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Diagnosis of nonalcoholic fatty liver disease

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Abstract

The diagnosis of nonalcoholic fatty liver disease (NAFLD) relies on confirmation of the presence of hepatic steatosis in more than 5% of hepatocytes by either imaging or histology without demonstrable secondary cause such as alcohol consumption, medications, hereditary or autoimmune disorders. NAFLD begins with simple steatosis that may later progress to steatohepatitis and end-stage liver cirrhosis. The differentiation of simple steatosis from steatohepatitis carries a prognostic value. Patients with steatohepatitis are at high risk of both liver and cardio-vascular related morbidity and mortality. NAFLD should be clinically suspected in individuals diagnosed with metabolic syndrome and metabolic risk factors. Liver enzymes are not reliable in diagnosing either hepatic steatosis or steatohepatitis, but the presence of abnormal liver functions should alert the clinician to arrange NAFLD workout. Advanced imaging techniques and various scoring systems are helpful to detect the extent of the disease non-invasively. Although, liver histology is considered the gold standard investigation in diagnosing and staging the disease, the liver biopsy is invasive and technically demanding.

Key words: Non-alcoholic fatty liver disease, NAFLD, steatohepatitis

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become one of the major medical problems around the globe due to looming obesity epidemic and associated metabolic derangements.[1] NAFLD is a spectrum of disease starting from simple steatosis, steatohepatitis (NASH), and fibrosis to NASH cirrhosis.[2] Both NASH and resultant cirrhosis increases the risk of hepatocellular carcinoma.[3] Detection of hepatic steatosis and the absence of an identifiable secondary cause are the two criteria needed to diagnose the NAFLD. Hepatic steatosis is defined as the presence of fat infiltration in more than 5% of hepatocytes.[4] NASH is diagnosed when there is lobular inflammation and hepatocyte damage (ballooning) in addition to hepatic steatosis.[4]

Individuals with NAFLD are generally asymptomatic while few may present with fatigue and right upper quadrant discomfort.[5] History and examination should include risk factor identification for steatosis which is the steppingstone for further investigations for NAFLD. The diagnosis of steatosis always depends on the selection of appropriate investigations and their interpretation. In practice, the incidental finding of abnormal liver enzymes or detecting steatosis on imaging, should alert the clinician for further investigations to detect NAFLD. Patients with NASH in addition to having a substantial risk of liver cirrhosis and hepatocellular carcinoma, also carry a high risk of cardiovascular morbidity and mortality.[6] Therefore, it is prognostically beneficial to differentiate simple steatosis from steatohepatitis.

Effective screening and timely diagnosis followed by appropriate lifestyle interventions may halt or even reverse the progression of the disease and related complications.[7] In clinical practice, laboratory parameters and hepatic imaging are widely used as noninvasive indirect evidence to confirm the diagnosis of NAFLD.[8] Diagnosis of NAFLD consists of following steps: screening for hepatic steatosis when it is

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clinically warranted, confirming the diagnosis of steatosis, excluding other causes of steatosis, and diagnosing different stages of NAFLD (simple steatosis, NASH, stages of fibrosis and its complications).

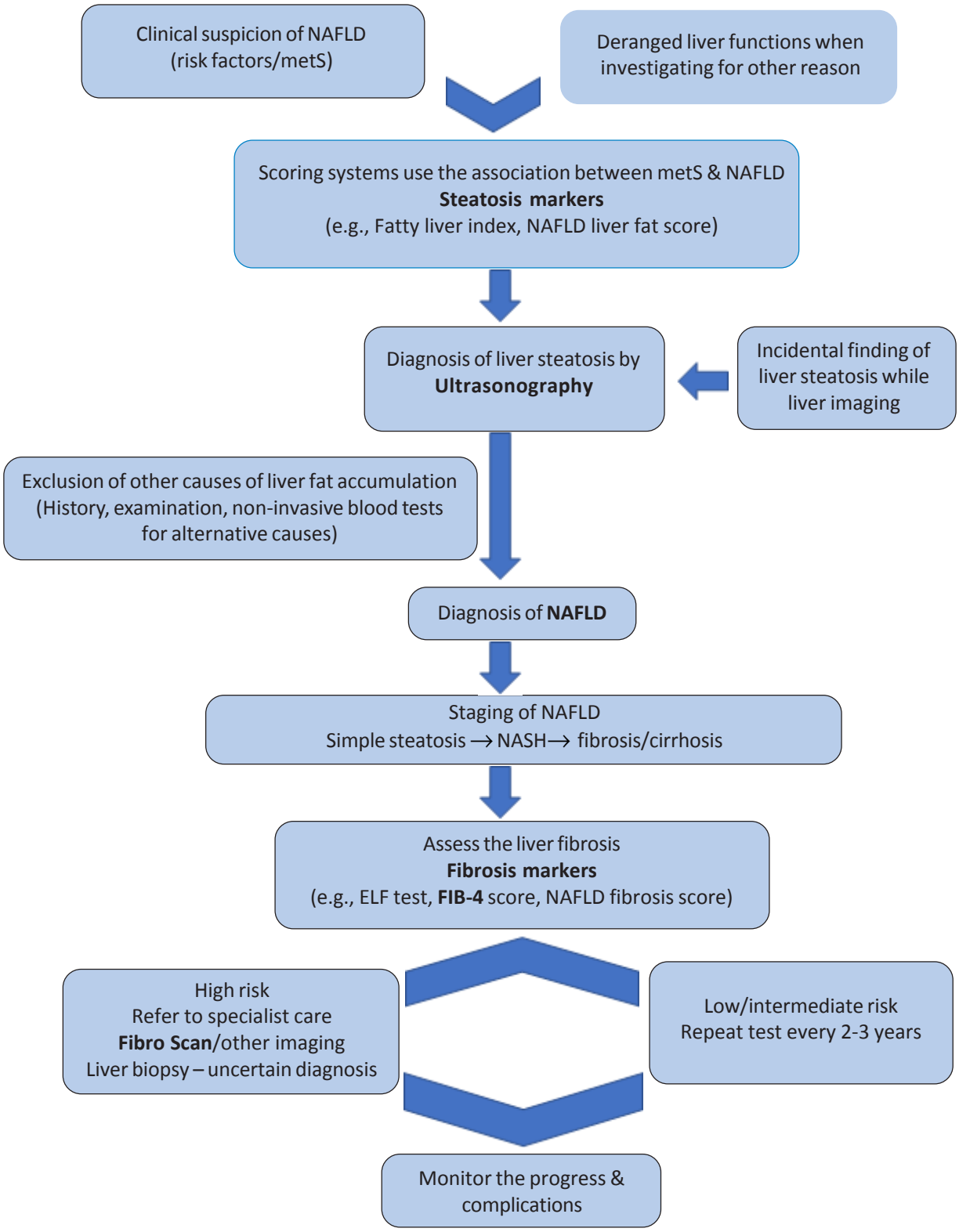

Figure 1. Screening and diagnosis of NAFLD[26].

Exclusion of other causes

Hepatic steatosis is seen in variety of conditions. Therefore, an alternative cause should be excluded before attributing it to NAFLD. It is mandatory to exclude a significant alcohol consumption in the history. A significant alcohol consumption is defined as weekly consumption of more than 14 units for men and more than 7 units for women.[9]

Other common causes of steatosis are medications, viral hepatitis, and autoimmune liver disease. Though rare, haemochromatosis, coeliac disease and Wilson's disease also need to be excluded.[4]

Panel of frequently performed investigations include serology for viral hepatitis, profile of auto-antibodies, serum ferritin, transferrin saturation, serum ceruloplasmin and urinary copper excretion[10].

Autoimmune liver disease is diagnosed when autoantibodies [antinuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA)] are present in significant titre in the blood. Autoantibodies in low titre can be detected in patients with NAFLD, in whom a normal serum IgG level is found when compared to autoimmune hepatitis.[11] Patients with NAFLD can have raised ferritin levels due to background inflammation and insulin resistance. But there is normal transferrin saturation and serum iron.[12] A transferrin saturation of <45% rules out haemochromatosis. When the diagnosis of NAFLD is uncertain, a liver biopsy should be considered.[12]

Risk factors and screening

Metabolic syndrome is strongly associated with NAFLD. Metabolic syndrome consists of glucose intolerance, abnormal lipid levels (elevated serum triglyceride and low HDL), high blood pressure and central obesity. The presence of insulin resistance in metabolic syndrome is suggested to be the crucial factor in the development of NAFLD.[13] Almost one third of patients with NAFLD fulfil the criteria of metabolic syndrome while more than 90% of patients found to have at least one metabolic derangement seen in metabolic syndrome.[13] Higher the number of metabolic risk factors, higher the risk of developing steato-hepatitis.

Guidelines usually do not recommend population screening for NAFLD as it is not cost effective.[4] When there are identifiable metabolic risk factors, it is prudent to be vigilant about the NASH and fibrosis. People with increased risk of NAFLD are identified as follows: age more than 50 years, body mass index more than 35kg/m^2, metabolic syndrome, and type 2 diabetes mellitus for more than 10 years duration.[14] American Diabetes Association (ADA) recommends screening patients who have impaired fasting glucose levels and type 2 diabetes mellitus with increased ALT levels or fatty infiltration in liver in the ultrasound scan.[15]

Non-invasive investigations

Liver enzymes in diagnosing and staging the disease

Although, an incidental finding of deranged liver functions in an otherwise healthy individual raises the suspicion of hepatic steatosis, the abnormal liver transaminases are not a frequent finding in NAFLD. Normal alanine transaminase (ALT) levels are seen in almost 80% and more than 50% of individuals with NAFLD and NASH respectively.[16] When derangements in liver functions occur in NAFLD, only mild elevation of transaminases and/or gamma-glutamyl transferase are seen.[16] Characteristically in early stages, rise in ALT predominates over the rise in aspartate transaminase (AST). With the occurrence of significant fibrosis and cirrhosis, there is a drop in ALT levels and the reversal of AST/ALT ratios. Therefore, ALT is not a helpful guide in either the diagnosis of the steato-hepatitis or staging and assessing the severity of the disease.[17] Hence, liver enzymes are not reliable surrogate markers of steatosis or the inflammation.

Scoring systems in predicting hepatic steatosis

Unavailability of reliable single blood test has led the scientific community to study combination of several blood tests to develop scoring systems to diagnose NAFLD. Such scores may help in reliably identifying different stages and the extent of the disease. NAFLD fat score is one such scoring system and it uses combination of laboratory markers [fasting serum AST, AST/ALT ratio (AAR), fasting serum insulin] and the clinical history [type 2 diabetes and existence of metabolic syndrome].[18] Fatty liver index is a scoring system which uses BMI, waist circumference, serum gamma-glutamyl transferase and triglyceride levels in predicting the hepatic steatosis. However, both NAFLD fat score and fatty liver index are not suitable for the staging of the disease.[18]

Differentiating NASH from simple steatosis

The liver biopsy identifies the progression of simple steatosis to NASH. However, the liver biopsy is not suitable as a routine diagnostic procedure due to invasiveness, cost, and magnitude of the disease burden in the community.

Only few biomarkers have shown promising results at laboratory level in differentiating simple steatosis from NASH. As the inflammation is associated with increased apoptosis in NASH, markers of apoptosis are useful in identifying hepatic inflammation and cellular damage. Hepatocyte apoptosis releases cleaved fragments of Cytokeratin-18 (CK-18) which is a major intermediate filament protein in hepatocytes to the bloodstream.[19] ELISA test is used to detect CK-18 in patients with NASH.[20]

Another serum biomarker which is produced during matrix turnover, named terminal peptide of procollagen III (PIIINP), also reflect the inflammation of the hepatocytes, and can be used to identify NASH.[21] The cost and the availability of these tests limit their use only to few centres. At present, these non-invasive methods are not readily available in Sri Lanka for clinical use.

Identifying and staging the liver fibrosis

Liver fibrosis is an independent predictor of long-term clinical outcome and prognosis of NAFLD.[22] Therefore, when evaluating the NAFLD outcome, the assessment of the extent of fibrosis is considered important.

Identified non-invasive scoring systems that have been validated to stage the fibrosis in NAFLD are NAFLD fibrosis score, FIB-4 score and BARD score. These non-invasive scoring systems are also called surrogate markers of fibrosis.[17] Each system possesses their advantages and disadvantages in predicting the severity. Each system is further categorised to low, intermediate, and high risks according to individual marks they carry. The advanced fibrosis can be excluded safely when they carry low scores, but further tests are needed to evaluate liver fibrosis in individuals with intermediate and high scores.[18] Two yearly follow up with serum fibrosis markers is indicated for low-risk group while specialist referral for further evaluation is recommended for moderate-high risk individuals.[23]

European guidelines recommend calculating surrogate markers of fibrosis for every NAFLD patient, in order to rule out significant fibrosis. They recommend arranging Fibro Scan for individuals in whom the significant fibrosis cannot be ruled out, and also recommend referring them to specific centres for liver biopsy when significant fibrosis is confirmed.[23]

Both, FIB-4 index and NAFLD fibrosis score are accurate and validated globally but may overpredict the risk in old age. FIB-4 index is widely used to exclude advanced fibrosis since it is simple and inexpensive. FIB-4 index uses age of the patient, serum AST and ALT levels and platelet count to calculate the score. Latest evidence suggests that subsequent staging by second group of tests is more accurate after triaging by the FIB-4 index.[24]

The enhanced liver fibrosis (ELF) test is proposed as a non-invasive test to assess the advanced fibrosis in NAFLD. When combined, ELF test can be used as a second line test for intermediate group of FIB-4 index. It measures three direct markers of fibrosis: Hyaluronic acid (HA), procollagen III amino-terminal peptide (PIIINP), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1).[25] (Table 1).

Although few other biochemical markers of fibrosis have been introduced, those are not readily available for use at a large scale. PRO-C3 is a such marker of type III collagen formation, and its level proportionately increases with the extent of fibrosis. Therefore, PRO-C3 is considered as an independent marker of advanced fibrosis. A PRO-C3-based fibrosis algorithm (ADAPT) was introduced to identify advanced hepatic fibrosis in high risk NAFLD patients. Patient's age, presence of diabetes, PRO-C3, and platelet count are the main parameters used in ADAPT.[26]

High-risk NASH (NAFLD activity score [NAS] ≥4 and significant hepatic fibrosis stage ≥ 2) patients can be diagnosed using NIS4 score. It is a novel non-invasive diagnostic tool based on serum biomarkers miR-34a-5p, alpha-2-macroglobulin, YKL-40 (a chitinase-like glycoprotein), and HbA1c.[27]

Imaging

Diagnosing the hepatic steatosis

Imaging techniques are used to diagnose steatosis and to detect the extent of fibrosis. Ultrasonography is the widely acceptable first-line imaging modality that provides a qualitative assessment of fatty infiltration of the liver. Ultrasonography is useful as it is non-invasive, cost-effective, radiation-free, and an easily available imaging technique. It carries technical limitations in some individuals when there is central obesity. Steatosis can be reliably diagnosed by ultrasonography when fat infiltration occurs in more than 33% of hepatocytes, but it can be missed when there is lesser degree of steatosis.[28] Hence, ultrasonically normal liver does not exclude the mild steatosis of the liver. Another limitation of ultrasound scanning is that it does not differentiate steatosis from NASH or fibrosis and early cirrhosis.[29]

Table 1. Non-invasive tests for NAFLD diagnosis and staging[18,26]

Score	*Indices*
Scores predicting the hepatic steatosis (association between NAFLD & metS) – steatosis markers	
NAFLD liver fat score	metabolic syndrome type 2 diabetes fasting serum insulin fasting serum AST AST/ALT ratio (AAR)
Fatty liver index	BMI waist circumference gamma-glutamyl transferase triglycerides
Scores predicting the stage of the fibrosis – fibrosis markers	
BARD score	BMI Type 2 diabetes AST/ALT ratio
NAFLD fibrosis score (NFS)	Age Hyperglycaemia BMI Platelet count Albumin AST/ALT ratio
FIB-4 score	Age AST ALT Platelet count
ELF test	Hyaluronic acid (HA) Procollagen III amino-terminal peptide (PIIINP) Tissue inhibitor of matrix metalloproteinase 1 (TIMP-1)
NIS4	miR-34a-5p alpha-2-macroglobulin YKL-40 (a chitinase-like glycoprotein) HbA1c

A quantitative assessment of steatosis is not accurate and operator variability is seen in the reporting.[30]

Computer tomography (CT) can be used to diagnose steatosis accurately when there is moderate-severe disease. It carries the risk of ionizing radiation which would limit its use for monitoring of the disease progress.[31]

Magnetic resonance imaging (MRI) techniques are considered the most accurate non-invasive measures of steatosis.[32] The accuracy of the conventional MRI is limited by signal interference caused by protons in the fat. Therefore, modified techniques are developed to overcome those limitations. Magnetic resonance spectroscopy (MRS) measures hepatic proton density fat fraction (PDFF), which is an objective biomarker of liver fat content.

Restricted availability of CT and MRI scans preclude the routine use of those imaging modalities for the diagnosis of NAFLD.[33]

Identifying the NAFLD progression

Next steps of management depend on the diagnosis and staging of the liver fibrosis. Liver stiffness measurements (LSM) are used as a surrogate marker to diagnose liver fibrosis.[34] Transient elastography (TE) techniques such as FibroScan gives a LSM using pulsed-echo ultrasound and it is increasingly becoming popular. TE is a useful screening test to monitor the progression of the disease.[15] It accurately detects and reliably excludes the advanced fibrosis.[35] FibroScan has few limitations: less reliable in individuals with central obesity and increased waist circumference, its accuracy depends on the experience of the operator, and limited availability of the technique.

Another imaging technique, acoustic radiation force impulse (ARFI) generates shear waves using conventional B-mode ultrasonography and indirectly measures the elasticity of the liver tissue.[36] The velocity of the shear waves is proportionate to the degree of liver fibrosis.

Liver biopsy

Liver biopsy is the gold standard investigation that asses the hepatic steatosis, hepatocellular injury, inflammation, and fibrosis of hepatocytes histologically.[37] Lobular inflammation in addition to steatosis differentiates NASH from simple steatosis.[38] It is an expensive, painful, and an invasive procedure with a significant risk of morbidity and mortality. Sampling variability and operator variability are also considered as its limitations. The biopsy sample does not represent global disease since the liver fat infiltration, inflammation and fibrosis may not be uniform throughout the liver. The heterogeneity of liver involvement causes false negativity despite the presence of the disease.[39]

NAFLD activity score (NAS) is a scoring system which is used for prognostication using a histological grading and staging system. There are newly introduced similar systems to accurately detect NASH such as SAF score which represents steatosis (S), activity (A) and fibrosis (F).[40,41]

However, non-invasive strategies are accurate in most patients with NAFLD to diagnose and stage the disease. Liver biopsy is indicated in diagnostic dilemmas when history is unreliable, and results of non-invasive strategies are inconclusive. Non-invasive tests are not validated for the diagnosis of NASH, liver biopsy is an essential investigation for the diagnosis of NASH because it is the only procedure that reliably differentiates simple steatosis from NASH despite limitations due to sampling variability.[42]

European clinical practice guideline for management of NAFLD recommend that NASH has to be diagnosed by a liver biopsy showing steatosis, hepatocyte ballooning and lobular inflammation.[43]

Conclusion

A simple non-invasive investigation for screening NAFLD is not available yet. Until such test is available, screening for metabolic risk factors is useful to identify patients who need further investigations. Scoring systems predicting steatosis are used to screen individuals with metabolic risk factors. Ultrasonography is the first line investigation that reliably diagnose hepatic steatosis when there is more than one-third liver fat infiltration. Once NAFLD is diagnosed, staging the disease, and identifying the complications are currently recommended. Several non-invasive blood tests, imaging studies and scoring systems have been evaluated to diagnose and to stage the disease. But reliable non-invasive tests are yet to be explored to diagnose NASH. Non-invasive scoring systems (e.g., FIB-4,NFS) which stage fibrosis are widely used in NAFLD management algorithms. Despite being invasive and expensive, the liver biopsy remains the gold standard investigation for NAFLD in selected individuals.

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