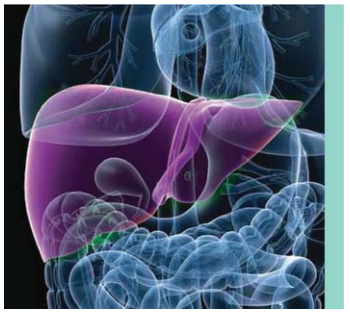

Putting It All Together: Noninvasive Diagnosis of Fibrosis in Nonalcoholic Fatty Liver Disease in Adults and Children

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Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the obesity and metabolic syndrome epidemics. It now affects up to 45% of adults and 10% of children in the United States.[1,2] Recent studies have shown clearly that the stage of fibrosis in adults with NAFLD is the most important histological feature in predicting long-term outcomes and the development of liver-related complications.[3,4] Despite the paucity of data regarding the natural history of pediatric NAFLD, its progression to cirrhosis and end-stage liver disease requiring liver transplantation in children and young adults is well documented.[5] Several studies have clearly shown that children with NAFLD may have advanced fibrosis (stage 3-4) on liver biopsy, making the identification of this high-risk group a top priority. Given the high prevalence of NAFLD in children and adults, there is an urgent need to find safe and cost-effective alternatives to biopsy to determine the stage of liver fibrosis. In this review, we will briefly discuss the noninvasive diagnosis of liver fibrosis in adults and provide details on the current status of noninvasive testing in children and adolescents.

NONINVASIVE DIAGNOSIS OF LIVER FIBROSIS IN ADULT NAFLD

Multiple noninvasive tests have been developed and validated in the adult NAFLD population to predict the stage of fibrosis.[6] These tests are being widely used by

Abbreviations: ALT, alanine aminotransferase; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; CK18, caspase-cleaved cytokeratin 18; ELF, enhanced liver fibrosis; FIB-4, Fibrosis-4; GGT, gamma-glutamyl transpeptidase; HA, hyaluronic acid; HCC, hepatocellular carcinoma; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score; PNFS, pediatric NFS; TE, transient elastography; VCTE, vibration-controlled transient elastography.

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TABLE 1. SUMMARY OF DIFFERENT SEROLOGICAL AND IMAGING TESTS TO DIAGNOSE FIBROSIS IN CHILDREN WITH NAFLD

Marker	Interpretation	Accuracy	Cost
PNFS	≥26% gives specificity of 92% for predicting advanced fibrosis	AUROC of 0.74 for advanced fibrosis	+
	≤8% gives sensitivity of 97% for ruling out advanced fibrosis		
HA	≥1200 ng/mL: absence of fibrosis was unlikely, 7% (95% CI: 1%-14%)	AUROC of 0.95	++
	≥2100 ng/mL: made F2-F4 likely, 89% (95% CI: 75%-100%).		
ELF	≥9.28 = presence of any fibrosis	AUROC of 0.92 for detecting any fibrosis	++
	≥10.18 = presence of significant fibrosis	AUROC of 0.98 for detecting significant fibrosis	
	≥10.51 = presence of advanced fibrosis	AUROC of 0.99 for detecting advanced fibrosis	
TE	5-7 kPa: F1-F4	AUROC of 0.977 for detecting any fibrosis	+++
	7-9 kPa: F2-F4	AUROC of 0.992 for detecting significant fibrosis	
	>9 kPa: F3-F4	AUROC of 1.000 for detecting advanced fibrosis	
MRE	Liver stiffness value of 2.71 kPa gives sensitivity of 88% and specificity of 85% for ≥ F2 fibrosis	AUROC of 0.92 for detecting significant fibrosis	+++
ARFI	ARFI cutoff of > 2.0 m/s for ≥ F3 fibrosis	100% sensitivity, 39% specificity	+++

Abbreviations: CI, confidence interval; TE, transient elastography.

gastroenterologists and hepatologists to risk-stratify patients with NAFLD without the need for liver biopsy. These tests can be divided into one of three categories: simple fibrosis scores that can be calculated from readily available clinical variables, complex fibrosis scores that rely on measuring serum biomarkers of fibrosis and extracellular matrix turnover, and imaging studies that are based on measuring liver stiffness as an indirect way to determine fibrosis stage.

The two most validated simple fibrosis scores in adults are the Fibrosis-4 (FIB-4) index (includes aspartate aminotransferase [AST], alanine aminotransferase [ALT], platelet count, and age; http://gihep.com/calculators/hepatology/fibrosis-4-score/) and the NAFLD fibrosis score (NFS; age, impaired fasting glucose/diabetes, body mass index, platelets, albumin, and AST/ALT ratio; http://nafldscore.com/). NFS has two cutoff values: < -1.455 to predict the absence of advanced fibrosis (F0-F2) and >0.675 to predict the presence of advanced fibrosis (F3-F4). Complex fibrosis scores include the European liver fibrosis panel (includes three fibrosis biomarkers: hyaluronic acid [HA], tissue inhibitor of metalloproteinase 1, and aminoterminal peptide of procollagen III) and the FibroTest (includes five biomarkers: haptoglobin, α2-macroglobulin, apolipoprotein A1, total bilirubin, and gamma-glutamyl transpeptidase [GGT]). Liver stiffness measurement by vibration-controlled transient elastography (VCTE) or FibroScan (Echosens, Paris, France) is one of the most commonly used imaging studies to stage fibrosis in hepatology clinics around the United States. A special probe (XL) was developed for obese patients with NAFLD. Other imaging modalities include acoustic radiation force impulse (ARFI) and magnetic resonance elastography (MRE).

NONINVASIVE DIAGNOSIS OF LIVER FIBROSIS IN PEDIATRIC NAFLD

Significant progress has been made in the field of noninvasive diagnosis of fibrosis in pediatric hepatology. Table 1 provides an overview of the use and accuracy of different noninvasive tests to diagnose liver fibrosis in pediatric NAFLD. However, it is important to realize that, at the present time, liver biopsy remains the only reliable method to stage fibrosis in children with NAFLD.

Fibrosis Scores. In a study that included pediatric patients with biopsy-proven NAFLD, our group has clearly shown that simple fibrosis scores that were developed and validated in adult patients with NAFLD (including FIB-4 index and NFS) are not accurate in predicting fibrosis stage in children.[7] Indeed, there was no significant difference in FIB-4 index and NFS values in children with and without advanced fibrosis. Therefore, by using a large cohort of children (n = 242) with NAFLD, we developed the pediatric NFS (PNFS), which includes ALT, alkaline phosphatase, platelet count, and GGT and can be calculated using the following online calculator:

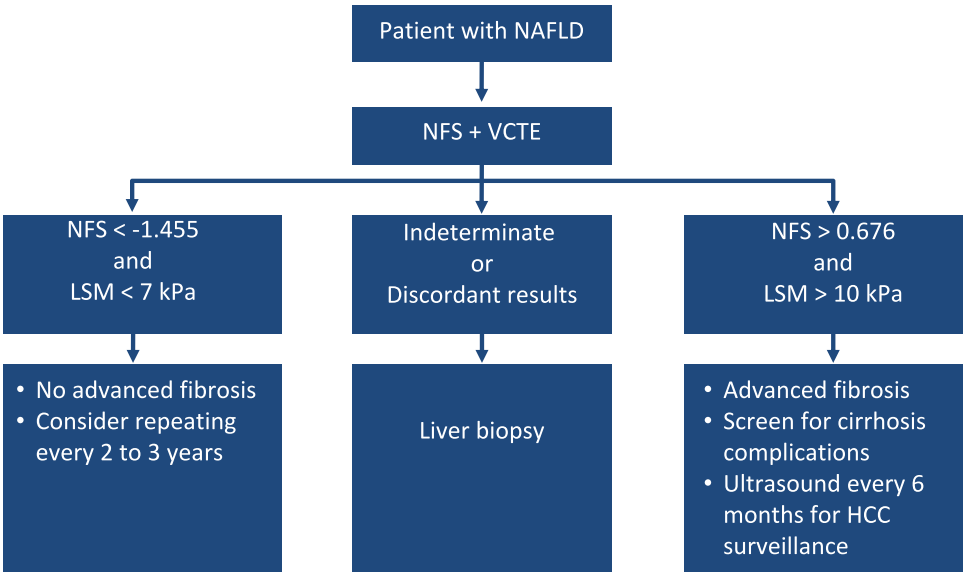

FIG 1 Algorithm used to determine the presence of advanced fibrosis in adults with NAFLD.

http://www.r-calc.com/calculator.aspx?calculator_id=JYAV-KOWT. However, it is critical to understand the limitations of this score, including the relatively small number of patients with advanced fibrosis (36/242) and the lack of external validation to date.[8]

Fibrosis Biomarkers. Caspase-cleaved cytokeratin 18 (CK18) fragments are considered a serum marker for hepatocyte apoptosis, a process that can activate the hepatic stellate cells to produce liver fibrosis. Fitzpatrick et al.[9] analyzed CK18 levels in 45 children with biopsy-proven NAFLD and found it to be a good marker to predict the presence of significant fibrosis. Similar results were replicated by Lebensztejn et al.[10] in a cohort of 52 children with NAFLD showing higher CK18 levels in those with fibrosis; however, the accuracy of CK18 to predict the presence of fibrosis was modest with an area under the receiver operating characteristic curve (AUROC) of 0.666. Our group conducted the largest study (n = 201) to assess the use of CK18 as a biomarker of liver fibrosis in children with NAFLD.[11] CK18 fragment levels were significantly higher in children with any fibrosis compared with those without fibrosis (304.6 versus 210.4; $P < 0.001$). CK18 level demonstrated good accuracy for prediction of any fibrosis (F1-F3) with an AUROC of 0.75. On multivariate logistic regression analysis, the combination of CK18 and waist circumference percentile generated an AUROC of 0.842 for prediction of any fibrosis.

w?>HA is an extracellular matrix glycosaminoglycan that is produced by activated hepatic stellate cells and is considered a direct biomarker of liver fibrosis. Conflicting results have been presented on the role of HA in predicting fibrosis in pediatric NAFLD, and more studies are needed to establish its role as a noninvasive biomarker.

Similar to adult studies, the enhanced liver fibrosis (ELF) panel showed excellent accuracy for predicting fibrosis in a cohort of 112 children with NAFLD (AUROC of 0.92 for any fibrosis); however, these results need further external validation.[12]

Imaging Studies. The same imaging modalities used routinely in adults to assess for liver fibrosis are being studied in the pediatric age group. Nobili et al.[13] measured liver stiffness by VCTE in 52 children with NAFLD and showed excellent accuracy for predicting advanced fibrosis (F3-F4) with an AUROC of 1.000. It is important to note that the study included only five children with advanced fibrosis. MRE was recently studied in a pilot project that included 35 children with different liver disease including NAFLD with promising results.[14] ARFI and other ultrasound-based methods to determine liver stiffness are being validated in the pediatric population. We need to establish specific cutoff values for children of different ages and different liver diseases before the wide use of these imaging techniques can be recommended in routine clinical practice.

CONCLUSION

Significant progress has been made in noninvasive diagnosis of hepatic fibrosis in adults with NAFLD, and the majority of patients can be risk-stratified without the need for liver biopsy. In our practice, we determine the NFS and liver stiffness measurement by VCTE in each patient with NAFLD with the results being consistent with one of three scenarios as shown in Figure 1: [1] both tests indicate the absence of advanced fibrosis → advanced fibrosis excluded, repeat testing in 2 to 3 years; [2] both tests indicate the presence of advanced fibrosis → advanced fibrosis confirmed, start screening for hepatocellular carcinoma (HCC) and consider screening for varices; or [3] the tests are discordant → perform liver biopsy to stage fibrosis.

The situation is different in children with NAFLD, for whom liver biopsy remains the gold standard for staging fibrosis. Adult fibrosis scores are not useful in children with NAFLD. Fibrosis biomarkers need further validation in different and larger cohorts. Imaging studies are promising, but pediatric-specific cutoffs need to be established. In conclusion, the validation of noninvasive markers and imaging studies in children with NAFLD is urgently needed to stage the severity of fibrosis and determine response to new therapeutic agents.

CORRESPONDENCE

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