

Non-alcoholic Fatty Liver Disease: A Review

Mamun Al Mahtab, Sheikh Mohammad Fazle Akbar

Mamun-Al-Mahtab, Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Sheikh Mohammad Fazle Akbar, Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan

Correspondence to: Mamun-Al-Mahtab, MBBS, MSc, MD, FACG, Associate Professor of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. shwapnil@agni.com

Received: July 20, 2012 Revised: September 8, 2012

Accepted: September 10, 2012

Published online: March, 2013

ABSTRACT

Although being recognized rather late non-alcoholic fatty liver disease (NAFLD) appears to be major cause of liver related mortality and morbidity globally. Data source: Review of published literature on NAFLD. Review of literature suggests that we now have broad based understanding about different aspects of NAFLD ranging from aetiology to pathogenesis and prognosis to treatment. A lot still needs to be done specially to diagnose non-alcoholic steatohepatitis (NASH) non-invasively as well as to develop specific pathogenesis directed therapy for this apparently benign, but deadly disease.

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Key words: Liver; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis

Mahtab MA, Fazle Akbar SM. Non-alcoholic Fatty Liver Disease: A Review. *Journal of Gastroenterology and Hepatology Research* 2013; 2(3): 439-444 Available from: URL: <http://www.ghrnet.org/index./joghr/>

INTRODUCTION

NAFLD represents a spectrum of conditions characterized histologically by macrovesicular hepatic steatosis and occurs in those who do not consume alcohol in amounts generally considered to be harmful to the liver. There are two histologic patterns of NAFLD, namely fatty liver alone (NAFL) and NASH^[1,2].

Traditionally, fatty disorders of the liver have been classified as alcoholic or non-alcoholic. NAFLD is associated with numerous

etiologies and the underlying mechanisms as well as the natural history of the disease may vary with specific etiology. Using the term "non-alcoholic" to describe fatty liver disease associated with all of these etiologies renders the condition heterogeneous in terms of etiology and possibly, natural history as well as response to therapy^[3].

HISTORY OF DISCOVERY OF NAFLD

The association of macrovesicular steatosis of liver with inflammatory changes and fibrosis in obese subjects has been known for several decades. However, it was largely ignored as a clinical entity until several reports documented the development of liver failure in some patients following surgical jejunio-ileal bypass for morbid obesity. The hepatic histology in such patients was indistinguishable from that seen in alcoholic hepatitis and included macrovesicular steatosis, Mallory bodies, balloon degeneration, hepatocyte necrosis and fibrosis. Similar hepatic lesions subsequently were described in obese patients who had neither abused alcohol nor undergone weight-loss surgery and also in diabetic individuals. In 1980, Ludwig and colleagues introduced the term NASH to describe these histologic findings in those who did not consume alcohol^[4].

RISK FACTORS

Obesity, non-insulin dependent (type 2) diabetes mellitus (DM) and hyperlipidemia are coexisting conditions frequently associated with NAFLD^[5]. The reported prevalence of obesity in several series of patients with nonalcoholic fatty liver disease vary between 30-100%, prevalence of type 2 DM vary between 10-75% and prevalence of hyperlipidemia vary between 20-90%. Some children with NAFLD have type 1 diabetes mellitus. The prevalence of NAFLD increases by a factor of 4.6 in obese people.

Regardless of body mass index, the presence of type 2 DM significantly increases the risk and severity of NAFLD. Truncal obesity seems to be an important risk factor for NAFLD, even in patients with normal body mass index (BMI). About 50% patients with hyperlipidemia have NAFLD on ultrasound. Hypertriglyceridemia, rather than hypercholesterolemia, may increase the risk of NAFLD.

NAFLD may affect persons of any age and has been described in

most racial groups. In most series, the typical patient with NAFLD is a middle-aged woman, but some have found a higher prevalence of NAFLD in males than in females^[6].

EPIDEMIOLOGY OF NASH

NASH has been reported worldwide, although geographic variations in prevalence are evident. NASH is the histologic diagnosis in 7-11% of patients undergoing liver biopsy in the United States and Canada^[4,7], but is found in only 1.2% patients undergoing liver biopsy in Japan^[8]. In a histologic study, NASH was documented in 26% of 81 non-alcoholic patients with marker-negative abnormal liver function test results^[9]. NASH may be even more prevalent among asymptomatic patients with abnormal liver function tests, negative viral markers and negligible alcohol intake, because many of these patients do not undergo liver biopsy^[10]. The prevalence of NASH in the general population has not been defined. NASH has been described in obese adolescents as well as adults. Most cases however occur in persons in 4th to 6th decades of life. Women are 60-83% of patients with NASH in most clinical studies.

The most important epidemiologic features of NASH are listed in table 1. Obesity is the condition most often reported in association with NASH. Obesity is described in 40-100% patients with NASH. Type 2 diabetes, hyperglycemia and glucose intolerance have been described in 20-75% adult patients with NASH. The spectrum of NAFLD, including NASH, has been associated with insulin resistance and hyperinsulinemia, even in lean subjects with normal glucose tolerance suggesting that insulin resistance may be the primary phenomenon in the development of NAFL and NASH^[10]. Hypertriglyceridemia, hypercholesterolemia, or both are present in 20-81% of patients with NASH. Most patients with NASH actually have multiple risk factors including obesity, type 2 DM and hyperlipidemia.

Other metabolic, surgical and genetic conditions are also associated with NASH. Jejunio-ileal bypass once a popular treatment for morbid obesity, is associated with 40% incidence of liver function abnormalities post-operatively and with severe NASH and hepatocellular failure in up to 6% of patients. NASH has also been described after bilio-pancreatic diversion, extensive small bowel resection, gastroplasty for morbid obesity and prolonged total parenteral nutrition. NASH has been associated with limb lipodystrophy, Weber-Christian disease, small intestinal diverticulosis with bacterial overgrowth, abetalipoproteinemia and therapy with diethylaminoethoxyhexestrol, amiodarone, perhexilinemaleate, tamoxifen, isoniazid and synthetic steroids. NASH has also been described as a consequence of chronic exposure to petrochemical substances in the workplace. The conditions associated with NASH are summarized in table 2.

CLINICAL AND LABORATORY FEATURES

NAFLD is asymptomatic in a large proportion of patients^[11]. Symptoms described include vague right upper quadrant pain, fatigue and malaise. NAFLD is often discovered incidentally during evaluation for an unrelated medical condition. Most patients with NASH have elevated liver function test results and/or hepatomegaly, the later has been described in upto 75% of patients.

Mild to moderate elevations of serum aminotransferase levels are present in 70-100% of patients with NASH. There is no significant correlation between the degree of serum aminotransferase elevation and the histologic features. Serum levels of aspartate transaminase (AST) are characteristically lower than those of alanine transaminase

Table 1 Important epidemiologic features of NASH.

Important epidemiologic features of NASH	
Female sex	60%-83%
Obesity	40%-100%
Type II diabetes mellitus, hyperglycemia or glucose intolerance	20%-75%
Hyperlipidemia	20%-81%

Table 2 Conditions associated with NASH.

Conditions associated with NASH	
Acquired metabolic conditions	Obesity Diabetes mellitus Hyperlipidemia Rapid weight loss Total parenteral nutrition Acute starvation
Inborn errors of metabolism	Wilson disease Abetalipoproteinemia Tyrosinemia Hypobetalipoproteinemia
Surgical procedures	Jejunioileal bypass Biliopancreatic diversion Extensive small bowel resection Gastroplasty for morbid obesity
Drugs/toxins	Amiodarone Glucocorticoids Perhexiline maleate Synthetic estrogens Tamoxifen Diethylaminoethoxyhexestrol Isoniazid Industrial exposure to petrochemicals
Miscellaneous factors	Partial lipodystrophy Jejunal diverticulosis with bacterial overgrowth

Table 3 Histologic features of NASH.

Histologic features of NASH	
Features present in all or most cases	Macrovesicular steatosis Parenchymal inflammation Hepatocyte necrosis Ballooning hepatocyte degeneration
Features observed with variable frequency	Perivenular, perisinusoidal or periportal fibrosis Mallory bodies Glycogenated nuclei Councilman bodies Lipogranulomas Stainable hepatic iron

(ALT), which contrasts with the pattern usually seen in alcoholic hepatitis. A small percentage of patients with NASH may have low-titer ($\leq 1/320$) antinuclear antibody (ANA) positivity. Serum and hepatic iron stores may be elevated in patients with NASH.

HISTOLOGY

NASH is indistinguishable histologically from alcoholic hepatitis. The major histologic features of NASH are summarized in table 3. The fatty changes characteristic of NASH can affect the hepatic lobules either diffusely or primarily in the central zones^[12]. Lobular inflammation of varying degrees is present in all cases and may consist of lymphocytes, other mononuclear cells and neutrophils. Glycogenated nuclei are present in 35-100% cases of NASH. Hepatocyte ballooning and/or hepatocyte necrosis of varying degrees are usually present.

Mallory bodies, which may be small, sparse and inconspicuous, have been described in 10-100% of patients with NASH. Mallory bodies may be more prominent in severe NASH. Councilman bodies and lipogranulomas have been identified infrequently. Stainable iron may be present in 15-65% patients with NASH. Pericellular, perisinusoidal and periportal fibrosis have been described in 37-84%

of patients with NASH. Fibrosis is most prevalent in zone 3. The extent of fibrosis may vary considerably, ranging from delicate strands surrounding small veins or groups of cells producing the so-called chicken wire fibrosis to densely fibrotic septa with distortion of the hepatic architecture. Cirrhosis is found on initial biopsy in 7-16% of patients with NASH. Histologic evidence of significant fibrosis or cirrhosis correlates poorly with clinical features and laboratory data^[13].

Pathogenesis of NASH

Obesity has emerged as a major health problem worldwide. Between 1980-2000, obesity rates doubled among adults and in the United States; about 60 million adults or 30% of the adult population are now obese. Obesity is closely associated with NAFLD, which is also becoming endemic.

The spectrum of NAFLD extends from simple hepatic steatosis through NASH to cirrhosis. The development of steatosis, steatohepatitis, fibrosis and cirrhosis is most likely the result of multiple metabolic abnormalities taking place in an environment of genetic predisposition. Many individuals with NASH are asymptomatic; however, over time (i.e. 5-10 years), chronic sub-clinical NASH incites a fibrotic response in a sizable subgroup, leading to bridging fibrosis in up to 50% and cirrhosis in as many as 10% of individuals with biopsy-proven NASH. The rate of liver-specific mortality in patients with NAFLD-induced cirrhosis appears to be similar to that of well-compensated patients who develop cirrhosis from other chronic liver diseases (i.e. about 10% per decade)^[14].

Obesity-related NAFLD is intimately related to metabolic syndrome, which is a constellation of disorders that includes obesity, type 2 DM, insulin resistance (IR) and dyslipidemia^[15]. It is estimated that 75% of type 2 DM patients have some form of fatty liver^[16]. Clinical pathologic studies in patients with NASH, as well as treatment trials with insulin-sensitizing agents, have suggested that IR and hyperinsulinemia are primary abnormalities in NAFLD.

The pathogenesis of NASH is not fully understood, and the 'two-hit' hypothesis^[17] remains the prevailing theory. Prolonged over-nutrition leads to accumulation of free fatty acids (FFAs) and triglycerides within the liver (steatosis, first hit). The progression of NAFL to NASH is associated with other factors (second hit), such as oxidant stress, mitochondrial injury, fatty acids lipotoxicity, innate immunity and inflammatory cytokines. All these contribute to the pathogenesis of NASH.

Fatty acids and NASH

Lipid accumulation in the hepatocytes (steatosis) is a characteristic histological feature of NAFLD. Steatosis results from increased fatty acid influx or impaired fatty acid utilization in the hepatocytes. Increased levels of free fatty acids (FFA), besides mediating IR, can be directly toxic to hepatocytes. Current concepts of the cellular toxicity of fatty acids are complex. However, there are two major mechanisms of fatty acid toxicity in the pathogenesis of NASH. The direct mechanism involves direct cytotoxicity of the fatty acids on the hepatocytes because of excessive intracellular fatty acid. The indirect mechanism involves cytotoxic effects of lipid peroxidation of fatty acids^[14,18].

Oxidant stress and NASH

Oxidant stress is thought to be a crucial mechanism of hepatocellular injury in NASH. There are many animal and human studies that correlate markers of oxidant stress with the presence of NASH and

many sources of oxidant stress in NASH, including cytochrome P450, peroxisomal β -oxidation, mitochondrial dysfunction and inflammatory cytokines. Antioxidant (vitamin E) has been shown to be beneficial in NASH^[14,19].

Mitochondrial dysfunction and NASH

Adenosine triphosphate (ATP) is the key element in the maintenance of cellular integrity. Mitochondrial dysfunction can impair ATP production and lead to hepatocellular injury. There is evidence of reduced hepatic ATP levels in animal models of NASH and in NASH patients themselves, there is a significant delay in ATP recovery after depletion, as measured by PNMR spectroscopy. Mitochondrial injury may be one cause of reduced hepatocellular ATP stores in NASH. Structural studies have shown mitochondrial crystal formation in NASH, which can lead to mutation and loss of the mitochondrial DNA that is essential for mitochondrial and hepatocellular viability^[14].

Innate immune system, inflammatory cytokines and NASH

The liver is a major component of the innate immune system, housing large numbers of tissue macrophages and specialized lymphocytes, such as natural killer T (NKT) cells. NKT cells can be directly cytotoxic, killing target cells directly via perforin or FasL-dependent mechanisms or indirectly by elaborating interferon (IFN)- γ . NKT cells also perform important immune regulatory functions, balancing the local production of Th-1 (pro-inflammatory) and Th-2 (anti-inflammatory) cytokines by other cells in their immediate microenvironment. NKT cells arise in the thymus and migrate to peripheral tissues, such as the liver and pancreas, where they accumulate in large numbers^[20]. Reducing the size of the NKT cell population in the pancreas or liver promotes Th-1 polarization of other resident, cytokine-producing mononuclear cells and increases apoptosis of pancreatic β cells and hepatocytes.

Although it is widely acknowledged that TNF- α expression increases in obesity, increased TNF- α production has been also described in obese patients with NASH. A Spanish study correlated liver disease severity with TNF- α gene expression in the adipose and liver tissues of 52 obese patients. TNF- α mRNA was over-expressed in the adipose tissues and livers of patients with NASH and was higher in patients with significant hepatic fibrosis^[21].

It has been demonstrated that liver enzymes improve in NASH patients who were treated with oral probiotics (mixtures of 'non-pathogenic' bacterial strains) in order to modify their intestinal flora, which supports the role of gut bacteria in the pathogenesis of human NASH.

METHODS TO MEASURE FIBROSIS

Measurement of fibrosis not only helps to stage the severity of disease, it allows serial determination of disease progression. The level of fibrosis may play an important role in clinical management and determine patients' prognosis. For example, aggressive therapy is more appropriate in HCV-infected patients with advanced fibrosis. Further, the fibrosis progression rate is an important predictor of the time to develop cirrhosis^[22].

It is essential to measure fibrosis accurately, given the growing prospect of anti-fibrotic therapies and the need to track their efficacy. Moreover, with growing evidence that fibrosis is reversible, methods will need to assess both progression and regression accurately. For example, specific therapy leads to a reduction in fibrosis in a number of diseases, including autoimmune liver disease, hepatitis C, hepatitis B and others. Percutaneous liver biopsy has traditionally

been considered to be the gold standard test to assess liver fibrosis. However, a variety of non-invasive tests have been advanced as potential alternatives to biopsy. These include clinical signs, routine laboratory tests, quantitative assays of liver function, markers of extracellular matrix synthesis and/or degradation and radiologic imaging studies. In addition to individual indicators of fibrosis, combination tests and a number of models for predicting liver fibrosis have been developed.

The ideal method to measure fibrosis would be simple, noninvasive, reproducible, inexpensive, accurate, and readily available. Unfortunately none of the currently available approaches fulfills all of these criteria.

NON-INVASIVE MARKERS OF FIBROSIS

Routine laboratory tests

Wai *et al.*^[23] (2003) constructed a simple model utilizing routine laboratory data. The authors devised a novel index, termed the AST to platelet ratio index, or APRI, which is the AST level/upper limit of normal (ULN) divided by the platelet count (109/L) multiplied by 100. The sensitivity and specificity for fibrosis of the APRI value depended on the cut-offs used. Using an APRI value of 1.50, the positive and negative predictive values for significant fibrosis (Ishak score=3) were 91% and 65%, respectively, whereas for cirrhosis and an APRI of 2.00, the positive and negative predictive values were 65% and 95%, respectively. Although the APRI is attractive because of its simplicity, it can neither definitively diagnose nor exclude cirrhosis and it will not identify patients with early fibrosis.

More complicated algorithms based on commonly available laboratory tests include the 'Fibrotest' reported by the French MULTIVIRC group^[24]. This group used mathematical modeling to develop an algorithm including five different markers to predict fibrosis (the markers selected were α_2 -macroglobulin, haptoglobin, GGT, apolipoprotein A1 and total bilirubin). This index predicted a specific biopsy category in 46% of patients^[25] and has been validated in a number of hepatitis C patient cohorts. The addition of ALT to the marker panel allows for prediction of METAVIR necro-inflammatory activity^[26]. The panel has also been examined in other liver disease cohorts^[27,28]. Limitations of this panel in fibrosis include false positive results due to increases in bilirubin or decreases in haptoglobin, for example from hemolysis secondary to ribavirin therapy. Likewise, false positive results may also occur in situations where there is hyperbilirubinemia, such as Gilbert's disease and cholestasis. Acute inflammation may also affect the results of the test owing to changes in α_2 -macroglobulin or increases in haptoglobin. Currently, it is unclear whether the 'fibrotest' assay meets sufficiently rigorous criteria, given a predictive value of only 46%, for routine clinical use.

Tests using extracellular matrix/fibrosis markers

Analyses of serum markers of extracellular matrix/fibrosis include many proteins important in fibrogenesis, ECM constituents (i.e. fibronectin, collagen I, collagen IV, collagen VI, amino terminal propeptide of type III collagen (PIIINP), tenascin, and hyaluronic acid, metalloproteinases, inhibitors of matrix metalloproteinases (i.e. TIMP-1, TIMP-2), and other proteins, peptides and cytokines. Although many tests have been studied individually, they are generally not sensitive for detection of fibrosis.

Tests using combinations of extracellular matrix and/or routine markers

A combination test including hyaluronic acid, TIMP1, and α_2 -macroglobulin was examined in a cohort of 294 patients with HCV

infection and subsequently validated in a second cohort of 402 patients^[29]. This had a combined AUROC of 0.831 for METAVIR F2-F4 fibrosis. The positive and negative predictive values were 74.3% and 75.8% respectively, with an accuracy of 75%. This three-marker panel thus may help differentiate patients with HCV infection with moderate/severe fibrosis from those with no/mild fibrosis, although it was not possible to differentiate specific stages accurately.

Another combination test was developed by the European Liver Fibrosis (ELF) Study Group^[30]. This group examined collagen IV, collagen VI, PIIINP, matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 9 (MMP-9), TIMP-1, tenascin, laminin and hyaluronic acid (HA). The study was unique in that it examined patients with a wide variety of liver diseases, including those with chronic hepatitis C virus infection ($n=496$), alcoholic liver disease ($n=64$), non-alcoholic fatty liver disease ($n=61$), chronic hepatitis B virus infection ($n=61$), primary biliary cirrhosis or primary sclerosing cholangitis ($n=53$), recurrent liver disease post-orthotopic liver transplantation ($n=48$), autoimmune hepatitis ($n=45$), hemochromatosis ($n=32$), cryptogenic cirrhosis ($n=19$), both hepatitis B and C ($n=4$) and other or no known diagnosis ($n=138$); the cohort also had a wide distribution of fibrosis stages (Scheuer fibrosis stages were as follows: stage 0=24.6%; stage 1=35.5%; stage 2=13.4%; stage 3=14.9%; and stage 4=1.8%). An algorithm was developed that detected the upper third of fibrosis groups (Scheuer stages 2, 3, and 4) with a sensitivity of 90% and accurately detected the absence of fibrosis (Scheuer stages 0, 1), with a negative predictive value for this level of fibrosis of 92%. The AUC of a receiver operating characteristic (ROC) plot was 0.804. Interestingly, the addition of clinical chemistry tests including liver function tests, or hematological indices including platelet count and prothrombin time, did not improve test performance. The test appeared to be best in patients with hepatitis C, NAFLD and alcoholic liver disease. The inclusion of patients with multiple etiologies of liver disease, although appealing, has the potential to limit the accuracy of these and other panels, as the characteristics of specific assays may be disease specific. Another model, including AST, cholesterol and insulin resistance (as well as age and an estimate of past alcohol intake) in patients with HCV, found that the sensitivity for detection of advanced fibrosis depended on the index value used. At a low probability index, the sensitivity for predicting significant fibrosis was high, but specificity was low, while at a high probability index, sensitivity for significant fibrosis was low, but specificity was high.

Proteomics

With the recent explosion in proteomics, proteomic approaches have attempted to identify unique protein fingerprints in patients with liver disease. Various platforms are available, including those that measure protein expression, protein-protein interactions or even enzymatic activity. The majority of approaches have used high throughput technologies to identify novel protein expression patterns^[31]. Another report in patients with HCV fibrosis identified several serum proteins to be differentially regulated^[32]. In this study, patients with advanced fibrosis had elevated levels of α_2 -macroglobulin, haptoglobin, and albumin, but apolipoprotein A1, apolipoprotein A-IV, complement C4, and serum retinol-binding protein were reduced.

Another approach has included measurement of labeled N-glycans found in serum. The technique exploits the ability to analyze the desialylated total serum N-glycome on a DNA analyzer. The authors focused on cirrhosis (primarily ethanol induced), demonstrating unique patterns of serum N-glycans in those with cirrhosis compared to those with chronic liver disease alone. It was postulated that in

cirrhotic livers characteristic N-glycans with a bisecting GlcNAc residue were prominent. In normal liver, the enzyme responsible for this modification, N-acetylglucosaminyl transferase III (GnT-III), is found only in non-parenchymal cells, but in regenerating liver (two-thirds partial hepatectomy) this enzyme is produced in hepatocytes. Thus, GnT-III expression is presumably a manifestation of hepatocellular regeneration, reflected by regenerative nodules. This approach was most sensitive for the detection of cirrhosis and was also able to exclude cirrhosis with great accuracy. When combined with the commercially available Fibrotest, this test had 100% specificity and 75% sensitivity for diagnosing compensated cirrhosis^[33].

Imaging tests

A wide variety of radiographic tests have been used to image patients with fibrosis/cirrhosis. Included in this group are ultrasound, CT, and MRI. In general, these tests are capable of detecting evidence of portal hypertension, thus they have the ability to detect advanced disease. As currently used in clinical practice, however, they are insensitive for the detection of moderate degrees of fibrosis. Transient elastography, which uses pulse-echo ultrasound acquisitions to measure liver stiffness and predict fibrosis stage, has gained interest as a method to quantify fibrosis as it appears that liver 'stiffness' may accompany the fibrogenic response (Wells 2005;). In a prospective multicenter study of 327 chronic HCV patients, the AUROCs for 98 METAVIR stage F2-F4 and cirrhosis were 0.79 and 0.97, respectively^[34]. In a separate study of 183 chronic HCV patients, transient elastography compared favorably with the Fibrotest and APRI (AUROC for F2-F4=0.83, 0.85 and 0.78, for transient elastography, Fibrotest and APRI, respectively). When transient elastography was combined with the Fibrotest, the predictive value for fibrosis stage F2-F4 was improved, with an AUROC of 0.88^[35]. Transient elastography (Fibroscan) reportedly offers good reproducibility with low inter- and intra-observer variability. The procedure is performed by obtaining multiple validated measurements in each patient, further reducing the potential for sampling errors. The depth of measurement from the skin surface is between 25-65 mm, raising the possibility that this technique may be difficult to use in obese patients or those with ascites. However, newer probes are being developed for obese patients and further investigation is expected. Finally, it would theoretically be desirable to utilize advances in the molecular understanding of liver fibrosis to image the liver. For example, the number of activated stellate cells, which reflect fibrogenic activity, might be identified by tagging them with cell-specific markers. Alternatively, matrix or matrix turnover could be labeled using molecular tools. Although such approaches are appealing, they remain experimental at present.

Tests of liver function

A variety of liver function tests have been used to assess liver fibrosis and cirrhosis. Such tests generally measure advanced disease and several depend on perfusion, such as indocyanine green, sorbitol and galactose clearance tests or tests such as the ¹³C-galactose breath test and the ¹³C-aminopyrine breath test that depend on the functional capacity of the liver. Another test, the MEGX test, which measures monoethylglycineylidide (MEGX) formation after the administration of lidocaine, depends upon the activity hepatic cytochrome P450 3A4 isoenzyme, which catalyzes oxidative N-de-ethylation of lidocaine to MEGX. The MEGX test has a sensitivity and specificity in the 80% range for distinguishing chronic hepatitis from cirrhosis in comparison to standard liver tests. Unfortunately,

although the MEGX test and other function tests may predict prognosis in cirrhotic patients, they are insensitive for quantifying fibrosis in patients with less advanced disease.

Liver biopsy

Percutaneous liver biopsy has traditionally been considered to be the gold standard test to measure fibrosis. Although there is great experience with liver biopsy, this procedure is time consuming, inconvenient, uncomfortable, invasive and makes both patients and physicians anxious. Further, liver biopsy can be associated with substantial sampling-error. In a recent study in which 124 patients with chronic HCV infection underwent laparoscopy-guided biopsy of each the right and left hepatic lobes, 33.1% had a difference of at least one histologic stage (modified Scheuer system) between the two lobes. Furthermore, in 18 study subjects, a stage consistent with cirrhosis was found in one lobe, whereas stage 3 fibrosis was reported in the other. Finally, in 10% of subjects, stage 0-2 disease was identified in one lobe and stage 3-4 fibrosis was found in the other. Similar variability was reported in another study in patients with fatty liver disease.

There are several other limitations of liver biopsy. Quantification of fibrosis in biopsies is subject to significant interobserver variation. In chronic hepatitis C, for example, standardized grading systems, including Knodell, METAVIR, Scheuer or Ishak are concordant in only 70-80% of samples. Specimen quality is very important, with smaller samples leading to an underestimation of disease severity.

Another major problem with using liver biopsy or serum markers to quantify fibrosis is that all of the currently utilized grading systems use a simple linear numerical scoring approach, implying that they represent linear changes in fibrosis content.

CONCLUSION

Since NAFLD was appreciated as an independent disease entity, our understanding about different aspects of the disease ranging from aetiology to pathogenesis and prognosis to treatment has broadened. However a lot still needs to be done specially to diagnose NASH non-invasively as well as to develop specific pathogenesis directed therapy for this apparently benign, but deadly disease.

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Peer reviewer: Yoshihiro Kamada, MD, PhD, Assistant Professor, Department of Gastroenterology and Hepatology, Osaka University, Graduate School of Medicine, 2-2, K1, Yamada-oka, Suita, Osaka 565-0871, Japan.