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REVIEW ARTICLE

# Non-alcoholic Fatty Liver Disease Diagnosis, Grading and Staging; A Simplified Tool for Clinicians

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**Abstract**

Non-alcoholic Fatty Liver Disease (NAFLD) is currently reliably and increasingly being diagnosed and classified by non-invasive methods in comparison with standard invasive methods. Various permutations and combinations has been studied and explored with different clinical sign and symptoms with or without lab profiles to closely approach the precise diagnosis. It is indeed the need of the hour to understand the most suitable non-invasive clinical modality in a given set of patient profile to reach to the finest and closest diagnosis without compromising the outcome as far as the patient management is concerned. This could well be learnt progressively to more and more effectively use of these non-invasive tools and avoiding cumbersome liver biopsy in parallel

Fatty liver is frequently used terminology in the day to day reporting of routine and focused abdominal imaging. This leaves a good amount of stress and alarm on patients and their kin's mind. The most frequently asked question after these reports is 'what is the current status and what next ?' Various clinical methods extending from a single parameter to combination of various clinical laboratory profile has been tried to answer the question precisely. There are a lot of scores and system which exists in the literature but 'which one to use and where to use' is sporadically arranged making it difficult in usability. This is to simplify the same and make better understanding of the topic for the clinicians to objectively answer all the queries related to stage, grade and diagnose of this entity. Furthermore it is attempted to reliably use the non-invasive modalities of staging and grading to replace cumbersome liver biopsy without compromising the clinical benefits out of management dependent on these parameters .

## CONVENTIONAL METHODS

NAFLD should strongly be suspected in patients with deranged liver enzymes and/or who have features of metabolic syndrome. However, before labelling a person as NAFLD extensive/detail history (alcohol abuse, drug intake) must be taken and serological studies (viral and autoimmune hepatitis, alpha-1-antitrypsin deficiency, hemochromatosis and Wilson's disease) should be done.

NAFLD patients are mostly asymptomatic; however, asymptomatic liver enzyme elevation gives a clue to the diagnosis. Often, hepatic steatosis is detected incidentally on liver ultrasound. NAFLD is typically seen in obese populations who might complain of pain in right hypochondrium and fatigue. Few of the patients go on to progress to chronic liver disease and cirrhosis. Dorsocervical lipohypertrophy has been reported to be strongly associated with severity of steatohepatitis in a recent study[1]. Few terms need clarification before proceeding further as in below table[1].

**Table 1:** Various simplified definitions related to Non-alcoholic fatty liver disease

| **Non-alcoholic Fatty Liver (NAFL)related terminology** | |
|---|---|
| NAFLD | Comprises gamut of condition, ranging from fatty liver to steatohepatitis to cirrhosis in non-alcoholic non-drug abuse individuals. |
| NAFL | Hepatic steatosis without hepatocellular injury(ballooning) or fibrosis. |
| "Non-alcoholic steatohepatitis (NASH)" | Hepatic steatosis with hepatocyte injury (ballooning) with or without fibrosis. |
| NASH Cirrhosis | Cirrhosis with current or previous histological evidence of NASH |
| Cryptogenic Cirrhosis | Cirrhosis without obvious etiology. |
| NAFLD Activity Score (NAS) | Combination of steatosis, inflammation and ballooning scores. It is a useful tool to measure changes in liver histology in patients with NAFLD in clinical trials. |

**Source:** Modified from HEPATOLOGY, June 2012 (Aasld Practice Guideline for NAFLD).

### Biochemical

Almost sixty six percent of NAFLD patients have normal liver enzymes at a given time and even in patients with normal liver enzymes, a spectrum of histological abnormalities can be seen[2]. ALT is generally more than AST, however AST/ALT greater than 1.0 is indicative of advanced disease[3] ALP and GGT are also raised and might be markers of increased mortality risk[4]. Similarly low albumin and hyperbilirubinemia are seen in advanced liver disease which is a consequence of NAFLD[5]. Elevated Serum Ferritin (in one-half of the patients) and transferrin saturation (in one-tenth) are also seen[3].

Fatty liver Index score made up of BMI, Triglycerides, GGT, and waist circumference has been used to predict fatty liver on ultrasound with AUROC of 0.84[6].

### Ultrasound

Ultrasound detects hepatic steatosis reliably. On Ultrasound, fatty liver is seen as a bright liver with echogenicity of liver more than right kidney. USG has specificity of 85-95% for fat detection, while sensitivity varies with amount of fat (55% for 10-20% fat and 80% for greater than thirty percent fat, giving overall sensitivity of 65%-95%).The grading system of steatosis is as follows:

- *Mild steatosis*: increased echogenicity of liver, normally seen diaphragm and intrahepatic vessels.
- *Moderate steatosis*: moderate increase echogenicity, mildly obscured visualization of diaphragm and intrahepatic vessels.
- *Severe steatosis*: marked increase in echogenicity, obscured penetration, poor or non-visualization of diaphragm and intrahepatic vessels.

In a recent study, using liver biopsy as gold standard, USG had a sensitivity of 64% and specificity of 91% for diagnosis of NAFLD which changes to 91% and 93% respectively once the fat content increases to greater than thirty percent as discussed above[7].

Ultrasound however loses its sensitivity in morbidly obese. It can't differentiate fat from both fibrosis and focal fat sparing and focal fatty change may give a pseudo tumour appearance. Ultrasound is operator dependent and also poor at quantifying small changes in fat and hence not suitable for longitudinal studies[8].

### Staging of NAFLD

Once diagnosis of NAFLD is made, the next step is to make an assessment of severity. It has been traditionally been done with liver biopsy which we will discuss first.

### Liver biopsy

Liver biopsy is not routinely performed in NAFLD. It is indicated generally when there are signs of chronic liver disease (CLD), splenomegaly, cytopenia, deranged iron studies, diabetes mellitus, and/or significant obesity in person aged 45 or more.

The histological spectrum of NAFLD ranges from

steatosis to cirrhosis as summarized in following (Fig. 1). Histological NASH is usually milder as compared to alcoholic steatohepatitis, so abundant neutrophils and Mallory's bodies should lead to suspicion of alcohol abuse. However it is impossible to distinguish them with hundred percent certainties based on histology alone. It is possible that changes of steatohepatitis may be absent in advanced disease.

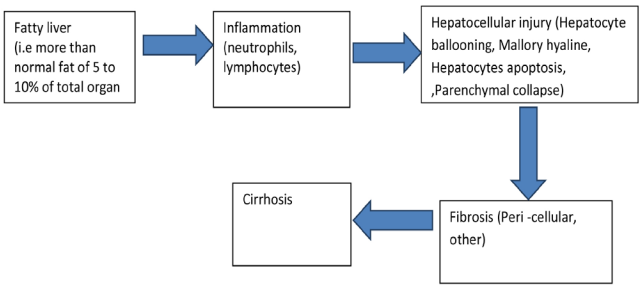


**Fig. 1:** showing spectrum of Histological changes in Non-alcoholic Fatty Liver Disease

## Steatosis

There is usually evidence of steatosis (>5%), and it can exist in variable combinations of macro vesicular (more common, with large fat vacuoles in cytoplasm and eccentric nucleus, caused due to triglyceride accumulation) and micro vesicular type (central nucleus, multiple small fat vacuoles in cytoplasm, caused due to mitochondrial injury)(9).

**Table 2:** showing aetiology associated with Macro and Micro vesicular steatosis

| **Usual causes of Macro-Vesicular Steatosis** | **Usual causes of Micro-Vesicular Steatosis** |
|---|---|
| Obesity | Acute Fatty Liver of pregnancy |
| Diabetes Insipidus | Drugs (valproate, Nucleoside Analogs) |
| Protein-Calorie Malnutrition | Toxins |
| Total parenteral Nutrition | Total Parenteral Nutrition |
| Drugs and Toxins | Rey's Syndrome |
| Metabolic Disorders (Wilson Disease) | Viral infections |
| Infections (Hepatitis C) | |

These types of steatosis can give a clue to the cause of NASH as below Table 2.

Similarly, localization of steatohepatitis also gives a clue to the cause of NAFLD as shown in Table 3.

**Table 3:** Showing zone based classification of NAFLD

| Zone* 1 predominance | Zone III predominance |
|---|---|
| Paediatric NAFLD | Adult NAFLD |
| Hepatitis c | Drugs |
| TPN | Metabolic Abnormalities |
| Cachexia | |
| AIDS | |
| Cystic fibrosis | |
| Steroids | |
| Amiodarone | |

(*Description of Zones: Zone 1, closest to the terminal branches of the portal venule and hepatic arteriole. This zone first receives oxygen, hormones, and nutrients from blood. Zone 3 is furthest from the distributing vessels; between zones 1 and 3 is the intermediate zone 2.)

- Zone 3 predominance: adult NAFLD, many drugs, metabolic abnormality
- Zone 1 predominance: paediatric NAFLD, hepatitis C, TPN, cachexia, AIDS, cystic fibrosis, steroids, amiodarone

Sometimes adult NAFLD may have diffuse steatosis as well with no zonal predominance.

*Inflammation*

Some patients of steatosis end up as fibro inflammatory lesions while others go on to develop fibrosis directly. Inflammation in NASH may be:

- Lobular: acute (neutrophilic) or Chronic (mononuclear cells)
- Portal: generally chronic (typically seen in three settings of NASH: severe steatohepatitis of adult or children, resolution of NASH following treatment and paediatric liver biopsies),

Acute (PMN infiltrates) in portal tracts generally points towards alcoholic etiology or biliary obstruction rather than NASH.

In NASH, Kupffer cells aggregates increase also and are localized in Zone 3 instead of Zone 1, where they are usually seen.[10]

## Hepatocellular Injury

Three histological features are representative of hepatocellular injury.

A. ballooning. B. acidophil bodies (apoptosis) c. spotty necrosis

Ballooning is generally seen in Zone 3, is sometimes challenging to recognize and considered a required feature for distinction between steatohepatitis (with ballooning) and steatosis (without ballooning).

Apoptosis and spotty necrosis however are not limited to zone 3.[11]

**Fibrosis**

Peri-sinusoidal (chicken wire) fibrosis in zone 3 is the earliest stage of fibrosis in NAFLD. Gradually it may extend to portal, periportal and central areas[12].

Taking into account all these histological changes scoring system has been established for NASH which is as follows:

**Grading and staging of NAFLD**

Staging and grading of NAFLD is done based on quantity of fat accumulation, evidence of necrosis and inflammation with their cellular and distribution detail and distribution and amount of fibrosis. The same has been summarised and depicted in table 4 given below.

**Table 4:** showing principle of staging and grading based on biopsy

| GRADING NAFLD | STAGING NAFLD |
|---|---|
| I. Macro vesicular steatosis<br><br>Grade 0: none<br>Grade 1: up to 33 percent (%)<br>Grade 2: 33 %-66 %<br>Grade 3: >66 %<br>II.Necro-inflammatory activity<br>Grade 1 (mild) ≤66 %, zone 3 ballooning occasionally found, sporadic acinar neutrophils (PMN) ± lymphocytes, minimal inflammation of porta<br>Grade 2 (moderate) Definite zone III ballooning & occasional presinusoidal fibrosis, intra-acinar PMNs,portal and intra-acinar inflammation<br>Grade 3 (severe) Pan-acinar steatosis, widely distributed with PMNs, ballooning, intra-acinar and portal inflammation | I. Stage 1 focal or extensive presence of Zone III presinusoidal/peri- cellular fibrosis;<br>II. Stage 2 Zone III presinusoidal/peri -cellular fibrosis with focal or extensive **periportal fibrosis**<br>III. Stage 3 Zone III presinusoidal/peri -cellular fibrosis and portal fibrosis with focal or extensive **bridging fibrosis**<br>III. Stage 4 Cirrhosis |

Apart from the above grading and staging system proposed by American Gastroenterological Association (AGA), several systems have been suggested for histological assessment of NAFLD, of which Kleiner NAFLD activity score is most well validated and established[13]. It is a composite score consisting of Degree of steatosis (0-3), Lobular inflammation (0-3), Hepatocyte ballooning (0-2) plus additional score for fibrosis. A score of ≥ 5 suggests probable or definite NASH while <3 indicates against NASH. However, despite liver biopsy being gold standard, inter observer variability remains a cause of concern[14].

Apart from these conventional methods of diagnosis and evaluation, unconventional non-invasive techniques also exist which has been gaining in popularity. Such assessments can provide information on amount of liver fibrosis and/or NASH. Fibrosis is said to have stronger impact on outcome than inflammation. Some authors have advocated, non-invasive scoring systems for fibrosis and inflammation as more accurate measure of global liver fibrosis severity than liver biopsy which samples only 0.02% of entire organ[15].

**Newer non-invasive methods**

There are two important aspects to be looked while staging NAFLD patients:

A. Level of Fibrosis
B. Level of inflammation/ballooning

Various non -invasive tools have been developed many more would be in the pipeline to stage NAFLD without compromising the true picture of the same.

**Assessment of fibrosis**

**1. Scoring systems:**

They have been found more helpful in diagnosing advanced fibrosis[16] and might miss mild to moderate ones.

**A) BARD [body mass index (BMI):** Aspartate aminotransferase/alanine aminotransferase (AST/ALT), diabetes] Ratio as depicted in the table below table 5 combines simple parameters to give significant information.

According to original methods, a total of 2-4 points indicate significant fibrosis. It is simple to use for physicians working at primary and secondary care level, and ever since its use in the primary study[17]; It has been validated[18] and promoted in review of assessing fibrosisnon-invasively[19] with Sensitivity, Specificity, Negative predictive value (NPV) and Positive predictive value (PPV) of 89%, 44%, 95%, 25% respectively.

**Table 5:** Showing calculation of fibrosis score by body mass index (BMI), Aspartate aminotransferase/ alanine aminotransferase (AST/ ALT), diabetes (BARD ratio).

| Parametrs | Scores |
|---|---|
| Body mass index(BMI)<28 kg/m$^2$ | 0 |
| BMI ≥28 kg/m$^2$ | 2 |
| AST/ALT ratio < 0.8 | 0 |
| AST/ALT ratio ≥ 0.8 | 2 |
| Type 2 diabetes | 1 |

### (B) AST to Platelet Ratio Index (APRI)

The APRI is calculated as mentioned below:

$$APRI = \frac{\text{AST level / Upper level of normal AST in IU/L}}{\text{Platelet count } (10^9/l)} \times 100$$

It has been mostly used in chronic hepatitis. But there is later validation in many studies that it could be used as well for diagnosis of advanced fibrosis. Use of APRI in combination (such as Fibro Test, an algorithmic approach) may result in higher diagnostic accuracy than using alone. A simplified and useable interpretation has been depicted below in the table 6.

**Table 6:** Showing Aspartate aminotransferase to Platelet Ratio Index (APRI) for calculating fibrosis and cirrhosis

| APRI score | Sensitivity (%) of fibrosis | Specificity(%) fibrosis |
|---|---|---|
| >0.5 | 81 | 50 |
| >1 | Sensitivity (%) of cirrhosis | Specificity (%) cirrhosis |
| | 76 | 71 |
| >2 | 46 | 91 |

### C) AST/ALT ratio

Its advantage is ease of calculation and it is a component of various scoring systems. Using cut off of 0.8 it has sensitivity 74%, specificity 78%.

### D) Fibrosis-4 (FIB-4)

The FIB-4 combines biochemical values (platelet count, ALT and AST) and age(table 7). It had good predictability for advanced fibrosis. It has been used for fibrosis secondary to hepatitis C and NASH. This indicates the level of fibrosis/ scarring of the liver. Fib 4 score = (Age x AST)/[Platelet count x (square root of ALT)]

**Table 7:** Showing prediction of fibrosis by Fibrosis-4(platelet count, ALT and AST) score

| FIB-4 score | Interpretation |
|---|---|
| < 1.45 | Absence of advanced fibrosis |
| between 1.45 - 3.25 | Inconclusive |
| > 3.25 | Presence of advanced fibrosis |

This suggests that a FIB-4 index outside the value of 1.45-3.25 is a reliable method for assessing liver fibrosis and is proven to be concordant with Fibro Test.

### E) GGT

Using a cut off of greater than 96.5 U/L GGT predicts advanced fibrosis with 83% sensitivity, 69% specificity[20].

### F) Fibro meter

Fibro meter NAFLD is different from original fibro meter test and it consists of seven variables (age, weight, fasting glucose, AST, ALT, ferritin, platelet count).

0.4184 glucose [mmol/l] + 0.0701 AST [U/l] + 0.00008 ferritin [µg/l] − 0.0102 platelet [g/l] − 0.0260 ALT [U/l] + 0.0459 body weight [kg] + 0.0842 age [years] + 11.6226. Applying this formula, it's value ranges from 0 to 1; with value, more than 0.715 strongly co-relates well and reliably with presence of significant fibrosis. It has AUROC 0.943 for significant fibrosis[41].

### G) NAFLD Fibrosis score

It is a panel comprising of six variables of age, hyperglycaemia, BMI, platelet count, albumin and AST/ALT ratio.

Formulae for NAFLD fibrosis score

-1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × Impaired fasting glucose (IFG)/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×109/l) – 0.66 × albumin (g/dl)

By applying high cut off of 0.676, it had PPV of 90% by applying low cut off -1.45, it had NPV of 93% in original study[16] and pooled AUROC, sensitivity and specificity of (0.85,0.90 and 0.97) in other studies[21].

## 2. Biomarkers

### A. European Liver Fibrosis test( ELF Test)

It is a panel of automated immunoassay of markers of matrix turnover in serum (hyaluronic acid, Tissue inhibitor of metalloproteinases 1 (TIMP-1) and Procollagen 3 N-terminal peptide (P3NP) used in combination with age[22]. It is a useful diagnostic tool when added with other simple

markers like BMI and platelet[23] and a useful prognostic tool as well for predicting morbidity or mortality at follow up[24].

**B) Fibro test**

The test involves assessment of alpha-2 macroglobulin, alpha-2 globulin (haptoglobin), gamma globulin, apolipoprotein A1, gamma glutamyl transferase and total bilirubin. Sensitivity and specificity for detection of significant fibrosis (F2 or greater) are approximately 75 and 85 percent, respectively [25].

**C) Other biomarkers**

Type VI collagen 7 S domain, Hyaluronic acid, serum laminin have been used with varying success in many studies (26)

**3. Radiological assessment**

**I) Fibro scan:** Based upon elastography, measures liver tissue stiffness (LSM) noninvasively and very quickly. Using a usg probe of low frequency of 50 MHz. Waves transmitted into the liver induces other waves called as shear waves. The velocity of this wave correlates directly with liver stiffness: (27) the is the liver, the faster the shear waves. Results are expressed in kilopascals (kPa) Interpretation has been suggested as follows: <7.9, steatosis; 7.9-9.6, indeterminate (biopsy recommended); >9.6, NASH (≥ F3 Fibrosis) (28).

This test has some limitations. The technique may be limited in patients with ascites or those with morbid obesity since fluid and adipose tissues attenuate the elastic wave. In addition, results are not reliable in patients with acute viral hepatitis. Fibro scan has been successfully validated in NAFLD[29] and a recent meta-analysis showed pooled AUROC, sensitivity and specificity of 0.94, 0.94 and 0.95 respectively.

Fibro scan also evaluates steatosis by new parameter called as"controlled Attenuation Parameter".

II) MR Elastography has also been attempted with excellent results and appears to have a lot of promise[30].

Assessment of NASH Inflammation and steatosis

**1) Biomarkers**

**I) NASH test:** Steato test combines ten blood tests with age, BMI and gender to predict steatosis better than ultrasound, gamma-glutamyl transferase (GGT) or ALT with AUROC of 0.8[31].

**ii) Cytokeratin-18 (CK-18):** Cytokeratin-18 is a marker of apoptosis and hence it is significantly higher in biopsy proven NASH than control with an AUROC 0.83 for NASH diagnosis[32]. These findings have been validated in other studies (96-103) and a meta-analysis where AUROC, Sensitivity and specificity for NASH using CK-18 was 0.82, 0.78 and 0.87 respectively.

**2) Radiology**

**a) Contrast enhanced Ultrasound**

Using levovist as contrast, ultrasound has been shown to reliably distinguish (AUROC 100%) between simple steatosis and NASH as deposition of levovist micro bubbles decreases in liver parenchyma of NASH patients due to changes inKupffer cell function and peri-cellular and periportal fibrosis.[33]

**b) CT scan**

Yield of unenhanced CT is similar to ultrasound in NAFLD. Severity of liver to spleen attenuation ratio (L/S) attenuation ratio (low attenuation of steatotic liver in contrast to spleen) is directly proportional to severity of steatosis.[34] A CT L/S cut off value of 0.8 yields specificity and sensitivity of 100% and 82% respectively for diagnosing micro vesicular steatosis amounting 30% or more. However due to radiation risk CT scan is infrequently used for diagnosis of NASH.

The final remarks for evaluation and assessment as quoted from word gastroenterology organisation release that "screening for NASH/advanced liver disease in the general population is not justified but it should be sought in all patients who present with risk factors for NASH".

## CONCLUSION

With rising prevalence of metabolic syndrome in Urban India and Western World, NAFLD threatens to be a significant cause for morbidity and mortality from liver disease and associated cardiovascular diseases and cancers. While steatosis has a benign prognosis, a substantial amount of patient population will go on to develop NASH, Cirrhosis and Hepatocellular carcinoma (HCC). Although liver biopsy remains gold standard, a creative use of various scoring system, biomarkers and fibro-scan can be used to rule out severe NAFLD with reasonable accuracy and hence decrease number of liver biopsies, while still not compromising on patient care. While lifestyle modifications, mainly diet and exercise, should be instituted in every patient of NAFLD, It is patients with advanced course diagnosed either non-invasively or with liver biopsy that should be treated aggressively.

**No Disclosures**

**No conflict of interest**

## REFERENCES


1. Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of non-alcoholic fatty liver disease. Hepatology 2010; 52: 894–903.
2. Cheung O, Kapoor A, Puri P, *et al.* The impact of fat distribution on the severity of non-alcoholic fatty liver disease and metabolic syndrome. Hepatology 2007; 46: 1091–100.
3. Fracanzani AL, Valenti L, Bugianesi E, *et al.* Risk of severe liver disease in non-alcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology 2008; 48: 792–8.
4. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with non-alcoholic steatohepatitis. Hepatology 1999; 30: 1356–62.
5. Ghouri N, Preiss D, Sattar N. Liver enzymes, non-alcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. Hepatology 2010; 52: 1156–61.
6. Sumida Y, Yoshikawa T, Okanoue T. Role of hepatic iron in non-alcoholic steatohepatitis. Hepatol Res 2009; 39: 213–22.
7. Balkau B, Lange C, Vol S, Fumeron F, Bonnet F. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study. BMC Gastroenterol 2010; 10: 56.
8. Mottin CC, Moretto M, PadoinAV,*et al.* The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. ObesSurg 2004; 14: 635–7.
9. Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. AJR Am J Roentgenol 2007; 189: W320–3.
10. Pessayre D, Fromenty B. NASH: a mitochondrial disease. J Hepatol 2005; 42: 928–40.
11. Poonawala A, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. Hepatology 2000; 32: 689–92.
12. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002; 36: 1349–54.
13. Paradis V, Zalinski S, Chelbi E, *et al.* Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology 2009; 49: 851–9.
14. Kleiner DE, Brunt EM, Van Natta M, *et al.* Design and validation of a histological scoring system for non-alcoholic fatty liver disease. Hepatology 2005; 41: 1313–21.
15. Younossi ZM, Gramlich T, Liu YC, *et al.* Non-alcoholic fatty liver disease: assessment of variability in pathologic interpretations. Mod Pathol 1998; 11: 560–5.
16. Janiec DJ, Jacobson ER, Freeth A, Spaulding L, Blaszyk H. Histologic variation of grade and stage of non-alcoholic fatty liver disease in liver biopsies. ObesSurg 2005; 15: 497–501.
17. Angulo P, Hui JM, Marchesini G, *et al.* The NAFLD fibrosis score: a non-invasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007; 45: 846–54.
18. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008; 57: 1441–7.
19. Ruffillo G, Fassio E, Alvarez E, *et al.* Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in non-alcoholic fatty liver disease. J Hepatol 2011; 54: 160–3.
20. Poynard T, Ratziu V, Charlotte F, *et al.* Diagnostic value of biochemical markers (NashTest) for the prediction of non-alcohol steato hepatitis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 2006; 6: 34.
21. Abiru S, Migita K, Maeda Y, *et al.* Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. Liver Int 2006; 26: 39–45.
22. Shimada M, Kawahara H, Ozaki K,*et al.* Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of non-alcoholic steatohepatitis. Am J Gastroenterol 2007; 102: 1931–8.
23. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology 2004; 40: 46–54.
24. Argentou M, Tiniakos DG, Karanikolas M, *et al.* Adipokine serum levels are related to liver histology in severely obese patients undergoing bariatric surgery. ObesSurg 2009; 19: 1313–23.
25. Arvaniti VA, Thomopoulos KC, Tsamandas A, *et al.* Serum adiponectin levels in different types of non-alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. Acta GastroenterolBelg 2008; 71: 355–60.
26. Targher G. Relationship between high sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease J Hepatol 2006; 45: 879–81
27. Sumida Y, Nakashima T, Yoh T, *et al.* Serum thioredoxin levels as a predictor of steatohepatitis in patients with non-alcoholic fatty liver disease. J Hepatol 2003; 38: 32–8.
28. Malik R. The clinical utility of biomarkers and the non-alcoholic steatohepatitis CRN liver biopsy scoring system in patients

with non-alcoholic fatty liver disease. J GastroenterolHepatol 2009; 24: 564–8.

29. Mitry RR. Noninvasive diagnosis of non-alcoholic fatty liver disease using serum biomarkers. Hepatology 2007; 46: 2047–8.
30. Papatheodoridis GV. Serum apoptotic caspase activity in chronic hepatitis C and non-alcoholic fatty liver disease. J ClinGastroenterol 2010; 44: e87–95.
31. Tsutsui M, Tanaka N, Kawakubo M, *et al.* Serum fragmented cytokeratin 18 levels reflect the histologic activity score of non-alcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. J ClinGastroenterol 2010; 44: 440–7.
32. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as non-invasive biomarkers for non-alcoholic steatohepatitis: a multicentre validation study. Hepatology 2009; 50: 1072–8.
33. Iijima H, Moriyasu F, Tsuchiya K, *et al.* Decrease in accumulation of ultrasound contrast microbubbles in non-alcoholic steatohepatitis. Hepatol Res 2007; 37: 722–30.
34. Seung Soo Lee and SeongHo Park, Radiologic evaluation of non-alcoholic fatty liver disease
35. World J Gastroenterol. 2014 Jun 21; 20(23): 7392–7402.