Thank you for reviewing our paper, here are our responses:

1. Anonymous 03805231 - the new proposition of nomenclature (MAFLD) must be cited in the introduction:
   - Answer: 157 - 162

2. Anonymous 03805231 - table 3 is not cited in the text. please provide:

   - Answer: 377 - 378

3. Figure 1 does not aggregate information. I suggest to suppress this figure:

   - Answer: Figure 1 was used in addition to statement 170 – 171. Ensured color blind compliance.

4. The title of the manuscript does not mention the treatment of NAFLD, focusing in the progression of the disease. So I suggest to withdraw this part talking about treatment

   - Answer: The treatment section was removed as recommended and the references were adjusted accordingly.

5. Please provide the decomposable figure of Figures, whose parts are movable and editable. So you can put the original pictures in PPT and submit it in the system. Please provide the decomposable figure of all the figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. - image files.ppt" on the system. Make sure that the layers in the PPT file are fully editable. For figures, use distinct colors with comparable visibility and consider colorblind individuals by avoiding the use of red and green for contrast. Please read these four important guidelines carefully and modify your figure(s) accordingly: First, all submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes. Second, for line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes. Third, please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor. Fourth, in consideration of color-blind readers, please avoid using red and green for contrast in vector graphics or images.
   - Answer: File was created and uploaded with the 4 recommendations as stated

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Clinical Indicators for Progression of Nonalcoholic Steatohepatitis to Cirrhosis

Tasur Seen, Muntazir Ali Sayed, Muhammad Bilal, Jonathan Vincent Reyes, Priyanka Bhandari, Vennis Lourdusamy, Ahmed Al Khazraji, Umer Syed, Yasar Sattar, Raghav Bansal

Tasur Seen, Division of Internal Medicine, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Priyanka Bhandari Division of Internal Medicine, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Muntazir Ali Sayed Division of Internal Medicine, R.C.S.M. Government College, Maharashtra 416013, India

Muhammad Bilal Division of Gastroenterology, Hepatology and Endoscopy, Pakistan Institute of Medical Sciences, Islamabad

Jonathan Vincent Reyes Division of Internal Medicine, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Vennis Lourdusamy Division of Gastroenterology, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Ahmed Al Khazraji Division of Gastroenterology, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Umer Syed Division of Gastroenterology, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Yasar Sattar Division of Internal Medicine, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, Elmhurst, NY 11373, United States

Raghav Bansal Division of Gastroenterology, Icahn School of Medicine, Elmhurst Hospital, Elmhurst, NY 11373, United States

ORCID number: Tasur Seen (0000-0001-5841-0211), Priyanka Bhandari (0000-0002-8085-5564), Muntazir Ali Sayed (0000-0003-4537-2513), Muhammad Bilal (0000-0002-5945-0968) Jonathan Vincent Reyes (0000-0002-2572-6436), Vennis Lourdusamy (0000-0002-2902-0537), Ahmed Al Khazraji (0000-0001-5428-9848), Umer Syed (0000-0001-8842-2950), Yasar Sattar (0000-0002-1304-5748), Raghav Bansal (0000-0003-2182-3076)

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Corresponding Author: Ahmed Al Khazraji Division of Gastroenterology, Icahn School of Medicine, Elmhurst Hospital and Mount Sinai Hospital, 79-01 Broadway, Elmhurst, NY 11373, United States. alkhazra@nychhc.org

ABSTRACT

Non-alcoholic Fatty Liver Disease, NAFLD, is a disease spectrum characterized by fat accumulation in hepatocytes presenting as hepatic steatosis to advance disease with active hepatic inflammation, known as nonalcoholic steatohepatitis (NASH). Chronic steatohepatitis will lead to progressive hepatic fibrosis causing cirrhosis and increased risk for developing hepatocellular carcinoma (HCC). Fatty liver disease prevalence has increased at alarming rates

alongside obesity, diabetes and metabolic syndrome to become the second most common cause of cirrhosis after alcohol related liver disease worldwide. Given this rise in prevalence, it is becoming increasingly more important to find non-invasive methods to diagnose disease early and stage hepatic fibrosis. Providing clinicians with the tools to diagnose and treat the full spectrum of NAFLD will help prevent known complications such as cirrhosis and HCC and improve quality of life for the patients suffering from this disease.

This article discusses the utility of current non-invasive liver function testing in the clinical progression of fatty liver disease along with the imaging modalities that are available. Additionally, we summarize available treatment options including targeted medical therapy through four different pathways, surgical or endoscopic intervention.

Keywords: Nonalcoholic fatty liver disease; Steatosis; Hepatitis; Cirrhosis; Hepatocellular carcinoma; Liver function tests; Imaging; Histopathology

CORE TIP

Fatty liver disease rates along with obesity, diabetes and metabolic syndrome continue to increase and now is the second leading cause of cirrhosis secondary to alcohol related liver disease. The need for consistent and readily available methods to accurately diagnose and stage hepatic fibrosis becomes increasingly necessary. With an up to date armamentarium to diagnose and treat the full spectrum of NAFLD will decrease complications such as cirrhosis and HCC and will improve the likelihood for patients to have a higher quality of life.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) was first introduced by Schaffner and Thaler in 1986[1]. They assembled a group of non-alcoholic patients with liver diseases and biopsy specimens of liver pathology similar to that of alcoholic liver disease. They defined these subsets of patients as NAFLD. Over the last 20 years, the “non-alcoholic” portion of the diagnosis has been heavily criticized, as it carries an unfavorable connotation for patients that may

negatively impact their overall care. In 2019, a group of international experts suggested the term metabolic (dysfunction) associated fatty liver disease “MAFLD” as a more appropriate diagnosis for NAFLD. As the underlying pathology is more related to metabolic dysfunction rather than the exclusion of alcohol. [2] Over the decades, NAFLD has grown to become the second most common cause of liver cirrhosis after alcohol related liver disease. The prevalence of NAFLD has grown every year in the United States (U.S.) secondary to a rise in diabetes, obesity and metabolic syndrome, with an incidence of 31% in 2012 as opposed to 18% in 1988–1991[3, 4]. NAFLD refers to a spectrum of liver injury due to accumulation of triglycerides in hepatocytes presenting as a spectrum of conditions, ranging from a simple hepatic steatosis characterized by fat accumulation in the absence of hepatic inflammation to a more severe disease form characterized by active hepatic inflammation, also known as NASH. Progressive hepatic inflammation will lead to cirrhosis and increase the risk of developing HCC as shown in [Figure 1]. Up to 1/3 of NAFLD patients will have NASH which is a risk factor for fibrosis progression, and approximately 40% of NASH patients will experience fibrosis progression [5]. The recent estimated annual progression of fibrosis from NAFLD is up to 0.09% with an incidence of advanced fibrosis as 70 per 1000 patients [6]. The high prevalence of NASH among the biopsied patients could be explained secondary to the indication for biopsy in these patients with elevated LFTs, and the data cannot be extrapolated to the subset of NAFLD patients with normal LFTs where biopsy is not performed often. In the same study, the prevalence of NASH in patients without indication for biopsy was 6.7% [5]. The annual incidence of hepatocellular carcinoma (HCC) in NAFLD patients is 44% per 1,000 person-years. NAFLD-related HCC amounts to about 2% to 4% of annual cases [7].

Liver biopsy is not always performed and the diagnosis is often made with available non invasive tests including blood tests and elastography (MRE, Fibroscan). Advantages of fibroscan, other than being a non invasive modality that helps in sequential assessment of progression or regression of steatosis/fibrosis, include elimination of sampling error experienced by liver biopsy. Liver biopsy is the gold standard in confirming the diagnosis of NAFLD and allowing accurate hepatitis fibrosis staging. The major histologic features include steatosis, lobular inflammation, and cytological ballooning; these findings help in grading and staging the disease [8, 9]. Moreover, the diagnostic tests especially non-invasive fibrosis assessment testing helps to monitor NAFLD stages to prevent disease progression and diagnose cancer early. Our review article discusses the indicators that help in understanding the progression of the disease including symptomatic worsening, liver function testing, imaging, and histopathological changes.

REVIEW

NAFLD encompasses a spectrum of conditions which ranges from bland hepatic steatosis to steatohepatitis causing hepatic fibrosis which will lead to cirrhosis, liver failure and increase the risk of HCC. The risk factors of fatty liver disease are similar to those of metabolic syndrome which leads to insulin resistance [5]. This includes diabetes mellitus, dyslipidemia and elevated body mass index (BMI). It is important to distinguish simple hepatic steatosis, which carries very low risk of developing chronic disease and cirrhosis versus NASH which carries a risk of progressive fibrosis, cirrhosis, liver failure and HCC. Overall, one fifth of NASH patients can progress to advanced hepatic fibrosis [10-12]. Hence, the assessment of the degree of hepatic fibrosis with noninvasive diagnostic panels and imaging is important in monitoring disease progression. Several scoring systems and specialized biomarkers have been developed by combining various serologic and clinical parameters for the prediction of fibrosis in NAFLD [13-18]. Despite the advancement of many diagnostic noninvasive fibrosis assessment modalities, one fourth of advanced fibrosis NASH patients can be misclassified as mild hepatic fibrosis [14].

The mortality of NAFLD is not merely targeting the liver. The majority of NAFLD patients are at risk of developing atherosclerotic coronary artery disease (CAD) carrying higher mortality rate approaching [19]. Understanding patients' risk factors and stage of hepatic fibrosis can help predict patients' clinical outcomes [10] . Multiple clinical indicators and serological markers of disease progression remains an area of intensive clinical and basic science research till this day (Table 1, Table 2).

ROLE OF NONINVASIVE LIVER FUNCTION TESTING IN CLINICAL PROGRESSION OF NASH

CLINICAL ASSESSMENT IN PROGRESSION OF NASH

NASH is a histological diagnosis characterized by hepatocytic inflammation that may progress to fibrosis. Hepatic fibrosis divided into four stages. Stage I describes as mild hepatic fibrosis, stage II moderate hepatic fibrosis, stage III moderate to severe fibrosis, and stage IV severe or advanced fibrosis. It is crucial to identify advanced fibrosis stage as these patients are at-risk to develop decompensated cirrhosis and end-stage liver disease. A number of clinical factors help clinicians to predict the likelihood of the patient progressing into devastating categories of this disease.

ROLE OF LIVER CHEMISTRY IN THE CLINICAL PROGRESSION OF NAFLD

Liver chemistry test identify active hepatic inflammation. This includes alanine aminotransferases (ALT) and aspartate aminotransferases (AST), alkaline phosphatase (ALP) and direct and indirect bilirubin. Other laboratory data should be monitored in NASH patients are platelet count and coagulation panel, fasting blood glucose and glycosylated proteins and lipid panel. Serum hyaluronic acid tissue metalloproteinase, and type 4 collagen are serological markers help in assessing fibrosis stage.

AST and ALT: In a cross-sectional study, Martin-Rodrigues *et al* [20] reported that serum ALT level is the most predictive laboratory investigation for NAFLD. The AST-ALT Ratio (AAR) is higher in increased liver fat content, fibrosis, and other metabolic derangements like diabetes and dyslipidemia. Steatosis or steatohepatitis can be observed, but nevertheless patients have normal serum ALT levels [8]. An AAR>1 is consistent with a diagnosis of NASH. This forms the basis of several other laboratory combinations that may indicate the progression of NAFLD and diagnosing liver fibrosis including BAAT (which uses BMI, age, ALT, and triglycerides), BARD (which uses BMI, AST:ALT, and diabetes), and FIB-4 scores [21, 22]. The FIB-4 score is a simple, noninvasive and inexpensive test superior to BAAT and BARD scores in monitoring the progress of NASH [17]. The FIB-4 score is reliable in ruling out advanced fibrosis in patients with histological evidence of NAFLD who had normal or increased levels of ALT, thus decreasing the need for invasive liver biopsy with sensitivity 84-94% [18].

Alkaline phosphatase (ALP): few subsets of NASH patients present with an isolated ALP elevation [23]. Cholestasis also has been noted on histology in NASH [23]. Elevated ALP should be accompanied by an increase in γ-glutamyltransferase (GGT) enzyme suggesting hepatic inflammation. Otherwise elevated ALP without GGT elevation are seen in pregnancy, muscular disease and bone disease such as Paget's disease.

Bilirubin: bilirubin is synthetic marker for liver function alongside with PT/INR. Also, it is a part of various scoring system used to estimate the degree of fibrosis. Demir *et al* [24] introduced the Non-Invasive Koeln-Essen-Index (NIKEI) score which uses age, aspartate aminotransferase (AST), AST/alanine aminotransferase (ALT) ratio, and total bilirubin. In a prospective study by Ratziu *et al* [25], the diagnostic utility of FibroTest, a noninvasive marker of fibrosis, was determined in a sample of 170 patients with NAFLD. The FibroTest includes α2-macroglobulin, apolipoprotein A1,

haptoglobin, total bilirubin, and γ-glutamyl-transpeptidase. Ratziu concluded this simple and noninvasive quantitative estimate of liver fibrosis reliably predicts advanced fibrosis [25]. Hepascore, a combination of bilirubin, γ-glutamyl-transpeptidase, hyaluronic acid, and 2-macroglobulin together with age and sex, is an accurate and reliable panel in predicting different stages of fibrosis. However, the limitation of this study included validation of this score among only patients with hepatitis C [21]. NIKEI had superior negative predictive value for advanced fibrosis compared to the FIB-4 score (which uses age, aspartate aminotransferase [AST], alanine aminotransferase [ALT], and platelet counts) [24].

Platelet count: Platelet count has great value in assessing degree of fibrosis. Thrombocytopenia occurs in cirrhosis secondary to thrombopoietin deficiency and splenic sequestration from underlying splenomegaly occurring from portal hypertension. Platelet level has been used in combination with other biochemical parameters such as in AST/platelet ratio index and FIB-4 score to monitor liver disease progression [21]. Kawamura established the Fibrosis Score for NASH (FSN), a new scoring system specific to the fibrotic stage of NASH [26]. FSN can accurately predict the fibrotic stage and distinguishes patients with advanced fibrosis of NASH. The platelet albumin AAR (PLALA) score is unique in that it distinguishes cirrhosis in NAFLD compared to most other fibrosis scoring systems. Each factor (platelet count <15.3 104/μL; albumin < 4g/dL; AAR > 0.9) is awarded 1 point, and a PLALA score of 2 or 3 may be predictive of cirrhosis in patients with NAFLD [27]. The PLALA score may be an ideal scoring system for detecting cirrhosis in NAFLD patients with sufficient accuracy and simplicity for clinical use. MPV was elevated in NASH and advanced liver fibrosis (stages 3–4) patients, making MPV a noninvasive, novel marker to predict advanced disease. Another study looked into the performance of red cell volume distribution width-to-platelet ratio in predicting liver fibrosis in patients with NAFLD [27]. This ratio was both correlated and able to predict liver fibrosis.

Fasting blood glucose and glycosylated protein: In their observational cohort of 118 patients, assessing the clinical determinants of fibrosis progression rate in NAFLD patients with baseline and follow-up histological evaluation. Advanced fibrosis is more likely to be found in patients with underlying type 2 diabetes[28]. These patients had histological evidence of more inflammation in the fibrous portal areas in those already developing cirrhosis than those at an earlier stage of the disease. Furthermore, this study also observed that type 2 diabetes can drive fibrosis in the absence of hepatic inflammation.

Glycosylated albumin to glycosylated hemoglobin ratio: Glycosylated albumin (GA) and glycosylated hemoglobin (HbA1c), which are indicators of glycemic control, show a strong relationship with advanced liver fibrosis. The GA/HbA1c ratio, which is typically 3 in a healthy individual, is higher in liver fibrosis patients. Patients with chronic liver diseases have reduced albumin turnover resulting in an elevated level of GA. Also, they have a reduced erythrocyte lifespan which accounts for changes in the increased ratio. The GA/HbA1c ratio's accuracy in detecting liver fibrosis might be limited by other concurrent diseases that can affect plasma and hemoglobin levels [25]. The HOMA-insulin resistance score is a somewhat rigorous and reliable scoring system that indicates NAFLD progression using a formula that involves insulin levels and fasting glucose to calculate insulin resistance. [28]. The HOMA-insulin resistance score has a high sensitivity for NASH. The NAFLD fibrosis score (NFS) is a noninvasive score (using age, albumin, AST/ALT ratio, BMI, the presence of diabetes or impaired fasting glucose, and platelet count) most predictive of mortality in NASH compared to NAFLD [29]. As of 2015, the NFS score was endorsed by the American Association for the Study of Liver Diseases (AASLD), American College of Gastroenterology (ACG), and the American Gastroenterological Association (AGA) as a screening guideline in clinical practice. These screening tools may be more important in detecting NASH in people with diabetes.

Hyaluronic acid tissue metalloproteinase: A high level of hyaluronic acid (hyaluronate) tissue metalloproteinase 1 has been indicative of fibrosis. The European liver fibrosis scoring system has indicators for cellular matrix activities including age, the amino-terminal peptide of procollagen III, tissue metalloproteinase 1 inhibitor, and hyaluronic acid [22].

Type IV collagen: The FSN score, which includes type IV collagen 7S, platelet count, AST, and ALT, has been more efficient in distinguishing the advanced fibrosis stages 3–4 of NASH compared to other scoring systems including APRI (AST to platelet ratio index), NAFLD Score, FIB-4 Index, BARD, and NIKEI [26]. The nonalcoholic steatohepatitis, ferritin, insulin, and type IV collagen 7S (NAFIC) score, and modified NAFIC score were proven to be clinically useful in screening for fatty liver patients [30].

Albumin: Bazick *et al.* [5] demonstrated that serum albumin gets reduced drastically in patients presenting with NASH. Their clinical variable could be used to guide clinical decision making about referring patients with diabetes and NAFLD to hepatologists [5].

Prothrombin time: In a study by Assy *et al* [31], up to 46% of patients with NAFLD showed thrombotic risk factors. The presence of thrombotic risk factors correlated with the extent of

hepatic fibrosis. This is consistent with known coagulopathy in those with altered synthetic function due to hepatic fibrosis.

ROLE OF IMAGING TECHNIQUES IN THE CLINICAL PROGRESSION OF NASH

Noninvasive techniques such as US, CT, magnetic resonance imaging, and proton magnetic resonance spectroscopy can detect hepatic steatosis but cannot reliably distinguish simple steatosis from NASH [32].

Liver Ultrasonography (US)

US is the preferred cost effective method in the U.S. for screening patients with suspected NAFLD. The findings on US include: diffuse increase in echogenicity of the liver parenchyma, hepatomegaly and vascular blunting [33] The sensitivity of US in detecting hepatic steatosis up to 94%. The sensitivity decreases as the degree of steatosis dropped below 30% [33-35]. US cannot differentiate between simple hepatic steatosis versus NASH. Thus, laboratory serological and histological data is helpful in pointing towards NASH. [22].

Magnetic Resonance Elastography (MRE)

MRE is a special type of MRI performed with a contrast material to produce detailed images of the small intestine. MRE equivalent of transient elastography (TE) has recently demonstrated excellent diagnostic accuracy. It has shown a sensitivity and specificity of 98% and 99%, respectively, for detecting all grades of fibrosis [35]. Huwart *et al* [36] conducted a prospective blind comparison of magnetic resonance elastography (MRE), US elastography, and APRI (AST to platelet ratio index) in a study of 141 patients who underwent liver biopsy for chronic liver disease. They found MRE was associated with a higher technical success rate than US elastography [36]. This study also showed that MRE did not affect hepatic stiffness.

Fibroscan

Transient Elastography (Fibroscan, Echosens, Paris, France) is a noninvasive method of assessing liver fibrosis. It can be performed at the bedside or in the outpatient clinic. It employs US-based technology to measure liver stiffness and has been validated for use in patients with chronic hepatitis C and B [37, 38]. However, studies have shown good results in patients with NAFLD [39, 40]. In only 5% of the cases, it has failed to show any readings. This is mostly seen in obese patients. This limits the TE's utility in the NAFLD cohort. However, a recently introduced XL probe may reduce this problem [41]. In a meta-analysis for NASH with advanced fibrosis, pooled AUROC

(area under the receiver operating characteristic curve), sensitivity and specificity of NFS, and Fibroscan are 0.85 (0.80–0.93), 0.90 (0.82–0.99), 0.97 (0.94–0.99), and 0.94 (0.90–0.99), 0.94 (0.88–0.99) and 0.95 (0.89–0.99), respectively [16]. Fibroscan is validated in NAFLD and represents a useful tool for rapid, noninvasive assessment of liver fibrosis and determining the need for biopsy. As this modality evaluates liver stiffness (related to fibrosis, inflammation, and portal hypertension), Fibroscan values should be interpreted in context of the morphological, biological, and clinical data.

ROLE OF LIVER BIOPSY IN THE CLINICAL PROGRESSION OF NASH

A percutaneous liver biopsy is currently the gold standard to assess hepatic fibrosis and inflammation in chronic liver disease [42]. However, liver biopsy is an invasive procedure with associated costs, complications, and inherent inaccuracy due to sampling error and inter-observer and intra-observer variability in histopathological interpretation [42, 43]. Despite the criticism of liver biopsy's associated risks, there are 3 basic histological systems that can be used to monitor the progression of NASH. These systems are the steatosis activity and fibrosis score, the NASH activity score, and the Brunt system that grades and stages NASH [Table 3] [28, 44]. Due to the risks and limitations associated with liver biopsy, it is controversial to perform liver biopsy on every patient suspected of having NAFLD. Therefore, it cannot be considered a "screening" tool [45]. However, there are studies that support the importance of liver biopsy. An older study, by Skelly *et al* [46] showed that biopsy on 354 patients with abnormal liver tests- 66% had fatty liver, 50% of those had steatohepatitis, and approximately 19% of the remaining biopsies had other treatable causes diagnosed by the pathology evaluation. This included autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), hemochromatosis and alcoholic liver disease (ALD). An adequate liver biopsy, with appropriate clinical history, interpreted by a trained liver pathologist, is not only pivotal for an accurate and complete diagnosis (or exclusion) of NAFLD (or NASH), but also is optimal for obtaining detailed information regarding disease pattern, severity and fibrosis. It not only provides important information with respect to subtypes, potential future risks, possible etiology, and natural history of disease, but also sets the groundwork for future molecular studies and clinical trials, assisting clinical colleagues and patients with treatments and follow-up.

CONCLUSION

NASH-related cirrhosis is the most common cause of chronic liver disease and indication for liver transplant. The increasing number of affected people imposes a strain on available organs. There

are many comorbidities and risk factors implicated in NASH severity and progression to chronic liver disease.

Due to the increasing prevalence of NAFLD in the population, there is an increasing need to find non- invasive methods to diagnose and stage NAFLD. The ideal test should be reproducible, cheap, and able to diagnose full spectrum of NAFLD, predict fibrosis, and reflect changes that occur with treatment. Preliminary evaluation includes clinical presentation with consideration of comorbidities and liver function test in the blood. Noninvasive imaging such as MRE and fibroscan can provide objective measures of liver steatosis and stiffness in patients without advanced fibrosis or cirrhosis. Due to the limitations, risks and cost of liver biopsy- it cannot be used as a screening test, although is typically relied upon to confirm the diagnosis. Several different methodologies including imaging modalities, serum markers and combined tests are currently being investigated.

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Figure 1 Histologic progression of nonalcoholic fatty liver disease from simple steatosis to cirrhosis.

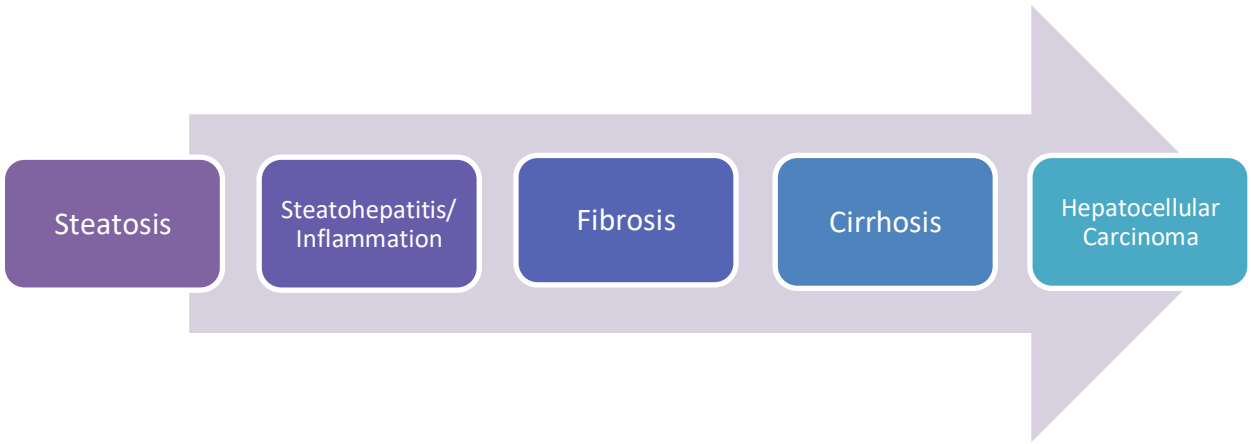

Figure 2. The spectrum of nonalcoholic fatty liver disease

Abbreviations: NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma.

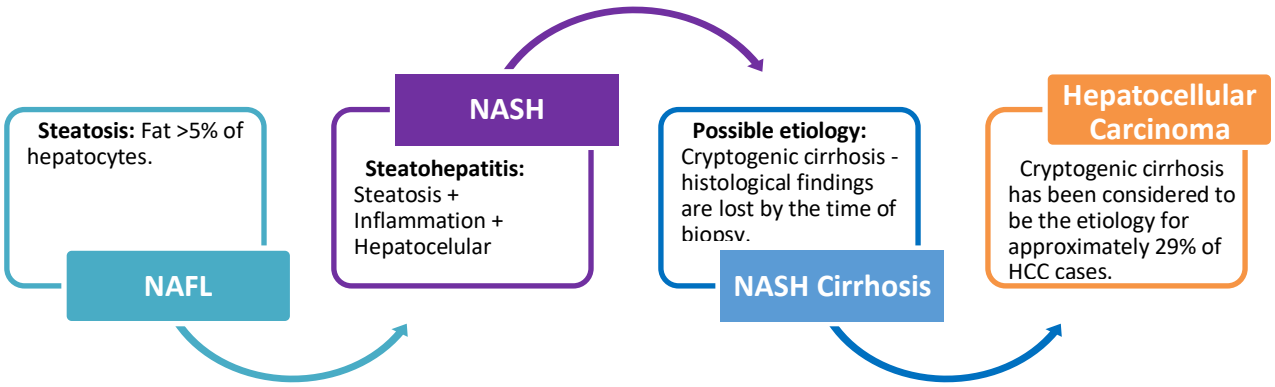

Table 1. Role of noninvasive liver function testing in clinical progression of NASH

Sr. No.	Indicator	Author Name	Journal	Year	Results
1	Bilirubin	Demir M	PLOS One	2013	Total bilirubin was identified as a significant predictor of advanced fibrosis and used to construct the NIKEI score which can reliably exclude advanced fibrosis in subjects with NAFLD.
		Ratziu V	BMC Gastroenterol	2006	FibroTest which includes total bilirubin in its panel is a simple and noninvasive quantitative estimate of liver fibrosis which reliably predicts advanced fibrosis.
		Adams LA	Clin Chemistry	2005	Hepascore, a model of 4 serum markers plus age and sex provides clinically useful information regarding different fibrosis stages among hepatitis C patients.
2	Serum Aspartate and Alanine aminotransfe rase (AST/ALT)	Martin-Rodriguez JL	Medicine (Baltimore)	2017	Serum ALT level is the most predictive laboratory investigation for the NAFLD. The AST-ALT Ratio (AAR) is higher

				in increasing liver fat content, fibrosis and other metabolic derangements like diabetes and dyslipidemia.
	Enomoto H	World J Gastroenterol	2015	The AST/ALT Ratio (AAR) > 1 is consistent with NASH.
	Arora A	J Clin Exp Hepatol	2012	AAR > 1 may indicate the progression of NAFLD and aid in diagnosing liver fibrosis.
	Shah AG	Clin Gastroenterol Hepatol	2009	The FIB-4 score composed of age, AST and ALT and platelet counts is an invasive and inexpensive method which has shown superiority to BAAT (BMI, Age, ALT, Triglycerides) and BARD (BMI, AST:ALT, Diabetes) scores in monitoring the progress of NASH.
	McPherson S	Eur J Gastroenterol Hepatol	2013	The FIB-4 score was reliable in ruling out advanced fibrosis in patients with histological evidence of NAFLD who had

						normal or increased levels of ALT, thus decreasing the need for invasive liver biopsy.
3	Platelet Count	Enomoto H	World Gastroenterol	J	2015	A reducing level of platelet count has been well documented in advancing liver diseases.
		Kawamura Y	Hepatol Int		2015	FSN score of 17 variables including platelet count could accurately predict fibrotic stage and discriminates patients with advanced fibrosis of NASH.
		Kessoku T	World Gastroenterol	J	2014	PLALA Score is a very unique scoring system as it has shown usefulness in distinguishing cirrhosis in NAFLD when compared with most fibrosis scoring systems
		Abdel-Razik A	Eur Gastroenterol. Hepatol.	J	2016	Mean Platelet Volume (MPV) is a noninvasive novel marker to predict advanced disease as it was increased in

					NASH patients and advance liver fibrosis.
		Cengiz M	Eur J Gastroenterol. Hepatol.	2015	Red cell volume distribution width-to-platelet ratio (RPR) was both correlated and able to predict liver fibrosis. It may reduce liver biopsy in NAFLD.
4	Fasting blood glucose and glycosylated protein:	Pelusi S	PLOS One	2016	Nonalcoholic steatohepatitis with greater degree of fibrosis was discovered in patients with insulin resistance. Type 2 diabetes in patients with NAFLD tends to drive the rate of fibrosis.
5	Hyaluronic acid (hyluroante) tissue metaloprotei nase	Arora A	J Clin Exp Hepatol	2012	European Liver Fibrosis score ELF scoring system has indicators for cellular matrix activities including Hyaluronic acid (hyluroante) tissue metalloproteinase which has been indicative of fibrosis.
6	Type IV collagen	Nakamura A	J Diabetes Investig	2013	NAFIC Score including type IV collagen 7S and

					Modified NAFIC score were proven to be clinically useful in screening for NASH in NAFLD patients.
7	Glycosylated Albumin to Glycosylated Hemoglobin Ratio	Hu K-C	World Journal of Gastroenterology	2014	HOMA-IR score indicates NAFLD progression using a formula that involves insulin levels and fasting glucose to calculate insulin resistance (IR). The score has a high sensitivity for NASH.
		Stål P	World Journal of Gastroenterology	2015	NAFLD fibrosis score (NFS), a non- invasive score which includes the presence of diabetes or impaired fasting glucose is the most predictive of mortality in NASH as compared to NAFL patients
8	Prothrombin time	N Assy	World Journal of Gastroenterology	2005	Increase prothrombin time is usually associated with cirrhotic changes.
9	Albumin	Jessica Bazick	Diabetes care	2015	Serum albumin gets reduced in patients progressing to NASH and fibrosis from NAFLD.

Table 2. Role of imaging techniques in clinical progression of NASH

Sr. No.	Imaging Modality	Author Name	Journal	Year	Results
1	Ultrasound	Sanyal AJ	Gastroenterology	2002	Ultrasonography (US) is currently the preferred method in United States for screening asymptomatic patients with elevated liver enzymes and suspected NAFLD with sensitivity in detecting steatosis varying between 60–94%.
2	Magnetic Resonance Elastography	Iijima H	Hepatol Res	2007	Magnetic Resonance Elastography has excellent diagnostic accuracy with sensitivity and specificity of 98% and 99%, respectively, for detecting all grades of fibrosis.
		Huwart L	Gastroenterology	2008	Magnetic resonance elastography was associated with a higher technical success rate than US elastography
3	Fibroscan	Wong VW	Gut	2012	Transient elastography had shown good results in patients with NAFLD. It is a non-invasive method of assessing liver fibrosis which can be performed

				at the bedside or in the outpatient clinic.
	Wong VW	Hepatology	2010	Transient elastography had shown good results in patients with NAFLD. It is a non-invasive method of assessing liver fibrosis which can be performed at the bedside or in the outpatient clinic.
	Castéra L	Gastroenterology	2005	Fibroscan has now been validated in NAFLD, and represents a useful tool for rapid, non-invasive assessment of liver fibrosis and determining the need for biopsy. Nonetheless, fibroscan values should be interpreted in consonance with clinical, biological, and morphological data.

Table 3. NASH Activity Score; Steatosis, Activity, and Fibrosis Score; and Brunt Grading and Staging systems.

NASH Activity Score	**Steatosis, Activity and Fibrosis Score**	**Brunt Grading and Staging**
Steatosis grade 0-3	Steatosis S0-S3	Grade 1 (Mild)
Lobular inflammation 0-3	Activity A1-A3	Grade 2 (Moderate)
Ballooning 0-2	Lobular inflammation 0-2	Grade 3 (Severe)
Fibrosis 0-4 (grade 1 has subgrade A,B,C)	Ballooning 0-2	Stages fibrosis
	Fibrosis F0-F4	Stage 1-4