Steatosis affects the sensitivity but not the specificity of non-invasive fibrosis tests in non-alcoholic fatty liver disease – implications for screening strategies

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List of abbreviations in the order of appearance: NAFLD: non-alcoholic fatty liver disease**;** NASH: non-alcoholic steatohepatitis; FIB-4: Fibrosis 4 index; NFS: NAFLD fibrosis score

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The clinical and financial burden of non-alcoholic fatty liver disease (NAFLD) is rising, with a global prevalence of approximately 25% (1). NAFLD encompasses a wide disease spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. The progression to advanced fibrosis occurs in only a small subset of patients, but represents a poor prognostic indicator, associated with increased liver-related mortality (2). Screening for liver disease severity is therefore vital to appropriately select patients who require dedicated hepatological follow-up.

Although histology remains the gold standard for disease staging (3), the high prevalence of NAFLD and relatively low severity in the majority of patients, makes liver biopsy an inappropriate first-line diagnostic tool. In recent years, there has been a rapid surge in the development of non-invasive tests for staging of hepatic fibrosis in NAFLD. The strength of a non-invasive test for NAFLD lies in its ability to accurately risk-stratify patients, enabling improved selection of those requiring secondary care referral or further investigations (4).

The Fibrosis 4 index (FIB-4) and NAFLD fibrosis score (NFS) are the most commonly used simple non-invasive scores for fibrosis assessment in NAFLD and are composed of readily available clinical and laboratory variables. They are designed to assess the presence of advanced fibrosis (≥F3) and have dual cut-offs, namely a high cut-off with high sensitivity and a low cut-off with high specificity. Their main utility is in ruling out advanced fibrosis with excellent negative predictive value/likelihood ratio. Therefore, they are increasingly being used as early screening tools in patients with NAFLD for the exclusion of advanced fibrosis. A meta-analysis of the diagnostic accuracy of non-invasive fibrosis tests in NAFLD, incorporating dual cut-off values for FIB-4 and NFS, showed the low cut-off values have excellent specificity (0.97 for both FIB-4 and NFS) and the high cut-off values have relatively high sensitivity (0.84 for FIB-4, 0.80 for NFS) for ruling out and diagnosing advanced fibrosis

respectively (5). A proportion of patients falls in an indeterminate category and needs further testing.

Transient elastography (Fibroscan) is the most validated elastography-based technique for fibrosis assessment in NAFLD (6). Acoustic Radiation Forced Impulse (ARFI) is an alternative elastography technique, which has comparable diagnostic accuracy to Fibroscan for the detection of advanced fibrosis and cirrhosis (7). Falsely elevated liver stiffness measurements (LSM) can occur in a range of conditions, including acute hepatitis, extrahepatic cholestasis, congestive heart failure, hepatic amyloidosis, and recent food intake (8). Obesity and the presence of steatosis can also influence diagnostic accuracy, prompting the development of a dedicated XL probe for obese patients (9).

In their study of 315 Asian patients with biopsy-proven NAFLD, Joo and co-authors, compared the diagnostic accuracy of a variety of non-invasive tests to detect advanced fibrosis and the potential influence of steatosis and other metabolic comorbidities such as obesity and the presence of metabolic syndrome. These tests included acoustic radiation force impulse imaging (ARFI) to obtain LSM, AST to ALT ratio (AAR), AST to PLT ratio index (APRI), FIB-4, NFS and BARD index (Body Mass Index, AST/ALT ratio, Diabetes) (10).

The cohort in the study included patients with an appropriate spectrum of disease; F1-F2 and advanced fibrosis (≥F3) were present in 65.4% and 17.4% of patients respectively, while steatosis severity was equally distributed with a third of patients having mild, moderate and severe steatosis respectively.

Dual cut-off values were used for FIB-4 and NFS to ‘rule in’ or ‘rule out’ patients with advanced fibrosis (10). Comparing the areas under receiver operating characteristic curve (AUROC), the authors identified FIB-4 to have the best diagnostic accuracy for ruling out advanced fibrosis (NPV 94%, AUROC 0.87). The study confirmed that negative predictive

values (NPV) of FIB-4, NFS and ARFI were all high, in contrast to relatively modest sensitivities and positive predictive values (PPV), therefore these tests appear best placed in clinical practice to indicate the absence of advanced fibrosis rather than to diagnose it. As already established, NFS and FIB4 were significantly better than APRI, AAR and BARD (11) and interestingly they had similar negative predictive values with ARFI (10).

The severity of radiological steatosis was the only independent factor affecting the AUROC of FIB-4 and NFS, while the presence of metabolic syndrome did not significantly affect them. Although the AUROC of ARFI numerically decreased with increasing degrees of steatosis, this did not reach statistical significance. Importantly, while the sensitivities, positive predictive values (PPV) and AUROC of these tests were lower in the context of severe steatosis, their NPV were relatively unchanged, permitting their use as screening tests for the exclusion of advanced fibrosis. On the other hand, caution is required to avoid overestimating positive results and LSM in patients with severe steatosis (10).

The authors acknowledge several study limitations, including the cross-sectional study design and failure to include patented non-invasive direct serum markers of fibrosis such as the Enhanced Liver Fibrosis (ELF) panel and FibroTest. The study is also limited to an Asian patient population and thus may not be readily applicable to other patient groups.

The study by Joo showed for the first time that the presence of steatosis affects the sensitivity of FIB4 and NFS in patients with NAFLD, and should be taken into account when interpreting their results. Perhaps most importantly, steatosis does not affect the specificity of NFS and FIB4 and thus their main utility in selecting patients who do not have advanced fibrosis. The effect of steatosis follows earlier reports that these scores should be adjusted in patients who are above 65 years of age and are unreliable in patients younger than 35 years (12). Therefore, as with elastography techniques, factors affecting the diagnostic

accuracy of simple non-invasive tests are increasingly emerging. This key finding is summarized in Figure 1.

This study also confirms the effect of steatosis in LSM measurements as already reported with Fibroscan using the M probe; Petta et al. assessed the impact of steatosis severity on LSM, in 253 patients with biopsy-proven NAFLD (13). Higher median LSM values, assessed by Fibroscan, and a higher rate of false positive LSM results were observed in patients without significant fibrosis (F0-F2) who had severe histological or radiological steatosis, indicating an overestimation of LSM in this context. A potential explanation is that fat droplets in hepatocytes influence the architectural structure of the liver, affecting the propagation time of the vibratory wave transmitted by Fibroscan. In patients with advanced fibrosis, the specificity of LSM was relatively unchanged despite the presence of severe steatosis, supporting the use of Fibroscan as a first-line exclusion tool for advanced fibrosis even in the presence of severe steatosis (13). The diagnostic accuracy of ARFI in lesser fibrosis stages was not available in this study to corroborate these findings. Incorporating the controlled attenuation parameter (CAP) has been suggested as a potential way to overcome the influence of steatosis on the diagnostic accuracy of transient elastography. In a study of 324 patients with NAFLD, higher CAP values were associated with increased rates of false positive LSM, determined by the Fibroscan M probe, particularly in patients with lower stages of fibrosis (F0-2) (14). It was therefore proposed that combining LSM and CAP values may avoid overestimation of liver fibrosis assessed by transient elastography in patients with severe steatosis (14). Future studies are required to validate this.

In conclusion, the study by Joo confirms the excellent negative predictive value of FIB-4 and NFS in ruling out advanced fibrosis in patients with NAFLD, which is not affected by the presence of severe steatosis, thus supporting their use as 'rule out' tests. However, the rate of false positive readings, assessed by simple non-invasive tests or ARFI to a lesser extent, does appear to increase in the context of severe steatosis, therefore caution should be applied when interpreting results in this patient group. Correcting LSM for the presence of steatosis may potentially help to improve diagnostic test accuracy particularly in lesser fibrosis stages, but further investigation is required.

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Figure 1. Pitfalls when using simple non-invasive fibrosis tests in patients with non-alcoholic fatty liver disease (NAFLD); the presence of steatosis influences the sensitivity but not the specificity of the test. Cut-off adjustment is required if age>65 years.