



NONALCOHOLIC STEATOHEPATITIS (NASH)

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Non Invasive Biomarkers Of Non-Alcoholic Steatohepatitis and Liver Fibrosis

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Introduction

The dramatic increase in the prevalence of non-alcoholic steatohepatitis (NASH) world-wide¹⁻² and the invasive nature of the liver biopsy as a diagnostic procedure, has necessitated the development of new non-invasive biomarkers.

Although liver biopsy is still considered the gold standard method for diagnosing NASH, the dramatic rise in NASH prevalence means that the resources needed to perform liver biopsies on every patient would be enormous and impractical. The biopsy procedure is invasive for the patient and may be associated with clinical complications such as trauma and bleeding. Additionally, it presents several limitations in terms of accuracy, reproducibility and poor diagnostic performance since a small sample of the liver parenchyma may not be representative of the pathology in the rest of the liver tissue.³⁻⁴ Therefore, the development and validation of noninvasive biomarkers, has become a main focus of interest for screening, stratifying severity and monitoring progression of NASH and liver fibrosis in recent years.

Plasma biomarkers of inflammation, fibrosis, apoptosis and oxidative stress associated with the pathophysiological processes in NASH have been studied and validated in recent years.⁵⁻⁷ One of the main determinants of NASH prognosis is the identification of fibrosis stage and rate of progression.⁸⁻¹⁰ In this sense, the classification of no/minimal fibrosis, significant fibrosis, severe fibrosis or cirrhosis is very useful for selecting patients for treatment studies and monitoring progression/regression of the disease. Current non-invasive methods for assessing fibrosis range from biomarker assays to advanced imaging techniques such as transient elastography.

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Medpace provides customized, high quality central laboratory services to pharmaceutical and biotech clinical development industries. Our four wholly-owned laboratories offer full-service support to seven continents for phase I-IV studies. We have extensive experience from small and simple clinical trials to those that are large, global, and complex.

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With laboratories in the US, Europe, China and Singapore, Medpace Central Laboratories has the global reach and capabilities to conduct NASH studies in concert with Medpace CRO or as standalone service.

Our test menu includes many validated biomarkers associated with NASH: cytokeratin-18 fragments (M30 and M65), apolipoprotein A1, apolipoprotein B, leptin, adiponectin, resistin, free fatty acids, ghrelin, hsCRP, interleukin-6, and tumor necrosis factor-alpha as well as validated assays used in NASH fibrosis scores such as Fibrotest/FibroMax and ELF (Enhanced Liver Fibrosis). In addition, Medpace Central Laboratories can validate new biomarkers quickly and efficiently – typically in an industry leading 10-12 weeks.

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- US and global experience that spans 14 countries, 1,300 subjects and 150 sites
- Wholly-owned imaging core lab to support NAFLD/NASH studies, ensuring imaging components such as MRE, MRI, PDFF, MRS and LMS are seamlessly integrated into the complex structure of the overall trial
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Non Invasive Approaches for the Diagnosis and Monitoring of Non-Alcoholic Steatohepatitis

Inflammation biomarkers

General markers of inflammation such as TNF- α , interleukin(IL) 6, high sensitivity C-reactive protein, monocyte chemoattractant protein-1 (MCP-1), and ferritin, have been reported to be elevated in patients with NASH.¹¹⁻¹² Adipokines and other cytokines have been shown to correlate well with the presence and severity of the disease. In particular, high plasma levels of tumor necrosis factor α (TNF- α) and low levels of adiponectin are found in NASH patients and are associated with a greater degree of liver damage.¹³⁻¹⁶ Adiponectin modifies insulin receptor function and influences hepatocellular free acid metabolism. Circulating levels of adiponectin are negatively associated with insulin resistance, type 2 diabetes and dyslipidemia. Recent findings from a meta-analysis¹⁷ showed that adiponectin is a biomarker of non-alcoholic fatty liver disease (NAFLD) progression to NASH. Adiponectin - leptin ratio in combination with insulin resistance index gave an area under the receiver operating characteristic (AUROC) curve of 0.82 for prediction of disease.¹⁸ Resistin was reported¹⁹ to correlate well with NASH severity in a study of 91 patients. IL 6 and IL8 have also been studied and found to have an AUROC of 0.8 for the prediction of NASH.^{20, 21} The ghrelin-ghrelin O-acyltransferase (GOAT) system has recently been found to play a crucial role in the development of steatosis and its progression to NASH. Furthermore, a significant correlation has been described between ghrelin expression and NASH.

Oxidative stress biomarkers

Oxidative stress is an important mechanism in the pathogenesis of NASH. Malonaldehyde, thiobarbituric acid reactive substances (TBARS) and oxidized low density lipoprotein (LDL) have been associated with the oxidative stress process in patients with NASH.²²⁻²⁴

Apoptosis biomarkers

Markers of apoptosis have been shown to be very useful in differentiating simple steatosis from NASH.²⁵ One of the most promising biomarkers associated with the degree of hepatocyte apoptosis is cytokeratin-18 (CK-18). Activation of caspase 3 results in cleavage of CK18 and circulating levels of CK18 fragments have been investigated and associated extensively with the diagnosis of NASH. Levels of CK-18-M30 fragments have been reported to correlate well with the severity of NASH and with histological progression of the disease.²⁶⁻²⁹ A few studies suggested that CK-18 fragments may have a better performance for the diagnosis of NASH when combined with other tests (e.g. computed tomography (CT) scan, fibroblast growth factor 21, etc.).³⁰ CK18-M65 levels (antibodies which recognize uncleaved CK18) have been also reported to be useful for detecting NASH.³¹

Biomarkers of fibrosis

The severity and progression of liver fibrosis plays a key role for predicting diseased outcomes and for making therapeutic decisions in NASH patients.³³⁻³⁵ In this sense, the development of non-invasive markers of hepatic fibrosis is extremely valuable to identify NASH patients that may progress to cirrhosis. On the other hand liver biopsies have shown poor diagnostic performance for assessing hepatic fibrosis.³²

Several non-invasive methods have been used to measure the degree of extracellular matrix (ECM) turnover as predictors of fibrosis in patients with NASH. Constituents of the extracellular matrix represent a more direct method of assessing fibrogenic activity, as a dynamic process rather than a static one.

Combinations of both clinical markers and ECM turnover are used as NASH fibrosis scores and include the FibroTest and the Enhanced Liver Fibrosis test (ELF). Fibrotest is an algorithm of 13 markers derived from regression analysis including haptoglobin, α 2-macroglobulin, apolipoprotein A1, bilirubin, γ -glutamyl transpeptidase, age and gender^{35, 36}. The use of Fibrotest in NAFLD has shown a sensitivity of 92% and a specificity of 98% for advanced fibrosis detection.⁴¹

The ELF test provides a single score by combining, in an algorithm, the quantitative measurements of three direct serum markers, including hyaluronic acid (HA: a glycosaminoglycan that is produced by hepatic stellate cells), amino-terminal pro-peptide of type III pro-collagen (PIIINP: a marker of early fibrogenesis and inflammation), and tissue inhibitor of metalloproteinase 1 (TIMP-1: which is the circulating inhibitor of matrix metalloproteinases (MMP) enzymes that can enhance fibrogenesis). Studies have confirmed that the ELF test can accurately determine the degree of liver fibrosis³⁷⁻³⁹ with a good discrimination of the different fibrosis stages (mild moderate, severe liver fibrosis and cirrhosis) showing a sensitivity of 0.90 and a specificity of 0.63 for identifying severe fibrosis.⁴⁰ Therefore, the ELF test may be useful to evaluate the impact of treatment targeted to the underlying causes, and in the development of new treatments, such as anti-fibrotic drugs.

Imaging Techniques

Magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) is the imaging technique with the best accuracy and typically used in NASH clinical research settings. This imaging technique is useful to determine hepatic fat content.⁹⁶⁻⁹⁹ Other imaging techniques for the evaluation of hepatic fibrosis are magnetic resonance elastography⁴² and ultrasound-based transient elastography.⁴³ The rationale for these methods is that the collagen deposition associated with fibrosis produces a lattice-like framework that imparts stiffness to the pressure compliance of the liver. Transient elastography (TE) has been well validated showing overall a good accuracy and an excellent diagnostic capability across different liver diseases for cirrhosis.⁴⁴

Emerging Biomarkers

In the past years, a great effort has been made for the identification and validation of novel biomarkers to assess NASH using high-throughput analysis based on genomics, proteomics, and metabolomics.

Several studies have shown differences in the expression of a panel of genes when comparing NASH patients to controls⁽⁴⁵⁾ or patients with other cirrhosis etiologies. Some of the upregulated gene sets found in NASH included those responsible for the platelet derived growth factor, hepatic nuclear factor 3 and the smad4 pathways.⁴⁶

One of the polymorphisms showing stronger association with NASH is patatin-like phospholipase domain-containing 3 (PNPLA3) rs738409. It has been observed that individuals with the rs738409 polymorphism presented a significantly increased risk for developing NASH.⁶¹

There is a different protein expression profile in the serum of NASH patients vs normal and simple steatosis patients.^{47,48} Some of the proteins that showed increased expression in NASH disease include lumican, (a keratan sulphate proteoglycan involved in collagen cross-linking and epithelial-mesenchymal transition^{49,50} and hemoglobin.⁵¹

Changes in glycosylation in serum can be particularly useful as biomarkers of liver dysfunction, since most glycoproteins are made in the liver. In addition, the asialoglycoprotein receptor and the mannose/O-linked betaN-acetylglucosamine receptor in liver are important in clearing aberrantly glycosylated proteins from the serum. Thus, the N-glycome profile will reflect any changes in the liver and can be very valuable biomarker of NASH disease. The GlycoCirrhotest,⁵² the GlycoFibrotest,⁵³ and the GlycoHCC test⁵⁴ are tests that are associated with the detection of cirrhosis, fibrosis and hepatocellular carcinoma respectively, based on differences in N-glycome patterns.

Circulating microRNAs (miRNAs) are very stable in the blood and can be easily quantitated, constituting promising clinically-useful biomarkers of liver injury in the NASH disease. miR-122 is the most abundant miRNA in the adult human liver and its expression is strongly decreased in NASH patients compared to healthy individuals.^{55,56} Several studies have been described an association between miR-122 and advanced fibrosis in the liver.⁵⁷ The expression of miR-21 in the liver has been shown to progressively increase with progression of NAFLD disease.⁵⁸ Some studies reported a correlation between histological results from NASH patients and miR-16 circulating levels.⁵⁹ miR-34a has been found to regulate cell death, oxidative stress and metabolism in the liver and correlates with NAFLD histologic severity.^{56,60}

About the Author

Dr. Hermida received her PhD in Molecular and Cellular Biology from the University of Navarra. She then worked at the Institute of Pharmacology and Therapeutics (University of Brussels) as postdoctoral researcher and, subsequently, joined Medpace as Clinical Laboratory Associate Director. Dr. Hermida has contributed greatly to the understanding of the pathophysiologic process of fibrosis in the context of cardiovascular disease and the development of antifibrotic drugs that are currently in clinical trials.

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