



## Review Article

# Diagnosis of Non-alcoholic fatty liver disease

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Steatosis;  
 Hhepatitis;  
 HAIR score;  
 NASH test;  
 Fibrosis score;  
 BAAT score

**ABSTRACT**

Non Alcoholic Fatty Liver Disease is the deposition of fat in liver in absence of excessive of alcohol consumption. Non Alcoholic Fatty Liver Disease ranges from simple steatosis to Nonalcoholic steatohepatitis and cirrhosis. Most cases (90%) of Non Alcoholic Fatty Liver Disease have simple steatosis with benign prognosis. Ten to thirty percent of Non -Alcoholic Fatty Liver Disease progresses to NASH and 25-40% of Nonalcoholic steatohepatitis undergoes progressive liver fibrosis. Ultimately 20-30% of Nonalcoholic steatohepatitis will go into cirrhosis during their lifetime. Nonalcoholic steatohepatitis cirrhosis has higher chances of (2.6% per year) going into hepatocellular carcinoma. There are several risk factors noted for Non Alcoholic Fatty Liver Disease. Some of which includes increasing age, metabolic syndrome, dietary factors etc. Investigations regarding liver function test can be divided into invasive and noninvasive types. Under invasive procedures comes liver biopsy and non-invasive includes radiological tests and various biochemical tests. This article tries to analyze different scoring systems and their significance in diagnosing steatohepatitis and fibrosis.

**INTRODUCTION**

Fatty liver or hepatosteatois is referred to as fat accumulation in liver that exceeds 5-10% by weight of the organ.<sup>1</sup> There are several causes of fatty liver, alcohol is the commonest and well known since several years. However, in recent years due to life style changes and environmental factors nonalcoholic causes of fatty liver have risen. In all cases demonstrating elevation of liver function test results, Non Alcoholic Fatty Liver Disease (NAFLD) is noted in 90% of cases.<sup>2</sup> It is one of the leading causes of abnormal liver function test (LFTs) in countries like UK and China.<sup>3,4</sup>

In general, NAFLD is the deposition of fat in liver in absence of excessive of alcohol consumption. More specifically, American Association for the Study of Liver

Diseases (AASLD) has proposed that NAFLD is diagnosed when following 4 criteria are met<sup>5</sup>:

- 1) Fatty change of liver is observed by imaging or histologically,
- 2) No marked alcohol drinking habit is present (ethanol intake of <210 g/week for men and <140 g/week for women),
- 3) No presence of other factors inducing fatty change of the liver and
- 4) No concomitant factors causing chronic liver disease are present.

Non Alcoholic Fatty Liver Disease ranges from simple steatosis to Nonalcoholic steatohepatitis (NASH) and cirrhosis. Most cases (90%) of NAFLD have simple steatosis with benign prognosis. Ten to thirty percent of NAFLD progresses to NASH and 25-40% of NASH undergoes progressive liver fibrosis.<sup>6</sup> Ultimately 20-30% of NASH will go into cirrhosis during their lifetime.<sup>7</sup>

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NASH cirrhosis has higher chances of (2.6% per year) going into hepatocellular carcinoma. In association with other liver diseases contributing to NAFLD, the disease progression is more rapid.<sup>8</sup> Most common cause of death associated with NAFLD is cardiovascular disease (28% of total deaths) followed by extra hepatic malignancies (25% of total deaths) and liver disease (13% of total deaths) respectively.<sup>9</sup> Nonalcoholic steatohepatitis has a high risk of liver disease-related deaths such as from hepatic cirrhosis and hepatocellular carcinoma.<sup>10</sup>

Nonalcoholic steatohepatitis can be differentiated from other NAFLD related conditions only in liver biopsy. Its microscopic findings should fulfill 3 following criteria:<sup>10</sup>

- 1) Macrovesicular fatty change of hepatocytes
- 2) Inflammatory cell infiltration and
- 3) Ballooning degeneration of hepatocytes.

Differential diagnosis of secondary Hepatic steatosis can be divided in macrovesicular and microvesicular types. Macrovesicular type which is relevant to NAFLD includes, excessive alcohol consumption, Hepatitis C (genotype 3), Wilson's disease, lipodystrophy, starvation, rapid weight loss, insulin resistance, syndrome X (obesity, diabetes, hypertriglyceridemia, hypertension), total parenteral nutrition, abetalipoproteinemia, Medications (e.g. amiodarone, methotrexate, tamoxifen, corticosteroids) and inflammatory bowel diseases.<sup>5</sup>

## RISK FACTORS

There are several risk factors noted for NAFLD. Some of which includes: age (higher risk with increasing age), metabolic syndrome (70-90% have NAFLD, metabolic syndrome is also a parameter having value as an independent predictor of fibrosis), gender (men>Female, however, women have higher risk of advanced fibrosis), ethnic groups (higher in Hispanics and lower in Blacks), dietary factors (High cholesterol, high saturated fats, high fructose intake and low carbohydrate intake. Caffeine on the other hand may be protective), obstructive sleep apnoea (risk of fibrosis is increased) and genetic factors (Patatin-like phospholipase domain-containing 3 (PNPLA3) gene).<sup>6</sup> Others with emerging association are polycystic ovary syndrome, hypothyroidism, hypopituitarism, hypogonadism and pancreato-duodenal resection.<sup>5</sup> Among these risk factors the metabolic syndrome has special importance. It is diagnosed when three or more of the following features are present:<sup>6</sup> (Table 1)

A third of NAFLD cases have full metabolic syndrome and >90% have at least one of the criteria. Also the severity of NASH is directly proportional to severity of metabolic syndrome and these patients have high chances of going into fibrosis.<sup>6</sup>

**Table 1: Diagnostic features of metabolic syndrome**

Central obesity	(waist circumference $\geq 94$ cm for men and $\geq 80$ cm for women),
Impaired fasting glucose	( $>5.6$ mmol/L or no treatment)
Hypertriglyceridemia	( $>1.7$ mmol/L or no treatment),
Low HDL cholesterol and	( $<1.0$ mmole/L for men or no treatment, $<1.3$ mmol/L for women or no treatment)
Hypertension	( $>135/85$ mmHg or no treatment).

However, for NAFLD, a disease (prevalence of 10-24% worldwide and as high as 57.5% to 74% in those who are obese)<sup>2</sup>, the diagnosis to be made on an invasive procedure makes it likely to have sampling errors, costly and is not possible to be performed in all patients.

## INVESTIGATIONS

Investigations regarding liver function test can be divided into invasive and noninvasive types. Under invasive procedures comes liver biopsy and non-invasive comes rest.

### I. LIVER BIOPSY

Liver biopsy is usually indicated in cases where we find diagnostic uncertainty or if non-invasive staging is indeterminate. Liver biopsy remains the gold standard for the diagnosis of NAFLD.

## MICROSCOPIC DIAGNOSIS

The histopathological finding of NASH includes fat deposition, inflammatory cell infiltration (neutrophil and lymphocytes) in lobules, ballooning degeneration, Mallory-Denk bodies, pericellular fibrosis, sinusoidal fibrosis, giant mitochondria, eosinophilic necrosis and iron deposition. However, all of the specific findings are not available for the diagnosis and so over the years few pathological criteria for nonalcoholic steatohepatitis have been proposed. The initial one was proposed in 1999, by Motteoni et al, which included Type 1: steatosis alone, Type 2: steatosis with inflammation, Type 3: steatosis with hepatocyte ballooning and Type 4: Type 3 with Mallory-Denk bodies or fibrosis.<sup>15</sup>

The stratification was further studied for several years and the prognostically two groups had different outcomes they were: type 1 plus type 2 and type 3 plus type 4. Disease related mortality ranged in former from 1.7% to 2.7% and in later from 11% -17.5%. (d) The problem lied in the subjective variation of grading the balloon degeneration, which differentiated type 2 from type 3. Hence a proposal of another grading was made in 2005, by the Nonalcoholic Steatohepatitis Clinical Research Network (NASH-CRN) which graded steatosis (0-3), inflammation (0-3) and Hepatocyte ballooning (0-2) aiming at the type 2 and type 3 of Matteoni classification. The total score was divided into two groups as lower than 2 and more than 5 as Non

NASH and NASH. Cases in between were considered borderline. Validation studies later showed that it was more objective and reproducible, required no special staining, was applicable for pediatric NASH, and useful for assessing therapeutic effects in clinical studies. However, it cannot diagnose the burnt out NASH in which inflammation has resolved after treatment and only fibrosis remains as fibrosis is not considered in the classification.<sup>16</sup> Motteoni et al, classification is said to have more diagnostic and prognostic significance than NAS classification. However, NAS classification is recommended for larger clinical studies and for monitoring the short term therapeutic effect of drugs.<sup>10</sup> Recently Younossi et al, have proposed that NASH is diagnosed as (1) any degree of steatosis along with centrilobular ballooning and/or Mallory-Denk bodies or (2) any degree of steatosis along with centrilobular pericellular/perisinusoidal fibrosis or bridging fibrosis.<sup>17</sup> This classification tries to eliminate the subjective variation of balloon degeneration of the Motteoni et al, study and also incorporated the burnt out NASH by including fibrosis.

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## **DRAWBACKS**

### **Sampling error:**

It is reported that only 1/50000 of the whole liver tissue is sampled if only one biopsy is taken.<sup>10</sup> Hence it is advised that 2 or more samples with thick needle and of a length approximately 15-16 mm or more.<sup>5,10,11</sup> In various studies done, the reporting of fatty change (78%) had higher consistency than fibrosis (41%) and balloon degeneration (18%) (needed for diagnosis of NASH). Also fibrosis stage varied by one or more stage between right and left lobe of liver.<sup>10</sup> Hence, AASLD had recommended the biopsy of right lobe biopsy first, and rebiopsy from the left lobe after treatment when left lobe is biopsied before treatment.<sup>10,12</sup>

### **Inter and intra-observer variation:**

This inter-observer variation was measured in several studies. Efforts were made in training the histopathological observation. However, the pre and post training values did not differ much.<sup>10</sup>

### **Complications and risks:**

Complications of biopsy includes: Pain (in 20-84%, depending on whether mild pain is included or not), serious complication (0.3-0.57%) to mortality (0.01%). The complication can be reduced by performance of the procedure by trained and experienced personal and using aspiration type biopsy needle.<sup>10,13,14</sup>

## **II. IMAGING**

Ultrasonography is one of the common diagnostic modality used in NAFLD however its sensitivity is low when fatty changes are less than 20-30% and has subjective variation.<sup>18,19</sup> Various (0-3) grades of steatosis have been proposed based on visual analysis of the intensity of the echogenicity. Grade I is characterized by just increased in echogenicity. When the echogenic liver obscures the echogenic walls of portal vein branches, it is grade II. In grade III the echogenic liver not only obscures portal vein branches but also the outline of diaphragm.<sup>20</sup> These are however subject to inter-observer variation. The sensitivity of USG in detecting hepatic steatosis ranges from 60 to 94% and the specificity from 84 to 95%.<sup>21-23</sup>

Contrast enhanced ultrasound has recently been proposed to differentiate between NASH and simple steatosis.<sup>24</sup> Fibroscan (Transient elastography) gives an account of "liver stiffness measurement" (LSM) has a high AUROCs for detection of  $\geq$  stage 2 fibrosis,  $\geq$  stage 3 fibrosis and cirrhosis of 0.84, 0.93 and 0.95 respectively.<sup>6</sup> However, the results may be invalid in older patients (>52 years) and with central obesity or type 2 diabetes.<sup>25</sup> CT scan is more objective and can measure visceral fat. Fatty liver is diagnosed when liver to spleen ratio is <0.9, however its sensitivity is low.<sup>26,27</sup> MRI has shown promising results but its use is limited to research.<sup>10</sup>

## **III. NON INVASIVE DIAGNOSTIC METHODS**

Considering the risk of liver biopsy, a noninvasive diagnostic tool for the diagnosis and monitoring of NAFLD would be of great value. An ideal test should be simple, provides incremental information to currently available diagnostic tools, easy to measure and handle, cost-effective, accurate for diagnosis of NASH or stage of fibrosis, accurate for risk stratification and monitoring response to therapy and validated in multiple large and prospective trials.<sup>27</sup>

### **LIVER FUNCTION TEST**

Liver enzymes are normal in up to 78% of cases of NAFLD.<sup>7,28</sup> The AST and ALT elevation is typically found to be mild and is usually not more than four times the upper limit of normal. As the disease progresses from fibrosis to cirrhosis ALT falls and AST may rise.<sup>6</sup> The AST/ALT ratio of less than 1 can indicate no or minimal fibrosis and more than 1 that of development of cirrhosis. However, this is not very sensitive. Gamma Glutamyltransferase (GGT) is increased in NAFLD. It is associated with increased mortality and also is a good predictor of advanced fibrosis. It cannot, however, be taken as NAFLD's sole diagnostic tool.<sup>29</sup> It has been reported that among cases with normal liver function tests (LFT), 70-80% of subjects with central obesity and around 50-80% with type 2 diabetes have evidence of NAFLD on

imaging. Hence dismissing patients as normal based on LFT will lead to mismanagement of NAFLD/NASH.

### NON INVASIVE INVESTIGATION IN DIFFERENTIATING SIMPLE STEATOSIS FROM NASH:

Following investigations (as shown in table 2) have been evaluated in several studies as soul modality in diagnosis and management of NAFLD. However, these studies are mostly done in western population, in severe obesity and weight loss related clinical studies and no proper validation studies done. Apart from individual tests several scores have been proposed by various groups across the world in evaluation of simple steatosis and NASH. Most of the scores have combination of investigations and clinical signs.

**HAIR score:** ( three scored components) includes hypertension (HTN), ALT level, and insulin resistance.<sup>30</sup> The presence of at least 2 factors have high sensitivity and specificity in predicting NASH

**Mayo Clinic study:** Any three of the six criteria should be met for diagnosis: 1. age  $\geq$  50 years old, 2. female sex, 3. BMI  $\geq$  30 kg/m<sup>2</sup>, 4. AST  $\geq$  45 IU/L, 5. ALT/AST ratio  $\geq$  0.8, and 6. hyaluronic acid  $\geq$  55 ng/mL<sup>31</sup>

Palekar et al. studied 6 different variables: age, gender, AST, BMI, AST/ALT ratio(AAR) and serum hyaluronic acid. Sensitivity and specificity for the diagnosis of NASH was 74% and 66% respectively.<sup>32</sup> A recently proposed equation ( $2.627 \times \ln [AST] + 2.13$  for DM)<sup>33</sup>

**NASH clinical scoring system** includes: HTN, type 2 DM, AST  $\geq$  27 IU/L, ALT  $\geq$  27 IU/L, sleep apnea syndrome, and race (other than blacks).<sup>34</sup>

**Nice's Model:** CK18, ALT, and the presence or absence of metabolic syndrome is scored.<sup>35</sup>

Few Japanese studies have proposed scores for the differentiation. **NAFIC score**, comprises three items – ferritin [200(female) or 300 (male) ng/ml as one point], fasting insulin [10microU/ml or higher as point one], and type 4 collagen 7S [5.0 ng/mL or higher as point one]. NAFIC score of 2 or more has higher changes of NASH. This study had an advantage of being validated by a large study and is done in an Asian population. However, the costs of these tests are high, it's utility in other Asian or non-Asian population is not known.<sup>36</sup>

All of these tests need more validation studies in a larger scale and in specific target population.

### NON INVASIVE INVESTIGATIONS IN DIAGNOSIS OF NASH WITH ADVANCED FIBROSIS:

**Table 2: Individual tests as tool for evaluation of steatosis and NASH**

Biomarkers	Study results
<b>Hepatocyte apoptosis:</b> <sup>10,29,30</sup>	
Cytokeratin-18	Validation study done. not recommended by AASLD
Homocysteine levels,	
Serum prolidase enzyme activity catalysis,	
Plasma pentraxin 3 levels and Tissue polypeptide specific antigen.	
<b>Inflammation:</b> <sup>29</sup>	
TNF alpha	Most of the studies not show clear utility values in investigation related to NAFLD/NASH
Adiponectin,	
C-reactive protein,	
IL-6,Leptin,	
CC-chemokine ligand 2 and Hyaluronic acid	
<b>Fibrosis:</b> <sup>29</sup>	
Type IV collagen 7S,	Fibrosis: <sup>29</sup> Type IV collagen 7S, Hyaluronic acid, Platelet
Hyaluronic acid,	
Platelet	
<b>Oxidative stress:</b> <sup>29</sup>	
Lipid peroxidation products,	Mixed results
Vitamin E levels,	
Copper-to-zinc superoxide dismutase,	
Glutathione peroxidase, Thioredoxin levels	

Fibrosis can be staged according to **Burnt's criteria** into<sup>37</sup>

- Stage 1- zone 3 perisinusoidal fibrosis;
- Stage 2- zone 3 perisinusoidal fibrosis with portal fibrosis
- Stage 3- zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis; and
- Stage 4- cirrhosis.

According to **Kleiner's classification** fibrosis is classified into 4 stages with stage I subdivide into three substages: Substages 1a zone 3 perisinusoidal fibrosis with delicate collagen deposition, 1b are zone 3 perisinusoidal with dense collagen deposition and Substages 1c portal or periportal (representing the pediatric pattern of fibrosis). Stage 2 indicates Zone 3 and periportal fibrosis. Advanced fibrosis is classified as Stage 3 or 4.<sup>38</sup> Limitations of these classification include: no account of mixed portal/central lesions and does not evaluate remodeling. New scoring system, modified Laennec scoring system, has been proposed however it lacks validation, does not evaluate etiology and remodeling/regression.<sup>38</sup>

Several scoring systems have been proposed regarding fibrosis in NASH. These include:

A French group proposed the **BAAT score** (0-4 points): 1



point each is assigned to BMI  $\geq 28$  kg/m<sup>2</sup>, ALT 2 or more times greater than the normal upper limit, age  $\geq 50$  years old, and TG  $\geq 1.7$  mmol/L. In this system the negative predictive value (NPV) for a score of 0-1 point was 100% in cases with fibrosis of stage 2 or higher.<sup>10</sup>

**FibroTest** takes in to account bilirubin,  $\gamma$ GT,  $\gamma$  globulin, haptoglobin, and  $\alpha$ 2-macroglobulin and is proposed by the same French group.<sup>10</sup>

**Mayo Clinic** has proposed the **NAFLD fibrosis** score (NFS) [ $= -1.675 + 0.037 \times \text{age (year)} + 0.094 \times \text{BMI (kg/m}^2) + 1.13 \times \text{IFG/DM (with = 1, without = 0)} + 0.99 \times \text{AAR} - 0.013 \times \text{platelets (PLT)} (\times 109/\text{L}) - 0.66 \times \text{Alb (g/dL)}$ ]. The score is interpreted as low (NFS  $< -1.455$ ), intermediate and high (NFS  $> 0.676$ ) scores.<sup>39</sup>

**BARD score** with one point to BMI  $\geq 28$  kg/m<sup>2</sup>, two points to AAR  $\geq 0.8$ , and one point to DM, respectively. It is reported that with score of 2 or higher the possibility of Stage 3 or 4 is very high.<sup>40</sup> It is seen that with this test, even with mild disease the score is of  $\geq 2$  due to obesity and diabetes.<sup>6</sup>

**FIB-4 index**, calculated as:  $[\text{age (year)} \times \text{AST (IU/L)}] / [\text{PLT (109/L)} \times \text{ALT (IU/L)}]^{41}$

AST to platelet ratio index (APRI)  $\{[(\text{AST level/upper limit of normal AST})/\text{PLT (109/L)}] \times 100\}$ . Useful for predicting significant fibrosis due to NASH<sup>42</sup>

Combined interpretation of PLT and AAR (PAAR) is another useful parameter in that patients with platelet count of 195000 or greater along with an AAR below 0.8 have very low possibility of having Stage 3 or higher fibrosis.<sup>43</sup> Platelet count alone cannot be well correlated with fibrosis as the count is relatively higher with advanced fibrosis.<sup>10</sup>

McPherson et al made a comparison of five scoring systems, AAR, APRI, BARD, NFS, and the FIB-4 index which involved 145 English NAFLD patients.<sup>42</sup> They concluded that FIB-4 index was the most favorable (0.86), followed by AAR (0.83), NFS (0.81), BARD (0.77), and APRI (0.67) [within the bracket are the area under the receiver operating characteristic curve (AUROC) values]. Positive predictive values of the FIB-4 index and of NFS are promising and are 75% and 79%, respectively.

In a validation study of 827 cases with biopsy proven NAFLD fibrosis, AUROC of BARD score was 0.81 for stage 3-4 fibrosis. Similarly the validation studies done for NAFLD fibrosis score (733 cases) and FIB-4 score (541 cases) showed an AUROC of 0.88 and 0.80 respectively for stage 3-4 fibrosis.<sup>40,41</sup>

## CONCLUSION

NAFLD is the deposition of fat in liver in absence of excessive of alcohol consumption.

Non Alcoholic Fatty Liver Disease ranges from simple steatosis to Nonalcoholic steatohepatitis (NASH) and cirrhosis. Most cases (90%) of NAFLD have simple steatosis with benign prognosis. Ten to thirty percent of NAFLD progresses to NASH and 25-40% of NASH undergoes progressive liver fibrosis. Ultimately 20-30% of NASH will go into cirrhosis during their lifetime. Various noninvasive modalities are used in the diagnosis of NAFLD and its various stages. However combination of different modalities both invasive and noninvasive is required for the ultimate diagnosis and proper management of the patient with non-alcoholic fatty liver disease.

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