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REVIEW

Current status and agenda in the diagnosis of nonalcoholic steatohepatitis in Japan

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Abstract

Nonalcoholic fatty liver disease (NAFLD), a manifestation of metabolic syndrome, includes a wide range of clinical entities from simple fatty liver, a benign condition, to nonalcoholic steatohepatitis (NASH), a condition which can progress to cirrhosis, hepatocellular carcinoma and hepatic failure. The diagnosis of NASH requires no history of previous or current significant alcohol consumption and no evidence of other chronic liver diseases. Ethanol intake levels of 20 g daily (or 140 g weekly) are endorsed as the acceptable threshold to define no nalcoholic patients. Liver biopsy is the current gold stan dard for the diagnosis of NASH and provides progno stic information. Histopathological diagnosis of NASH is based on the following 3 features: (1) hepatic macrovesicular steatosis; (2) lobular inflammation; and (3) ballooning degeneration of hepatocytes. It is impractical to biopsy every patient with suspected NAFLD. Although highly accurate and affordable noninvasive screening tools can differentiate NASH from NAFLD, no imaging studies or laboratory tests are able to precisely diagnose NASH. There is no universal agreement regarding the indications for liver biopsy in NAFLD patients. In Japan, liver biopsies are considered in patients with suspected NAFLD based on several criteria including low platelet counts, elevated fibrosis markers, increasing age and other deciding parameters. Further studies are needed to establish a suitable scoring system that can distinguish steatohepatitis from simple steatosis.

Key words: Insulin resistance; Liver biopsy; Oxidative stress; Fibrosis

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INTRODUCTION

In 1980, Ludwig *et al*[1] coined the term nonalcoholic steatohepatitis (NASH) to describe the morphologic pattern of liver injury in 20 patients evaluated at the Mayo Clinic. These patients had histological evidence of alcoholic hepatitis on liver biopsy but no history of alcohol abuse. Nonalcoholic fatty liver disease (NAFLD) represents a wide spectrum of conditions ranging from simple fatty liver which in general follows a benign non-progressive clinical course to NASH, a more serious form of NAFLD that may progress to cirrhosis and end-stage liver disease[2]. NAFLD is strongly associated with obesity and metabolic syndrome. Today, NAFLD is the most common chronic liver disease (CLD) in the Western world[3,4] and in the

Asia-Pacific Region[5,6]. Although the true prevalence of NAFLD or NASH remains to be established, current best estimates make the prevalence of NAFLD 9%-30% and of NASH 1%-3% in Japan[7]. On imaging findings consistent with steatosis, a diagnosis of NAFLD can be made with a reasonable degree of confidence if a history of significant alcohol consumption and other causes of liver disease are excluded. Until recently, liver biopsy was the only method for differentiating NASH from simple fatty liver. This review paper discusses the clinical features and diagnostic challenges for NASH in Japan.

LABORATORY STUDIES/IMAGING

Liver transaminase levels are mildly elevated (usually < 3-5 x the upper limit of normal) in NASH patients. Although aminotransferase levels are elevated in the majority of patients, normal values do not exclude the presence of necroinflammatory changes or fibrosis[8-10]. The ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) is usually < 1 but this ratio increases as the level of fibrosis progresses[11-13]. Serum alkaline phosphatase (ALP) and γ-glutamyl transferase (γGT) may also be mildly elevated. Other abnormalities including hypoalbuminemia, a prolonged prothrombin time and hyperbilirubinemia may also be found in patients with cirrhotic-stage NASH. Increased serum ferritin levels are often seen in NASH patients but transferrin saturation is almost normal[14]. Although markers of insulin resistance and hepatic fibrosis seem to be higher in NASH than in simple fatty liver, currently laboratory studies cannot truly confirm a diagnosis of NASH.

Imaging tests [such as ultrasound (US), computed tomography (CT) or magnetic resonance imaging] may reveal fat accumulation in the liver but their sensitivity is low. Furthermore, these imaging studies cannot differentiate NASH from simple fatty liver[15]. Although US is an acceptable first-line screening procedure for NAFLD in clinical practice, it underestimates the prevalence of hepatic steatosis when there is < 20%-30% fat[15,16]. According to a study from Japan[17], US could more accurately identify the presence of steatosis in NASH patients than CT but the sensitivity of US for detecting steatosis was reduced, especially in patients with advanced histological fibrosis. Although other modalities such as transient elastography (Fibroscan, EchoSens, Paris, France), contrast enhanced US and Xenon CT are reported to be promising for distinguishing between simple fatty liver and NASH, there are no established noninvasive methods of evaluation available for patients with NAFLD. In Japan, contrast enhanced US with Levovist (Schering AG, Berlin, Germany) can identify patients with NASH among those with NAFLD[18,19]. Yoneda *et al* recently reported that elastography techniques such as transient elastography and acoustic radiation force impulse elastography have been shown to be useful for estimating liver fibrosis in NAFLD patients[20,21]. With high negative predictive value and modest positive predictive value in French and Chinese cohort of NAFLD patients[22], transient elastography is useful as a screening test to exclude advanced fibrosis. Although these techniques are painless, rapid, have no associated complications and are, therefore, very easily accepted by patients compared to liver biopsy, it may be difficult to distinguish between simple fatty liver and NASH with mild fibrosis.

DIFFERENTIAL DIAGNOSIS

A complete laboratory evaluation to exclude other causes of liver disease should also be performed. This includes screening for common causes such as viral hepatitis B and hepatitis C virus (HCV) as well as less common causes including autoimmune disorders, celiac disease and genetic conditions such as Wilson's disease, hemochromatosis and alpha-1-antitrypsin deficiency. Other liver diseases, hepatic malignancies, hepatobiliary infections and biliary tract disease should also be excluded[4,5]. Thus, hepatitis B surface antigen, anti-HCV, anti-nuclear antibody (ANA) and anti-mitochondrial antibodies (AMA) should be measured to rule out these diseases. Elevated serum autoantibodies are common in patients with NASH/NAFLD. Although low titers of ANA positivity are seen in up to a third of patients with NASH/NAFLD, ANA titers greater than 1:320 are generally rare. Therefore, ANA positivity does not always exclude NASH/ NAFLD[23-25]. Low titers of anti-smooth muscle antibody (ASMA) and AMA have also been reported in patients with NASH/ NAFLD[16,18]. In patients with suspected NAFLD, if ANA or ASMA titers are greater than 1:160 and 1:40 respectively, a liver biopsy should be considered to exclude the presence of autoimmune hepatitis[26]. Among NAFLD patients with ANA positivity, potential risk factors such as female sex, obesity, insulin resistance and severe fibrosis have been found in some studies although no consensus has been established. Familial hypobetalipoproteinemia (FHBL), a hereditary disorder characterized by decreased plasma concentrations of low-density lipoprotein (LDL) cholesterol and apolipoprotein B (Apo-B), is classified as one of the causes of NAFLD[27,28]. Regarding lipids, it is worth measuring serum levels of Apo-B in patients with no obvious risk factors for NAFLD or with low levels of LDL and HDL cholesterol to look for evidence of FHBL. In Japan, a case of FHBL with cryptogenic cirrhosis showing recurrent NASH after undergoing living donor liver transplantation was reported[29].

Due to its high prevalence, it is now recognized that NAFLD/NASH can occur together with other CLDs. In chronic hepatitis C, and possibly ALD and hemochromatosis, NAFLD can exacerbate liver damage[30-32]. The diagnosis and management of NAFLD with other CLDs remains unresolved. The nomenclature "NASH" may be changed for these reasons as proposed by Brunt[33]. This strongly argues for a change in nomenclature (such as metabolic fatty liver disease and metabolic steatohepatitis) which would drop the "negative" definition of "non-alcoholic" and would recognize the likely causal role of insulin resistance in NAFLD/NASH.

MEANING OF "NONALCOHOLIC" LIVER DISEASE

The diagnosis of NASH requires no history of significant alcohol consumption. There is no consistent agreement regarding the definition of significant alcohol consumption. According to the Italian Association for the Study of the Liver Expert Committee[34] and the position statement on NAFLD/NASH based on the European Association for the Study of the Liver (EASL) 2009 special conference[35], European hepatologists suggested a daily alcohol consumption 20 g in women and 30 g in men as the optimal cutoff values of "non-alcoholic". According to the American Gastroenterological Association (AGA) institute technical review on nonalcoholic fatty liver disease[3] and the summary by the American Association for the Study of Liver Diseases (AASLD)[4], a daily alcohol consumption of > 20 g/d is commonly used as exclusionary criteria; however, the validity of these cutoffs is unknown. In contrast, intake levels of 20 g/d (140 g weekly) for men, and 10 g/d (70 g weekly) for women have been endorsed as the acceptable thresholds to define "non-alcoholic" in the guideline proposed by the Asia-Pacific Working Party for NAFLD (APWP-NAFLD)[5] and by the National Institutes of Health Clinical Research Network[36]. The reason that a small amount of alcohol intake is permitted in the diagnosis of NASH is based on the fact that intake levels above the defined thresholds (> 20 to 40 g/d in males and > 10-30 g/d in females) are toxic for the liver[37-39] and because modest alcohol consumption is thought to reduce the prevalence of NAFLD by improving insulin resistance[40-42]. At the 45th Annual Meeting of the Japan Society of Hepatology (JSH) in June 2009, a consensus was reached that alcohol intake levels of 20 g/d (140 g/wk) were accepted as the optimal cut-off values of "non-alcoholic"[43]. It is often difficult to differentiate NASH from ALD. Conventional markers such as mean corpuscular volume, γGT and AST/ALT ratio are not useful and specific serum markers for chronic alcohol abuse are of limited value. Measurement of carbohydrate-deficient transferrin levels (CDT) is the most widely used and perhaps the most specific serum marker for detecting chronic alcohol abuse. Serum CDT levels were known to be lower in patients with alcoholic hepatitis than those with NASH[44]. Practical clinical use of this marker is questionable because it can be measured only in research laboratories. A careful history of alcohol intake is essential to exclude alcohol-induced fatty liver disease (AFLD) because the histological features of AFLD and NAFLD are indistinguishable for pathologists. It is difficult to distinguish between these two entities, especially in those with obesity and associated metabolic risk factors because AFLD and NAFLD commonly occur in this population. The diagnosis and treatment of this condition is still unclear.

HISTOLOGICAL DIAGNOSIS

Currently, histological assessment of liver biopsy specimens remains the gold standard for the diagnosis of NAFLD. There are a constellation of histological findings in NASH with no single pathognomonic lesion. The principal histological features of NASH include the presence of macrovesicular fatty changes of hepatocytes with displacement of the nucleus to the edge of the cell, ballooning degeneration of hepatocytes and a mixed lobular inflammation. Other features such as perisinusoidal/peri-cellular fibrosis, Mallory-Denk bodies (MDB), megamitochondria, acidophil bodies, glycogenated nuclei and hemosiderosis can be found. Bridging fibrosis and cirrhotic changes may be present in the advanced fibrotic stage. In spite of considerable efforts, there is still no international agreement regarding the histopathological criteria that firmly define NASH. Therefore, a large amount of uncertainty exists between pathologists and clinicians. Moreover, borderline lesions of the two entities exist in clinical practice. In 1999, Matteoni *et al*[45] divided NAFLD into four categories or types based on the presence of steatosis, lobular inflammation, hepatocyte ballooning and MDB/fibrosis (Table 1). As originally reported, after a median follow-up period of 8.17 years, liver-related mortality of NALD (type 3 or 4) was 11% versus 1.7% in NAFLD (type 1 or 2)[46]. A more recent study with a median follow-up period of 18.5 years showed that liver-related mortality of NALD (type 3 or 4) increased to 17.5% in comparison with only 2.7% in NALD (type 1 or 2)[32]. NAFLD (type 3 or 4) is now considered to be a single group that represents NASH[47]. These findings confirm that, with longer follow-up periods, more NASH patients develop liver-related deaths. It also confirms that most patients with non-NASH are not at similar risk of liver-related deaths. Long term follow-up studies have never been performed in Japanese patients with NAFLD. It is expected that prospective studies in Japan will confirm these observations. On the basis of this classification system, hepatocyte ballooning should be considered as a more specific histological feature for the diagnosis of NASH. However, the presence or absence of hepatocyte ballooning is influenced by the var iability in pathologists' interpretation. To account for this, another scoring system has been developed by the Na tional Institute of Diabetes and Digestive and Kidney Diseases. The score, named NAS (NAFLD Activity Score), is the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2). NAS does not include fibrosis (Table 1). A NAS of ⩾ 5 is almost always associated with the diagnosis of NASH and cases with a NAS of < 3 are largely considered to be "non-NASH". Patients who had scores of 3 or 4 are reported to be borderline[33]. The system is simple and requires only routine histochemical stains with reasonable inter-observer reproducibility. This score is valuable for quantifying histological changes, especially in clinical trials, but its generalizability and diagnostic utility are unknown. Clinically important differences exist between community general pathologists and expert hepatopathologists in assessing NAS[48]. The primary purpose of NAS is to assess overall histological change; it was not designed to replace

Table 1 The pathological criteria for the diagnosis of nonalcoholic fatty liver disease

Matteoni's classification[45]				
Type	Histological findings	Liver related deaths (mean observation period) 8.17 years[31]	18.5 years[32]	Diagnosis
Type 1	Fatty liver alone	1.70%	2.70%	non-NASH
Type 2	Fat accumulation and lobular inflammation			
Type 3	Fat accumulation and ballooning degeneration	11.00%	17.50%	NASH
Type 4	Type 3 and either Mallory-Denk body or fibrosis			
NAFLD activity score (NAS)[47]				
Item	Definition		Score	Diagnosis
Steatosis	< 5%		0	Total score
	5%-33%		1	0-2: non-NASH
	> 33%-66%		2	3-4: borderline
	> 66%		3	5-8: NASH
Lobular inflammation	No foci		0	
	< 2 foci per 200 × field		1	
	2-4 foci per 200 × field		2	
	> 4 foci per 200 × field		3	
Ballooning	None		0	
	Few balloon cells		1	
	Many cells/prominent ballooning		2	

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis.

the pathologist's determination of steatohepatitis or to represent an absolute severity scale. In some patients with cirrhosis, the features of steatosis and necroinflammatory activity may no longer be present (so called "burned-out NASH"). NAS has some limitations in diagnosing NASH in such patients. However, APWP-NAFLD suggests that use of NAS should be encouraged for routine reporting as well as research studies[5]. At the 45th Annual Meeting of the JSH in June 2009, it was agreed that a diagnosis of NASH should be based on the following three features: (1) hepatic steatosis (> 5%-10% of hepatocytes affected); (2) lobular inflammation with mononuclear cells and/or neutrophils; and (3) ballooning degeneration of hepatocytes[43]. The presence of fibrosis or MDB is not essential for a diagnosis of NASH[4,5,43]. A universally accepted histological grading and staging system for steatohepatitis does not exist. The first histological scoring system for NASH was proposed by Brunt *et al*[49]; its design was based on a model used in other CLDs and included three qualitatively assessed grades of necroinflammatory activity (based on degrees of steatosis, ballooning and inflammation) and four stages of fibrosis. Unfortunately, this system applied only to NASH and it is not applicable to the entire spectrum of NAFLD.

INDICATIONS AND LIMITATIONS OF LIVER BIOPSY

Liver biopsy remains the best diagnostic tool for confirming NASH as well as the most sensitive and specific means of providing important prognostic information. Liver biopsy is also helpful to determine the effect of medical treatment given that there is poor correlation between histological damage and the results of liver tests or imaging studies. However, it is not practical to biopsy every patient with suspected NAFLD. The AGA states that the decision to perform a liver biopsy in a patient with suspected NAFLD and the timing of the biopsy must be individualized and should include the patient in the decision making process[3]. According to the AASLD, firm recommendations of when to perform a liver biopsy in the routine clinical setting have not yet been developed and management decisions will continue to be tailored to individual patients[4]. According to APWP-NAFLD[5], liver biopsy is not usually required for diagnosis of NAFLD. However, it should be considered in cases where: (1) there is diagnostic uncertainty; (2) patients are at risk of advanced hepatic fibrosis (in the absence of clinical or imaging evidence of cirrhosis); (3) in those enrolled in clinical trials; and (4) because of reduced risk and grea ter convenience in those already undergoing laparoscopy for another purpose (e.g. cholecystectomy, gastric banding). Based on the EASL 2009 special conference[35], liver biopsy may be restricted to cases where non-invasive methods suggest advanced fibrosis and to cases with indeterminate or discordant results, thus deemed insufficient to exclude advanced fibrosis. During elective surgical procedures such as bariatric surgery and cholecystectomy, they suggest that a liver biopsy be performed. At the 45th Annual Meeting of the JSH in June 2009, it was agreed that liver biopsies are considered in patients with suspected NAFLD based on several criteria including low platelet counts, elevated hepatic fibrosis markers, increasing age and other deciding parameters. However, the optimal cut-off values of these parameters have never been established[43]. In this way, no guidelines or firm recommendations have yet been made as to when and for whom it is necessary. Arguments against routine liver biopsy include the generally benign course of the disease in most cases, lack of established effective therapies and the risks of biopsy. As a single percutaneous liver biopsy yields only a minute percentage (1/50000 or 0.002%)

Table 2 Noninvasive biomarkers previously studied or currently under evaluation

Insulin resistance	**Inflammation and apoptosis**
HOMA-IR	TNF-α
Leptin	hsCRP
Adiponectin	CK-18 fragments
	TNF-α/adiponectin ratio
Hepatic fibrosis	Interleukin-6
Hyaluronic acid	CC-chemokine ligand-2
Type Ⅳ collagen 7S	
TGFβ	**Endocrine**
	DHEA-S
Oxidative stress	
Ferritin	**Imaging studies**
TBARS	US elastography
Oxidized-LDL	Fibroscan
Total antioxidant response	ARFI
Total lipid peroxide levels	Contrast enhanced US
Thioredoxin	MRI (SPIO, Gd-EOB-DTPA)
Advanced glycation end products	

HOMA-IR: homeostasis model assessment for insulin resistance; TGFβ: transforming growth factor β; TBARS: thiobarbituric acid-reacting substance; LDL: low-density lipoprotein; TNF: tumor necrosis factor; hsCRP: high sensitivity C-reactive protein; CK: cytokeratin; CCL2: CC-chemokine ligand-2; DHEA-S: dehydroepiandrosterone-sulphate; ARFI: acoustic radiation force impulse elastography; SPIO: super-paramagnetic iron oxide; US: ultrasound; MRI: magnetic resonance imaging.

of the total hepatic tissue, paired biopsies have been evaluated in several published studies. Several recent studies have highlighted its sampling variability, although this may be attenuated with good core biopsy samples[50,51]. According to two studies[52,53], a difference of one stage of fibrosis or more was seen in 30%-41% of paired biopsies. In contrast, recent data have shown that significant sampling variability exists for inflammatory changes rather than steatosis or fibrosis[54]. In addition to the sampling variability noted above, variability in pathologists' interpretation also exists for liver inflammation compared to steatosis or fibrosis[54,55]. It is obvious that liver biopsy is an invasive procedure, stressful for patients and their physicians and is associated with potential significant complications such as pain, hemorrhage and so on[26,56,57]. Finally, another important limitation of liver biopsy relates to the fact that histological analysis remains subjective, influenced by the skill and experience of the examining pathologist. Overall, a large amount of confusion continues to exist between pathologists and clinicians for this condition.

NONINVASIVE TESTS FOR DISTINGUISHING NASH FROM NAFLD

Currently, liver biopsy is the gold standard for diagnosis but there is an increasing requirement for simple, less invasive, highly accurate and affordable screening tools. Moreover, given the extremely high prevalence of NAFLD in the general population, a liver biopsy is poorly suited as a diagnostic test for NAFLD. A variety of clinical parameters, indicators of insulin resistance, oxidative stress[58], inflammation, fibrosis, apoptosis and endocrine function have been explored to distinguish between simple fatty liver and NASH (Table 2). As we previously reported, thioredoxin (TRX), an oxidative stress-inducible thiol-containing protein which has important roles in redox regulation, is also significantly elevated in the NASH patients' serum compared to those with simple fatty liver or healthy subjects[59]. Advanced glycation end products (AGEs), final reaction products of protein with sugars, are elevated in NASH patients compared to simple steatosis or healthy people[60] and are decreased after the treatment with atorvastatin[61]. Dehydroepiandrosterone (DHEA), the most abundant steroid hormone, has been shown to influence sensitivity to reactive oxygen species, insulin sensitivity and expression of peroxisome proliferator-activated receptor alpha. Low levels of circulating sulfated-DHEA (DHEA-S) might have a role in the development of advanced NASH[62]. This was confirmed by our validation study using a Japanese population with NAFLD[63]. Elevation of serum ferritin levels, a marker of iron storage, is associated with NASH. We previously reported high frequencies of hyperferritinemia and increased hepatic iron stores in Japanese NASH patients[59]. Yoneda *et al*[64] also have reported that measurement of serum ferritin is useful to distinguish NASH from NAFLD. In the Japanese population, however, the frequency of HFE mutation (hemochromatosis gene) is known to be extremely rare. This mutation does not have a role in hepatic iron overload in Japanese NASH[65]. Serum ferritin levels have been found to be a significant independent predictor of severe fibrosis in 167 Italian NAFLD subjects[66] but this has not been confirmed by other studies. In Western countries, mildly increased serum ferritin does not necessarily indicate coexisting iron overload. Recently, it is noteworthy that serum ferritin is closely associated with insulin resistance and can be considered a marker for metabolic syndrome[67]. Elevated serum ferritin in NASH may be derived from multiple factors such as hepatic iron accumulation but also hepatic inflammation, highly expressed cytokines, oxidative stress and so on[14]. Apoptosis has an important role in the pathogenesis of NASH. Caspase generated cytokeratin 18 (CK-18), a protein in involved in apoptosis, is elevated in patients with NASH compared to those with simple fatty liver and normal subjects[68]. Also, in Japan, this marker is useful for assessing and monitoring the histological activity of NAFLD[69]. Kitade *et al*[70] reported that significant development of hepatic neovascularization was observed in NASH and CK-18 levels were also positively correlated with the degree of neovascularization. These provocative preliminary data deserve further study but it may be too optimistic to assume that a single biomarker can reliably predict histology in NAFLD, a condition with relatively complex phenotype and multiple comorbidities. Currently it is not routinely available as a laboratory test. These tests are inconclusive in many patients and have not been fully validated in patients with NAFLD.

In an effort to improve to accurately diagnose NASH

Table 3 Panel markers for nonalcoholic steatohepatitis diagnosis

Index	Author (Nation)	Paper	Parameter	Patient selection	*N*	AUROC
HAIR score	Dixon JB (Australia)	Gastroenterology 2001	HTN, ALT, insulin resistance (1/QUICKI)	patients with BMI > 35 undergoing laparoscopic banding	105	0.900
	Palekar NA (USA)	Liver Int 2006	Age, female, BMI, AST, AST/ALT ratio, HA	biopsy proven NAFLD	80	0.763
NashTest (NT)	Poynard T (France)	BMC Gastroenterol 2006	Age, sex, height, weight, TG, AST, ALT, TC, α2-macroglobulin, apolipoprotein A1, haptoglobin, γGT, T-Bil	biopsy proven NAFLD	257	0.790
	Gholam (USA)	Am J Gastroenterol 2007	AST, DM	Severely obese subjects (BMI ≥ 40 kg/m^2) undergoing Rouxen-Y gastric bypass surgery	97	0.820
NASH clinical scoring system	Campos GM (USA)	Hepatology 2008	HTN, DM, AST, ALT, sleep apnea, non-black	Patients undergoing laparoscopic banding	200	
NAFIC score	Sumida Y (Japan)	J Gastroenterol, in press	Ferritin, fasting insulin, type Ⅳ collagen 7S	Biopsy proven NAFLD	177[1] 442[2]	0.851[1] 0.782[2]

HA: hyaluronic acid; TG: triglyceride; TC: total cholesterol; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; HTN: hypertension; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index; γGT: γ-glutamyl transferase; T-Bil: total bilirubin; DM: diabetes mellitus; AUROC: area under the receiver operating characteristic curve; [1]Estimation group; [2]Validation group.

Table 4 Panel markers for fibrosis in nonalcoholic fatty liver disease

Index	Author (Nation)	Paper	Parameter	Patient selection	*N*	Stage	AUROC
	Angulo P (USA)	Hepatology 1999	Obesity[1], age, AST/ALT ratio, DM	NASH	144	F0-2 *vs* F3-4	
BAAT	Ratziu V (France)	Gastroenterology 2000	BMI, age, ALT, TG	Patients with BMI>25, raised transaminse	93	F0-1 *vs* F2-4	0.840
FibroTest (FT)	Ratziu V (France)	BMC Gastroenterol 2006	Age, sex, α2-macroglobulin, , apolipoprotein A1, haptoglobin, T-Bil, γGT	Biopsy proven NAFLD	267	F0-2 *vs* F3-4	0.840
N score	Miyaaki H (Japan)	Liver Int 2008	Female, age, DM, HT	Biopsy proven NAFLD	182	F0-2 *vs* F3-4	0.780
NAFLD fibrosis score	Angulo P (USA, UK, Australia, Italy)	Hepatology 2007	Age, BMI, AST/ALT ratio, IFG/DM, platelet count, albumin	Biopsy proven NAFLD	480[2] 253[3]	F0-2 *vs* F3-4	0.880[2] 0.820[3]
ELF panel	Guha IN (UK)	Hepatology 2008	TIMP1, HA, P3NP	Biopsy proven NAFLD	192	F0 *vs* F1-4 F0-1 *vs* F2-4 F0-2 *vs* F3-4	0.820 0.900 0.930
BARD score	Harrison SA (USA)	Gut 2008	BMI, AST/ALT, DM	Biopsy proven NAFLD	827	F0-2 *vs* F3-4	0.810[2] 0.780[3]
FIB4 index	Shah (USA)	Clin Gastroenterol Hepatol 2009	Age, AST, ALT, PLT	Biopsy proven NAFLD	541	F0-2 *vs* F3-4	0.802
FibroMeter	Calès (France)	J Hepatol 2009	Glucose , AST, ferritin, PLT, ALT, BW, age	Biopsy proven NAFLD	235	F0-1 *vs* F2-4	0.936[2] 0.952[3]
PAF	Hossain (USA)	Clin Gastroenterol Hepatol 2009	Male , Caucasian, DM, ALT, AST	Biopsy proven NAFLD	432	F0-1 *vs* F2-4	0.742

[1]BMI > 31.1 (male), 32.2 (female); [2]Estimation group; [3]Validation group; TG: triglyceride; IFG: impaired fasting glycemia; TIMP1: tissue inhibitor of metalloproteinases; HA: hyaluronic acid; P3NP: type Ⅲ procollagen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index; PLT: platelets; γGT: γ-glutamyl transferase; T-Bil: total bilirubin; DM: diabetes mellitus; BW: body weight; AUROC: area under the receiver operating characteristic curve; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis.

noninvasively and determine the stage of fibrosis, several groups have used different combinations of clinical and biochemical markers to generate various clinical scoring systems. A proprietary algorithm that provides an estimate for either NASH diagnosis (Table 3) or the presence and extent of fibrosis (Table 4) have also been developed. It is uncertain whether these scoring systems will be useful for Asian/Japanese patients because almost all of the proposed scoring systems have been based on Western subjects and the definition of severely obese differs between the West and Japan. In fact, many previous studies have reported that "overweight" Asians who are not "morbidly obese" by Western standards generally have a higher risk of developing lifestyle related diseases such as metabolic syndrome, type 2 diabetes and fatty liver disease. Most noninvasive diagnostic tools for NASH are developed from studies using small sample sizes and also lack rigorous external validation. Although serological hepatic fibrosis markers such as hyaluronic acid[71] or type Ⅳ collagen 7S[72] are expected to be able to differentiate the advanced stage from mild fibrosis, there are no favorable serological markers to distinguish early stage NASH from simple steatosis without inflammation and fibrosis. In Japan, Shimada *et al*[73] suggested that

Table 5 NAFIC score[80]

Variables	Cut-off values	Score values
Ferritin (Female/Male) (ng/mL)	200/300	1 point
Insulin (μU/mL)	10.0	1 point
Type Ⅳ collagen 7S (ng/mL)	5.0	2 point

combinations of type Ⅳ collagen 7S, adiponectines and HOMA-IR are useful to distinguish early stage NASH from simple steatosis in Japanese NAFLD patients.

Recently, four new scoring systems have been described, the NAFLD fibrosis score[12], enhanced liver fibrosis score[74], BARD score[13] and FIB-4 index[75]; all are based on relatively large sample sizes and show encouraging results. However, according to a study of 122 Japanese NAFLD patients by Fujii *et al*[76], when a BARD score of 2 or more was used, the area under the receiver operating characteristic curve (AUROC) was 0.73 with an odds ratio (OR) of 4.9 for the detection of advanced fibrosis. It was concluded that the BARD score is less predictive of advanced fibrosis in Japanese NAFLD patients, mainly because they are not as obese as in Western countries. In Japan, Fujii *et al* showed that noninvasive laboratory tests designed to predict cirrhosis in patients with HCV such as AST/ALT ratio, age-platelet index, AST-to-platelet ratio index, cirrhosis discriminant score and the hepatitis C antiviral long-term treatment against cirrhosis model are also useful in patients with NASH[77]. The N score (the total number of the following risk factors: female > 60 years, type 2 diabetes and hypertension), established on the basis of a multicentre study of 182 Japanese NAFLD patients in Nagasaki[78], is very simple tool to use in practice. These promising models will need to be validated by external investigators before they are recommended for wide clinical use. However, the question is what stage of disease should be distinguished by using these parameters or scoring systems[79]. The majority of studies concentrate on the distinction of severe fibrosis but separation of the milder forms of fibrosis and NASH from simple steatosis is required to support emerging therapeutic trials. We have, therefore, constructed a simple clinical scoring system of three variables; serum ferritin, fasting insulin and type Ⅳ collagen 7S, based on the multiple regression analysis on data from 177 biopsy-proven Japanese NAFLD. These three variables were combined in a weighted sum [serum ferritin ⩾ 200 ng/mL (female) or 300 ng/mL (male) = 1 point, fasting insulin ⩾ 10 lU/mL = 1 point and type Ⅳ collagen 7S ⩾ 5.0 ng/mL = 2 points] to form an easily cal culated composite score for predicting NASH, called the NAFIC score (Table 5). According to our validation study of 442 Japanese patients with biopsy-proven NAFLD from the Japan Study Group of NAFLD (JSG-NAFLD) including eight hepatology centers in Japan, AUROC was the greatest for NAFIC score among several previously established scoring systems for detecting NASH but also for predicting significant or severe fibrosis[80]. Our results suggest that liver biopsies can be avoided in NAFLD patients with a NAFIC score of 0 or 1 because they are likely to have NAFLD without advanced fibrosis. In contrast, liver biopsies should be recommended in NAFLD patients with an NAFIC score of ⩾ 2 to assess the extent of hepatic fibrosis and predict prognosis. Thus, the diagnostic algorithm for NASH diagnosis in Japan proposed by JSG-NAFLD is shown in Figure 1. The present results need to be validated in independent populations by other investigators before wide clinical use since it is unknown whether our score can be applicable for NAFLD patients of other races/ethnics.

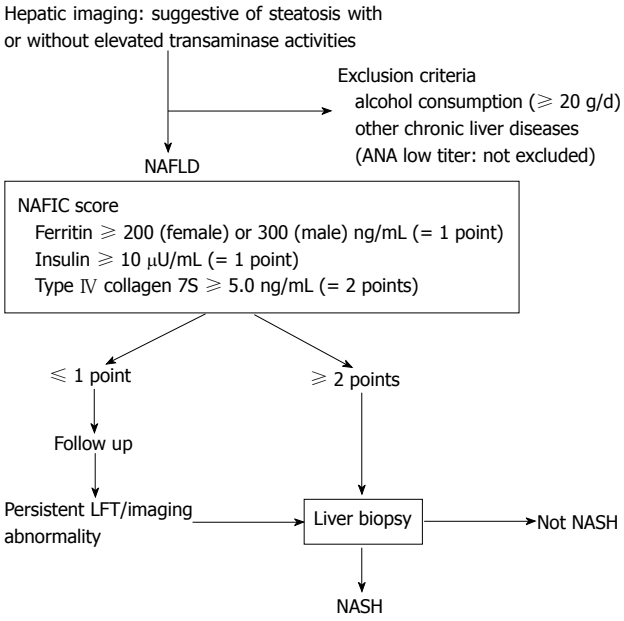

Figure 1 Diagnostic algorithm for nonalcoholic steatohepatitis diagnosis proposed by Japan Study Group of nonalcoholic fatty liver disease. NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis.

CONCLUSION

NAFLD, a manifestation of metabolic syndrome, is a leading cause of CLD worldwide. NASH, the progressive form of NAFLD, can progress to cirrhosis, hepatic failure and hepatocellular carcinoma. It is important to identify patients with NASH. However, there is no simple test to reliably detect NASH apart from liver biopsy. The clinical spectrum of NAFLD warrants continued research to determine its pathogenesis and to improve diagnostic modalities. It is hoped that improved imaging techniques, the discovery of serum biomarkers and the development of clinical algorithms will enable a more accurate diagnosis of NASH without the need for a liver biopsy.

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