Biomarkers & diagnostics: Growing influence on drug development for NAFLD/NASH

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Biomarkers and diagnostics are playing an ever-increasing role in drug development. Personalized medicines, defined as drug therapies used alongside specific diagnostic tests, topped 40% of all FDA approvals in 2018. Across biopharma, drug developers increased their utilization of selection biomarkers, significantly improving the probability of success for their clinical programs.[1,2] While the first, and perhaps best known examples are in oncology, biomarkers as candidate diagnostics are becoming increasingly important in complex, multifactorial chronic diseases including non-alcoholic fatty liver disease (fat accumulation in the liver not due to alcohol overconsumption, or NAFLD) and non-alcoholic steatohepatitis (NASH).

Advancing precision medicine for NAFLD/NASH

The statistics are staggering. Nearly one-third of U.S. adults, and nearly one-quarter of adults worldwide, are thought to have NAFLD.[3] Depending upon a complex combination of genetic and environmental risk factors, between 11% and 40% of individuals with NAFLD will progress to NASH, in which inflammation, hepatocyte ballooning and fibrosis markedly affect liver function.[3] The rise in pediatric NAFLD is particularly alarming, as it is estimated that nearly a quarter of children diagnosed with NAFLD (10% of the pediatric population) have already progressed to NASH.[4] With NASH patients significantly predisposed to hepatocellular carcinoma,[5] and NASH already the leading cause of liver transplantation among females and the second leading cause overall,[6] the gravity of this burgeoning epidemic is clear. None of this has been lost on drug developers; dozens of biopharma companies, from small to large, have recognized that new treatments for NASH are a significant opportunity to improve outcomes of large, global populations.

Despite the large numbers of individuals thought to be affected, patient identification remains one of the key challenges in NASH drug development. Most clinical trials use a variety of liver biopsy-based criteria to identify and stratify eligible patients for enrollment.[7] However, biopsies are invasive, uncomfortable, and risky for patients, and expensive for investigators (an estimated $3,000-$5,000 per patient).[8,9] Recent publications have highlighted variability in biopsy-based disease diagnosis and staging introduced by both the fine needle sampling, as well as intra- and inter-reader differences between interpreting pathologists.[10-11] This variability has contributed to challenges inherent in measuring the impact of new candidate therapies, and is being recognized as untenable for future clinical care once new NASH drugs are available.[12-14]
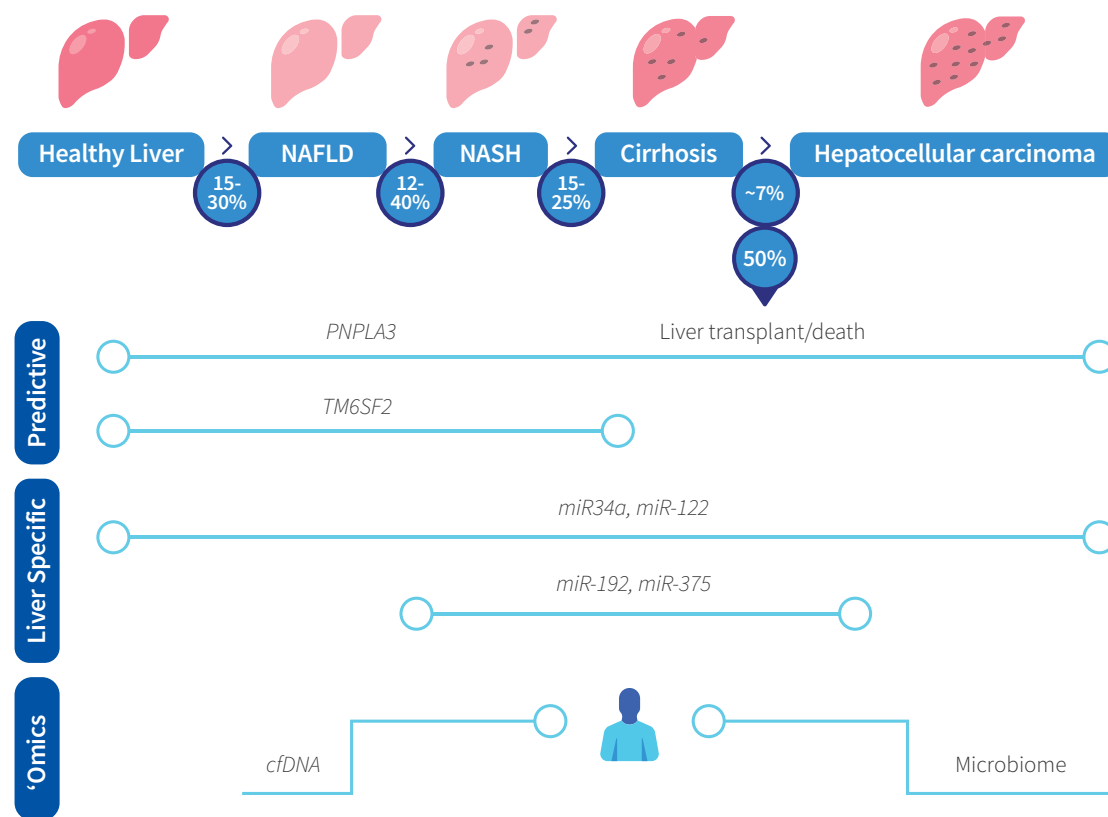
The challenge with liver biopsies highlights the need for noninvasive tools for refining clinical trial enrollment, as well as predicting and monitoring therapeutic response. Non-invasive tests can also serve as important, accessible tools for clinicians in patient education, relevant for both clinical trial and clinical care setting. If patient screening could begin with a noninvasive test, results from which were used to then characterize an individual as either at higher vs. no or minimal risk of disease progression, a prospective pool of trial enrollees could be enriched prior to undergoing biopsy. By avoiding unnecessary biopsy screening in a larger population, this approach would be significantly less expensive and far safer for patients. It is notable that, in the future, noninvasive testing, including circulating biomarkers and imaging methodologies are widely anticipated to replace liver biopsy for NASH diagnosis in clinical care, simply due to the scale of this global health problem.[12-16]

Current biomarkers in NAFLD/NASH

Noninvasive techniques to detect and monitor NAFLD/NASH often include estimating liver fat with ultrasound or magnetic resonance-based technologies. Alternative measures of liver stiffness: Magnetic Resonance Elastography (MRE) and Vibration-Controlled Transient Elastography (VCTE), such as FibroScan®, are being explored by investigators for clinical trial screening and enrollment, particularly with later stage drug candidates with anti-fibrotic mechanisms of action.[15,17] While emerging data suggests that these are helpful tools, neither method has the sensitivity nor the consistency needed to detect early NASH with mild to moderate fibrosis.[15]

However, some novel biomarkers that could help identify NASH are emerging. Pro-C3 (N-terminal pro-collagen III peptide, Nordic Biosciences) has been identified from robust collagen and extracellular matrix research to show promise in both differentiating fibrotic from non-fibrotic patients and reflecting treatment-mediated improvements in histological endpoints.[18-20] Similar data has recently been published examining Siemens' Enhanced Liver Fibrosis (ELF) test, consisting of three biomarkers of fibrosis [hyaluronic acid, procollagen III N terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP1) combined into a single score.[21] Alleles of the PNPLA3 and TM6SF2 genes seem to predict disease progression rate.[22-24] Researchers have also looked at "omics" approaches, exploring liver-specific miRNAs, circulating free DNA, and sequencing-based interrogation of the microbiome.[25-27]

Examining multivariate tests for predicting NASH progression

The complexity of NASH pathophysiology has highlighted the utility of multivariate tests to help predict NASH disease status. These can include standard clinical chemistry analyses as well as clinical features, such as age, gender, BMI and biomarkers. Examples include: the aforementioned ELF score, BioPredictive's FibroTest (FibroSure), FIB-4, NAFLD Fibrosis Score (NFS), BARD, APRI, Hepascore and the lipid/amino acid "omic" OWLiver test. While most of these tests have been well-validated in hepatitis/fibrosis, their utility in NASH could benefit from additional clinical validation.

In the ongoing search for noninvasive biomarkers, it is important that a biomarker distinguish between individuals with the greatest risk of progression from those with fairly stable disease, and/or determine which subjects would benefit from a particular intervention. To be of maximum utility, such a biomarker test should perform with equivalent predictive power across patient subpopulations, regardless of co-morbidities.
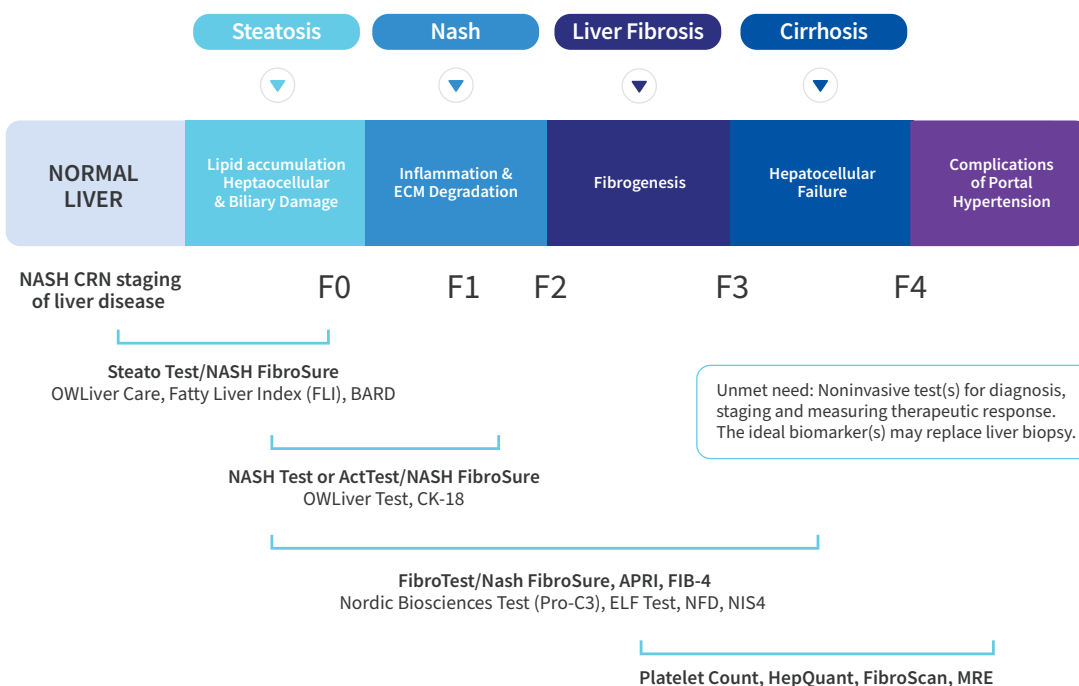
By combining the sensitivity and specificity of a test, the Area Under the Receiver Operating Characteristic (AUROC) scoring system can be used to determine how well a biomarker or multivariate test can distinguish between two diagnostic groups (e.g. diseased vs. normal, or "at risk" vs. not "at risk"). AUROC scores range from 0-1.0, where 1.0 (100%) is a perfect test.

One example of a test in transition from use for hepatitis to NASH is FibroSure and its suite of NASH-related tests. FibroSure is in wide use by hepatologists, with approximately 170,000 tests conducted at Labcorp per year. This test includes 10 biomarkers in combination with age and gender. It provides a well-established, quantitative, surrogate marker of liver fibrosis, hepatic steatosis and inflammation. Data continues to emerge for NASH utility, with a recent study showing an AUROC = 0.69 - 0.73 for a study including diabetics from multiple ethnic groups.[28]

Enhanced Liver Fibrosis (ELF) is another test with rising popularity. ELF is good at diagnosing severe fibrosis in patients with chronic hepatitis B, hepatitis C and HIV and has recently been shown to diagnose advanced fibrosis in subjects with NAFLD as well.[29,30] However, test scores are independently influenced by age and gender, which may confound interpretation of results in the mild to moderate fibrosis range.[31,32] With reported AUROC ranging from 0.82 to 0.90, ELF is increasingly being used in NASH trials for patient screening and as a surrogate marker for fibrosis. Preliminary results are encouraging.[33-35]
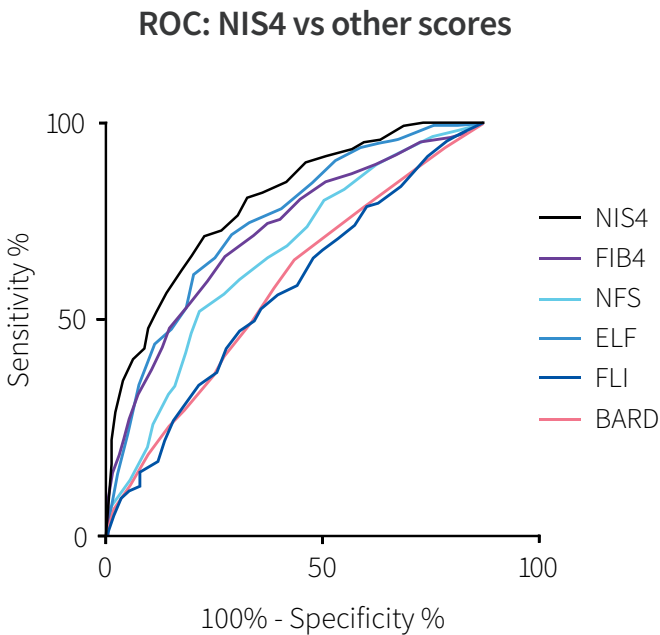
Exploring assays and biomarkers across the NAFLD/NASH continuum

Since NAFLD is a progressive disease, it is important to understand the utility of tests for staging NAFLD and where they are applicable along the continuum.

	Steatosis	Nash	Liver Fibrosis	Cirrhosis
NORMAL LIVER	Lipid accumulation Heptaocellular & Biliary Damage	Inflammation & ECM Degradation	Fibrogenesis	Hepatocellular Failure	Complications of Portal Hypertension

NASH CRN staging of liver disease: F0 F1 F2 F3 F4

Steato Test/NASH FibroSure
OWLiver Care, Fatty Liver Index (FLI), BARD

Unmet need: Noninvasive test(s) for diagnosis, staging and measuring therapeutic response. The ideal biomarker(s) may replace liver biopsy.

NASH Test or ActTest/NASH FibroSure
OWLiver Test, CK-18

FibroTest/Nash FibroSure, APRI, FIB-4
Nordic Biosciences Test (Pro-C3), ELF Test, NFD, NIS4

Platelet Count, HepQuant, FibroScan, MRE

While there are several tools that can provide information at later stages of the disease, there is a clear need for a noninvasive, widely accessible diagnostic tool that can be used to identify patients with early NASH, and in particular, those at risk for more rapidly progressing fibrosis. While drug developers are actively looking for technologies that improve patient identification in the clinical trial setting, they are also interested in tools which can translate and offer clinical value in the real world once drugs are approved.

As part of a comprehensive set of NASH biomarker tests, Labcorp has implemented – through an agreement between Labcorp and the late-stage biopharmaceutical company, GENFIT – an innovative noninvasive, multi-parametric blood-based biomarker test called NIS4TM.[36]

ROC: NIS4 vs other scores

Legend:
- NIS4
- FIB4
- NFS
- ELF
- FLI
- BARD

Y-axis: Sensitivity %
X-axis: 100% - Specificity %

NIS4 has been validated for the identification of NASH patients at an increased risk of progression to cirrhosis (NAS≥4 and F≥2) using measurements from four individual analytes:

miR-34a-5p (serum)

alpha-2 macroglobulin (A2M) (serum)

chitinase-3 like 1 protein (CHI3L1) (serum)

HbA1c (whole blood)

Preliminary data supports use of NIS4 as a progression and prognostic biomarker and suggests that NIS4 may outperform other existing noninvasive tests and scores for patients with "at risk" NASH.[37] Clinical data generated with NIS4 has demonstrated higher overall diagnostic performance (AUROC=0.82) in a head-to-head comparison against other noninvasive tests including FIB4, NFS, and ELF.[38] Importantly, recent data has shown that NIS4 maintains its predictive value across sub-populations, including obese/non-obese and diabetic/non-diabetic, distinguishing this test from others.[38,39]

Partnerships to Support Better Decision Making with NASH: Clinical Trial to Clinical Care Biomarker data generated in the clinical trial setting drives drug development decision making as our industry expands its understanding of a biomarker's future potential as a diagnostic test. These candidate diagnostics will help further define NAFLD/NASH at the molecular level, opening up new avenues for improved medical treatment. In the case of NASH, new in vitro diagnostics will enable earlier patient identification and the need for potential therapeutic intervention, of critical importance to such a global medical concern.

Making headway in NAFLD/NASH – and many other rapidly evolving areas of unmet medical need – will require coordinated efforts and collaborations between sponsors, diagnostic developers, CROs, and ultimately, clinicians. Through these partnerships, disease-defining tools that can advance the use of novel treatments and bring critical medicines to patients faster.

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