tests. Overall, TE has a 90% negative predictive value (NPV), a sensitivity of 88%, a positive predictive value (PPV) of <65%, and places the fewest patients (43.6%) in what is considered “the grey zone” compared to blood tests. Furthermore, TE has been shown to also have prognostic ability. Since TE has diagnostic accuracies of 80.8%, it is thus able to categorize patients into subgroups found to be have different prognoses. [47]

Using a “stiffness” cutoff of 3.63 kPa, MRE sensitivity has a sensitivity of 0.86 (95% confidence interval [CI]: 0.65-0.97), a specificity of 0.91 (95% CI: 0.83-0.96), a PPV of 0.68 (95% CI: 0.48-0.84), and a NPV of 0.97 (95% CI: 0.91-0.99) with an area under the curve (AUC) of advanced of 0.924 for diagnosing advanced fibrosis.) [39] In addition, the use of 3D MRE has shown that at 40Hz and a “stiffness” cutoff of 2.43 an AUC of 0.962 for diagnosing advanced fibrosis. [46] In fact, in a recent study comparing ARFI based versus MRE-based NAFLD fibrosis assessment, MRE was significantly better. [45] Further, MRE was significantly better than TE when
diagnosing cirrhosis.[48] There are several caveats that must be addressed before using MRE. These caveats include: the cost of the procedure, the patient size, the presence of claustrophobia or the presence of metal implants. (46,48)

Although TE or other ultrasound-based tests are more accessible and easier to use, they are limited when used in patients with obesity, ascites, or acute inflammation. [34] However, even though, the 2D and 3D MRE is able to overcome all these issues except for iron overload or acute inflammation, it is limited by the having restricted accessibility at many centers especially worldwide and the required expertise needed to obtain adequate results in the setting of 3D MRE. So, as of now, accurate imaging is a trade-off between specificity, accessibility, and ease of use such that as specificity goes up accessibility and ease go down. It is also reasonable to use ultrasound based tests in non-obese individuals and consider using MRE in individuals with obesity especially morbid obesity. [34,38,45] Further research is needed to quantify the exact trade off that occurs when one imaging technique is traded off for the other.

**Noninvasive 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 levels, those that include components of metabolic syndrome, measuring circulating keratin18 fragment levels as well as tests based on soluble markers such as Fibrometer, microRNA panels, and lipidomic panels. [49-61]

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. [49] Another study found that individuals with NAFLD can have normal ALT levels as the disease progresses. [62]

Feldstein et al., in their seminal work, found that circulating levels of cytokeratin-18 (CK-18) fragments were predictors of NASH in patients with NAFLD. [53] Since the release of their observations, there has been intense investigations which have unequivocally found that increased circulating levels of CK-18 fragments are associated with NASH. [54,55] However, at the same time, there are a number of issues with this diagnostic method (lack of a commercially available clinical test, poor reproducibility with limited sensitivity/specificity at the individual patient level [51] and a lack of a clear cut-off point) which limit its clinical utility at the present time. [62]

Others have looked into combining these different measures to diagnose NASH. Loomba and colleagues examined the efficacy of a panel of eicasanoids in the detection of NASH using a lipidomics based approach and showed excellent diagnostic accuracy ranging between 0.9-1.0. [63]. These data need to be confirmed in larger, multicenter studies.

The NASH Test combines demographic characteristics (age, gender, and BMI), serum parameters (aminotransferases and lipids), and alpha-2 macroglobulin, ApoA1, and haptoglobin. The NASH Test sensitivity is 33% with a specificity of 94% indicating it has a good negative predictive value (NPV) for NASH of 81%. [54]. Another combined test, The NASH Diagnostics Panel, which uses the presence of CK-18 fragments, adiponectin, and resistin initially performed well but in a larger study was not found to be as effective. [55] The NAFLD Diagnostic Panel, used CK-18 fragments in combination with the presence of type II diabetes mellitus, triglycerides, and gender, but it did not perform any better than the NASH Diagnostics Panel [56].
On the other hand, the OXNASH score, which uses AST, age, BMI and a ratio of 13-hydroxy-octadecadienoic acid (13-HODE) to linoleic acid (LA), correlates with histologic features of NASH and provides AUC’s of 0.730 [95% CI (0.637, 0.823)] for inflammation; followed by ballooning of 0.723 [95% CI (0.630, 0.816)], steatosis 0.705 [95% CI (0.570, 0.840)]; and fibrosis 0.673 [95% CI (0.577, 0.770)] [57, 58]. The Hepascore, derived from age, gender, bilirubin, gamma glutamyltransferase [GGT], hyaluronic acid, a-2 macroglobulin, when compared to a simple non-invasive score [BARD Index (BMI, AST, ALT, Diabetes)] performed reasonably well in identifying fibrosis F2-F4 with AUC’s of 0.73 to 0.91 with more accuracy noted for patients with a fibrotic stage of 4. [47,59] The Fibro meter NAFLD (Age, weight, fasting glucose, AST, ALT, ferritin and platelet count) performs reasonably well in identifying mild to moderate fibrosis. The positive predictive value (PPV) for correctly identifying F=1 was 84.9% for the Fibro meter NAFLD with a negative predictive value (NPV) of 66.7% and a diagnostic accuracy of 80%. The PPV for F=3 was found to be 74.5% with a NPV of 86.2 and a diagnostic accuracy of 82.1%. [64] Several prognostic scores (Palekar score, Shimada index, Nice model, Gholam’s model), have also been developed with all performing somewhat similarly with AUC ranging from 0.76 to 0.90 [60,61].

Metabolic syndrome is another commonly used index to identify individuals with NAFLD at risk for NASH. Several studies have found a significant relationship between the increasing number of metabolic syndrome components and the likelihood of NASH in patients with NASH. (65-67) Yet, what has not been explored is the combination of metabolic syndrome, levels of ALT and age to predict NASH in NAFLD leaving another area of further exploration.

With these continuing challenges in correctly diagnosing NASH, the NAFLD scientific community needs to reevaluate the need for predicting NASH in patients with NAFLD. Should
we instead focus on NASH with stage $\geq$ 2 fibrosis as it is the sub-phenotype that is primarily targeted in Phase 2B and Phase 3 clinical trials?

**Serum Fibrosis Markers in Non-alcoholic Steatohepatitis**

Since stage of fibrosis is the most important predictor of outcome [8, 10, 68] a great deal of effort has focused on determining presence of fibrosis. In this context, NAFLD biomarkers can target domains that can be defined as the following: 1) diagnostic markers, reflecting current stage of fibrosis; 2) prognostic markers, stratifying individuals by fibrosis progression risk, discriminating fast vs. slow progressors and/or predict long-term outcomes and hard endpoints; and 3) monitoring markers, that may be used to track disease progression or treatment response. Such biomarkers should be at one of four qualification levels: 1) exploration (early-phase experimental biomarkers), 2) demonstration (“probable valid” biomarkers), 3) characterization (“known valid” biomarkers), and 4) surrogacy (registerable “surrogate endpoint”). [69-82] Although there has been some progress in biomarker development for detection of advanced fibrosis, existing biomarkers are generally at the first two qualification levels and need further independent validation.

Serological markers for the evaluation of liver fibrosis may be divided into ‘indirect’ markers (that reflect alterations in hepatic function but not collagen turnover, e.g. ALT, AST and platelet levels) and ‘direct’ markers (that directly measure aspects of extracellular matrix (ECM) deposition and/or turnover). [69, 71] Furthermore, novel kinetic biomarkers using deuterated water based approaches are emerging to assess dynamic changes in the fractional synthesis rate of collagen turn over in the liver in patients with NAFLD. [83]

**Indirect Markers and ‘Simple Panels’**
Significant hepatic fibrosis can lead to hepatocellular dysfunction and portal hypertension, which are reflected by changes in standard biochemical and haematological parameters. These tests, alone or combined as ‘simple panels’, are potentially attractive clinical tools as they are inexpensive and many indices are already routinely measured in patients with liver disease. [70]

The results of a head-to-head comparison in a large cohort with biopsy-proven NAFLD patients have been published. In general, simple panels have a relatively robust NPV so they can reliably exclude advanced fibrosis but have poor PPV (ranging from 27-79%). [71] Using such tests may help to mitigate the healthcare burden such a large ‘at risk’ population places on resources by not allowing those considered to have ‘low-risk’ scores to undergo further investigation. As liver fibrosis progress towards advanced fibrosis/cirrhosis serum ALT levels tend to fall whereas AST levels remains stable or increases, leading to an increase in the AST/ALT ratio. This phenomenon is exploited as a component of many of the simple panels.

The NAFLD Fibrosis Score (NFS) is calculated using six routinely measured parameters found to be independently associated with advanced fibrosis on multivariate analysis. [62] By applying a low cut-off (<1.455), advanced fibrosis can be excluded with high accuracy (NPV 93%) whilst a high cut-off threshold (>0.676) offers accurate detection of advanced fibrosis (PPV 90%). [62] Use of this score has been suggested to reduce the need for liver biopsy by ~75%. Despite extensive external validation, NFS is not as robust in predicting advanced fibrosis in NAFLD. Nevertheless, NFS can accurately exclude advanced fibrosis in NAFLD and is clinically useful.

The FIB4 Score is one of the best performing simple non-invasive tests for advanced fibrosis in NAFLD. A score of <1.3 has a 90% NPV for stage 3-4 fibrosis, whilst a score of >2.67 has an 80% PPV with only a quarter of the cohort being unclassified 1.3 or above 2.67. [73] Other studies have also found that the FIB-4 score narrowly out performs other simple non-invasive tests in predicting advanced fibrosis. [71] The specificity for advanced fibrosis using the FIB-4 and NFS declines with age, becoming unacceptably low in patients aged over 65-years;
however, age-adjusted lower cut-offs (NFS <0.12 and FIB4 <2) have been derived that maintain the high NPV and so help to exclude advanced fibrosis in those aged ≥65-years. [74] It is also important to note that these scores may not be helpful in the younger age group and perform poorly with relatively low AUC. In this context, the exact cut off threshold and validity of these non-invasive tests in clinical setting require further external validation before its full clinical use can be recommended. [75-79]

**Direct Markers: Collagen Turnover**

When the severity of ongoing liver injury exceeds that of hepatic regeneration, hepatocytes are replaced by an extra cellular matrix (ECM) composed of collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Candidate biomarkers derived from these processes are appealing targets and are currently an area of active investigation. [75,76]

One such biomarker is hyaluronic acid (HA). HA production is increased when collagen synthesis is accelerated so this is a marker of increased ECM production. Similarly, liver fibrosis results in the deposition of collagen and release of pro-peptides, predominantly Pro-Collagen III (PIIINP). The terminal peptide of PIIINP correlates with the NAFLD activity score (NAS), and its constituent components (P<0.001) where a threshold of 6.6 ng/mL provides a NPV for advanced fibrosis of 95%-97% and 100% for cirrhosis. [75] The Enhanced Liver Fibrosis (ELF®) test is a commercial panel of markers focusing on matrix turnover comprised of tissue inhibitors: matrix metalloproteinase 1 (TIMP 1), HA, and aminoterminal peptide of pro-collagen III (P3NP). [76,80] When compared with the NAFLD Fibrosis Score this test performed only marginally better for severe fibrosis (AUROC 0.93 vs 0.89) and moderate fibrosis (AUROC 0.90 vs 0.86), but combining the two modalities enhanced efficacy (AUROC 0.98 for severe fibrosis and 0.93 for moderate fibrosis). [81] Fibrotest® is a commercial panel, with a reported AUROC of 0.75-0.86 for F2-F4 and 0.81-0.92 for F3-F4. [81] Other commercial assays currently in development...
with promising preliminary results detect pathologically modified proteins generated by specific proteases such as the specific collagen fragments of Pro-C3 and Pro-C6 using proprietary Protein Fingerprint™ ELISA assays. [82]

**Other Promising Experimental Markers: Genetics/Epigenetics, Metabolomics, and Lipidomics:**

Inter-patient variation in NAFLD progression risk is, at least in part, determined by genetic modifiers that influence individual response to environmental (diet, lifestyle) factors. [22]

Mounting evidence indicates that epigenetic factors such as differential DNA methylation and circulating cell-free DNA methylation signatures in plasma, may potentially stratify patients with NAFLD into mild versus severe fibrosis. [83,84] MicroRNA (miRNA) is another genetic marker that appears to be relatively stable and can be detected in plasma following release from injured tissue and may serve as another disease biomarker. [85-87] These modalities remain highly experimental however and require further validation.

**Summary:**

Over the last forty years, NAFLD has evolved from an unrecognized entity to a heterogeneous collection of overlapping liver diseases with a common phenotype of having hepatic steatosis. Although NAFLD is quite common, affecting approximately 25% of the world’s adult population, it is increasingly clear that subjects with NASH and especially those with significant fibrosis are at the greatest risks for excess mortality and adverse clinical outcomes as well as impairment of PRO and significant economic burden.

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 is considered the current imperfect “gold” standard for diagnosing NASH and staging fibrosis, it is an invasive procedure with some variability in assessment of the key features of NASH.
Therefore, a number of serum markers, radiographic modalities, and noninvasive predictive algorithms have been or are currently undergoing investigation. To date, most of these modalities suffer from suboptimal performance. However, MRI-PDFF seems to be the most accurate modality for detecting hepatic fat while MRE seems to be the most accurate test for staging liver disease. Thus, a combination of MRI-PDFF and MRE may provide a relatively accurate method to risk stratify subjects with NAFLD. But, availability and cost of these modalities presents a major challenge to most clinical practices.

As a result of the limitations of the non-invasive tests and the radiographic imaging, the regulatory authorities [Federal Drug Administration (FDA) and European Medical Agency (EMA)] now require histologic endpoints for approval of drugs and diagnostic modalities. As such, until non-invasive measures are perfected and robustly validated the diagnosis of NAFLD may continue to be underestimated and the development of therapeutic options may be hindered.
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Figure Legends:

Figure 1. Stages of fibrosis in NAFLD according to the NASH CRN staging system (Masson trichrome stains). Collagen is blue with this method.

Stage 1 (left) – Centrilobular perisinusoidal fibrosis. Blue stained collagen fibers outline the sinusoids surrounding the central vein in the center of the field.

Stage 2 (left middle) – Centrilobular perisinusoidal fibrosis and periportal fibrosis. Delicate collagen fibers are deposited around the sinusoids in the upper part of the field while denser collagen expands the portal tract in the lower part of the field.

Stage 3 (right middle) – Bridging fibrosis. A vascularized septum of fibrous tissue transects the hepatic parenchyma,

Stage 4 (right) – Cirrhosis. Nodules of hepatocytes are surrounded by variable-size fibrous septa.

Figure 2: Novel 3-D MRE and Diagnosis of NASH and Advanced Fibrosis
Table 1. Histologic features of NASH CRN NAFLD Activity Score (NAS) and fibrosis staging (27)

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<th>Feature</th>
<th>Definition</th>
<th>Score or Code</th>
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<tr>
<td>Steatosis Grade</td>
<td>Low- to medium-power evaluation of parenchymal involvement by steatosis</td>
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<td></td>
<td>&lt;5%</td>
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<td>&gt;66%</td>
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<td>Lobular inflammation</td>
<td>Overall assessment of all inflammatory foci per 200X field</td>
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<tr>
<td></td>
<td>Many cells/prominent ballooning</td>
<td>2</td>
</tr>
<tr>
<td>NAFLD Activity Score</td>
<td>Sum of Steatosis + Lobular Inflammation + Ballooning</td>
<td>0 to 8</td>
</tr>
<tr>
<td>Fibrosis Stage</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Perisinusoidal or periportal</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mild, zone 3, perisinusoidal</td>
<td>1A</td>
</tr>
<tr>
<td></td>
<td>Moderate, zone 3, perisinusoidal</td>
<td>1B</td>
</tr>
<tr>
<td></td>
<td>Portal/periportal</td>
<td>1C</td>
</tr>
<tr>
<td></td>
<td>Perisinusoidal and portal/periportal</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bridging fibrosis</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 1

338x190mm (96 x 96 DPI)
Figure 2

338x190mm (96 x 96 DPI)