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Non-invasive liver fibrosis tests in non-alcoholic fatty liver disease

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List of abbreviation

Non-alcoholic fatty liver disease (NAFLD); non-invasive tests (NITs); NAFLD Fibrosis Score (NFS); Fibrosis 4 (FIB-4); ultrasound (US); magnetic resonance elastography (MRE); liver stiffness measurement (LSM); Vibration Controlled Transient Elastography (VCTE); negative predictive value (NPV); predictive positive value (PPV); compensated advanced chronic liver disease (cACLD); clinically significant portal hypertension (CSPH)

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease worldwide[1]. Liver fibrosis is the main prognostic factor for liver-related events in NAFLD and, together with necroinflammation and ballooning, is the main feature toward which new therapeutic agents are currently being developed.[2] Advanced fibrosis (≥F3) is an independent risk factor for development of both liver-related events and non-liver related morbidity and mortality.[3]

Although liver biopsy is still considered the gold standard for fibrosis assessment, several easy-to-use and well-performing non-invasive tests (NITs) have been developed in the last decade and are increasingly used in clinical practice.[4]

NITs can be categorized in serum tests and imaging modalities. Serum markers are either indirect or direct. Indirect serum markers consist of combinations of routine laboratory parameters with demographic characteristics such as age or the presence of diabetes. Direct serum markers target the fibrogenic process and the extracellular matrix turnover. NAFLD Fibrosis Score (NFS) and Fibrosis 4 (FIB-4) are the most validated and widely used indirect biomarkers. They have been developed with two cut-offs: a lower one, with high sensitivity, to rule-out and a higher one, with high specificity, to diagnose advanced fibrosis. More recently, it was shown that increasing age impairs the sensitivity of NFS and FIB-4. Thus, new higher rule-out cut-offs have been proposed for patients older than 65 years (2.0 for FIB-4, 0.12 for NFS).[5] The ELF test and more recently pro-C3 either alone or in algorithms (ADAPT-C3) are the most widely used direct serum tests.

Besides serum biomarkers and scores, ultrasound (US) and magnetic resonance elastography (MRE) techniques perform liver stiffness measurement (LSM) that is closely, although not exclusively, linked to liver fibrosis. US elastography methods (Vibration Controlled Transient Elastography [VCTE, Fibroscan™], acoustic radiation force impulse, point shear wave elastography and two-dimensional shear wave elastography are reliable, widespread and affordable

techniques.[6] Although Fibroscan™ is the most validated method, several US machines manufacturers developed elastography software on their devices allowing stiffness assessment during routine US examination. MRE assesses fibrosis panoramically in the whole liver and has a better performance in stratifying intermediate stages, although its high cost makes it feasible almost exclusively for research purposes.[4,7]

Although NITs clearly changed the clinical management of NAFLD/NASH, none of them is a perfect and infallible diagnostic and/or prognostic tool. Many factors can influence the reliability of measurements and should be considered. False positive results are common with NFS and FIB4 in patients>65 years old[5], with ELF in patients with extra-hepatic inflammatory or fibrotic diseases and with US-elastography techniques in patients with obesity, acute hepatitis or cholestatic diseases.[8]

In this scenario, combination of independent NITs, together with the use of specific tests in different clinical settings is highly recommended to optimize the diagnostic and prognostic stratification of NAFLD/NASH patients.[9]

In this editorial, we briefly examine the role of NITs in the investigation and management of patients with NAFLD in different clinical settings and scenarios (Table 1).

1. NITs in Primary Care Setting

The goal in primary care settings, facing populations with low prevalence of advanced fibrosis, is to perform a risk stratification that guides referrals in secondary care. NITs with high sensitivity, high negative predictive value (NPV), low cost and wide availability are the best choice in such settings for successfully ruling out advanced fibrosis.

FIB4 and NFS have high accuracy in ruling out advanced fibrosis with a sensitivity of 77% and 73% respectively (FIB4 < 1.3 or NFS < -1.455).[8] In patients with indeterminate scores (1.3<FIB4< 3.25 or -1.455<NFS<0.676) guidelines recommend a two-step approach consisting of an additional more specific test: Fibroscan (or other elastography techniques) or ELF. [4,10]

Whereas US-elastography does not yet have broad availability in primary care, ELF is available in most laboratories in western countries and its cost is affordable considering that it only needs to be performed on a selected population. A recent metanalysis confirmed that the diagnostic accuracy of ELF for advanced fibrosis is acceptable with an AUROC of 0.83. Among proposed cut-

offs (7.7 and 9.8 low and high cutoffs respectively), 9.8 has a sensitivity of 65% and specificity of 86% for advanced fibrosis. [11]

We recently showed that a two-step approach of FIB-4 followed by ELF resulted in a reduction of unnecessary referrals by 81% and a 5-fold increase in the diagnosis of advanced fibrosis compared to standard of care.[12] The recent EASL guidelines recommend that patients at risk of NAFLD with high FIB-4 or NFS and patients with intermediate FIB-4 or NFS and high Fibroscan (>8 KPa) or ELF test (>9.8) should be referred to secondary care for further evaluation.[4]

2. NITs in Secondary Care Setting

Patients referred to secondary care setting are more likely affected by NASH and advanced fibrosis than in the general population. NITs in this setting should guarantee high specificity and high predictive positive value (PPV) to reliably identify patients eligible for invasive diagnostic/prognostic and therapeutic interventions.

Whereas FIB-4 and NFS have a sub-optimal specificity for diagnosing advanced fibrosis, VCTE and other elastography techniques have a better performance in diagnosing patients with advanced fibrosis or compensated advanced chronic liver disease (cACLD).[7] The term cACLD has been introduced in the Baveno VI consensus and identifies asymptomatic patients with advanced fibrosis or cirrhosis who are at risk of developing CSPH.[13] Baveno VI and Baveno VII consensus proposed a low VCTE cut-off of <10kPa to rule out and a high cut-off of >15kPa to diagnose cACLD.[13,14] A recent real-world validation of those cut-offs, showed that the optimal cut-offs to diagnose cACLD in patients with NAFLD are <8 KPa (sensitivity >90%) and >12 KPa (specificity 88.2%). [15]

The Baveno VII consensus[14] and the recent EASL guidelines[4] agreed that LSM by VCTE is the reference NIT for the diagnosis of CSPH in patients with cACLD, although invasive HVPG measurement still remains the gold standard. In non-obese patients with NASH-related cACLD, LS by VCTE ≥25 kPa is sufficient to rule in CSPH with specificity and positive predictive value of >90%. Furthermore, either LS between 20-25 kPa with platelets count <150000/ml or LS between 15-20 kPa with platelets count <110000/ml signify high risk of clinically significant portal hypertension (CSPH) (>60%).[14,16,17]

2D-SWE diagnostic performance for CSPH is slightly inferior with an AUROC of 0.88 however there is a high variability of cut-offs in different studies. The 2D-SWE low cut-off of 14 kPa allow to rule-out CPSH with 91% sensitivity, whereas the high cut-off of 32 kPa has a specificity of 89% for diagnosing CSPH.[18]

NITs have also demonstrated a prognostic role in patients with NAFLD. A recent longitudinal study showed that APRI, NFS and FIB-4 changes over time are significantly associated with fibrosis progression. A unit change in FIB-4 or NFS reflect a mean fibrosis stage progression of 0.26 and 0.19, respectively[19]. Liver decompensation in patients with NAFLD-related cACLD is independently predicted by baseline LSM (VCTE) (HR 1.03, 95%CI 1.02-1.04, p<0.001), especially if LSM > 21kPa[13] (HR 3.71, 95%CI 1.89-6.78; p<0.001) and by Δ-LSM (median interval between baseline and follow-up LSM was 37 months, HR 1.56, 95%CI 1.05-2.51, p=0.04). Baseline LSM and Δ-LSM also independently predict HCC occurrence but not extrahepatic complications.[20]
In a retrospective longitudinal study with a mean follow-up of 81 months, both baseline NFS and FIB-4 predicted liver-related events (HR 2.77 and 1.68 respectively), whereas they had a good performance for HCC (HR=1.94) and overall mortality (HR=2.00) respectively.[21]

NITs are increasingly used in therapeutic clinical trials in NASH either to pre-select patients for liver biopsy or to assess therapeutic response. All phase IIb and III therapeutic trials in NASH require a liver biopsy for inclusion and also to evaluate surrogate endpoints of therapeutic response, namely resolution of steatohepatitis or improvement in fibrosis. Patient eligibility for trials is determined upon the presence of NASH (NAS≥4) with a fibrosis stage of 2 or 3; also defined “fibrotic NASH”. There are no validated NITs for the diagnosis of NASH, however new scores have been proposed for diagnosis of fibrotic NASH: MACK-3, NIS-4 and FAST.[4]
In terms of treatment response, improvement in NITs are secondary or exploratory endpoints in current clinical trials. In the lanifibranor phase IIb trial, there was a reduction of LSM (-1.7 and -1 kPa for 800mg and 1200mg arms respectively), whereas there were no significant changes in FIB4 and ELF.[22] In the obeticholic acid phase III trial, among patients in the high dose therapeutic arm (25mg), during 18 months of follow up there was a significant reduction of LSM (-10.7%) and FIB4 (-6.7%).[23] In the semaglutide phase II trial there was a dose-dependent reduction of both LSM (24%) and ELF (3.6%) in therapeutic arms whereas there wasn’t any change in the placebo arm.[24]
Further and larger studies are needed to confirm these results and to establish the role of NITs in

the assessment of the therapeutic response.

Conclusions

In conclusion, NITs have changed the management of patients with chronic liver diseases. In the field of NAFLD, future developments are likely to include their widespread use in risk stratification of patients in primary care settings and for the evaluation of treatment response when approved pharmacological treatment becomes available.

Table 1. Role of non-invasive tests in the management of patients with NAFLD in different clinical settings.

NIT	Primary Care	Secondary Care - Diagnosis	Secondary Care - Prognosis
FIB4 and/or **NFS**	High sensitivity and NPV[4,12] • Low cut-off (FIB4<1.3 or NFS < -1.455): Rule-out advanced fibrosis (NPV>90%) • Indeterminate result (1.3<FIB4<3.25* or -1.455<NFS<0.676*): Re-test with a more specific NIT (ELF or VCTE/2D-SWE) • High cut-off (FIB4>3.25* or NFS>0.676*): Referral to secondary care	Low specificity and PPV (<70%)[4] Their use would lead to false positive results and consequently inappropriate invasive tests and therapies	Baseline FIB4 and NFS are associated with development of liver related events. [21] Further studies are needed to better define and quantify prognostic role
ELF	Test patients with indeterminate result at FIB4 and/or NFS[12] • ELF<9.8: Rule-out advanced fibrosis • ELF>9.8: Referral to secondary care	Good diagnostic performance of advanced fibrosis. ELF>9.8 specificity 86%, ELF>11.3 specificity 96% Better performance if combined with other NITs (VCTE).[11]	Baseline ELF is associated with development of liver related outcomes.[25] Further studies are needed to better define and quantify prognostic role

VCTE (Fibroscan)	Not widely available in primary care clinics Training required	High specificity and PPV • LS < 8 kPa: rule out cACLD (sensitivity and NPV>90%) • 8<LS<12 kPa: indeterminate result. Consider diagnostic liver biopsy • LS > 12 kPa: diagnosis of cACLD (specificity 88.2%) Consider liver biopsy for experimental therapy eligibility	†15<LS<20 with platelets <110000/ml, and 20<LS<25 with platelets <150000/ml are at high risk of having CSPH[14] †LS>25kPa: diagnosis of CSPH (specificity and PPV >90%)[4,14,17] LS > 21 kPa and positive Δ-LSM are associated with higher risk of liver decompensation events in cACLD[20]
MRE	Costly and time consuming Not suitable for 1st line evaluation of general population	High diagnostic performance for advanced fibrosis (sensitivity of 0.905 and specificity of 0.933 for cut-off of 3.62 kPa)[4] Consider MRE in patients with indeterminate result at VCTE/2D-SWE Consider for research purposes	Prognostic role of MRE has been explored in few studies. Further studies are needed to better define and quantify prognostic role

**consider as upper limit for FIB4 2.0 and for NFS 0.12 in patients older than 65y*, †non-obese NAFLD patients

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