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Liver fibrosis in non-alcoholic fatty liver disease - diagnostic challenge with prognostic significance

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most

common liver disease in the Western world, with a prevalence of 20%. In a subgroup of patients, inflammation, ballooning degeneration of hepatocytes and a varying degree of fibrosis may develop, a condition named non-alcoholic steatohepatitis. Advanced liver fibrosis (stage F3) and cirrhosis (stage F4) are histologic features that most accurately predict increased mortality in both liver-related and cardiovascular diseases. Patients with advanced fibrosis or cirrhosis are at risk for complications such as hepatocellular carcinoma and esophageal varices and should therefore be included in surveillance programs. However, liver disease and fibrosis are often unrecognized in patients with NAFLD, possibly leading to a delayed diagnosis of complications. The early diagnosis of advanced fibrosis in NAFLD is therefore crucial, and it can be accomplished using serum biomarkers (*e.g.*, the NAFLD Fibrosis Score, Fib-4 Index or BARD) or non-invasive imaging techniques (transient elastography or acoustic radiation force impulse imaging). The screening of risk groups, such as patients with obesity and/or type 2 diabetes mellitus, for NAFLD development with these non-invasive methods may detect advanced fibrosis at an early stage. Additionally, patients with a low risk for advanced fibrosis can be identified, and the need for liver biopsies can be minimized. This review focuses on the diagnostic challenge and prognostic impact of advanced liver fibrosis in NAFLD.

Key words: Non-alcoholic fatty liver disease; Fibrosis; Mortality; Biomarkers; Elastography

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Core tip: Non-alcoholic fatty liver disease (NAFLD) has a prevalence of 20% in the Western world. A subgroup of NAFLD patients develops inflammation and fibrosis or cirrhosis. This condition, named non-alcoholic steatohepatitis, is associated with increased

mortality in liver-related and cardiovascular diseases. Advanced liver fibrosis is the histologic feature that most accurately predicts future morbidity; therefore, early detection of advanced fibrosis is crucial. Serum biomarkers, such as the NAFLD Fibrosis Score, Fib-4 Index or BARD, or non-invasive imaging techniques, such as transient elastography, may identify patients with a low risk for advanced fibrosis and minimize the need for liver biopsy.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the Western world. It has a global estimated median prevalence of 20%, ranging from 6.3% to 33% depending on the population, ethnicity, and assessment method for diagnosis^[1,2]. Most patients have "simple steatosis" or non-alcoholic fatty liver (NAFL) without inflammation, tissue damage or fibrosis. However, in a subgroup of patients, non-alcoholic steatohepatitis (NASH), fibrosis and/or cirrhosis may develop. The prevalence of NASH in the general population is unknown, but it is estimated to be 3%-5%^[1].

NAFLD is closely related to obesity, type 2 diabetes mellitus and dyslipidemia, with a prevalence ranging from 50% to 90% in these patient groups^[2-4]. The current dogma implicates that NAFL is a stable disease with or without a very slow, histologic progression over time, whereas NASH may advance to fibrosis and cirrhosis^[1,2,5-8]. However, several recent studies have challenged this view, demonstrating histological progression also in NAFL patients without histologic signs of NASH at baseline^[9-12].

It is crucial for clinical management to obtain a prompt diagnosis of patients with advanced fibrosis because they carry an increased risk for developing complications such as hepatocellular carcinoma (HCC) or esophageal varices^[5]. Consequently, patients with NAFLD who are diagnosed with advanced fibrosis or cirrhosis should be included in surveillance programs that utilize ultrasonography and endoscopy. In addition, recent data have noted that advanced fibrosis in NAFLD predicts not only liver-related mortality but also increased mortality due to cardiovascular events^[13]. Therefore, patients with an increased risk for future complications must be identified sufficiently early to enable closer monitoring compared with those with a more benign course.

The prevalence of NAFLD is increasing, possibly due to the growing number of obese individuals in the

Western world. In Ohio, United States, the number of patients with NAFLD among those listed for liver transplantation rose from 0% to 26% from 2000 to 2012. Similarly, the proportion of transplanted patients with NAFLD as the main diagnosis increased from 0% to 23.4% during the same time period^[14].

Fibrogenesis in NAFLD is a critical process that affects clinical management. This review focuses on the natural course, diagnostic challenge and prognostic impact of advanced liver fibrosis on NAFLD.

HISTOPATHOLOGIC CLASSIFICATIONS OF NASH AND FIBROSIS

The current definition of NAFLD requires evidence of hepatic steatosis without signs of secondary hepatic fat accumulation due to alcohol consumption, steatogenic medication or hereditary disorders^[5]. In the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines, NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury (ballooning of the hepatocytes), whereas NASH comprises the presence of hepatic steatosis plus inflammation with ballooning, with or without fibrosis^[5].

In a pioneering publication from 1999, Matteoni *et al*^[15] presented the first diagnostic criteria to categorize NAFLD into four different subtypes: NAFLD type 1 with fatty liver alone; type 2 with fatty liver plus lobular inflammation; type 3 with fatty liver plus ballooning degeneration; and type 4 with fat accumulation, ballooning degeneration and either Mallory-Denk bodies or fibrosis. In that study, fibrosis staging was not further evaluated. They demonstrated that cirrhosis developed in 21%-28% of patients whose liver biopsies displayed NAFLD type 3 or 4, whereas only 4% of patients with NAFLD type 1 and none of those with type 2 had cirrhosis development after a mean follow-up of 10 years. There was a trend for increased liver-related mortality in patients with subtypes 3 and 4 compared with subtypes 1 and 2. The subtypes 3 and 4 are those that we consider today to represent NASH^[16].

There was a need for a more quantifiable grading and staging system, which was addressed by Brunt *et al*^[17] during the same year. They developed a semi-quantitative system to grade NASH activity and stage NASH fibrosis. In their study, three grades of necro-inflammatory changes (mild, moderate and severe) were presented along with a staging score reflecting both the extent and location of fibrosis. Fibrosis Stage 1 encompassed perisinusoidal fibrosis, Stage 2 encompassed perisinusoidal with periportal fibrosis, Stage 3 included bridging fibrosis and Stage 4 included fully developed cirrhosis. The Brunt grading and staging system was based on the diagnosis of NASH depending on several histological features and not only one single attribute.

Table 1 Fibrosis staging in non-alcoholic fatty liver disease according to the Nonalcoholic Steatohepatitis Clinical Research Network Pathology Committee^[18]

Perisinusoidal or periportal fibrosis	1
Mild perisinusoidal fibrosis (zone 3)	1A
Moderate perisinusoidal fibrosis (zone 3)	1B
Portal/periportal fibrosis	1C
Perisinusoidal and portal/periportal fibrosis	2
Bridging fibrosis	3
Cirrhosis	4

However, an increasing need for a more detailed scoring system did emerge. Such a system should enable assessment of the various histologic features during therapy and encompass the whole spectrum of NAFLD. Thus, the NASH Clinical Research Network (CRN) Pathology Committee performed a thorough univariate and multivariate analysis on the associations between the different histologic features observed in NASH and the diagnosis of NASH according to the Pathology Committee. The result was a scoring system of both NASH activity (Grade), and collagen deposition plus architectural remodeling (Stage). The grading system, the NASH Activity Score (NAS), was the unweighted sum of three histological components: steatosis (0-3), lobular inflammation (0-3) and ballooning degeneration (0-2). It ranged from 0 to 8. NAS includes the features of active injury that are potentially reversible^[18]. Additionally, the fibrosis staging system of Brunt *et al*^[17] was further developed. In the NASH CRN system, the fibrosis score for stage 1 was subdivided into delicate (1A) and dense (1B) peri-sinusoidal fibrosis, whereas stage 1C was defined as portal fibrosis without concomitant peri-sinusoidal fibrosis^[18] (Table 1). The NASH CRN fibrosis staging system is one of the most validated systems currently available^[19].

NAS has become widely accepted and used in clinical trials^[20-22], and it is recommended as an endpoint in trials evaluating short-term treatments of NASH^[19]. Thus, NAS has proven useful for comparative analyses and interventional studies but less beneficial as a diagnostic tool of NASH because neither fibrosis nor the location of lesions is included^[23]. However, some authors still consider the numerical composite score of the NAS value to define whether NASH is present. However, in the original study by Kleiner *et al*^[18], 16% of patients with a $NAS \geq 5$ did not meet the diagnostic criteria for NASH. Thus, NAS cannot be considered as a substitute for the diagnosis of NASH^[23]. Additionally, NAS was shown to be a poor predictor of fibrosis progression; therefore, it has also been questioned as a suitable endpoint for clinical studies^[24].

Later, Younossi *et al*^[25] evaluated various pathologic criteria for the diagnosis of NASH, comparing inter-observer agreement and the ability to predict liver-related mortality. They demonstrated that the original

Matteoni criteria for NASH was a better predictor for liver-related mortality than both the Brunt criteria and NAS^[25]. In their study, fibrosis scoring was simplified into four categories: (1) centrilobular/perisinusoidal; (2) centrilobular plus periportal; (3) bridging fibrosis; and (4) cirrhosis. Among individual features, fibrosis stages 3-4 (advanced fibrosis) showed the best independent association with liver-related mortality. These data indicate that fibrosis is a better predictor of liver-related mortality than NAS, which only grades steatosis and necro-inflammatory activity^[25].

Recently, a new algorithm was developed by the Fatty Liver Inhibition of Progression Pathology Consortium based on a composite score evaluating Steatosis, Activity and Fibrosis (SAF score). Initially, this score was developed for classifying NAFLD in morbidly obese patients^[26], but it has now been validated in a cohort of patients with NAFLD and metabolic syndrome^[27]. In contrast to NAS, the SAF score separates steatosis from necro-inflammation, two features that may have distinct prognostic potential. The SAF scores steatosis (0-3), ballooning degeneration (0-2), lobular inflammation (0-2), and fibrosis (0-4). NASH is present when steatosis is present and when both features of activity (ballooning and lobular inflammation) display at least grade 1. Interestingly, independent from the classification of whether NASH is present, the overall histological severity of disease is scored separately as mild disease ($A < 2$, $F < 2$) or significant disease ($A \geq 2$, $F \geq 2$), also considering fibrosis staging. Therefore, NAFLD patients with less fat but still advanced fibrosis, and without necro-inflammation, would be classified as having "significant disease", even though they did not fulfill the criteria of NASH. Thus, the fibrosis component has an impact on the SAF score that may be relevant for long-term prognostication, although the association between the SAF score and long-term liver-related mortality has not yet been evaluated.

NATURAL COURSE OF FIBROSIS DEVELOPMENT IN NAFLD

Progression of liver fibrosis is observed in one-third of patients 4-5 years after the first liver biopsy. Variables associated with progression are obesity and body mass index (BMI)^[28]. In a study of 106 patients with NAFLD, fibrosis stage progressed in 37%, remained stable in 34% and regressed in 29%. Diabetes and body mass index were associated with fibrosis progression^[29]. In a meta-analysis comprising ten studies with 221 patients, 37.6% had progressive fibrosis over a mean follow-up time of 5.3 years. In this analysis, only age and inflammation in the initial biopsy were independent predictors of fibrosis progression^[30]. Thus, approximately one-third of NAFLD patients progress in the fibrosis stage during a five-year follow-up, some of whom have a more rapid course.

For a long time, patients with simple hepatic steatosis without inflammation were considered to have a benign course with little progression, whereas progression to cirrhosis was observed only in patients with steatohepatitis^[1,2,5-8,31,32]. However, this view has been modified in studies demonstrating that steatosis alone may progress to NASH with fibrosis^[12].

In a study from Hong-Kong, paired liver biopsies were evaluated, and 23% of patients with simple steatosis developed NASH over a three-year period, whereas the regression of NASH was only observed in one patient^[9]. Weight loss and reduction in waist circumference were associated with stable disease activity and non-progressive fibrosis.

In a study on 108 NAFLD patients who underwent serial liver biopsies with a median interval of 6.6 years, 42% had fibrosis progression. Diabetes was significantly associated with fibrosis development. There was no significant difference in the proportion exhibiting fibrosis progression between patients with NAFL or NASH at index biopsy (37% vs 43%)^[11].

In a recent study, 25 patients with NAFL and 45 patients with NASH and/or advanced fibrosis were followed with repeat liver biopsy for an average of 3.7 years. Among the patients with NAFL, 16 patients (64%) developed NASH, eight of which had severe ballooning and six with bridging fibrosis. Mild lobular inflammation or any degree of fibrosis conveyed a higher risk of progression than simple steatosis alone. Older age and deterioration of metabolic risk factors were associated with a more rapid progression^[33].

A recent meta-analysis evaluated 411 patients with biopsy-proven NAFLD from 11 cohort studies (150 patients with NAFL and 261 patients with NASH). In the whole cohort, 33.6% of patients had fibrosis progression. This result was also observed in patients with NAFL but at a slower pace. In those with NAFL, it took an average of 14.3 years to progress one stage in fibrosis score; however, in those with NASH, the time to progress with one stage was halved to 7.1 years^[10].

Taken together, the data indicate that fibrosis progression is also observed in patients with NAFL, particularly in those with mild inflammatory changes, delicate fibrosis, older age or deterioration of metabolic risk factors. However, patients with NASH have a more rapid course, with a significant risk for liver-related mortality^[6].

PROGNOSTICATION OF NAFLD

Several studies have evaluated the overall and disease-specific mortality in NAFLD. Liver disease is the third leading cause of death in NAFLD after cardiovascular disease and malignancy^[34]. In a 28-year follow-up of 118 Swedish patients with NAFLD, there was a 69% increased risk of death compared with the total population, which was adjusted for sex, age, and calendar period. Those with simple steatosis had a 55% increased risk; however, in those with

NASH, the risk was increased to 86%^[35]. In another Swedish cohort study of 129 patients with biopsy-proven NAFLD with a mean follow-up of 13.7 years, survival and causes of death were compared with a matched reference population. Mortality was increased in patients with NASH but not in those with NAFL. The major causes of death were cardiovascular and liver-related events^[31]. In a recent paper, these two cohorts were merged in a study comprising 229 patients with a mean follow-up of 26 years. In that study, advanced fibrosis (stage 3-4) was an independent predictor of overall and disease-specific mortality, whereas NAS > 4 was not associated with increased mortality^[13].

These results indicate that fibrosis has a strong association to long-term outcome, and they are in line with previous studies. The original NASH criteria presented by Matteoni *et al*^[15], which include fibrosis staging, shows a better association with liver-related mortality than both the NAS or Brunt criteria. When evaluating distinct pathologic features, advanced fibrosis shows the best independent association with liver-related mortality^[25].

Interestingly, non-invasive biomarkers of advanced fibrosis can also predict mortality. Three-hundred two patients with NAFLD were sub-grouped as low-risk (60%) and intermediate-to-high risk individuals (40%), according to the non-invasive NAFLD fibrosis score (NFS). In a multivariate analysis, a higher NFS at baseline was significantly predictive of death^[36]. In another retrospective, multicenter cohort study of 320 patients with biopsy-proven NAFLD, non-invasive scoring systems correlated with an increased risk for liver-related complications or death, and NFS had the best performance to identify patients at risk^[37].

Non-invasive biomarkers of liver fibrosis were also tested in 2312 patients with type 2 diabetes and/or dyslipidemia, and the patients were followed prospectively for 5-15 years. Biomarkers indicative of advanced fibrosis were associated with overall mortality in a multivariate Cox model^[38].

In studies from tertiary centers, selection bias leads to a high proportion of patients with advanced fibrosis or NASH. By contrast, population-based studies on NAFLD demonstrate a considerably smaller proportion of patients with advanced fibrosis. In the National Health and Nutrition Examination Survey conducted in 1988-1994, mortality data were followed-up through December 31, 2006. NAFLD was diagnosed on ultrasonography examination in 3792 individuals, comprising 34% of the total cohort, but the NAFLD fibrosis score indicative of advanced fibrosis (NFS > 0.676) was only observed in 3.2%, whereas 71.7% had NFS consistent with a lack of significant fibrosis (NFS < -1.455). After a median follow-up of 14.5 years, NAFLD in general was not associated with higher mortality. However, subjects with a high NFS indicative of advanced fibrosis had a 69% increase in mortality, mostly from cardiovascular events, and independent of other known risk factors^[39].

The major causes of death in NAFL are cardiovascular disease and cancer^[8]. In type 2 diabetes, the diagnosis of NAFLD is associated with an increased incidence of cardiovascular events, and this association was independent of other metabolic risk factors, suggesting that NAFLD by itself confers an increased risk for cardiovascular disease^[40]. Interestingly, 86% of the diabetic NAFLD patients had normal liver enzymes^[40]. The same authors also investigated carotid artery intima-media thickness (IMT) in patients with diabetes and NAFLD, and they found a strong association with the degree of hepatic steatosis, necroinflammation, and fibrosis. After adjustment for other potential confounders, the grade of NASH activity and stage of fibrosis independently predicted carotid IMT in a logistic regression analysis^[41].

Taken together, the data indicate that advanced fibrosis is a strong predictor of increased overall and liver-related mortality in NAFLD and that NAFLD itself is an independent risk factor for cardiovascular disease.

NON-INVASIVE DIAGNOSIS OF ADVANCED FIBROSIS IN NAFLD

Clinical and laboratory variables (serum biomarkers)

Clinical predictors of advanced fibrosis in NAFLD are male sex, Caucasian ethnicity, diabetes mellitus, obesity and increased aspartate transaminase (AST) or alanine aminotransferase (ALT) levels^[42,43]. However, there is a poor correlation between ALT levels and NASH, or the stage of fibrosis^[44]. In a study of 222 patients with NAFLD, 23% had normal ALT. The proportion of patients with advanced fibrosis was similar among those with normal and elevated ALT^[5].

AST is a better predictor for advanced fibrosis than ALT. In early studies on NAFLD, an AST/ALT ratio > 1 was found to be associated with advanced fibrosis^[43]. Another test that includes AST is the AST: platelet ratio index (APRI)^[45], with a negative predictive value of 94% to exclude advanced fibrosis (F3-4) in NAFLD. Another laboratory parameter related to fibrosis is serum ferritin. In a study of 628 patients with biopsy-proven NAFLD, elevated serum ferritin (> 1.5 × ULN) was associated with the diagnosis of NASH, high NAS, and development of advanced hepatic fibrosis^[46].

For clinical decision-making with the purpose of identifying patients with an indication for liver biopsy, several composite scores have been explored. In 2007, the NAFLD fibrosis score (NFS), based on six routine clinical parameters, was developed and validated in > 700 patients with biopsy-proven NAFLD^[47]. The parameters are age, BMI, the presence of diabetes or impaired fasting glucose, the AST/ALT ratio, platelet count and albumin. A score below -1.455 has a high negative predictive value to exclude advanced fibrosis (stage 3-4), whereas a score > 0.676 predicts advanced fibrosis. Only patients in the indeterminate

range between these two values need to undergo liver biopsy, thus avoiding up to 75% of biopsies^[47]. In a meta-analysis from 2010, the pooled AUROC, sensitivity and specificity of NFS for the detection of NASH with advanced fibrosis was 0.85 (0.80-0.93), 0.90 (0.82-0.99), and 0.97 (0.94-0.99)^[6]. The NFS is endorsed by current American guidelines as a screening test to exclude low-risk individuals from further investigations^[5].

Another simple score that was developed to exclude the presence of advanced fibrosis in patients with NAFLD is the BARD score. It is based on three variables combined in a weighted sum (body mass index ≥ 28 represents 1 point, the AST/ALT ratio ≥ 0.8 represents 2 points, and diabetes mellitus represents 1 point). A score of 2-4 had an odds ratio of 17 (confidence interval: 9.2-31.9) to determine advanced fibrosis and a negative predictive value of 96%.

The FIB-4 index was first developed for patients with hepatitis C and HIV but has been validated and compared with other non-invasive markers in a cohort of 541 NAFLD patients^[48]. FIB-4 is based on patient age, AST, ALT, and platelet count. This index was superior to both NFS and BARD in this specific cohort. An FIB-4 index ≥ 2.67 had an 80% positive predictive value, and a value ≤ 1.30 had a 90% negative predictive value to diagnose advanced fibrosis. These results were also confirmed in a Japanese study^[49].

Recently, a new non-invasive score, the non-invasive Koeln-Essen-index (NIKEI) based on age, AST, AST/ALT ratio, and total bilirubin, was compared with the FIB-4 index^[50]. NIKEI had a slightly better AUROC of 0.968 than 0.929 for the FIB-4 index. The authors concluded that NIKEI can reliably exclude advanced fibrosis in subjects with NAFLD, particularly if used in conjunction with the FIB-4 index.

More complex scores include markers related to matrix turnover. Guha *et al*^[51] developed the "Enhanced Liver fibrosis panel" (ELF), a panel of tissue inhibitor of matrix metalloproteinase 1 (TIMP 1), hyaluronic acid (HA), and aminoterminal peptide of pro-collagen III (P3NP). The ELF has an area under the curve (AUC) of 0.90 for distinguishing severe fibrosis. The addition of more variables from the NAFLD Fibrosis Score (NFS) improved the diagnostic performance of the ELF, yielding an AUC of 0.98, but these results have to be confirmed in larger studies. The ELF has also been validated in pediatric patients with NAFLD^[52].

Another composite score is the Hepascore, originally developed for chronic hepatitis C, which includes six variables (age, sex, α2- macroglobulin, hyaluronic acid, bilirubin, γ-glutamyltransferase)^[53]. The Hepascore seems to be more accurate than the BARD and APRI but is similar to the FIB-4 score^[53].

Proprietary panels have also been developed to evaluate fibrosis in NAFLD. First, the FibroMeter™ -NAFLD includes seven variables (age, body weight, ferritin, platelets, AST, ALT, and fasting glucose). Its AUROC to predict significant fibrosis (F2-4) was better

than that of the NAFLD fibrosis score, however with similar accuracy to predict cirrhosis^[54]. Second, the FibroTest™, which is based on a combination of age, gender, bilirubin, γ -glutamyltransferase, apolipoprotein A1, haptoglobin, and α 2-macroglobulin, has a performance similar to the FibroMeter™-NAFLD^[55].

Adams *et al*^[56] compared the performance of several scores and concluded that more complex scores (NFS, Fibrotest, Hepascore) perform better than simple ones (BARD). However, all scores based on biochemical parameters have modest accuracy for determining significant fibrosis (F2-4) with predictive values less than 90% in the majority of subjects, whereas the accuracy to exclude advanced fibrosis (F3-4) is better^[56].

Which of these scores should be used in clinical practice? All of them have high negative predictive values to exclude advanced fibrosis^[57-59]. Proprietary tests and more complex panels have the disadvantage of not being easily accessible in clinical everyday practice, whereas calculators for NFS, BARD score and the FIB-4 index are easily found on the Internet. In current American guidelines, the NFS is recommended^[5,59], but some authors claim that BARD is easier to estimate than NFS^[60], whereas other support FIB-4^[61] or a combination of FIB-4 and BARD in a stepwise fashion^[62].

Transient elastography

Transient elastography (TE; Fibroscan™) was first developed for the assessment of liver fibrosis in patients with chronic hepatitis C, in which it showed a good correlation with the METAVIR fibrosis stage^[63]. The Fibroscan™ probe creates a low-frequency (50 Hz) elastic shear wave, which propagates through the liver tissue. The velocity of the shear-wave is measured and is directly related to tissue stiffness, which, in turn, is associated with the stage of fibrosis. Transient elastography is a quick and easy method, with a short procedure time and yielding immediate results. TE has been evaluated in patients with NAFLD in several studies^[64-70]. In a meta-analysis, the pooled AUROC, sensitivity and specificity values of Fibroscan™ for the detection of NASH with advanced fibrosis were 0.94 (0.90-0.99), 0.94 (0.88-0.99) and 0.95 (0.89-0.99), respectively^[6]. In another meta-analysis, transient elastography had an AUROC of 0.84-1.00 to exclude advanced fibrosis^[71]. It had a high negative predictive value and a modest positive predictive value, indicating its usefulness as a screening test in the decision-making for liver biopsy. The cut-offs for excluding advanced fibrosis differ between various diagnoses. In NAFLD, liver biopsy may be considered in patients with a liver stiffness greater than 7.9 kPa using the M-probe (7.2 kPa with the XL-probe), a cut-off above which advanced fibrosis may occur^[65].

The major pitfall for the use of transient elastography in NAFLD is the high failure rate due to

invalid measurements in patients with high BMI and/or central obesity^[67]. Failure rates lie approximately within 14%-17% using the standard (M-) probe^[67,69,72] but can be improved to < 2% using the XL-probe^[69]. Comparative studies on the M- and XL-probes show that the stiffness values with the XL-probe in general are 1.7 ± 2.3 kPa lower than those with the M-probe^[66]. Therefore, separate cut-off values have been suggested for the XL-probe^[57].

In a comprehensive review, Castera *et al*^[57] suggest the sequential use of serum markers and elastography to predict the severity of fibrosis and help decision-making on whom to perform a liver biopsy for the staging of fibrosis (Figure 1). First, the use of the NAFLD fibrosis score (NFS) is suggested in patients with suspected NAFLD, as recommended by both the AASLD and European Association of the Study of the Liver guidelines^[5,59]. Patients with intermediate NFS values (between -1.455 and 0.676) are further evaluated with transient elastography. A TE value < 7.9 kPa with the M-probe (< 7.2 kPa with the XL-probe) excludes advanced fibrosis with a negative predictive value of 89%-95%. These patients can be managed at primary care centers. However, a TE value > 9.6 kPa with the M-probe (> 9.3 kPa with the XL-probe) confirms advanced fibrosis with a positive predictive value of 72%^[57], and patients should be screened with endoscopy for esophageal varices and ultrasonography for hepatocellular carcinoma. Thus, applying this algorithm, liver biopsies are only needed in patients with an NFS value between -1.455 and 0.676 and a Fibroscan value between 7.9-9.6 kPa with the M-probe (7.2-9.3 kPa with the XL-probe)^[57].

Acoustic radiation force impulse imaging

Acoustic radiation force impulse imaging (ARFI) uses short-duration acoustic pulses that generate shear waves, which propagate through tissues and generate small tissue displacements^[73]. ARFI is easily applied in ultrasonography machines that are commercially available and slightly modified. Two studies compared ARFI with transient elastography and found similar diagnostic performance between the two methods^[74,75]. Cut-off values for advanced fibrosis in NAFLD have not yet been validated in larger studies.

Magnetic resonance elastography

In MR elastography (MRE), acoustic shear waves with frequencies between 40 and 120 Hz are generated by a pneumatic or electromechanical driver that is placed adjacent to the abdominal wall of the patient lying in supine position^[76]. A modified phase-contrast MRI sequence is used to image the propagation of the shear wave in the region of interest of the liver. The technique can be used on conventional MRI systems with additional hardware and software. A study from 2011 suggests that MRE could detect advanced fibrosis in patients with NAFLD with a high accuracy^[75]. In a

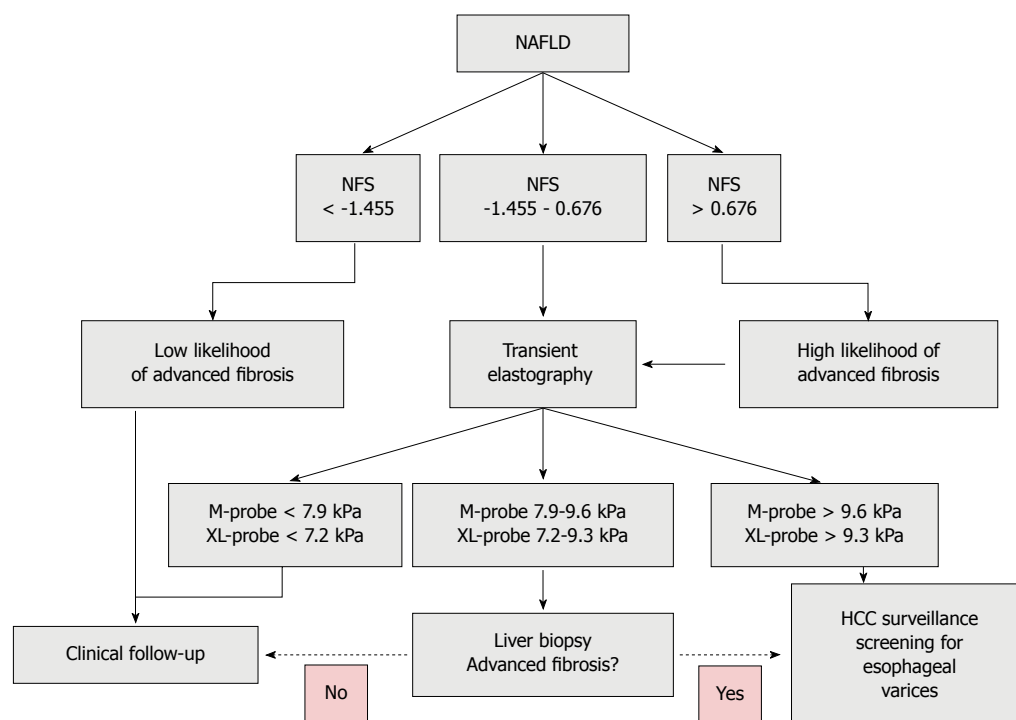


Figure 1 Proposed algorithm for the non-invasive diagnosis of advanced fibrosis in non-alcoholic fatty liver disease, as suggested by Castera *et al*^[87]. NFS: NAFLD Fibrosis Score; NAFLD: Non-alcoholic fatty liver disease; HCC: Hepatocellular carcinoma.

recent prospective study of 102 patients with biopsy-proven NAFLD, the MRE had a high diagnostic accuracy for predicting advanced fibrosis (AUROC 0.957)^[77]. The MRE technique is time-consuming and has a high cost; therefore, it has not yet been established in clinical practice.

SHOULD RISK GROUPS BE SCREENED FOR FIBROSIS?

One may argue that the treatment of NAFLD is the same regardless of whether fibrosis is diagnosed-*i. e.*, weight loss, increased physical activity and optimal glucose control if diabetes is present. Recent data demonstrate, however, that NAFLD patients with advanced fibrosis or cirrhosis have an increased risk for liver-related mortality, particularly the development of HCC. This risk is estimated to be > 2% per year if cirrhosis is present^[78]. Only small HCC tumors found at an early stage have a potential for cure, if treated with liver transplantation, hepatic resection or local ablation. Patients with NAFLD and advanced fibrosis should therefore be evaluated if they are candidates for HCC surveillance with semiannual ultrasonography investigations.

Presently, HCC surveillance is largely defective in patients with NAFLD. In a recent study on the use of HCC surveillance in clinical practice, the diagnosis of NAFLD increases the risk of not receiving surveillance more than two-fold^[79]. In more than one-third of HCC patients with NAFLD, surveillance is missed as a consequence of undiagnosed liver disease,

compared with only 7.5% in patients with hepatitis C. Furthermore, in NAFLD, only 13% of HCCs are discovered by surveillance compared with 35% in hepatitis C. Even if HCC can be encountered in non-cirrhotic livers^[80], the incidence increases with concurrent cirrhosis.

In a cohort of 1500 patients with hepatocellular carcinoma from Veterans Administration (VA) hospitals in the United States, NAFLD is the third most common risk factor for HCC and is observed in 8% of cases. Fifty-eight percent of NAFLD cases have underlying cirrhosis, and a lower proportion of these cases received treatment compared with HCV-associated HCC cases^[81]. Thus, undiagnosed liver disease or unrecognized advanced fibrosis is common in NAFLD, leading to a high proportion of HCC patients who can only be offered palliative treatments^[79].

One patient group in whom screening for NAFLD and advanced fibrosis would be feasible is type 2 diabetics. In a study of 1918 patients with diabetes, > 98% had reliable elastography measurements (1770 with the M probe and 114 with the XL probe). The proportion of patients with increased liver stiffness was 17.7%. Ninety-four patients underwent liver biopsy, and 50% of these had advanced fibrosis (F3-4)^[82]. Thus, in this cohort of patients with diabetes type 2 and without any known liver disease, 2.3% were found to have undiagnosed advanced fibrosis or cirrhosis due to NAFLD.

CONCLUSION

NAFLD is the most common liver disease worldwide,

and with an increasing incidence. NAFLD is associated with an increased mortality in liver-related and cardiovascular events, the risk of which is highest in those with NASH and advanced fibrosis. The single histopathologic feature with the greatest impact on mortality is liver fibrosis, which can be divided into four stages (F1-4). One aim is to discover significant fibrosis (F2-4) in time to intensify treatment and delay further progression. If advanced fibrosis or cirrhosis (F3-4) has developed, there is an increased risk for hepatocellular carcinoma, and these patients should be considered for HCC surveillance. Screening tests to exclude advanced fibrosis comprise non-invasive serum biomarkers (NAFLD Fibrosis Score, BARD or FIB-4 index) or non-invasive imaging techniques based on liver stiffness measurements (transient elastography, ARFI or MRE). With these tests, patients without a risk of advanced fibrosis can be excluded, and the need for liver biopsies can be minimized. Strategies should also be developed to identify NAFLD patients with significant fibrosis among risk groups-e.g., patients with type 2 diabetes and/or obesity.

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