# Non-invasive fibrosis assessment in non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is estimated to affect approximately 25% of the adult population worldwide.[1] NAFLD encompasses a histopathological spectrum of progressive pathologic conditions ranging from non-alcoholic fatty liver (NAFL) to steatohepatitis (NASH) and cirrhosis.[2]

One of the important features of this disease process is liver fibrosis, which is a pathological process often caused by different types of liver injury, leading to the formation of scar tissue. Some studies showed that NAFLD without advanced fibrosis has a much lower risk of developing liver-related complications and liver-related mortality compared to advanced NAFLD.[3] Although the current "gold standard" for staging liver fibrosis is the liver biopsy, the invasive nature of this method limits its utility in routine clinical practice. Additionally, the potential risks of acute complications limit the usefulness of liver biopsy as a screening tool, and there is consequently considerable research interest in finding suitable alternatives. A broad categorization of some of these alternative tests includes serum biomarkers of liver fibrosis, imaging techniques, genomic markers, or a combination of the above diagnostic tests, for example, Fibrometer-VCTE (using 'vibration-controlled transient elastography' technology together with serum biomarkers) and FAST score (test combining 'FibroScan' with 'aspartate transaminase' [AST] levels). More information regarding each specific test referenced in this article can be found in Supplementary Table 1 http://links.lww.com/CM9/A344 and Supplementary file 1, http://links.lww.com/SLA/C383.

Serum fibrosis biomarkers can be divided into direct or indirect biomarkers. This categorization largely depends on whether the tests refer to biological processes that are directly related to the fibrogenesis (e.g., hyaluronic acid [HA] and tissue inhibitor of matrix metalloproteinase 1 [TIMP-1]) or whether they represent indirect processes associated with risk factors for fibrosis (e.g., the aspartate transaminase-to-platelet ratio index [APRI]). Thus, the latter are not direct measurements of liver fibrosis, making them less accurate for assessing hepatic fibrogenesis compared to direct biomarkers. However, their wider availability to clinicians makes indirect biomarkers of fibrosis valid options for testing and screening patients with NAFLD. That said, direct biomarkers of fibrosis may not be liver-specific, since fibrosis from organs other than the liver may result in false-positive tests. Besides, both direct and indirect biomarkers of fibrosis can be influenced by inflammation, impaired biliary excretion, and decreased kidney function.[4]

Nonetheless, serum fibrosis biomarkers are cost-effective when compared to liver biopsy, have a small risk of sampling error, and can be repeated multiple times, allowing the monitoring of fibrosis. To further increase the accuracy of individual fibrosis biomarkers, researchers have proposed the use of panels by combining multiple biomarkers (as summarized in Supplementary Table 1 http://links.lww.com/CM9/A344). In general, most of these panels (e.g., the Fibrosis 4 [FIB4] index, NAFLD fibrosis score [NFS], and BARD index [components described in Supplementary Table 1 http://links.lww.com/CM9/A344]) do not have good diagnostic accuracy for diagnosing advanced fibrosis, but they have high negative predictive values (NPVs) (over 90%) and, therefore, can be used in clinical practice to rule out advanced fibrosis.[5] Besides, serum biomarker panels are continuously being refined, with a good example being the BARDI score (an enhanced version of the BARD score that adds international normalized ratio [INR] to the panel). The BARDI has better accuracy (with an area under the receiver operating characteristic [AUROC] of 0.88) than

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the BARD score without adding substantial costs, and maintaining the simplicity and accessibility that made BARD a fairly good screening test.[6]

In addition, in the last 5 years, a lot of promising other serum biomarker panels have been developed. The Hepamet score is capable of accurately discriminating advanced fibrosis using common clinical/biochemical parameters while showing improved diagnostic accuracy amongst NAFLD patients aged >65 years.[7] The ADAPT algorithm is a recently proposed panel that combines age, pre-existing diabetes, serum plasma collagen type III (PRO-C3), and platelet count.[8] This algorithm has been developed in an Australian cohort of 150 patients with biopsy-proven NAFLD and then validated in an international cohort of 281 patients with NAFLD. The accuracy of this algorithm is satisfactory (AUROC of 0.86 for the derivation cohort and AUROC of 0.87 for the validation cohort), and it can accurately identify patients with cirrhosis.[8] Currently, our group has developed a nomogram with an improved staging of fibrotic NASH by combining MACK-3[9] (i.e., a promising blood test combining the homeostatic model assessment of insulin resistance [HOMA-IR], serum AST, and cytokeratin-18 levels) with other independent predictors of fibrotic NASH (MACK-3, platelet count, and presence of metabolic syndrome).[10] When testing this novel nomogram against the original MACK-3 performance, our novel nomogram had higher accuracy for diagnosing fibrotic NASH than MACK-3 alone (AUROC about 0.80 vs. 0.75 for MACK-3), whilst also having an improved positive predictive value (PPV).

Unlike serum biomarker tests that measure the levels of certain markers and use algorithms, imaging techniques give a better idea of the area of the liver that is affected by fibrosis. Imaging techniques usually allow an accurate assessment of fibrotic changes in the liver. Currently, there are two different imaging techniques for measuring liver fibrosis or liver stiffness: magnetic resonance-based elastography (MRE) techniques and ultrasound-based elastography techniques.[11] These two imaging techniques can be also used in a complementary fashion. Point shear wave elastography (pSWE), which includes acoustic radiation force impulse (ARFI) imaging, or 2 dimensional (D)-SWE integrated into conventional ultrasound systems are some of the newest imaging approaches to elastography being studied at the moment. Vibration-controlled transient elastography (e. g. VCTE-Fibroscan) is an important ultrasound-based technique and it is currently the most commonly internationally used technique.[12,13] Both VCTE and MRE can provide extra information about the coexistence hepatic steatosis by using the controlled attenuation parameter (CAP) with VCTE or the computed proton-density fat fraction (PDFF) with MRE, respectively.[11,13]

The FibroMeter-VCTE algorithm (combining FibroMeter and Fibroscan) is a promising example of combining serum fibrosis biomarkers with imaging techniques to achieve better accuracy for predicting liver fibrosis in NAFLD (AUROC 0.97).[14,15] However, the major weaknesses of this algorithm are the lack of any external validation and the inclusion of only Caucasian individuals.[15] It is also important to compare this algorithm with other non-invasive tests of fibrosis (biomarkers or imaging techniques alone) in large and well-characterized international cohorts of patients, in order to undertake cost-effectiveness analyses.[15]

The FAST score is another example of a combination of serum biomarkers with imaging techniques, as it combines the VCTE data (FibroScan) with serum AST levels, allowing clinicians to accurately predict not only the severity of liver fibrosis, but also to identify patients with progressive NASH. In particular, the FAST score showed an AUROC of 0.75–0.95 for identifying patients with NASH with a NAFLD activity score (NAS) ≥4 and fibrosis ≥ F2[16] (this test is not mentioned in Supplementary Table 1 http://links.lww.com/CM9/A344 since, unlike the remaining tests, it does not individually stage liver fibrosis).

Beyond imaging tests and serum biomarkers of fibrosis, the development of genomics in the past decade has facilitated the discovery of several new markers. With the genomic information that can be obtained from transcriptomics, including the expression of non-coding RNAs (and more specifically microRNAs [miR]), it should be possible to further improve the screening tests for predicting disease progression in patients with NAFLD, since miRs provide insight into molecular signatures for processes such as liver fibrosis and inflammation.[17] Furthermore, identification of miRs may represent non-coding RNA from diseased tissue, which would be ideal for analyzing dynamic changes in the liver over time. miR-122 measurement is obtained by profiling microRNA and has an AUROC of 0.61 for predicting significant and advanced fibrosis in patients with NASH.[18] MicroRNA profiling is a recent technique based on genomics, and this form of profiling may have a key role in assessing cell-to-cell communication in liver tissue.

Another example of a genomic marker is methylated peroxisome proliferator-activated receptor γ (PPARγ), obtained by analyzing methylated DNA. Methylated PPARγ has been tested on a very small number of patients, and it is of uncertain diagnostic value in NAFLD. We suggest that further research in this area is needed since preliminary data suggests that this is maybe the most accurate non-invasive test to date for diagnosing advanced fibrosis (AUROC 0.91).[19]

The study of single nucleotide polymorphisms (SNPs) has also been a focus in multiple medical specialties. SNPs are single base pairs that are positioned within genomic DNA where different sequence alternatives exist for normal individuals in the population. These minor genetic differences might exert a large impact on the diagnostic effects of certain non-invasive tests of fibrosis and some publications have discussed their specific roles in the development of the disease as well (e.g., the patatin-like phospholipase domain-containing protein-3 [*PNPLA3*] rs738409 C>G p.I148M genetic variant).[20]

In conclusion, there has been a lot of effort to find acceptable non-invasive alternatives to liver biopsy for

diagnosing and staging liver fibrosis in NAFLD. To date, the best-validated simple non-invasive tests with the highest accuracy for ruling out advanced fibrosis are the FIB-4, NFS, and the Hepamet score. The VCTE (Fibroscan) is currently the best-validated imaging technique, and when used in conjunction with serum biomarker tests may be useful for identifying NAFLD patients who require a liver biopsy to more accurately stage the severity of fibrosis. To date, research has shown that combining different serum fibrosis biomarkers can improve accuracy and reliability and most panels mentioned in this article are either established tests that have undergone extensive validation, or are lesser-known potentially better tests that require further validation to prove their worth as diagnostic markers of liver fibrosis in NAFLD.

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## Conflicts of interest

None.

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