

# NAFLD/NASH and Non-Invasive Biomarkers Predicting Fibrosis

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## The Next Big Epidemic

It is estimated that 1 billion people world-wide are affected by non-alcoholic fatty liver disease (NAFLD), emphasizing the severity of this global epidemic. NAFLD describes a spectrum of metabolic disorders associated with the accumulation of excess fat within the liver (steatosis) which cannot be attributed to other factors of fat deposits such as heavy alcohol consumption or hereditary diseases. NAFLD is a strong risk factor for obesity, diabetes, metabolic syndrome, cardiovascular diseases and even cancer. Furthermore, steatosis can progress into non-alcoholic steatohepatitis (NASH), a more deleterious form of this metabolic disorder associated with liver inflammation and injury, with or without the development of fibrosis.

*Hepatitis: a medical condition characterized by inflammation of the liver.*

Over time, NASH can further degenerate into cirrhosis (chronic liver failure) and hepatocellular carcinoma (liver cancer), making NASH-related cirrhosis the third most common reason for liver transplants.

## Addressing an Unmet Need

Currently there is no NAFLD-specific treatment available. This is in part due to the slow progression of the disease, and lack of regulatory guidance regarding this indication. Although, guidelines for NAFLD therapeutic drug development have yet to be established, the Food and Drug Administration (FDA) along with the American Association for the Study of Liver Disease recommends the reversal of steatohepatitis without the advancement of fibrosis as a surrogate endpoint.

## What is Fibrosis?

Fibrosis is the formation of fibrous connective tissue as a result of injury, commonly referred to as scarring. When hepatocytes (liver cells) become inflamed in response to excess liver fat and cell death, they secrete toxic factors that induce the production of collagen (a connective tissue protein) and other fibrotic factors. Scarring can replace normal healthy liver tissue over the course of several months to years, resulting in stiffening of the liver, and can lead to liver failure and cirrhosis.

## Measuring Hepatic Scar

Fibrosis is measured histologically through tissue stains obtained from a small liver biopsy sample. A pathologist ranks the stage of fibrosis based on a 5-point scale from 0 (no fibrosis) to 4 (cirrhosis). This method is currently considered the gold standard for fibrosis determination, clinical diagnosis of NASH, and assessment of a treatment response. However, there are several shortcomings associated with this quite invasive technique. The biopsy surgery itself represents a source of risk and may even result in serious complications, especially in a compromised population. The biopsy is also impacted by sampling bias as fibrosis may not progress evenly across the organ, therefore one section may have a substantial difference in fibrosis and tissue damage than another. In addition, analysis of the degree of fibrosis is subject to individual interpretation. Finally, there are considerable costs associated with the surgery and assessments which may limit accessibility.

## Non-Invasive Alternatives

Advancements in imaging technology have flourished to support the determination of fibrosis through non-invasive techniques. Taking advantage of the consequence that fibrosis build up leads to liver stiffness, this parameter can be acquired and quantified by ultrasonography through transient elastography (TE) or acoustic radiation force impulse imaging (ARFI); or by magnetic resonance elastography (MRE).

*Elastogram: medical imaging tool that maps elastic properties of soft tissue.*

All three of these techniques rely on the emission of a shear wave (pulse) either mechanically induced or a high-frequency soundwave, which propagates into the liver. The velocity (speed) of the generated echo is proportional to liver stiffness and can be quantified. These methods have been validated against biopsy-proven NASH and are FDA supported to examine liver stiffness.

### Soluble Biomarkers of Fibrosis

While imaging tools require specialized equipment, soluble biomarkers can provide an alternative cost effective, non-invasive manner to monitor fibrosis development for diagnostic and therapeutic purposes.

*Soluble Biomarker: a measurable factor found in bodily fluids (blood, urine, saliva, etc.) from an individual whose presence is indicative of a specific phenomenon such as a disease.*

Many of these biomarkers have been confirmed against the gold standard biopsy.

Although not an exhaustive list, the following plasma biomarkers were found to be highly predictive of fibrosis in NAFLD subjects:

- \_ Cytokeratin-18 fragment – increases upon liver cell death and is associated with NASH inflammation and fibrosis stage
- \_ Hyaluronic acid – a marker of extracellular matrix turnover which is involved in collagen formation
- \_ Transforming growth factor  $\beta$  – activates collagen secretion and fibrosis

In addition, there are a number of soluble biomarkers able to distinguish between earlier stages of NAFLD development such as the progression from simple fatty liver to steatohepatitis.

### Algorithms Predictive of Fibrosis

There are also a host of open-source and proprietary predictive fibrosis and/or NASH algorithms based on a combination of serum liver function test, serum lipids, anthropometrics and key biomarkers. Again, many of these calculations have been validated against both biopsy and imaging studies, and show high sensitivity and specificity:

- \_ APRI (AST/Platelet Ratio Index)
- \_ BAAT (BMI, Age, ALT, Triglycerides)
- \_ BARD (BMI, AST/ALT Ratio, Diabetes)
- \_ ELF™
- \_ FIB-4 (Age, AST, ALT, Platelet)
- \_ FibroTest™
- \_ NAFLD Fibrosis Score
- \_ OWLiver®

### Applying Fibrosis Measurements in Early Clinical Research

An emerging trend in early clinical research is to examine pharmacodynamic effects in a patient population. In this respect, imaging tools and soluble biomarkers are extremely useful to evaluate NAFLD/NASH drug efficacy. These results can be instrumental in the “go/no-go” decision making process.

Furthermore, the prevention of fibrosis progression in NASH is thought to be a likely surrogate endpoint for late-stage clinical studies. Understanding early on, how an investigational product interacts with the fibrotic pathway may be valuable information for the development of therapeutic agents.

### Conclusion

NAFLD/NASH is viewed as the next major health epidemic and with no current medication on the market to treat this disease, it is expected to place a heavy burden on our healthcare systems. Presently, the standard of care for NAFLD/NASH includes invasive biopsy procedures for diagnosis and management. However, imaging techniques or soluble biomarkers can provide robust results that track disease progression as well as therapeutic outcomes over shorter periods, with the opportunity to assess drug efficacy earlier in clinical research.

### Further Reading

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