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The Diagnostic Evaluation of Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

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Review Article

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is an increasingly prevalent disease that has become the leading cause of liver-related morbidity and mortality in industrialized countries. It encompasses a spectrum of pathological manifestations that range from fatty infiltration without liver damage, to inflammation which can progress to fibrosis and cirrhosis. Individuals with features of metabolic syndrome are at high risk of developing NAFLD. A major challenge is to find the reliable non-invasive diagnostic tool for the different aspects of NAFLD, particularly steatosis, steatohepatitis, and fibrosis. Currently, a liver biopsy is the definitive diagnostic test, however it is invasive and carries the risk of overt complications, and provides information on a very small portion of the liver. It is non-practical to perform a liver biopsy on large numbers of patients, which underscores the need for the development of safe, cost-effective, accurate and reproducible screening tests that can allow for early detection, diagnosis and follow-up. Such tools would allow for early recognition, and when therapy is available, enable the initiation of treatment. In this review, we discussed the diagnostic tools in the evaluation of patients with NAFLD.

Keywords: Nonalcoholic Fatty Liver Disease; Nonalcoholic Steatohepatitis; Fibrosis; Diagnostic Modalities

Abbreviations: NAFLD: Nonalcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis; BMI: Basic Metabolic Index; T2DM: Type II Diabetes Mellitus; AASLD: American Association for The Study of Liver Diseases; PPV: Positive Predictive Value.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common etiologies of liver diseases in the United States [1]. NAFLD is a spectrum of pathological manifestations in non-alcoholic individuals which range from fatty infiltration of liver to steatohepatitis and cirrhosis. It is further categorized into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) based on histological findings. NAFL is defined as the presence of ≥5% hepatic steatosis without evidence of hepatocytic injury while NASH is defined as the presence of ≥5% hepatic steatosis and inflammation with hepatocyte ballooning injury with or without fibrosis [1,2]. NAFLD is a highly prevalent disease and has been reported to affect about one billion individuals in the world [3]. Recent studies suggest that up to 30 to 40% of Americans have NAFLD in the United States [4-9]. NAFLD is the leading cause of abnormal aminotransferase in industrialized countries [10]. Approximately 20% of NAFLD affected individuals are at risk of progression to NASH which is the second most common etiology for liver transplantation in the United States and is expected to be the leading cause in the next few years [11,12]. NAFLD can also lead to further complications such as end-stage liver disease, liver cirrhosis, and hepatocellular carcinoma. Screening for NAFLD is challenging because most patients are asymptomatic until the development of cirrhosis. Asymptomatic individuals come to attention due to blood tests performed for other indications [13]. Compared to the general population, patients with NAFLD have a significantly higher all-cause mortality, an increased incidence of cancer, diabetes and cardiovascular diseases, which is also the most common cause of death in pre-cirrhotic NAFLD [1,14-19].

Considering these challenges, there is a need to establish a practical and effective approach for the evaluation and early detection of NAFLD particularly in those individuals who are at risk of developing fibrosis. The common risk factors associated with NAFLD are high basic metabolic index (BMI), obesity, type II diabetes mellitus (T2DM), dyslipidemia, polycystic ovarian syndrome and MetS (presence of three or more of following is defined as MetS: 1. waist circumference >102 cm in men, > 88 cm in women; 2. triglyceride level ≥ 150 mg/dL; 3. HDL <40 mg/dL in men and < 50 mg/dL in women; 4. systolic blood pressure ≥ 130 mm Hg or diastolic pressure ≥ 85 mmHg; 5. fasting plasma glucose ≥ 110 mg/dL) [1,20]. Currently, routine screening of these patients is not recommended, however, a high index of suspicion in this population can improve the outcome of disease.

There is significant data about the genetic association of NAFLD, but at present due to the lack of large population studies screening of family members is not recommended [21-23]. Identifying NAFLD, particularly pre-cirrhosis, may minimize the progression to NASH, liver cirrhosis and hepatocellular carcinoma. There is a need for an effective screening strategy for the evaluation of NAFLD in the community due to the high prevalence of disease, increasing cost of diagnostic procedures, low predictive values of non-invasive tests, high risk of complications from invasive procedure (liver biopsy) [24]. The characteristics of a good screening and diagnostic tests are universal availabililty non-invasive, reproducible and cost-effective. It should diagnose a full spectrum of disease in addition to having high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) [25-27]. Unfortunately, there is not any single screening test for early detection of NAFLD. American Association for the Study of Liver Diseases (AASLD) published their practice guideline recently for the diagnosis and management of NAFLD [1]. In this review, we summarized the diagnostic tool and recent advances on diagnosis for early detection of NAFLD based upon current clinical practice guidelines [1].

Diagnosis and Recommendations

A large population of patients with NAFLD is asymptomatic. These patients are often identified with abnormal liver function test or liver imaging when performed for other purposes. Further testing should be performed in these asymptomatic patients with abnormal liver function test or who are found to have fatty liver or hepatic steatosis on liver imaging including ultrasound (US), computer tomography (CT) or magnetic resonance imaging (MRI) of liver. A high index of suspicion for NAFLD in patients with sign and symptoms of liver disease, abnormal liver function testing and fatty liver or steatohepatitis on liver imaging prompt further workup. Initial evaluation of subjects should be performed by ruling out other coexisting etiologies of hepatic steatosis and chronic liver diseases (CLD) including but not limited to viral hepatitis, alcoholic liver disease, Wilson disease, hemochromatosis, alpha 1 antitrypsin disease, autoimmune liver disease, acute fatty liver disease of pregnancy, HELLP syndrome, inborn metabolic diseases (cholesterol ester storage disease, lecithin cholesterol acyltransferase deficiency, Wolman's disease) and drug-

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induced liver disease. NAFLD patients should also be evaluated for commonly associated comorbidities including diabetes mellitus, hyperlipidemia, obesity, sleep apnea, hypothyroidism, and polycystic ovarian syndrome [1]. Currently, routine screening of high-risk patient or family members of NAFLD subjects is not recommended due to uncertainties of diagnostic test, lack of treatment options[1]. Also, long term benefits against cost-effectiveness of NAFLD screening in uncertain.

Non-Invasive Diagnostic Markers

Serum Markers

Transaminase levels: The transaminase is the most commonly used marker for the identification of liver disease. Majority of patients with NAFLD are incidentally diagnosed because of asymptomatic presentation of disease. NAFLD is the common reason for unknown elevation of liver enzymes. Individuals with persistent elevation of liver enzymes should be worked up for NAFLD after exclusion of alternative causes of liver diseases. The transaminases are not a sensitive or specific marker to diagnose NASH in patients with NAFLD and are usually normal in most of cases. A recent study revealed, no significant difference between normal and high levels of alanine aminotransferease (ALT) groups to diagnose NASH [28]. There was high specificity of elevated ALT versus normal ALT (28.9% vs 10.7%), however, sensitivity was found to be low in predicting NASH. There is no correlation between ALT and histological finding; therefore this modality is not useful to detect NAFLD and disease severity [29,30].

Circulating Keratin 18 Fragment Level (CK18): The caspase-cleaved fragmented CK-18 is an intermediate filament liver protein released into the bloodstream due to hepatocyte apoptosis. In suspected NAFLD patients, CK-18 fragment level is a reliable test to distinguish NASH from simple steatosis and can be detected with an ELISA [31,32]. Elevated CK-18 level is 66% sensitive and 82% specific to predicts NASH [33,34]. CK-18 levels correspond with the extent of ballooning degeneration of the hepatocytes which is also the histological hallmark of NASH [35]. This association allows the test to effectively differentiate between NASH and NAFLD. Aida et al determined a cut off value of CK-18 for NAFLD (230 I/U) and NASH (270 I/U). CK-18 is 89% sensitive, 65% specific, with a 34% positive predictive value (PPV) and 97% negative predictive value (NPV) to predict patients with NAFLD. Similarly, a cutoff value of 270 I/U is 64% sensitive, 76% specific with a 72%PPV and 67% NPV to predict NASH [36]. A meta-analysis of 10 studies showed superior diagnostic accuracy of CK-18 fragments than total CK-18 to predict NASH in NAFLD patients with AURCO of 0.8 for CK-18 fragment [37]. The limitations for using this test for the evaluation of NASH are low sensitivity, poor reproducibility and scarcity of commercial availability for CK-18. Although CK-18 is a promising biomarker for the identification of steatohepatitis, further validation needs to be established to use it as a diagnostic tool in routine clinical practice.

Adiponectin: Adiponectin is one of the anti-inflammatory and antifibrogenic protein acting on hepatocytes and hepatic macrophages [38-40]. Its protective role in liver injury is emerging from several studies over last few years. It is a good serum marker to predict inflammation and advanced liver fibrosis. In NASH, the upregulation of certain serum cytokines such as TNF-α, resistin, and leptin results in concomitant downregulation of protective cytokines like adiponectin42. A study showed association of low adiponectin level in NASH as compared to control group (5476vs 11,548 ng/ml, P = 0.00001).43 Adiponectin levels are inversely correlated with advanced liver fibrosis and inflammation (OR 8.0, P = 0.03, and OR 5.0, P = 0.009, respectively) 44, 45. Hypoadiponectinemia could be a marker of NAFLD and NASH, however, unlike NASH, no cut off for NAFLD has been suggested because of lack of significant amount of data42, 46. Non-availability of this test on a routine basis is one of the major limitations.

Procollagen Type III N-Terminal Peptide (PIIINP): PIIINP is a non-invasive marker for the monitoring and detection of liver fibrosis [46,47]. It also helps to distinguish simple steatosis from NASH by discriminating between different grades of NASH. PIIINP release into circulation in response to injury and is reflective of the degree of fibrosis at the site of diseases due to turn over of extracellular matrix [48]. To distinguish between simple steatosis, NASH or advanced fibrosis, Tanwar, et al. showed the correlation of PIIINP levels with degree of steatosis, lobular inflammation and histologic ballooning injury in liver. It showed an AUROC for PIIINP ranges from 0.77-0.82 to 0.82-0.84 in patients with F0-2 and F0-3 fibrosis respectively [49]. A recent study suggested the threshold of PIIINP ≥ 11 ng/ml which correlated with severe fibrosis and liver injury in 61 patients within a high-risk cohort of 467 tested [48]. This biomarker has promising effects to detect NAFLD patients who develop NASH or advanced fibrosis. Cost and commercial

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availability are main limitations of this serum marker for its wide application.

MicroRNAs (miRNAs) panel: miRNAs are noncoding small RNAs which regulates most of the cellular activities and post-translational gene regulation. miRNAs are found in abundance in the liver and have shown an impact on liver functions [50]. Both human and animal studies have shown the expression of specific miRNAs in NAFLD, NASH and metabolic syndrome [51,52]. Dysregulation of miRNAs could be noninvasive biomarkers for evaluation of patients with NAFLD and liver diseases. In a recent randomized control trial, role of miRNAs was evaluated in NAFLD patients. Serum levels of miR-122 was found to be 7.3 fold higher in NAFLD patients as compared to control group54. Higher levels of liver miR-122 and miR-34a were also found in NAFLD group compared to simple steatosis group. AUC value of miRNA panel was found to be significantly different from ALT in this study indicating miRNA panel is more sensitive and specific than ALT for diagnosis of NAFLD [53].

Radiographic Modalities

Ultrasonography (US): US of the liver is the most commonly used primary diagnostic tool for screening and evaluation of NAFLD. It provides quantitative assessment of fatty infiltration of liver as well as diagnostic information in the evaluation of NAFLD. It should be the first-line diagnostic imaging of liver to identify moderate to severe steatosis. US is 60-94% sensitive and 66-97% specific in the detection of steatosis [54,55]. It is a noninvasive, widely available and cost-effective diagnostic tool. The role of US is limited in the evaluation of NAFLD patients with BMI >40kg/m^2. The sensitivity of US in obese patients is only 86% [42,56]. US cannot detect mild stages of NASH or fibrosis in obese individuals where excessive abdominal fat interferes with the imaging quality [57]. A study on living donors for liver transplant showed the limitations of US to detect hepatic steatosis if it is less than 10% [58]. Hepatic steatosis between 10-19% and 20-29% was detected in 55% and 72% respectively in individuals with steatosis. Overall sensitivity and specificity of US is poor for detecting mild to moderate cases of hepatic steatosis, and cannot numerically enumerate the amount of triglyceride in the liver [59]. Ultrasounds can also be unreliable because they can suggest an increase in hepatic echogenicity secondary to extensive liver fibrosis, which can lead to inaccurate findings [60]. Supplemented with the fact that US has a low PPV, significant operator dependability, and inadequate assessment in morbidly obese individuals necessitates the need for alternative screening methods for the evaluation of NAFLD.

CT Scan/MRI/MRS: CT scan is one of the most accurate diagnostic tools in the evaluation of diffuse and focal fatty infiltration of liver in suspected NAFLD patients [61]. Hepatic steatosis can be detected by measuring the attenuation difference between liver and spleen tissues, using spleen as an internal control. The sensitivity and specificity of the CT scan range from 73-100% and 95-100% respectively for detection of moderate to severe steatosis [62]. The risk of radiation exposure, high cost and misdiagnosis of steatosis in the presence of other diffuse liver diseases are core limitations of CT scan for its extensive application in NAFLD patients.

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are more accurate markers to detect hepatic steatosis [63-65]. These modalities offer anatomical and biochemical information while assessing for NAFLD. MRI scan was found to be 85% sensitive and 100% specific for detecting mild steatosis while 80% sensitive and 95% specific for moderate to severe cases of hepatic steatosis [31,66]. Despite these advantages, there are multiple factors limiting the utility of MRI as a screening test. These factors are patient factors (lack of patient cooperation, claustrophobia, metallic implants and morbidly obese individuals who may not fit into the scanner), and imaging factors (high cost of imaging, long imaging time and long waiting list to scheduling imaging) [67].

Magnetic resonance spectrograph (MRS) is one of the most promising modalities for detection of hepatic steatosis. It measures the amount of water and hepatic fat contents by utilizing proton signals from acyl group of hepatic triglycerides. MRS findings of steatosis are accurately correlated with biochemical markers and histological evaluation of hepatic triglycerides [68]. However, MRS is expensive and does not provide information about necro-inflammation and fibrosis which currently confines it as a diagnostic tool for research purposes. Further studies are needed for its wide application as a screening test for the evaluation of NAFLD.

Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF): MRI-PDFF is the gold standard imaging biomarker for noninvasive quantification of liver fat in the liver and evaluation of hepatic steatosis in NASH66 [69-72]. MRI-PDFF technique measures the ratio

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of density of mobile fat proton and total density of proton from mobile fat and mobile water and helps in the quantification of fat which can be visualized on MRI imaging [73]. MRI-PDFF is one of emerging and non-invasive quantitative biomarker which is comparable to liver biopsy and histology to determine the changes in fat contents in NASH [69,71]. A multicenter randomized control trial determined the histological variants of hepatic steatosis in its subjects (based on the histological scoring system for NAFLD by the NASH Clinical Research Network) and concluded that MRI-PDFF was 83% sensitive and 90% specific for distinguishing grade 0-1 from grade 2-3 steatosis. Likewise, it was 84% sensitive and 90% specific for differentiating grade 0-2 from grade 3. They also suggest that this modality is efficacious in following patients undergoing NASH treatment and a subsequent improvement in grades of steatosis by keeping the histological grades as a reference point [74]. A retrospective study showed the AURCO of 0.95 for MRI-PDFF to differentiate moderate to severe steatosis from mild steatosis [75]. MRI-PDFF can be a remarkable tool for longitudinal assessment of NAFLD because of its high accuracy, reproducibility and strong correlation with histologic assessment of steatosis [76]. MRI-PDFF is a good marker of steatosis, however, it cannot assess liver fibrosis. It may not be feasible in individuals with obesity who cannot fit into MRI scanner, individuals with claustrophobia, and those with contraindications to MRI for example individuals with metallic implants. The major practical limitation of this modality is the high cost and limited availability on routine basis.

Transient Elastography (TE): TE is the measure of elasticity of liver tissue. Elasticity is measured by sending low-frequency shear waves from an ultrasound probe to the liver and by calculating the velocity of shear wave across the liver. It is a painless, non-invasive procedure, which can be performed in a short time and provides quick assessment of the liver stiffness which correlates with liver fibrosis, and guides whether liver biopsy needed or not [77]. A cutoff value of <5.5 kPa, rules out the liver fibrosis, however, the cutoff value >7.9kPa suggests significant fibrosis and a value > 13 suggests advanced fibrosis or cirrhosis. In a large study, TE has achieved an AUROC of 0.84, 0.93 and 0.95 in detection of F2, F3 and cirrhosis respectively in biopsy-proven NAFLD patients [78]. Using a cutoff value of 7.9 kPa in this cohort, 96% NPV was found to rule out F3-F4 but lower PPV value 53% at 7.9 kPa and 72% at 9.6 kPa was found. Food intake increases portal blood flow and liver stiffness which can result in an incorrect estimate of fibrosis. Controlled Attenuation Parameter (CAP) is a recent modification in elastography which can record level of fatty infiltration of liver. This modification allowed physicians to evaluate complete spectrum of NAFLD from simple fatty infiltration to NASH and cirrhosis. TE has limited ability to diagnose bridging fibrosis or cirrhosis due to higher false-positive rate and higher NPV than PPV79. High BMI and/or thoracic fold thickness also distort the results of TE in NAFLD. The use of XL probe in these individuals improved the effectiveness of elastography, however, failure rate is still 35% which necessitate further improvement in this technique [79]. TE cannot provide the operating physician with a concurrent sonographic illustration of the liver. Due to false estimation of liver elasticity, there is limited value of TE in the evaluation of patients with morbid obesity (BMI>35 kg/m^2), substantial ascites or type 2 diabetes which indicates the need for more advancement in this technique [80,81].

Magnetic Resonance Elastography (MRE): Is a new imaging modality that uses magnetic waves to generate images that provide information about the degree of liver stiffness. MRE is useful because it can accurately detect minimal and advanced scarring (F2-F4) of the hepatic tissues [82]. Scarring ≥F1 can be detected with 75% sensitivity, 86% specificity, 99% PPV and 85% NPV. Scarring ≥F2 can be detected with 87% sensitivity, 85% specificity, 88% PPV and 84% NPV. Similarly, scarring ≥F3 can be detected with 74% sensitivity, 87% specificity, 75% PPV and 81% NPV while scarring ≥F4 can detected with 91% sensitivity, 95% specificity, 59% PPV and 99% NPV [83]. MRE is especially useful because it can provide a numerical assessment on the extent of fibrosis, lipid accumulation and levels of iron stores in the liver [84]. Unlike Transient elastography, MRE can be performed in patients with significant obesity and ascites which adds to its clinical significance in screening for NASH and NAFLD. Adequate MRE results warrant certain duration of holding breath which may not be possible for some patients. The results can also be obscured in patients with concomitant hemochromatosis and iron overload in the liver tissue which can impede the travel of the MRE signal through the hepatic parenchyma83. Perhaps the most important limiting factor for MRE is the fact that this technique is new, costly and requires a certain high operational expertise which restricts its application to research and academic centers rather than the current clinical setup [85].

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Vibration Controlled Transient Elastography (VCTE): VCTE is a useful imaging modality for measuring the degree of hepatic fibrosis in individuals afflicted with NAFLD. This modality coalesces the findings of liver stiffness based on TE with biochemical indicators of NAFLD which include total platelet count, α2-macroglobulin, blood urea nitrogen (BUN), prothrombin index, ALT, AST, and gam-glutamyl transferase. This combination of two different parameters allows for an in-depth evaluation of the extent of liver damage through fibrosis [86]. While VCTE is not a diagnostic test, it can help in screening patients who would do well with a biopsy and similarly siphon patients with none to nominal fibrosis who do not warrant a biopsy at present. Formerly, VCTE was used specifically for the evaluation of severity of fibrosis in patients with hepatitis [87]. However, studies have been performed that elucidate its efficacy in assessing for fibrosis in NAFLD patients, which determined that VCTE has 70% sensitivity, 88% specificity in detecting F≥2 while detection of F≥3 had a higher sensitivity of 90% and a specificity 93% with an AUROC of 0.97 [88]. A prospective multicenter study undertaken to grade the accuracy of VCTE in quantifying the degree of steatosis and fibrosis concluded that the specificity of detecting for F>0 was 27% at a threshold of 4.6 kPa, 46% at a threshold of 5.5, 43 % at 6.1 kPa and 72% at 10.4 kPa. On the other hand, using higher cutoffs of 10.3, 11.6, 13.6 and 15.8 kPa, the resultant sensitivity in identifying similar benchmarks of fibrosis was 41%, 47%, 44%, and 59% respectively. This study also noted that VCTE was more specialized in the detection of advanced liver fibrosis in comparison to nascent forms of scarring in the hepatic parenchyma with an AUROC of 0.91[88]. This predilection to detect severe forms of fibrosis can be attributed to the fact that VCTE incorporates an analysis of biochemical indicators that are more pronounced with an increasing extent of tissue injury [87]. VCTE is also technically easy to perform, safe for frequent use and relatively inexpensive, which are added benefits to its use as a screening modality. Current guidelines recommended clinical use of both VCTE and MRE for evaluation of advanced fibrosis in patients with NAFLD [1].

Acoustic Radiation Force Impulse Imaging (ARFI) Elastography: ARFI elastography is a diagnostic tool used to measure the degree of fibrosis in patients with NAFLD. It amalgamates the use of elastography with B-wave ultrasonography [89]. Using the ultrasound probe, a specific area of interest in the liver is targeted and then subjected to shear waves produced by the probe (262 ms, 2.67 MHz). The shear waves are detected through the probe and quantified into the shear wave velocity (SVW). Based on the sonographic images, ARFI can provide a qualitative assessment on the degree of the liver fibrosis, while a quantification index, i.e. SVW, provides an arithmetical evaluation of the fibrosis [90]. The SVW has a mutual relationship with the extent of fibrosis; a higher SVW indicates a greater degree of fibrosis, as observed by the histopathological analyses of a subsequent biopsy specimen [91]. Based on these properties, ARFI can assess significant liver fibrosis in patients with NAFLD with 80% sensitivity and 85% specificity [92]. In a study of 54 NAFLD patients, AUROC for diagnosis F3-F4 was found to be 0.97 [93]. Increasing availability of ARFI on ultrasound machine can enable practical application of this technique to detect various stages of fibrosis. However, lack of expertise and confounding measurements of SVW based on presence of steatosis and inflammation underscores the need for future trials to legitimize this new modality in the clinical setting.

Scoring Systems: Several scoring systems are used to identify NAFLD and its progression to NASH and advanced liver fibrosis [94,95].

The NAFLD Fibrosis Score (NFS): NFS is commonly used scoring system to estimate advanced liver fibrosis. The NFS can only be used to determine the severity of liver fibrosis rather than diagnosis of NASH [2]. The NFS is based on the following parameters; age, body mass index (BMI), hyperglycemia, albumin, platelet count and AST/ALT ratio. Using these 6 parameters, Angulo et al. differentiate between advanced and minimal fibrosis with an area under the receiver operating characteristic curve (AURCO) 0.88 and 0.82 in the estimation and validation group respectively 96. They also determined a cut off value of NFS less than -1.455 to exclude and greater than 0.676 to predict advanced liver fibrosis. Using low cut off value, the fibrosis was excluded with high accuracy (NPV up to 93%), while fibrosis was diagnosed using high cutoff value with high accuracy (PPV up to 90%). Although the precision rate is very high to predict or exclude advanced fibrosis (≥ F3) using these cutoff values, however NFS does not clear the stage of liver fibrosis (F1-2) if the value is between -1.455 to 0.676. Liver biopsy is needed in these cases of intermediate stage of liver fibrosis. Further studies are needed to overcome these limitations of NFS in the differentiation of steatosis and NASH.

Fibrosis-4 (FIB-4) Index: FIB-4 index is a non-invasive method to determine advances fibrosis in NAFLD and is calculated by documenting the age of a subject and the

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values of aspartate aminotransferase (AST), ALT and platelet count obtained from a routine blood test, which emphasizes the ease of obtaining the FIB-4 score in a patient. A recent retrospective cohort study suggests that the FIB-4 index can provide a definitive diagnosis of NASH with a 67% sensitivity and 73% specificity. The same study also concluded that FIB-4 is also well equipped to identify NASH with mild fibrosis out of a NAFLD study population (57% sensitivity, and 75% specificity) [96]. A recent study determined the cutoff value of FIB-4 score for evaluation of advanced fibrosis [97]. A cutoff value of <1.45 excludes advanced fibrosis and has 74% sensitivity, 71% specificity, 22% PPV, 73% NPV giving an AUROC of 0.87. Similarly, a higher cutoff value > 3.25 predicts advanced fibrosis and has 26% sensitivity, 98% specificity, 75% PPV, 85% NPV giving an AUROC of 0.88. The efficiency of FIB-4 score between 1.45 to 3.25 in still undetermined and there is a scarcity of studies that have assessed the efficacy of FIB-4 in the clinical setting. Further studies need to be performed to determine its usefulness in diagnosing NASH and NAFLD. For detection of advanced liver fibrosis (F3) or cirrhosis (F4), both NFS and FIB-4 index are recommended to be useful tools in current guidelines [1]. Both NFS and FIB-4 are equivalent to MRE and better than other indices (like ASL/ALT ratio, BRAD score) for detection of advanced fibrosis in biopsy-proven NAFLD patients [83].

Comprehensive Index (CI): The comprehensive index (CI) combines six different serum biomarkers (weight, BMI, waist circumference, AST/ALT, triglycerides and fasting blood glucose) with different anthropometric denominations via a multivariate logistic regression analysis to detect NAFLD at an earlier stage. The sensitivity of CI was 90% while the specificity was 76% [99]. The CI can also take into account the development of single nucleotide polymorphisms (SNPs) in genes that regulate lipid metabolism. The incorporation of known gene mutations in CI can further enhance its sensitivity and specificity for detection of NAFLD. CI is unable to discern between various pathologic stages of NAFLD such as simple fatty liver, hepatic steatosis and its complicated advancements such as liver cirrhosis and HCC, which explains the narrow use of this index in current practice.

Fatty Liver Index (FLI): FLI is a simple and one of an accurate predictors of hepatic steatosis in the general population. It is based on an algorithm that accounts for four parameters including BMI, waist circumference, triglycerides, and γ-glutamyl transpeptidase. A study on 8626 patients determined the cutoff value of FLI in the evaluation of middle-aged and elderly patients with NAFLD [99]. A cutoff value of 30 was found to be promising in the identification of patients with NAFLD with 80% sensitivity, 72% specificity, giving an AUROC of 0.83 [99]. FLI is a practicable computing tool because it uses clinical and laboratory values that are readily performed in both inpatient and outpatient settings which enhances its applicability. This allows for effective screening of patients at risk of developing the disease and subsequent introduction of lifestyle modifications that can curb the development and/or progression of this ailment. It can also help in siphoning candidates with suspected NAFLD who can then take part in research models that target further screening, investigations and treatment [100].

Fibro Test: FibroTest is a noninvasive panel of serum markers to predict liver fibrosis with high NPV in advanced liver fibrosis [101-104]. The serum markers in this panel are haptoglobin, alpha2 microglobulin, total bilirubin, γ-glutamyl transpeptidase, and apolipoprotein A1. A recent study used FT to predict advanced fibrosis in NAFLD [105]. The authors found AURCO of 0.81-0.92 in detecting F3-4 fibrosis and 0.75-0.86 in predicting F2-4. They determined the cutoff value of 0.30 and 0.70 for advanced liver fibrosis with 90% NPV and 73% PPV. The diagnostic performance of Fibro Test was evaluated in a study of 600 biopsy-proven NAFLD patient by comparing FibroTest with BRAD score, FIB-4 index, and NFS107. The non-binary AUROC for FibroTest (0.877) was found to be superior to BRAD score (0.836), FIB-4 index (0.845) and comparable with NFS (0.866) [106]. Although FibroTest can detect liver fibrosis effectively, however, the routine application of this test is difficult due to unavailability of some of serum markers in most laboratories assay.

Enhanced Liver Fibrosis Panel (ELFP): ELFP is commercially available markers of matrix turnover including PIIINP, hyaluronic acid (HA) and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1). A recent study utilized ELFP in NASH patients showed AURCO of 0.90 and 0.82 in detecting stage F3-4 and F2-4 of advanced fibrosis respectively.108 The ELFP cutoff value -0.2070 was found to have 61% sensitivity and 80% specificity to rule out liver fibrosis in NASH patients. ELFP is better diagnostic panel than NFS for detection of moderate fibrosis (AUROC 0.90 vs 0.86) and severe fibrosis (AUROC 0.93 vs 0.89), combination of these tests performs even better than individual test for detection of moderate (AUROC 0.93) and severe fibrosis (AUROC 0.98) [107].

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BRAD Score: It is utilized to detect advanced liver fibrosis F3-4 in NAFLD. The BRAD score is based on BMI, AST/ALT ratio, and status of type II diabetes in suspected patients with NAFLD. A cutoff score value <2 is a reasonable predictor in exclusion of advances fibrosis (NPV 95-97%), while a cutoff score >2 is associated with advanced liver fibrosis F3-4 with sensitivity and specificity of 88% and 89% respectively, and an AURCO of 0.865.109 The BRAD has limited diagnostic value for detection of early stages of fibrosis and can only be utilized to predict severe fibrosis.

Invasive Diagnostic Tests

Liver Biopsy: Liver biopsy is still the gold standard diagnostic test to rule out liver fibrosis in patients with NAFLD43, [108,109]. It is the only test that can reliably differentiate between NAFL, NASH and can also identify the stage of liver fibrosis [110]. The general indications of liver biopsy are to confirm NAFLD, rule out other common causes of liver injury, and to determine the extent of liver damage for treatment and prognosis25. Liver biopsy is recommended in NAFLD patients with metabolic syndrome, those with high risk for developing steatohepatitis, liver fibrosis and to rule out other competing etiologies of steatosis and co-existing chronic liver diseases which cannot be ruled out without liver biopsy1, [111]. Liver biopsy is also recommended in suspected NAFLD patients with persistently elevated serum iron saturation and ferritin particularly in individuals with either homozygous or heterozygous mutation in C282Y HFE gene1. Liver biopsy is also recommended to be repeated after at least 5 years to follow up selected population who are at high-risk for progressive liver disease2.

Biopsy size is one of the most important parameters for the diagnosis of NAFLD and liver fibrosis. The average size of adequate liver biopsy must be 15 to 20 X 1.5 to 2 mm and should include 6 to 8 portal triads43. The selection of biopsy area is challenging due to uneven distribution of disease throughout the liver. Because biopsy specimen is only 1/50,000 of total liver tissue, therefore, an under-representation or over-representation of liver fibrosis can be obtained resulting in misclassification of severity of fibrosis in one-third of cases and exclusion of NASH in one-fourth of cases [112]. In a study conducted on sampling variability of liver biopsy in NASH, discordance was found in biopsies of two different sites for hepatocytes ballooning, steatosis, and inflammation [113]. In this study, 41% patients showed discordance of stage I or more of liver fibrosis. Another limitation of the liver biopsy is dependent on operator experience for interpretation [114]. Performing a liver biopsy from either of the two lobes also lead to variations in findings on an initial comparison. In comparison to the left hepatic lobe, the right hepatic lobe has more lobules per surface area which could represent a greater degree of disease severity [114]. Due to invasive nature of this procedure by using a large-bore needle, there are high risk of post procedural pain, bleeding, infection, pneumothorax and injury to other organs. The risk of bleeding ranges from 1 in 2500 to 10,000 biopsies for severe bleeding [115]. The risk of procedure related complications outweighs benefits when implementing liver biopsy on a large population of NAFLD.

Conclusion

With the advancement of serum biomarkers and imaging modalities in the past decade, a non-invasive assessment of patients with NAFLD/NASH has become a promising approach for early detecting of disease. Liver biopsy is still a gold standard diagnostic modality in the evaluation of NAFLD/NASH, however, the application of this modality is impractical for the screening of a large population of NAFLD. Further validation studies are required for clinical utilization of current non-invasive fibrosis biomarkers and imaging modalities for assessment of various stages of disease progression.

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Conflict of interest

The authors have no conflict of interest.

Author's Contribution

Muhammad Nadeem Yousaf: Manuscript writing
Usman Tariq, Salah Mankash, Fizah Chaudhary: Manuscript, data review and proof reading
Wajahat Z. Mehal: Manuscript writing and overall supervision.

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