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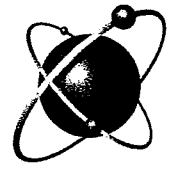
Imaging of Nonalcoholic Steatohepatitis: Advantages and Pitfalls of Ultrasonography and Computed Tomography

Maki Tobari, Etsuko Hashimoto, Satoru Yatsuji, Nobuyuki Torii and Keiko Shiratori



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□ ORIGINAL ARTICLE □

Imaging of Nonalcoholic Steatohepatitis: Advantages and Pitfalls of Ultrasonography and Computed Tomography

Maki Tobari, Etsuko Hashimoto, Satoru Yatsuji, Nobuyuki Torii and Keiko Shiratori

Abstract

Objective The present study was performed to clarify the ability of ultrasonography (US) and computed tomography (CT) to detect steatosis and advanced fibrosis in nonalcoholic steatohepatitis (NASH) patients, and to assess the influence of steatosis, fibrosis, and obesity on the radiological detection of steatosis and advanced fibrosis.

Methods One hundred and eighteen biopsy proven NASH patients underwent US and CT within 6 months before or after biopsy. The ability of US and CT to detect histological steatosis and advanced fibrosis was assessed. To evaluate whether fibrosis and obesity interfered with the detection of moderate to severe histological steatosis by US and CT, we analyzed 88 NASH patients with moderate to severe steatosis. To evaluate interference with the detection of advanced fibrosis by steatosis and obesity, we analyzed 59 NASH patients with advanced fibrosis.

Results The sensitivity of US for detecting moderate to severe histological steatosis in patients with mild histological fibrosis was 100%, but this was reduced to 77.8% in patients with advanced histological fibrosis (p=0.001). The sensitivity of CT was 69.8% in patients with mild histological fibrosis and 48.9% in those with advanced histological fibrosis (p=0.047). The sensitivity of US and CT for moderate to severe histological steatosis was similar in each body mass index group. The sensitivity for detecting advanced fibrosis was markedly decreased by severe steatosis and obesity in the case of both US and CT.

Conclusion If we are aware of these disadvantages of US and CT, it is useful for diagnosing steatosis and fibrosis in NAFLD patients.

Key words: NASH, NAFLD, ultrasonography, computed tomography

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is increasingly being recognized as the most common cause of chronic liver disease worldwide. NAFLD represents a wide spectrum of conditions, ranging from simple steatosis that generally follows a benign and nonprogressive clinical course to nonalcoholic steatohepatitis (NASH), which sometimes progresses to cirrhosis and hepatocellular carcinoma (HCC) (1-5). In Japan, annual health checks have shown that 10-30% of Japanese adults demonstrate evidence of NAFLD by ultrasonography (US) (6, 7). It has been reported that almost 10% of persons with NAFLD will have NASH, so the prevalence of NASH is estimated at 1-3% of the adult Japanese population, an extremely large number of potential patients. Unfortunately, there are no accurate noninvasive diagnostic methods for NASH, such as biochemical markers or imaging techniques, therefore liver biopsy is necessary to make a definite diagnosis, although the procedure is associated with pain, risks, high cost, and sampling errors (1, 2, 8-11).

In general practice, NAFLD (which includes simple steatosis and NASH) is a convenient term for the diagnosis and management of these patients. Diagnosis of NAFLD is based on the detection of steatosis by imaging techniques and the exclusion of other liver diseases, such as alcoholic liver disease or viral hepatitis. The response of NAFLD to

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treatment is usually evaluated by the changes in transaminases and from imaging findings. Therefore, imaging studies are extremely important, both for diagnosing NAFLD and for monitoring patients over time.

Abdominal US is currently the most common method employed for qualitative assessment of hepatic steatosis, because it is noninvasive, widely available, cheap, and provides useful information (12). Computed tomography (CT) scanning and magnetic resonance imaging (MRI) both seem to be sensitive techniques for the quantification of steatosis, but MRI is still less widely available and more expensive than CT. In general practice, therefore, detection of steatosis is generally done by US, after which CT is performed for more objective and quantitative assessment of the severity of steatosis based on the liver/spleen attenuation ratio (13).

Several studies have assessed the sensitivity, specificity, and positive and negative predictive value of US for detecting steatosis, and the reported sensitivity ranges around 80-100% (8-10, 12). In patients with morbid obesity, however, a sensitivity of less than 40% has been reported, presumably due to the technical difficulty of performing US in such patients (14). Moreover, recent studies have shown that US is not accurate for detecting hepatic steatosis in patients with chronic liver disease due to the presence of fibrosis (15). These factors often exist in NASH patients. Therefore, evaluation of the influence of obesity and fibrosis on the detection of steatosis is needed.

To date, few studies have involved the evaluation of steatosis in NASH patients by both US and CT (16-19). Assessment of the severity of hepatic fibrosis is essential for determining the prognosis and making treatment decisions in patients with NASH. It is also suspected that the detectability of fibrosis is decreased in NASH patients with severe steatosis. Accordingly, the present study was performed to clarify the ability of US and CT to detect steatosis and advanced fibrosis in NASH patients, and to assess the influence of steatosis, fibrosis, and obesity on the radiological detection of steatosis and fibrosis.

Methods

From 1990 to December 2007, 384 Japanese patients were diagnosed as having biopsy proven NASH at Tokyo Women's Medical University. Among them, 126 patients underwent liver biopsy, and also underwent US and CT within 6 months before or after biopsy. All patients gave informed consent to participation in the study. Eight patients were excluded due to a change in the severity of obesity (change of their BMI by more than 1) between the time of liver biopsy and imaging, leaving 118 patients for whom clinical data were collected retrospectively.

Diagnosis of NASH was based on the following criteria: 1) steatohepatitis on liver biopsy, 2) intake of less than 100 g of ethanol per week (confirmed by the attending physician and family members who were in close contact with the patient), and 3) appropriate exclusion of other liver diseases (viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, and metabolic liver diseases such as Wilson's disease and hemochromatosis) (1, 2).

A complete history was obtained and physical examination was performed in all patients. The following laboratory parameters were measured: albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltranspeptidase, platelet count, and prothrombin time.

All liver biopsy specimens were examined to assess the severity of steatosis and fibrosis, and the NAFLD activity score (NAS) was calculated (20, 21). Mild steatosis was defined as 10-29% of hepatocytes containing fat, moderate steatosis was 30-69% involvement, and severe steatosis was more than 70% involvement. Mild fibrosis was defined as F0-2 and advanced fibrosis was defined as F3-4 (bridging fibrosis and cirrhosis).

All 118 patients were examined by US and CT. US was used to detect the presence of steatosis and advanced fibrosis. Hepatic steatosis was defined as being present in patients with at least two of the following findings: increased hepato-renal contrast, liver brightness, deep attenuation, and vascular blurring. Advanced fibrosis was defined as existing in patients with at least two of the following: a blunt liver edge, surface nodularity, caudate lobe hypertrophy, a coarse echo pattern, increased definition of portal veins, splenomegaly, ascites, and varices (12, 22).

CT was performed with a multi-detector row helical scanner. On non-enhanced scans, the liver-to-spleen attenuation ratio was measured and the presence of steatosis was indicated by a ratio of less than 0.9 according Japanese criteria (13). Advanced fibrosis was defined by the presence of at least two of these features on CT scans: surface nodularity, a prominent caudate lobe associated with a shrunken right lobe, a decrease in the volume of the medial segment of the left lobe, splenomegaly, ascites, and varices (12, 13).

The ability of US and CT to detect mild to severe histological steatosis and advanced histological fibrosis was assessed and the sensitivity of each modality was calculated. Unfortunately, specificity could not be calculated because there were no control patients.

To evaluate whether fibrosis and obesity interfered with the detection of moderate to severe histological steatosis by US and CT, we analyzed 88 NASH patients with moderate to severe steatosis (Fig. 1). The patients were divided into a group with mild histological fibrosis and another group with advanced histological fibrosis to evaluate interference with the detection of moderate to severe histological steatosis by fibrosis. To examine the influence of obesity, we divided the patients into 3 groups according to the body mass index (BMI): BMI<25, BMI of 25-30, and BMI>30.

To evaluate interference with the detection of advanced fibrosis by steatosis and obesity, we analyzed 59 NASH patients with advanced fibrosis. The patients were divided into three groups with mild histological steatosis, moderate stea-

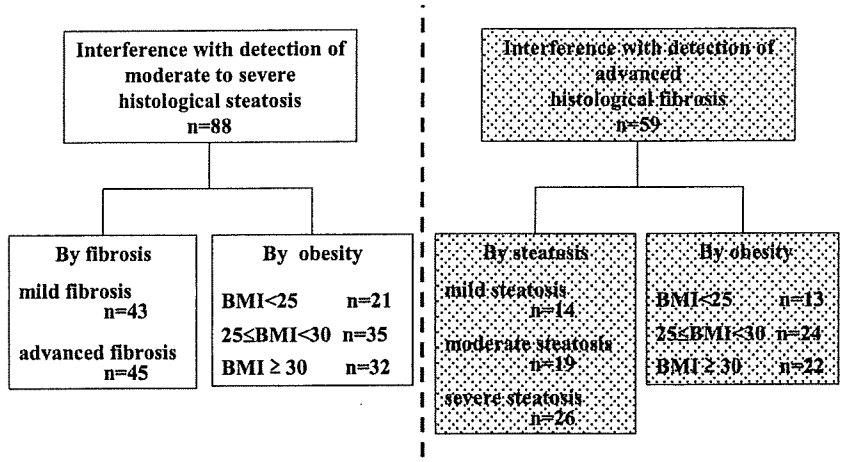

Figure 1. To evaluate whether fibrosis and obesity interfered with the detection of moderate to severe histological steatosis by US and CT, we analyzed 88 NASH patients with moderate to severe steatosis. To evaluate interference with the detection of advanced fibrosis by steatosis and obesity, we analyzed 59 NASH patients with advanced fibrosis.

Table 1. Patient Profile

		NASH n=118 Median (range or %)
Age (years)		54 (14~89y.o.)
Sex		M52 (44.1%) F66 (55.9%)
BMI (kg/m²)		27.3 (19.3~42.6)
Obesity (BMI≥25 kg/m²)		87 (73.7%)
Severe obesity (BMI≥30 kg/m²)		39 (33.1%)
Diabetes		58 (49.2%)
Hyperlipidemia		62 (52.5%)
Hypertension		53 (44.9%)
Alb	(g/dL)	4.3 (2.6-5.5)
T-bil	(g/dL)	0.6 (0.2-6.0)
AST	(IU/L)	51 (12-260)
ALT	(IU/L)	70 (10-302)
ALP	(IU/L)	265 (112-1407)
γ-GTP	(IU/L)	75 (17-1543)
Plt	(×10⁴/μL)	18.5 (3.7-45.1)
Prothrombin time	(%)	97 (45.5-100)
Fibrosis	F0-2	59 (50.0%)
	F3-4	59 (50.0%)
Steatosis	Mild	30 (25.4%)
	Moderate	25 (21.2%)
	Severe	63 (53.4%)
Activity	Mild	25 (21.2%)
	Moderate	59 (50.0%)
	Severe	34 (28.8%)

tosis, or severe steatosis. We also examined the influence of obesity.

Statistical analysis

Statistical analysis was done using SPSS software (version 11.0).

We assessed the relation between the liver/spleen attenuation ratio on CT and the severity of steatosis by analysis of variance (ANOVA), and we analyzed the sensitivity of detecting steatosis and fibrosis, as well as the influence of each factor by the chi-square test.

Results

Baseline demographic, clinical, and laboratory data for the NASH patients are shown in Table 1. The median age was 54 years, with a range of 14 to 89 years. There were 52 men and 66 women. Thirty-one patients had a BMI of less than 25, 48 patients had a BMI of 25-30, and 39 patients had a BMI of more than 30. Liver biopsy showed that 25.4% had mild steatosis, 21.2% had moderate steatosis, and, 53.4% had severe steatosis. In the case of fibrosis, 50.0% of NASH patients had mild fibrosis and 50.0% had

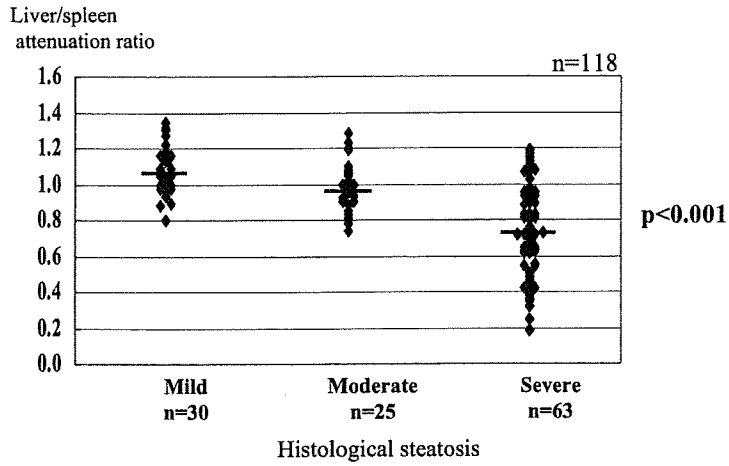

Figure 2. Scatter diagram of the liver/spleen attenuation ratio on CT in relation to the severity of histological steatosis. There was a significant difference of the liver spleen attenuation ratio between each stage of steatosis defined by liver biopsy (p<0.001).

advanced fibrosis. The NAFLD score was 3-4 in 4.2% and 5 or more in 95.8%.

Steatosis and imaging modalities

[Sensitivity of detecting steatosis]

Figure 2 displays the liver/spleen attenuation ratio obtained by CT in relation to the severity of steatosis. CT could precisely evaluate steatosis, and there was a significant difference of the ratio between each stage as defined by liver biopsy (p<0.001).

The sensitivity of detecting mild to severe steatosis in 118 NASH patients was 79.7% by US and 46.6% by CT. US was more accurate for identifying the existence of steatosis (p<0.001). US could not detect steatosis in 24 NASH patients, including 14 patients with mild histological steatosis and 10 patients with moderate histological steatosis. All 10 patients with moderate steatosis had advanced fibrosis and the US diagnosis was only advanced fibrosis in every case. Among the 14 patients with mild histological steatosis, 11 patients had advanced fibrosis and their US diagnosis was "advanced fibrosis without steatosis," while the other 3 patients had mild fibrosis and the US diagnosis was normal liver.

To evaluate the detection of each histological grade of steatosis, we analyzed 30 mild steatosis patients, 25 moderate steatosis patients, and 63 severe steatosis patients. US was more accurate for identifying the existence of each grade of steatosis. US detected 53.3% versus 10.0% for CT in patients with mild histological steatosis, while the rates were 64.0% versus 28.0% in moderate histological steatosis, and 98.4% versus 71.4% in severe steatosis. The difference between US and CT was statistically significant for each steatosis grade (p=0.0003 for mild steatosis, p=0.0107 for moderate steatosis, and $p \leq 0.0001$ for severe steatosis).

[Interference with detection of moderate to severe histological steatosis by fibrosis]

To evaluate interference with the detection of moderate to severe histological steatosis by fibrosis, we analyzed 88 patients with moderate to severe steatosis. Among them, 43 patients had mild fibrosis and 45 patients had advanced fibrosis.

The sensitivity of US for detecting moderate to severe histological steatosis in patients with mild histological fibrosis was 100%, but this was reduced to 77.8% in patients with advanced histological fibrosis (Fig. 3). The sensitivity of CT was 69.8% in patients with mild histological fibrosis and 48.9% in those with advanced histological fibrosis. The decrease in the sensitivity of detecting moderate to severe steatosis due to the presence of advanced fibrosis was statistically significant for both US and CT (p=0.001 for US and p=0.047 for CT). The extent of the decrease in sensitivity was similar for US and CT.

[Interference with the detection of moderate to severe histological steatosis by obesity]

Among the 88 patients, 21 patients had a BMI of less than 25, 35 patients had a BMI of 25 to 30, and 32 patients had a BMI of more than 30. The prevalence of advanced histological fibrosis was not significantly different in each BMI group (the prevalence of advanced histological fibrosis was as follows: BMI<25; 41.9%/25≤BMI<30; 50.0%/BMI ≥30; 56.4%; p=0.74). Figure 4 shows the detection of moderate to severe histological steatosis by US and CT. The sensitivity of US for moderate to severe histological steatosis was similar in each BMI group. CT could more accurately identify the presence of moderate to severe histological steatosis in obese patients (BMI>30), but the difference was not statistically significant.

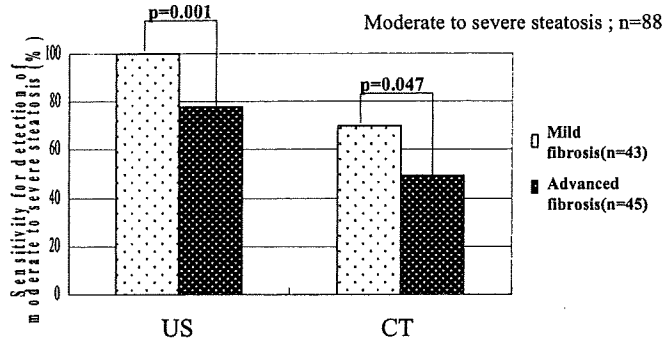

Figure 3. Comparison of the detection of moderate to severe histological steatosis between patients with mild fibrosis and advanced fibrosis. The decrease in the sensitivity of detecting moderate to severe histological steatosis due to the presence of advanced fibrosis was statistically significant for both US and CT.

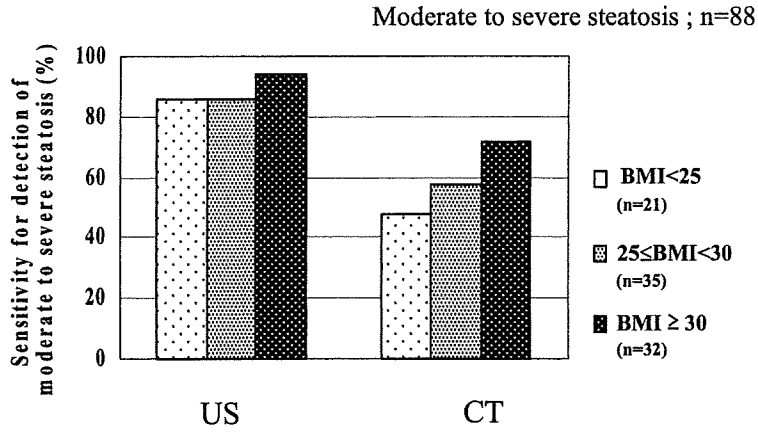

Figure 4. Detection of moderate to severe steatosis by US and CT according to BMI. The sensitivity of US for moderate to severe histological steatosis was similar in each BMI group. CT could more accurately identify the presence of moderate to severe histological steatosis in obese patients, but the difference was not statistically significant.

Fibrosis and imaging modalities

[Sensitivity of detecting advanced fibrosis]

To evaluate the detection of advanced histological fibrosis, we analyzed 59 NASH patients with advanced histological fibrosis. The sensitivity of detection was 59.3% for US and 71.2% for CT. The difference of sensitivity between US and CT was not significant, but CT was better able to detect advanced fibrosis.

[Interference with detection of advanced fibrosis by steatosis]

To evaluate interference with the detection of advanced histological fibrosis by steatosis, we analyzed 59 patients with advanced histological fibrosis. Among them, 14 patients had mild steatosis, 19 had moderate steatosis, and 26 had severe steatosis.

Interference with the detection of advanced histological fibrosis by steatosis is shown in Fig. 5. The sensitivity for detecting advanced fibrosis was markedly decreased by severe steatosis in the case of both US and CT (the sensitivity for mild/moderate/severe histological steatosis was 92.9/84.2/23.1% with US and 85.7/89.5/50.0% with CT). The loss of sensitivity was statistically significant for both modalities ($p<0.0001$ for US and $p=0.006$ for CT), but the decrease was greater for US.

[Interference with detection of fibrosis by obesity]

To evaluate interference with the detection of advanced histological fibrosis by obesity, we analyzed the patients with advanced fibrosis. The prevalences of severe histological steatosis were higher among the patients with BMI of more than 30. The difference was not statistically significant (the prevalence of severe histological steatosis was as follows: BMI<25; 41.9%/25≤BMI<30; 52.1%/BMI≥30; 64.1%; $p=0.18$). Among the 59 patients, 13 patients had a BMI of less than 25, 24 patients had a BMI of 25 to 30, and 22 patients had a BMI of more than 30. Detection of advanced histological fibrosis by US and CT is shown in

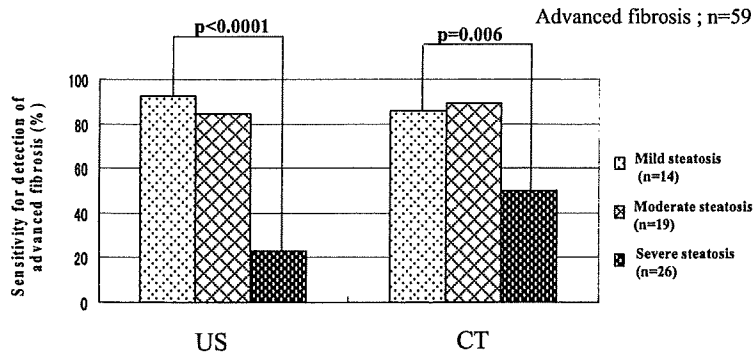

Figure 5. Comparison of the detection of advanced fibrosis in patients with mild, moderate, and severe steatosis. The sensitivity for detecting advanced fibrosis was markedly decreased by severe steatosis in the case of both US and CT.

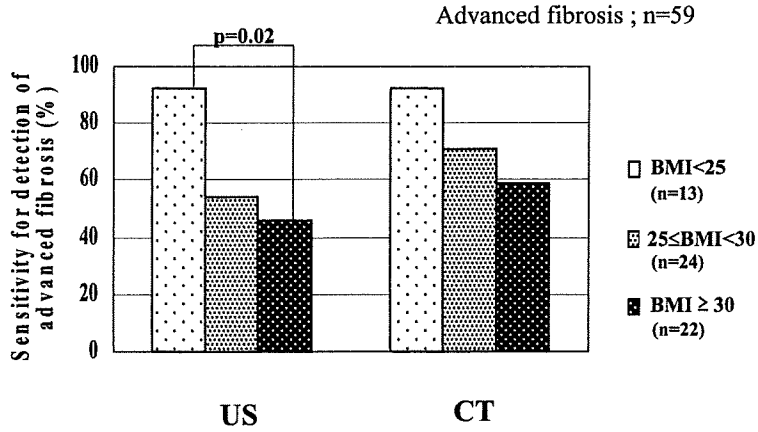

Figure 6. Sensitivity of US and CT for advanced fibrosis in relation to BMI. The sensitivity of both US and CT for detecting advanced fibrosis decreased as the BMI increased. The loss of sensitivity was statistically significant for US.

Fig. 6. The sensitivity of both US and CT for detecting advanced fibrosis decreased as the BMI increased. The sensitivity of detecting advanced fibrosis was as follows for US: BMI<25; 92.3%/25≤BMI<30; 54.2%/BMI≥30; 45.5%. For CT, it was: BMI<25; 92.3%/25≤BMI<30; 70.8%/BMI≥30; 59.1%. The loss of sensitivity was statistically significant for US (Fig. 6).

Discussion

NAFLD patients are usually asymptomatic and this condition is most often detected by US during an annual health check. Transaminases are not useful for making a diagnosis NAFLD because some patients have normal levels (6, 23). US also has several limitations because it is subjective, operator-dependent, poor at detecting mild steatosis, and poor at quantifying steatosis. Patients with detection of NAFLD by US underwent CT to confirm the presence of steatosis and to evaluate its severity by measurement of the liver/spleen attenuation ratio. Of course, CT has limitations with respect to the diagnosis of steatosis, including poor detection of mild steatosis, exposure to X-rays, and unavailability for patients with hemosiderosis (24). Moreover, both imaging modalities could not distinguish NASH from simple steatosis. Therefore, patients were evaluated to assess the need for liver biopsy to make a diagnosis of NASH. Liver biopsy is usually performed in NAFLD patients who are suspected to have other liver diseases and/or in patients suspected to have NASH. Therefore, it is important to determine the strengths and weaknesses of US and CT for confirming hepatic steatosis and fibrosis in NAFLD patients.

Several researchers have attempted to develop US grading systems in order to improve agreement between observers and decrease operator bias, and to develop US diagnosing systems for NASH (25, 26). However, even if the results are reproducible, it is very difficult to use such models in clinical practice. Since there is no correlation between the severity of steatosis and the severity of fibrosis in NASH patients, we think that detecting the presence of steatosis is sufficient (27, 28).

The present study revealed that US could more accurately identify the presence of steatosis in NASH patients than CT. In Japan, a liver/spleen attenuation ratio of less than 0.9 is defined as indicating steatosis (13). This definition is quite

strict, so the sensitivity of detecting steatosis by CT was relatively low. Detection of changes in the liver/spleen attenuation ratio is very useful for monitoring NASH patients, even if they have more than 0.9 liver/spleen attenuation ratio. The most important role of CT in NAFLD patients is quantitative assessment of the severity of steatosis by measurement of the liver/spleen attenuation ratio, as previously reported (12).

It is well known that mild steatosis is difficult to diagnose by US. In the present study, the sensitivity of US for mild steatosis in NASH patients with mild fibrosis was 81.3%. The high sensitivity of US for mild steatosis was probably achieved because most of our patients with mild histological steatosis had more than 20% of their hepatocytes containing fat. Interference by the presence of advanced fibrosis reduced the sensitivity of both US and CT for the detection of moderate to severe histological steatosis in NASH patients. It is important to know that the sensitivity of US for detecting moderate to severe steatosis in NASH patients with mild fibrosis was 100%, however in 22.2% of NASH patients with advanced fibrosis, even moderate to severe steatosis could not be detected by US. If liver biopsy was not done, these patients would be diagnosed as having idiopathic hepatic fibrosis. Interestingly, obesity did not interfere with the detection of moderate to severe histological steatosis by US or CT. Even when the focus was on the detection of severe histological steatosis, the results were similar for US and CT. According to our findings, it is not problematic to detect hepatic steatosis in obese Japanese patients.

Concerning fibrosis, CT could more accurately identify the presence of advanced fibrosis than US. The sensitivity of detecting advanced fibrosis by US or CT was markedly decreased in patients with severe steatosis and obesity (BMI> 25) and this decrease was more significant for US. It is important to recognize these weaknesses of US and CT.

Several reliable and noninvasive bio-markers for predicting the presence of advanced fibrosis or cirrhosis in NASH patients have been reported (28-31). We previously reported that measurement of hyaluronic acid can accurately identify advanced fibrosis in Japanese NAFLD patients, while the platelet count can be used to identify cirrhosis.

Therefore, imaging modalities might be used together with these markers to diagnose fibrosis more accurately.

The first limitation of our study is that we attempted to clarify the ability of US and CT for diagnosing steatosis and advanced fibrosis in NASH patients, but there were no controls. Therefore, we could not assess the specificity of the two imaging modalities for steatosis and fibrosis, so our study focused on sensitivity.

The second limitation is that the US data were interpreted by several radiologists, thus increasing inter-observer variability. However, we wanted to assess the validity of US in general practice, so the use of several radiologists was more appropriate.

We confirmed that US could more accurately identify the presence of steatosis in NASH patients than CT. Concerning interference with the detection of steatosis by advanced fibrosis, the decrease of detection was marked for both imaging modalities. However, obesity did not affect the detection of steatosis. The sensitivity of US and CT for advanced fibrosis was decreased markedly in patients with severe steatosis and obese patients, and this decrease was more marked for US. Awareness of these disadvantages of the common imaging modalities is useful for diagnosing steatosis and fibrosis in NAFLD patients.

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Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis

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Background. There have been few reports on hepatocellular carcinoma (HCC) in nonalcoholic steatohepatitis (NASH) and the natural history of NASH. Accordingly, we assessed the clinical features of HCC in NASH, the risk factors for HCC, and natural history of NASH with advanced fibrosis. ***Patients and methods.*** There were 34 NASH patients with HCC and 348 NASH patients without HCC. To clarify the clinical features of NASH patients with HCC and to determine the risk factors for HCC, we compared NASH patients with HCC with those without HCC. Univariate and multivariate logistic regression models were used for statistical analysis. A prospective cohort study of the outcomes of 137 NASH with advanced fibrosis was started in 1990. The impact of baseline risk factors on the development of HCC and survival was evaluated by Cox regression analysis. ***Results.*** In total, 88% of patients with HCC had advanced fibrosis, with a median age of 70 years. Older age, low level of AST, low grade of histological activity, and advanced stage of fibrosis were risk factors for HCC. A prospective cohort study showed that the 5-year cumulative incidence of HCC was 7.6%, and the 5-year survival rate was 82.8%. HCC was the leading cause of death. ***Conclusions.*** The present study confirmed that older age and advanced fibrosis were important risk factors for HCC, and that HCC was the major cause of mortality in NASH patients with advanced fibrosis. Regular screening for HCC is thus extremely important for NASH patients with advanced fibrosis.

Key words: nonalcoholic steatohepatitis (NASH), hepatocellular carcinoma (HCC), cirrhosis

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Introduction

Recently, lifestyle-related diseases (metabolic syndrome) such as diabetes mellitus, hypertension, and hyperlipidemia have become a leading public health problem because of their dramatic increase in both Western countries and Asia.[1,2] Nonalcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome. In Japan, annual health checks have begun to show 10%–30% of adults being diagnosed as having NAFLD.[3,4] Based on the prevalence of NAFLD, the prevalence of nonalcoholic steatohepatitis (NASH) is estimated to be 1%–3% of Japanese adults. Because of the dramatic increase in NASH patients in Japan, it will eventually become the most important cause of end-stage liver disease, as in the United States.

Because NASH features a wide range of severity from minimal fibrosis to cirrhosis, it is important to clarify the natural history of each stage of fibrosis in determining how to manage patients. Previous studies have shown that some patients develop hepatocellular carcinoma (HCC) without infection by any hepatitis viruses.[5–7] Growth factors associated with chronic inflammation, insulin resistance, and DNA mutation as a result of lipid peroxidation play significant roles in the development of HCC.

We have reported a case series of HCC in NASH patients and the natural history of NASH with advanced fibrosis.[8–12] Most NASH patients with HCC had cirrhosis. It is well known that cirrhosis is the most important risk factor for development of HCC among all causes of liver diseases. The same appears to be true of HCC patients with NASH. We also found that HCC was the major cause of mortality in NASH with advanced fibrosis. With the exception of one study, all previous studies found that cirrhotic NASH patients develop HCC within several years after the diagnosis of cirrhosis.[13–16] However, there have been few reports on HCC in NASH, and thus the natural history of NASH and the

natural histories of NASH and HCC in NASH remain unclear.

Accordingly, to clarify the clinical features of HCC in NASH and determine the risk factors for HCC, we compared NASH patients with HCC with those without HCC in a larger number of patients than in our previous study. A prospective study of the natural history of NASH patients with advanced fibrosis was started at Tokyo Women's Medical University Hospital in 1990. We also assessed the natural history of NASH with advanced fibrosis.

Patients and methods

Subjects

From 1990 to December 2007, 412 Japanese patients were diagnosed with biopsy-proven NAFLD at Tokyo Women's Medical University. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. A prospective cohort study of the natural history of NASH with advanced fibrosis was begun in 1990. We excluded 28 patients with simple steatosis and 2 patients with missing data or lack of informed consent; 382 NASH patients were therefore included in this study.

Definitions and criteria

A complete history was obtained and physical examination performed for all patients. The diagnosis of NASH was based on the following criteria: (1) detection of steatohepatitis on liver biopsy, (2) intake of less than 100 g ethanol per week (as confirmed by the attending physician and family members who were in close contact with the patient), and (3) appropriate exclusion of other liver diseases (such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, and metabolic liver diseases such as Wilson's disease and hemochromatosis).[17–19] All patients were negative for hepatitis B surface antigen and antibody to hepatitis C virus (HCV) and/or hepatitis C RNA on polymerase chain reaction analysis.

Obesity was defined as a body mass index (BMI) of more than 25 according to the Japanese Obesity Association criteria. The diagnosis of type II diabetes mellitus was based on the following Japanese criteria: random blood glucose >200 mg/dl or fasting glucose >126 mg/dl or hemoglobin A_{1c} >6.5% on two occasions, or current treatment for type II diabetes. Hyperlipidemia was diagnosed if the patient was being treated with lipid-lowering medications or had elevated levels of total cholesterol (>220 mg/dl) and/or triglycerides (>150 mg/dl) on at least three occasions. Hypertension was diagnosed if the patient was on antihypertensive therapy or had a blood pressure of more than 140/90 mmHg on at least three occasions. The following laboratory parameters were measured: albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), cholinesterase, iron, ferritin, platelet count, prothrombin time, and hyaluronic acid.

Histologically, the NAFLD activity score was calculated, and scores of more than 5 were defined as NASH.[20] Fibrosis was scored using a 5-grade scale: F0 = normal connective tissue; F1 = foci of perivenular or pericellular fibrosis in zone 3 or in zone 1; F2 = perivenular or pericellular fibrosis confined to zones 3 and 2, with or without portal/periportal fibrosis; F3 = bridging or septal fibrosis; and F4 = cirrhosis. A grade of mild, moderate, or severe necroinflammation (activity) was assigned based on the reviewer's overall impression after evaluating the specimens for ballooning degeneration, Mallory bodies, giant mitochondria, disarray of hepatocytes, lobular and portal inflammation, focal necrosis, Councilman bodies, lipogranulomas, and pigmented macrophages. Steatosis was graded as mild to severe: mild (affecting 10%–29% of hepatocytes); moderate (30%–69% of hepatocytes); or severe (>70% of hepatocytes).[21,22]

Of the NASH patients with HCC, seven patients whose liver biopsies showed cirrhosis at the time of initial liver biopsy did not undergo surgery for HCC or had not had a liver biopsy at the time of developing HCC. So, we did not have the histology at the time of development of HCC. Therefore, for analysis of inflammatory changes and steatosis, we used the histological data of the initial liver biopsy. Duration between the initial biopsies and development of HCC in these seven patients was less than 2 years.

Screening for HCC was performed in all patients by measuring serum alpha-fetoprotein and des-gamma-carboxyl prothrombin and by ultrasound (US) at the time of liver biopsy. NASH patients with advanced fibrosis were followed regularly, and screening for HCC was performed at least three times a year by measuring serum alpha-fetoprotein and des-gamma-carboxyl prothrombin and by performing US. Other imaging studies (US, computed tomography, magnetic resonance imaging, and selective hepatic arteriography) were performed in patients suspected to have HCC. HCC was diagnosed histologically or by detection of consistent findings on at least two radiologic modalities.[23]

During the follow-up period, weight was mainly controlled by diet and exercise in obese patients. Although several therapeutic approaches were done, no pharma-

cological therapy was proven to be effective for patients with advanced fibrosis. None of the patients was on hormone replacement therapy for menopause. None of the patients underwent bariatric surgery, and liver transplantation was performed for only one patient.

Statistical analysis

Analysis was performed with the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

Comparison of clinicopathological features between NASH patients with and without HCC

Univariate logistic regression analysis was used to compare baseline variables between NASH patients with and without HCC. To clarify risk factors for HCC, multivariate logistic regression analysis was performed.

Natural history of NASH with advanced fibrosis

The NASH patients with advanced fibrosis were followed until they died and were censused at the time of their last clinic visit. In the case of liver transplantation, the day of transplantation was handled as that of death. The primary outcomes were development of HCC, overall survival, and liver-related mortality. The time frame for each outcome was defined as the duration of time from liver biopsy to the onset of the relevant event. Time to failure (Kaplan–Meier) analysis was performed. The impact of baseline risk factors on the development of HCC, overall survival, and liver-related mortality were evaluated by Cox regression analysis. All parameters with a P value of less than 0.1 on univariate analysis were selected for multivariate analysis using a Cox proportional hazards model. P values of 0.05 or less were considered significant.

Results

Comparison of clinicopathological features between NASH patients with and without HCC

Twenty-three patients were diagnosed with concomitant HCC and NASH. During a mean follow-up of 40.3 months (range, 6–181.1 months), 11 patients developed HCC. Altogether, there were 21 men and 13 women with HCC.

Table 1 shows a comparison of the clinicopathological features of the 34 patients with HCC and the 348

Table 1. Comparison of clinicopathological features between nonalcoholic steatohepatitis (NASH) patients with and without hepatocellular carcinoma (HCC)

Variable	HCC (+) $n = 34$	HCC (–) $n = 348$
Age (years)	70 (54–89)	50 (10–84)
Sex (female)	38%	59%
Body mass index	26.1 (19.4–36.5)	26.6 (15.9–61.0)
Obesity (BMI > 25)	62%	69%
Diabetes	74%	43%
Hyperlipidemia	29%	62%
Hypertension	47%	33%
Laboratory data		
Albumin (g/dl)	3.8 (2.7–4.5)	4.3 (1.8–5.5)
Total bilirubin (mg/dl)	0.8 (0.2–3.1)	0.6 (0.2–7.9)
AST (IU/l)	42 (15–143)	52 (12–392)
ALT (IU/l)	37 (11–134)	84 (5–740)
Alkaline phosphatase (IU/l)	242 (145–1352)	245 (51–1407)
GGTP (IU/l)	102 (25–772)	71 (10–1543)
Cholinesterase (IU/l)	225 (111–353)	373 (65–668)
Iron (μg/dl)	101 (29–230)	103 (4–268)
Ferritin (ng/ml)	186 (23–1012)	213 (4–1237)
Platelet count ($\times 10^4/\mu l$)	11.7 (2.4–25.0)	22.1 (3.7–45.1)
Prothrombin time (%)	80.2 (40.0–100)	100 (35.7–120)
Hyaluronic acid (ng/ml)	184 (54–1060)	36 (2.9–1960)
Histological features		
Fibrosis F1–F2	12%	69%
Fibrosis F3–F4	88%	31%
Inflammation[a]	62%	82%
Steatosis[a]	59%	88%

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGTP, gamma-glutamyl transpeptidase
[a]Moderate to severe

Table 2. Comparison of clinicopathological features between NASH patients with and without HCC: univariate logistic regression

	P value	OR	95% CI
Age	0.000	1.129	1.082–10179
Diabetes	0.002	3.618	1.632–8.016
Hyperlipidemia	0.001	0.257	0.119–0.555
Albumin (g/dl)	0.000	0.257	0.142–0.465
AST (IU/l)	0.036	0.986	0.974–0.999
ALT (IU/l)	0.000	0.967	0.953–0.981
ALP (IU/l)	0.049	1.002	1.000–1.003
Platelet count ($\times 10^4/\mu l$)	0.000	0.830	0.779–0.884
Prothrombin time (%)	0.000	0.942	0.920–0.964
Hyaluronic acid	0.010	1.003	1.001–1.005
Fibrosis	0.000	4.309	2.721–6.822
Necroinflammation	0.000	0.265	0.143–0.492
Steatosis	0.000	0.285	0.181–0.454

OR, odds ratio; CI, confidence interval

patients without HCC. Table 2 lists factors significantly differing between NASH patients with and without HCC on univariate logistic analysis. The median age was 70 years in the case of the NASH patients with HCC (range, 54–89 years) and 50 years in the case of the NASH patients without HCC (range, 10–84 years). This difference was statistically significant. Male sex, diabetes mellitus, and hypertension were more common in the NASH patients with HCC. The difference in prevalence of diabetes between the groups was statistically significant. In contrast, the prevalences of obesity and hyperlipidemia were lower among the NASH patients with HCC. The difference in prevalence of hyperlipidemia between the groups was significant. The differences in albumin, AST, ALT, ALP, platelet count, prothrombin time, and hyaluronic acid level between the two groups were also statistically significantly. Stage of fibrosis was significantly higher in the NASH patients with HCC. In contrast, grades of activity and steatosis were significantly lower in NASH patients with HCC. On multivariate logistic regression analysis, older age [$P = 0.008$; odds ratio (OR) = 1.108; 95% confidence interval (CI), 1.028–1.195], low level of AST ($P = 0.027$; OR = 0.956; 95% CI, 0.919–0.995), low level of histological activity ($P = 0.010$; OR = 0.154; 95% CI, 0.037–0.638), and stage of fibrosis ($P = 0.001$; OR = 4.232; 95% CI, 1.847–9.698) were independent predictors of development of HCC (Table 3).

Characteristic features of HCC were as follows. Nine patients (26%) exhibited elevation of AFP. Twenty-four patients (70%) had a single nodule; there were two nodules in 3 patients (9%) and more than three in 7 patients (21%).

Natural history of NASH with advanced fibrosis

Our prospective cohort study consisted of 137 NASH patients with advanced fibrosis. During the median follow-up of 40.3 months among 118 patients with advanced fibrosis, who did not have HCC at the time of entry, the 5-year cumulative incidence of HCC was 7.6%. By outcome, 26 patients died, with death caused by liver failure in 7 patients, HCC in 12 patients, and other causes in 7 patients, including uterine cancer in 1 patient, pancreatic cancer in 1 patient, lung cancer in 1 patient, pneumonitis in 1 patient, cholangitis in 1 patient, cerebral infarction in 1 patient, and unknown cause in 1 patient. Liver-related deaths thus accounted for 19 (73%) deaths. The 5-year survival rate was 82.8%, and the 5-year survival rate for liver-related death was 85.9%. Unfortunately, Cox proportional hazard model analysis did not reveal any risk factors for development of HCC.

Table 3. Comparison of clinicopathological features between NASH patients with and without HCC: multivariate logistic regression

	P value	Odds ratio	95% CI
Age	0.008	1.108	1.028–1.195
AST	0.027	0.956	0.919–0.995
Activity	0.010	0.154	0.037–0.638
Fibrosis	0.001	4.232	1.847–9.698

CI, confidence interval

Discussion

HCC is the fifth most common cancer worldwide and the third most common cause of cancer mortality.[24] According to the most recent nationwide Japanese registration data, primary liver cancer ranked third for men and fifth for women as a cause of death from malignant neoplasm.[25] The latest nationwide report registered every 2 years by the Liver Cancer Study Group of Japan showed that hepatitis C was the most common underly-

ing liver disease in HCC.[26] Hepatitis C-related HCC accounts for 70% of all cases of HCC, followed by hepatitis B at 16%. However, hepatitis C-related HCC has recently been gradually decreasing and HCC in cases of liver disease of unknown cause gradually increasing. Obesity and diabetes are now widely recognized as significant risk factors for HCC.[27–30] These two major risk factors for HCC were the main causes of NASH. The increasing numbers of cases of HCC resulting from liver disease of unknown cause probably contribute to HCC in NASH.

We performed a large case-control study of NASH patients with and without HCC as well as a prospective cohort study on the natural history of NASH patients with advanced fibrosis who underwent follow-up using a predefined screening protocol for HCC at a single tertiary care hospital.

In the case-control study, we found that older age, low level of AST, histological low grade of activity, and histologically advanced fibrosis were significant risk factors for development of HCC in NASH on multivariate logistic regression analysis. According to the *P* value, older age and histologically advanced fibrosis were the strongest risk factors for the development of HCC. It is known that cirrhosis is present in about 80% of HCC patients with any underlying liver disease, and it is the most important single risk factor for HCC. Then, our NASH patients were divided by stage of fibrosis (F1 to F4) to assess the distribution of other risk factors. Figure 1 shows the age distribution of NASH patients by stage of fibrosis. Age was correlated with stage of fibrosis. Figure 2 shows the distribution of transaminases. F4 stage (cirrhosis) causes the levels of transaminases to decrease. Figure 3 shows the prevalence of histological activity by stage of fibrosis. The prevalence of mild activity in F4 stage (cirrhosis) was higher than in F2 and F3 stages. Figure 4 shows the prevalences of histological steatosis by stage of fibrosis.

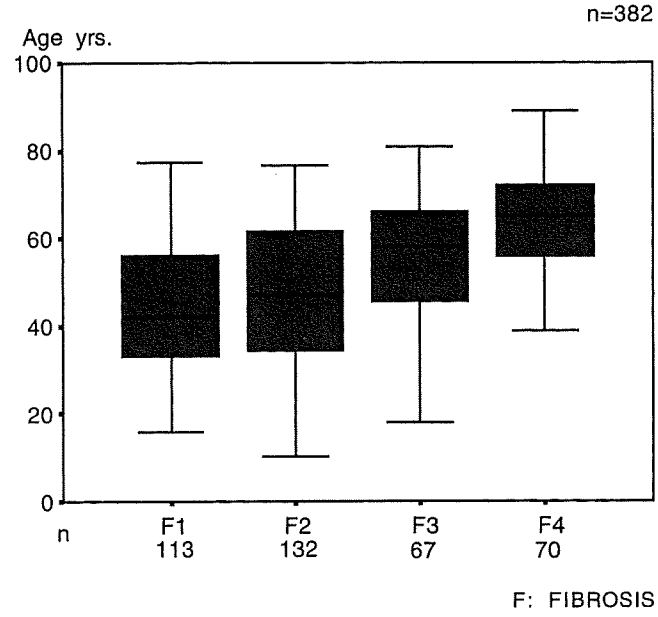

Fig. 1. Age (in years) distribution of NASH patients by stage of fibrosis (*F*). The age distribution and stage of fibrosis show a positive correlation

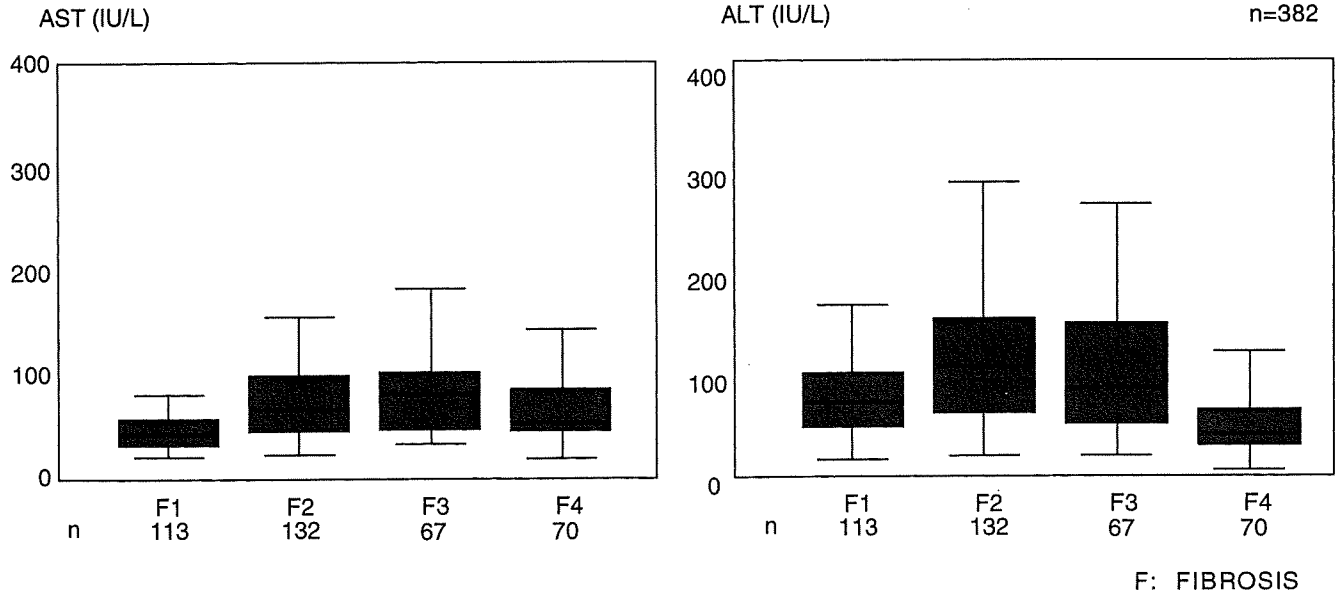

Fig. 2. Distribution of transaminases. A decrease in the levels of both aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) occurred in F4 stage cirrhosis

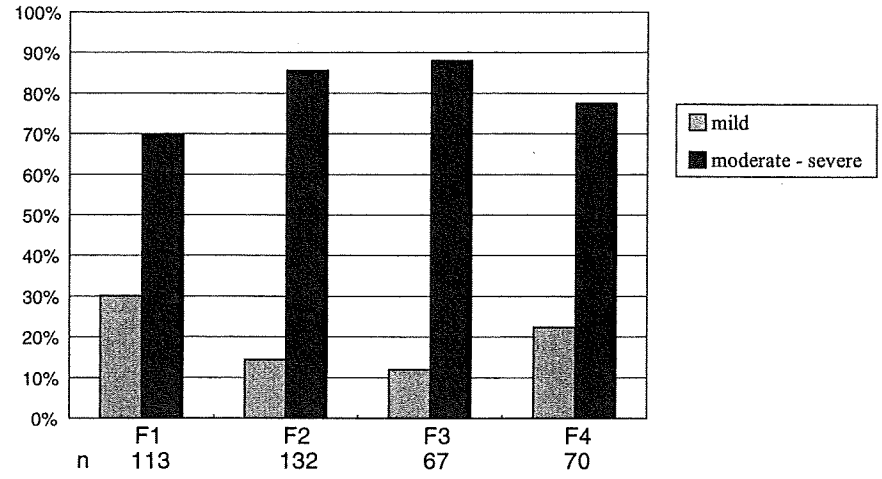

Fig. 3. Prevalence of histological activity by stage of fibrosis. The prevalence of mild activity in F4 stage (cirrhosis) was higher than in F2 and F3 stages

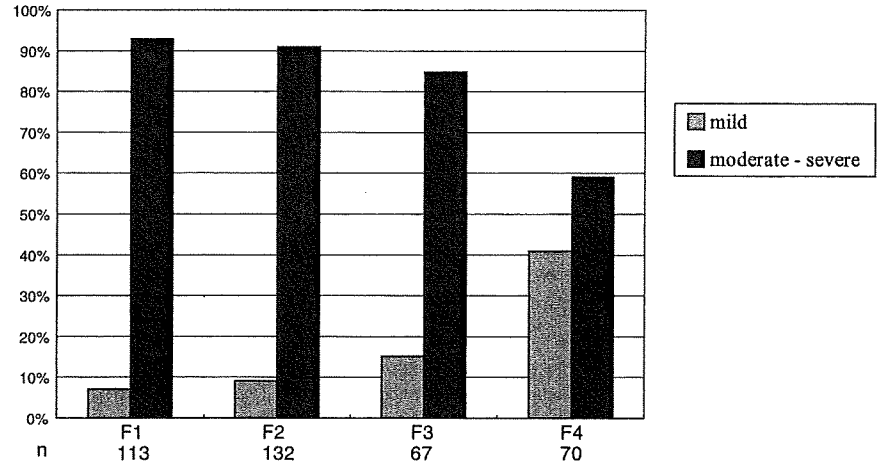

Fig. 4. Prevalence of histological steatosis by stage of fibrosis. A slight increase occurred in mild histological steatosis from F1 to F3 stages. However, a marked increase was evident from F3 stage to F4 stage

The prevalence of mild steatosis increased with each stage of fibrosis. These analyses showed that age, level of ALT, and grade of activity all correlated with each stage of fibrosis. In NASH patients, the severity of fibrosis and age are important risk factors for HCC, and this appears also to be the case for HCC patients with other causes of liver disease. In patients with cirrhosis from hepatitis C, high levels of transaminases are risk factors for the development of HCC; further studies are needed to clarify the reason for the conflicting result in our study. Histologically, when cirrhotic NASH patients progress to the end stage of their disease, characteristic features of NASH, including steatosis, necroinflammation, ballooning degeneration, and Mallory bodies, all disappear, in so-called burnt-out NASH. It is thus reasonable that a histological low grade of activity was selected as a risk factor for HCC. This factor also might be related to low levels of transaminases as a risk factor for HCC.

Our prospective cohort study confirmed that the occurrence of HCC was the strongest predictor of mortality. However, no risk factors for development of HCC and mortality were found on Cox regression analysis. Because our cohort consisted only of patients with advanced fibrosis, which is the strongest risk factor for development of HCC and mortality, statistical analysis was unable to detect additional risk factors in this group.

Another important finding of this study was that no patient among the NASH patients with advanced fibrosis died of heart disease, although several reports have indicated that NAFLD and NASH are risk factors for cardiovascular and cerebrovascular disease.[16] It is possible that patients with cardiovascular events are too ill to undergo liver biopsy. Because histological diagnosis is required for the diagnosis of NASH, the patients diagnosed with this condition consisted of only those with liver function sufficient to undergo liver biopsy. Our findings were thus affected by a certain degree of patient selection bias.

In this case-control prospective study, we found that older age and advanced fibrosis were important risk factors for development of HCC in NASH. The present study confirmed that occurrence of HCC was the stron-

gest predictor of mortality. These findings are likely to be helpful when devising therapeutic interventions for this patient population and also for the daily management of NASH patients. Regular screening for HCC is extremely important for NASH patients with these risk factors.

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EDITORIALS

Will non-invasive markers replace liver biopsy for diagnosing and staging fibrosis in non-alcoholic steatohepatitis?

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The recent dramatic increase of obesity and metabolic syndrome has caused a striking increase in non-alcoholic fatty liver disease (NAFLD) in both European and North American countries and Asia.[1]

Non-alcoholic fatty liver disease consists of two clinical entities: simple steatosis, which generally follows a benign non-progressive clinical course; and non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. NASH was characterized and defined by Ludwig *et al.* in 1980.[2] The concept referred to subjects who did not consume alcohol but had progressive liver disease with liver biopsy findings similar to those of alcoholic hepatitis, and documented the clinical correlation with obesity and lifestyle-related diseases such as type 2 diabetes and hyperlipidemia. NASH emerged as a clinicopathological concept and even now biopsy evaluation is considered the gold standard for a definitive diagnosis.

The principal histological features of NASH are as follows: presence of macrovesicular steatosis, ballooning degeneration of hepatocytes, mixed lobular inflammation, Mallory hyaline, megamitochondria, acidophilic bodies, and glycogenated nuclei. Perivenular and perisinusoidal–pericellular fibrosis are characteristic features of NASH fibrosis. These features are predominant in zone 3 (perivenular areas) and develop from zone 3 to the panacinar zone. However, many pediatric cases have atypical features such as more zone 1 steatosis, little or no ballooning or Malory hyaline, and more portal-based chronic inflammation and fibrosis.

In 1999, Matteoni *et al.* found that cirrhosis and liver-related mortality were related to the histological characteristics of NAFLD.[3] To evaluate the clinicopathological correlation, they divided 132 NAFLD patients into four categories based on the presence of specific histological lesions: type 1, fatty liver alone; type 2, fatty liver plus lobular inflammation; type 3, steatosis and ballooning degeneration; and type 4, as in type 3 plus either Mallory hyaline or fibrosis. They confirmed the benign course of patients who had steatosis with or without lobular inflammation (types 1 and 2 NAFLD) and a progressive course for patients who had type 3 or 4 NAFLD. They defined type 3 and 4 as NASH and emphasized the importance of ballooning degeneration, which is a structural manifestation of microtubular disruption and severe cell injury. Matteoni's system served to distinguish simple steatosis (type 1 and 2) from NASH (type 3 and 4), but did not include an assessment of NASH severity.

In evaluating chronic hepatitis, necroinflammatory grade and fibrotic stage are distinguished. On this conceptual basis, Brunt *et al.* proposed a grading (steatosis and necroinflammatory changes) and staging (fibrosis) system for a semiquantitative evaluation of NASH in 1999.[4] Unfortunately, this system applied only to NASH and it was not applicable to the entire spectrum of NAFLD.

In 2005, the NASH Clinical Research Network presented and validated a NAFLD activity score (NAS).[5] This score was based on the classification proposed earlier by Brunt *et al.*, it was applicable to adult and pediatric NAFLD patients and allowed the assessment of changes with therapy. The score is defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3) and ballooning (0–2); thus, NAS ranges from 0–8. A score of more than 5 correlated with a diagnosis of NASH, and biopsies with scores of less than 3 were diagnosed as 'not NASH'. Patients who had scores of 3 or 4 were borderline. Fibrosis was not included in NAS to maintain the paradigm of distinguishing grade from stage. Regarding stage, stage 1 was extended to include a distinction between delicate (1A) and dense (1B) perisinusoidal fibrosis in zone 3, and to detect portal-only fibrosis, without perisinusoidal fibrosis (stage 1C). Stage 2 was characterized by additional evidence of focal or extensive periportal fibrosis. Stage 3 was characterized by bridging fibrosis, and stage 4 was cirrhosis. Because NAS and staging allow the diagnosis of the entire range of NAFLD, it may prove useful in standardizing the histological diagnosis of NAFLD in clinical trials.

A liver biopsy remains the only reliable means to precisely diagnose NASH and establish the severity of liver injury and presence of fibrosis. However, liver biopsy has several drawbacks: it is an invasive, painful and costly procedure, with the possibility of sampling error and variability in pathologist interpretation. Moreover, given the extremely high prevalence of NAFLD in the general population, a liver biopsy is poorly suited as a diagnostic test for NAFLD. These shortcomings and drawbacks of liver biopsy, as recognized by a recent Asia–Pacific Consensus on NAFLD,[6] support the urgent need to find non-invasive markers for the assessment of NASH.

An ideal biomarker should be simple, accurate, reproducible, inexpensive and readily available. Currently, the NAFLD biomarkers have been evaluated for: (i) distinguishing NASH from NAFLD; and/or (ii) diagnosing advanced fibrosis or cirrhosis (Fig. 1). The study subjects have varied from the general population to NASH patients assessed in liver clinics.

Serum transaminases are usually mildly elevated in NAFLD, however, their utility for the diagnosis of NASH is poor. The process of development of NASH has been referred to as the two-hit hypothesis, with steatosis being the first hit and subsequent hepatocellular injury causing NASH the second hit. Insulin resistance, excess intracellular fatty acids, imbalance of cytokine production, oxidative stress, lipid peroxidation, iron overload, depletion of adenosine triphosphate and mitochondrial dysfunction are candidate pathways for induction of hepatocellular injury (NASH) in a steatotic liver. Accordingly, many studies have been focused on the second hit in NAFLD; namely, thioredoxin as a marker of oxidative stress, cytokines (tumor necrosis factor-α[7] and interleukin-6), adiponectin, leptin and C-reactive protein, among

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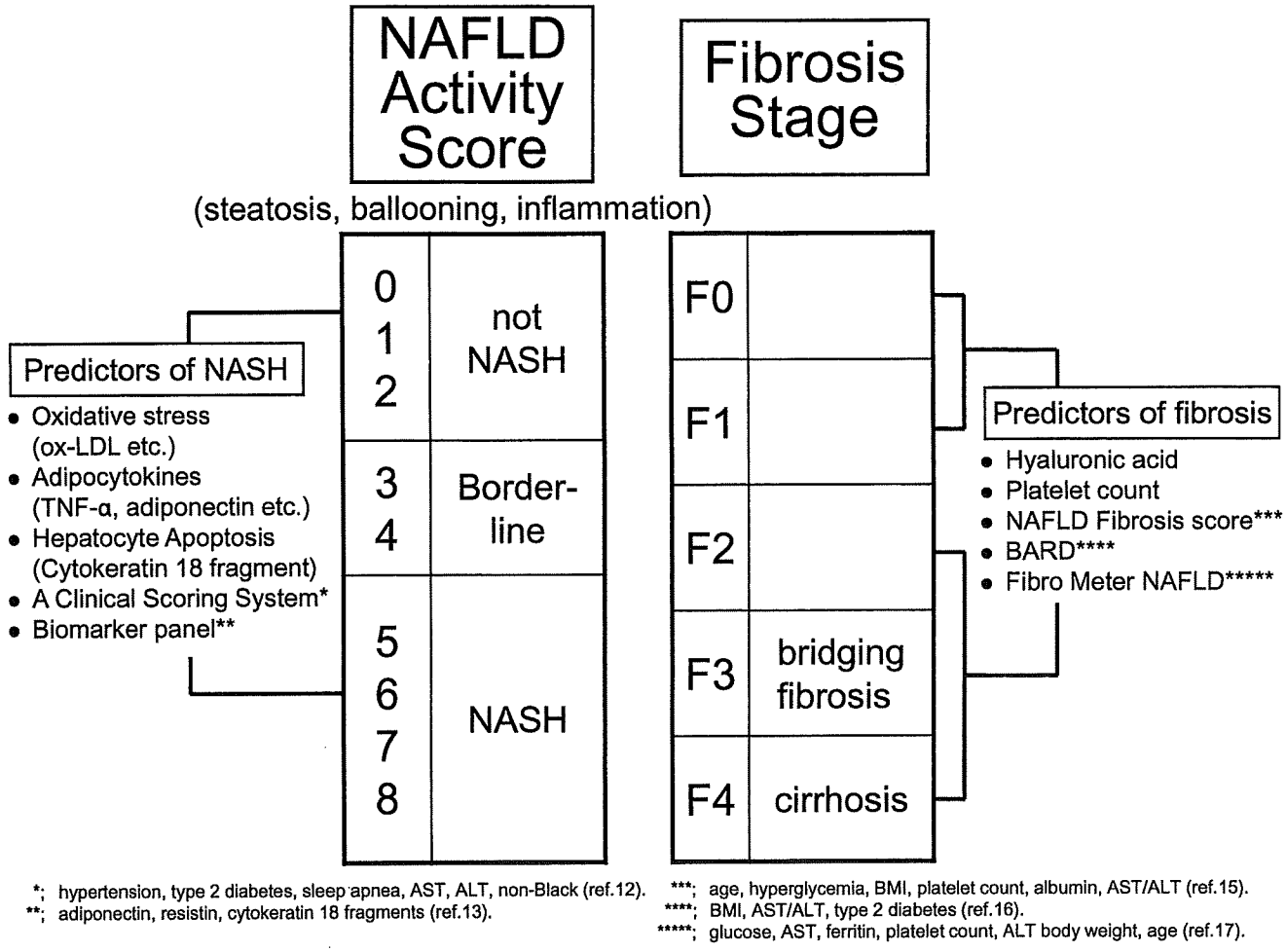

Figure 1 Serum and clinical predictors of NASH and/or fibrosis in NAFLD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; TNF, tumor necrosis factor.

others. The relationship between these markers and NASH is inconsistent, because they were found to correlate more closely with the degree of steatosis or necroinflammatory activity than with the fibrotic stage.

Hepatocyte apoptosis has been found to be a prominent feature of NASH. Wieckowska *et al.* reported that serum caspase 3-generated cytokeratin-18 (CK-18) fragment levels, a product of hepatocyte apoptosis in the injured liver, independently predicted the presence of NASH and correlated with the magnitude of hepatocyte apoptosis and disease severity.[8] This study was validated in a large number of NAFLD patients by the NASH Clinical Research Network.[9] Diab *et al.* also assessed the usefulness of plasma CK-18 fragments in a cohort of patients coming to bariatric surgery.[10] Their results support the potential utility of this test for diagnosis and staging of NAFLD. Moreover, they showed a significant decrease in CK-18 fragment levels in most patients 6 months after bariatric surgery.

The work by Malik and coworkers in this issue of the Journal reconfirms the usefulness of CK-18 fragments.[11] They evaluated the predictive value of serum markers to identify NASH and liver fibrosis. They evaluated CK-18 fragments, a hyaluronic acid tissue inhibitor of metalloproteinase 1, and YKL-40. They found that only CK-18 fragments could identify NASH. Accordingly, the CK-18 fragments are expected to become an ideal biomarker for NASH, although currently it is not routinely available as a laboratory test.

To increase the accuracy of non-invasive markers to diagnose NASH, multiple serum markers have been combined into mathematical models to obtain predictive scores. In 2008, two clinical scoring systems for predicting NASH in patients with morbid obesity have been reported. Campos *et al.* identified hypertension, type 2 diabetes, the presence of sleep apnea, elevated transaminases and non-black race as independent predictors of NASH.[12] The other one was described by Younossi *et al.*[13] It was based on serum levels of adiponectin, resistin and CK-18 fragments.

Concerning fibrosis, several routinely available laboratory tests serve to determine the cirrhotic stage. We have reported that elevation of serum hyaluronic acid levels is seen in stage 3 and 4 NAFLD patients, and platelet count decreased in stage 4 NAFLD patients; these findings indicate that hyaluronic acid can predict stage fibrosis

Journal of Gastroenterology and Hepatology **24** (2009) 501–508

at an earlier stage than platelet count.[14] Extracellular matrix proteins, such as hyaluronic acid and collagen components, have been examined as potential markers of liver fibrosis in NAFLD. Several models to predict the extent of fibrosis in NAFLD patients have been reported.[15–17] In general, most of these show similar accuracy for the detection of advanced fibrosis, but weak accuracy for that of mild fibrosis. This represents a limitation for the screening of patients with NAFLD. On the other hand, NAFLD patients with mild fibrosis might benefit the most from therapeutic interventions. Several imaging techniques have also been advocated as non-invasive diagnostic tests for NASH. In addition, ultrasonography-based transient elastography or FibroScan, have shown promising results regarding the severity of liver fibrosis.

In summary, liver biopsy currently remains the gold standard for the diagnosis of NASH, including assessment of the fibrotic severity of this common liver disease. In the future, improved understanding of the pathogenesis of NASH and new technologies, such as CK-18 fragments and serum hyaluronic acid, and FibroScan should contribute to the diagnosis of NASH and hopefully become a reliable non-invasive alternative to liver biopsy.

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Colorectal cancer in inflammatory bowel disease

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Colorectal (CRC) cancer is one of the most devastating complications of chronic colitis in the setting of inflammatory bowel disease (IBD). Only 1–2% of CRC occur in IBD patients but IBD is one of the major risk factors of CRC, and up to 15% of IBD mortality has been attributed to CRC.[1]

Current strategies in the reduction or management of colitis-associated CRC include chemoprophylaxis, colonoscopy surveillance of at risk individuals and proctocolectomy is a potentially curative treatment for those with precancerous dysplasia or early cancer. Colonoscopy surveillance is recommended after 8 to 10 years of extensive colitis and 12 to 15 years of left-sided colitis.[2] The detection of colorectal dysplasia is considered a strong predictor of CRC in IBD, and there may be some emerging evidence of improved survival in those who underwent surveillance.[3] The yield of detecting dysplasia is variable. Friedman *et al.* found a 25% rate of definite dysplasia after the tenth examination when surveillance is performed every 1–2 years.[4] Despite adherence to surveillance programs, CRC may still develop. Lutgens *et al.* showed that 17–28% of CRC are missed despite adherence to the British Society of Gastroenterology and American Gastroenterology Association guidelines, mostly due to CRC occurring prior to guideline-based surveillance starting points.[5] A high proportion of cancers are also found as interval cancers outside of surveillance programs, raising the possibility of missed early lesions during

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