Modification of a simple clinical scoring system as a diagnostic screening tool for non-alcoholic steatohepatitis in Japanese patients with non-alcoholic fatty liver disease

Akinobu Nakamura[1], Masato Yoneda[2], Yoshio Sumida[3], Yuichiro Eguchi[4], Hideki Fujii[5], Hideyuki Hyogo[6], Masafumi Ono[7], Yasuaki Suzuki[8], Takumi Kawaguchi[9], Noriaki Aoki[10], Takeshi Okanoue[11], Atsushi Nakajima[2], Shin Maeda[2], Yasuo Terauchi[1]*

ABSTRACT

Aims/Introduction: We reinvestigated the clinical usefulness of the modified NAFIC scoring system, modified by changing the weightage assigned to the fasting serum insulin level based on the importance of hyperinsulinemia in the pathogenesis of non-alcoholic steatohepatitis (NASH), in Japanese patients with non-alcoholic fatty liver disease (NAFLD) who had undergone liver biopsy.

Materials and Methods: The NAFIC score is conventionally calculated as follows: serum ferritin ≥200 ng/mL (female) or ≥300 ng/mL (male), 1 point; serum fasting insulin ≥10 μU/mL, 1 point; and serum type IV collagen 7 s ≥5.0 ng/mL, 2 points. A total of 147 patients with NAFLD who had undergone liver biopsies were included in the estimation group. To validate the modified scoring system, 355 patients from nine hepatology centers in Japan were also enrolled.

Results: In the estimation group, 74 (50.3%) patients were histologically diagnosed as having NASH, whereas the remaining 73 (49.7%) were diagnosed as not having NASH. As the percentage of NASH patients increased not only among participants with serum insulin levels greater than 10 μU/mL, but also in those with serum levels greater than 15 μU/mL, we advocated use of the modified NAFIC score, as follows: serum fasting insulin 10–15 μU/mL, 1 point and ≥15 μU/mL, 2 points. The modified NAFIC score showed improved sensitivity and negative predictive value for the diagnosis of NASH. This finding was also confirmed in the validation group.

Conclusions: The modified NAFIC scoring system could be a clinically useful diagnostic screening tool for NASH. (J Diabetes Invest, doi: 10.1111/jdi.12101, 2013)

KEY WORDS: Insulin, Non-alcoholic fatty liver disease

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) now ranks as one of the most frequently encountered liver diseases in both Western countries and Asia, including Japan[1,2]. The clinicopathological spectrum of NAFLD extends from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH). NASH is the more aggressive form of fatty liver disease, which can progress to cirrhosis and complications of cirrhosis, including hepatic failure and hepatocellular carcinoma[3]. A recently published meta-analysis concluded that NASH is associated with a higher overall and liver-related mortality as compared with SS[4]. Therefore, it is clinically important to distinguish NASH from SS.

Liver biopsy remains the gold standard for differentiating between NASH and SS, and clinical distinction between the two conditions in patients with NAFLD has been difficult. However, liver biopsy itself has significant limitations, as it is associated with pain, a relatively high risk of severe complications, sampling errors, a high cost and a high frequency of patient unwillingness to undergo this invasive test. Furthermore, with the increase in the estimated prevalence of NAFLD in the general population, it is impossible to carry out liver biopsy in

[1]Department of Endocrinology and Metabolism, [2]Division of Gastroenterology, Graduate School of Medicine, Yokohama City University, Yokohama, [3]Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, [4]Division of Hepatology, Saga Medical School, Saga, [5]Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, [6]Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, [7]Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi, [8]Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College, Asahikawa, [9]Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, [11]Hepatology Center, Saiseikai Suita Hospital, Suita, Japan; and [10]School of Biomedical Informatics, University of Texas Health Science Center at Houston, Houston, Texas, USA
*Corresponding author. Yasuo Terauchi Tel.: +81-45-787-2639 Fax: +81-45-784-3012
E-mail address: terauchi@yokohama-cu.ac.jp
Glycated hemoglobin is expressed by National Glycohemoglobin Standardization Program values
Received 30 January 2013; revised 22 March 2013; accepted 26 March 2013

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

all NAFLD patients to identify those with NASH[1,2]. Therefore, there is a need to develop simple and non-invasive tests that can allow accurate distinction between NASH and SS[5]. Although numerous non-invasive test panels consisting of combinations of clinical and routine laboratory parameters have been developed for the staging of liver disease[5,6], the NAFIC scoring system, based on the serum levels of ferritin, fasting insulin and type IV collagen 7S, has been recognized as a simple, and reasonably accurate and clinically useful tool for predicting the presence of NASH in Japanese patients with NAFLD[7].

We previously investigated the association between factors related to glucose tolerance and the severity of NASH in Japanese patients with NAFLD[8]. Although we found no correlations between the fasting plasma glucose and either the total NAFLD activity score or the stage of NASH, the fasting serum insulin level was significantly correlated with both, even after adjustments for age, sex and body mass index (BMI)[8]. This finding suggested a possible important role of hyperinsulinemia in the pathogenesis of NASH.

In the present study, therefore, we reinvestigated the clinical usefulness of the modified NAFIC scoring system, modified by changing the weightage assigned to the fasting serum insulin level based on the importance of hyperinsulinemia in the pathogenesis of NASH, in Japanese patients with NAFLD who had undergone liver biopsy.

MATERIALS AND METHODS

Patients

A total of 147 patients with liver-biopsy-confirmed NAFLD between 2004 and 2010 at Yokohama City University Hospital, Japan, were enrolled in the estimation group. Also, 355 patients with biopsy-proven NAFLD from 2002 to 2011 were enrolled from institutes affiliated with the Japan Study Group of NAFLD (JSG-NAFLD), represented by the following nine hepatology centers in Japan: Nara City Hospital, Hiroshima University, Kochi Medical School, Saga Medical School, Osaka City University, Kyoto Prefectural University of Medicine, Asahikawa Medical College, Kurume University and Saiseikai Suita Hospital in the validation group. A detailed history was obtained from every patient, and a physical examination was performed. The reported histological criterion for the diagnosis of NAFLD is the presence of macrovesicular fatty change in the hepatocytes, with displacement of the nuclei to the edges of the cells[9]. The criteria for exclusion from participation in the present study were: history of hepatic disease, including chronic hepatitis C or concurrent active hepatitis B (serum positivity for hepatitis B surface antigen), autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, hemochromatosis, α1-antitrypsin deficiency, Wilson's disease and current or past history of consumption of more than 20 g of alcohol daily. The present study was carried out with the approval of the institutional review board, and written informed consent was obtained from all patients.

Table 1 | Criteria of the original NAFIC scoring system and modified NAFIC scoring system

Clinical parameter	Definition	Point
Original NAFIC scoring system		
Insulin (μU/mL)	<10	0
	≥10	1
Ferritin (ng/mL)	<200 (female) or <300 (male)	0
	≥200 (female) or ≥300 (male)	1
Type IV collagen 7S (ng/mL)	<5	0
	≥5	2
Modified NAFIC scoring system		
Insulin (μU/mL)	<10	0
	10–15	1
	≥15	2
Ferritin (ng/mL)	<200 (female) or <300 (male)	0
	≥200 (female) or ≥300 (male)	1
Type IV collagen 7S (ng/mL)	<5	0
	≥5	2

Anthropometric and Laboratory Evaluation

The weight and height of the participants were measured with a calibrated scale after they had removed their shoes and any heavy clothing. Venous blood samples were collected after the participants had fasted overnight (12 h). Laboratory evaluations included measurements of the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), ferritin, type IV collagen 7 s and insulin in all patients; all of these parameters were measured using standard techniques. The original NAFIC score is a weighted sum of three clinical variables (serum ferritin ≥200 ng/mL [female] or ≥300 ng/mL [male], 1 point; serum fasting insulin ≥10 μU/mL, 1 point; serum type IV collagen 7 s ≥5.0 ng/mL, 2 points; Table 1)[7]. The BARD score is also a weighted sum of three clinical variables (BMI ≥28 kg/m^2, 1 point; serum AST/ALT ratio [AAR] ≥0.8, 2 points; diabetes, 1 point)[10]. Patients diagnosed as having diabetes before the start of the present study or whose glycated hemoglobin value was more than 6.5% were classified as having diabetes[11].

Histological Evaluation

All patients enrolled in the present study had undergone a percutaneous liver biopsy under ultrasound guidance. The liver biopsy specimens were stained with hematoxylin–eosin, reticulin and Masson trichrome stains, and all the specimens were examined by an experienced pathologist who was unaware of the clinical and biochemical data of the patients. All cases were classified as having steatosis or steatohepatitis on the basis of Matteoni's classification[12,13] (type 1, simple steatosis without inflammation or fibrosis; type 2, steatosis with lobular inflammation, but without fibrosis; type 3, additional presence of ballooned hepatocytes; type 4, presence of either Mallory's hyaline bodies or fibrosis). If Matteoni's classification was type 3 or 4, the patient was diagnosed as having NASH.

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

Statistical Analysis

The results are expressed as means ± standard deviation. Differences between two groups were analyzed for statistical significance by Student's *t*-test. The chi square test was used to analyze the significance of differences in the proportions among groups. To assess the accuracy of the clinical scoring system in differentiating between NASH and non-NASH, we calculated the sensitivity and specificity for each value, and then constructed receiver operating characteristic (ROC) curves by plotting the sensitivity against (1 − specificity) at each value. The diagnostic performance of the scoring systems was assessed by analysis of the ROC curves. The most commonly used index of accuracy was the area under the ROC curve (AUC), with values close to 1.0 showing high diagnostic accuracy. To evaluate the overall accuracy of the NAFIC score, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The Youden index was used to identify the optimal cut-off points. A *P*-value <0.05 was considered to show statistical significance.

RESULTS

Characteristics of the Patients in the Estimation Group

The clinical and biochemical characteristics of the patients in the estimation group are shown in Table 2. Of the 147 patients in this group, 74 (50.3%) were histologically diagnosed as having NASH (NASH group), whereas the remaining 73 (49.7%) were diagnosed as not having NASH (non-NASH group). There were no significant differences in the sex, the percentage of diabetes or AAR between the two groups, whereas age, BMI, and the serum levels of AST, ALT, ferritin, IV collagen 7S and insulin were significantly higher in the NASH group than in the non-NASH group. The breakdown of antidiabetic treatment in patients with diabetes is shown in Table S1.

Prediction of NASH by the NAFIC Score

The NAFIC scores ranging from 0 to 4, defined by the previous report[7], were calculated. The percentages of patients in the estimation group with NAFIC scores of 0, 1, 2, 3 and 4 were 27.9,

Table 2 | Clinical characteristics of the patients in the estimation group and in the validation group

	Non-NASH	NASH	*P*-value
Estimation group			
n	73	74	
Age (years)	46.4 ± 13.7	51.8 ± 14.8	0.0225
Sex (male/female)	45/28	38/36	0.2082
BMI (kg/m^2)	26.3 ± 5.0	28.9 ± 5.0	0.0028
Diabetes (yes/no)	15/58	21/53	0.2697
AST (IU/L)	39.4 ± 27.2	56.8 ± 29.2	0.0003
ALT (IU/L)	65.0 ± 45.9	91.9 ± 55.5	0.0017
AST/ALT ratio	0.67 ± 0.21	0.69 ± 0.24	0.6071
Ferritin (ng/mL)	212.3 ± 151.7	311.7 ± 276.2	0.0078
	172.0 (107.0; 254.0)	240.5 (102.3; 387.5)	
Type IV collagen 7S (ng/mL)	4.02 ± 0.87	4.98 ± 1.33	<0.0001
Insulin (μU/mL)	10.9 ± 7.1	16.3 ± 10.2	0.0002
	9.0 (7.3; 12.2)	13.5 (10.2; 19.5)	
NAFIC score	0.77 ± 0.89	2.08 ± 1.28	<0.0001
Validation group			
n	191	164	
Age (years)	48.1 ± 13.4	50.1 ± 14.4	0.1663
Sex (male/female)	131/60	84/80	0.0008
BMI (kg/m^2)	27.5 ± 3.8	29.2 ± 4.7	0.0003
Diabetes (yes/no)	63/128	88/76	0.0001
AST (IU/L)	43.5 ± 22.8	64.1 ± 39.0	<0.0001
ALT (IU/L)	81.0 ± 58.9	102.2 ± 63.2	0.0012
AST/ALT ratio	0.62 ± 0.22	0.68 ± 0.25	0.0149
Ferritin (ng/mL)	218.4 ± 229.5	237.6 ± 214.4	0.4186
	176.4 (93.2; 261.4)	193.3 (101.1; 304.4)	
Type IV collagen 7S (ng/mL)	3.82 ± 0.76	4.82 ± 1.47	<0.0001
Insulin (μU/mL)	12.9 ± 7.9	17.5 ± 10.2	<0.0001
	10.4 (7.7; 15.9)	15.5 (10.9; 20.8)	
NAFIC score	0.96 ± 0.93	1.76 ± 1.12	<0.0001

Values are expressed as means ± standard deviation, and the serum levels of ferritin and insulin are also expressed as median and interquartile range as follows; median (Q1; Q3). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; NASH, non-alcoholic steatohepatitis.

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

32.0, 20.4, 8.8 and 10.9%, respectively. The scores were significantly higher in the NASH group (2.08 ± 1.28) than in the non-NASH group (0.77 ± 0.89, $P < 0.0001$). Whereas the percentages of patients in the non-NASH group with NAFIC scores of 0, 1, 2, 3 and 4 were 45.2, 38.4, 13.7, 0 and 2.7%, respectively, the corresponding percentages in the non-NASH group were 10.8, 25.7, 27.0, 17.6 and 18.9% (Figure 1a). ROC curve analysis showed an AUC for this scoring system of 0.791; In contrast, the AUROC for the BARD scoring system was just 0.574. From the result of the Youden index derived from the ROC curve, the most appropriate cut-off value of the NAFIC score for the diagnosis of NASH was determined as 2. A 2 × 2 cross tabulation of the numbers of patients with and without NASH arranged in groups with NAFIC scores of <2 or ≥2 is shown in Table 3. At the cut-off value of the NAFIC score of 2, the sensitivity, specificity, PPV and NPV for the diagnosis of NASH were 63.5, 82.6, 79.7 and 69.3%, respectively.

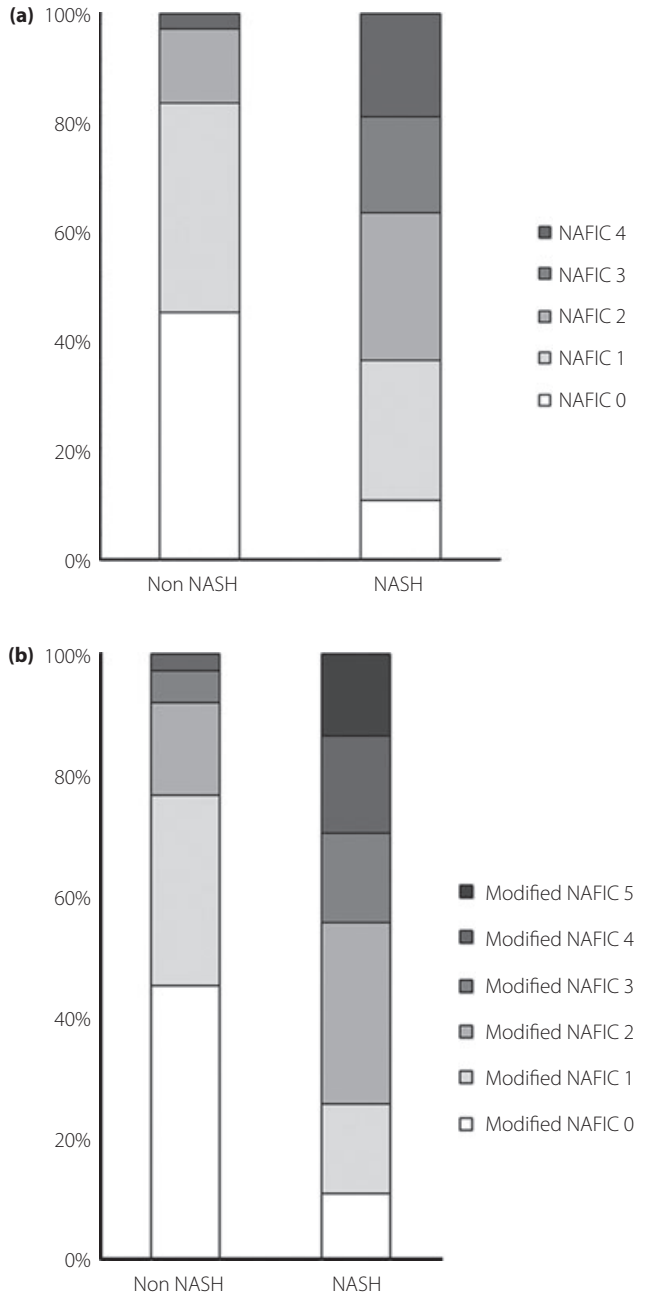

Figure 1 | Distribution of (a) NAFIC scores and (b) modified NAFIC scores in patients with and without non-alcoholic steatohepatitis (NASH) in the estimation group.

Table 3 | The 2 × 2 cross tabulations of the numbers of non-alcoholic steatohepatitis and non non-alcoholic steatohepatitis patients arranged in groups with NAFIC scores of <2 or ≥2 and modified NAFIC scores of <2 or ≥2

NAFIC score	0–1	2–4
Non-NASH	61	12
NASH	27	47
Modified NAFIC score	**0–1**	**2–5**
Non-NASH	56	17
NASH	19	55

$P < 0.0001$ with χ^2-test. NASH, non-alcoholic steatohepatitis.

Percentage of Patients with NASH in Relation to the Serum Insulin Levels

The percentages of patients with NASH according to the insulin levels in the estimation group are shown in Figure 2. The

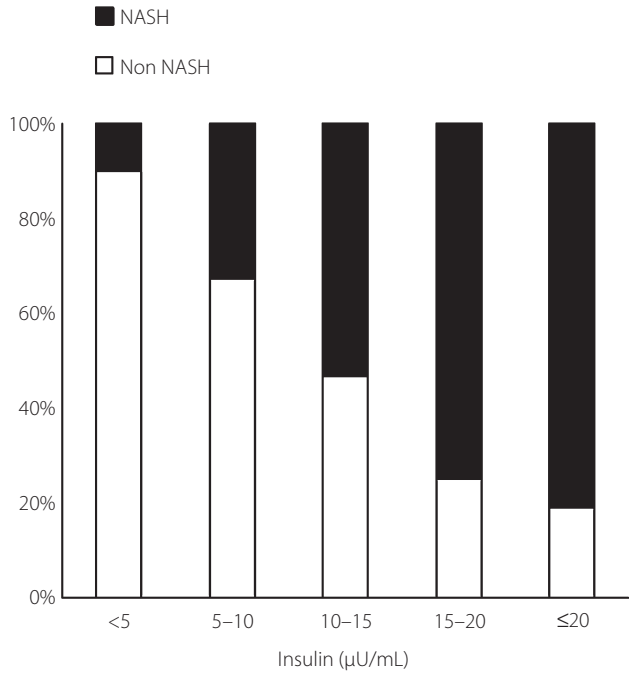

Figure 2 | Percentage of patients with non-alcoholic steatohepatitis (NASH) in relation to the insulin levels in the estimation group.

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

Table 4 | Odds ratios of non-alcoholic steatohepatitis estimated by serum insulin, ferritin, and IV collagen 7S levels

	Odds ratio*	95% Confidence interval
Insulin 10–15 μU/mL	2.81	1.26–6.27
Insulin ≥15 μU/mL	8.78	3.47–22.26
	Odds ratio†	95% Confidence interval
Ferritin ≥200 ng/mL (female) or ≥300 ng/mL (male)	2.65	1.30–5.40
IV Collagen 7S ≥5.0 ng/mL	13.14	4.34–39.80

*The odds ratio of non-alcoholic steatohepatitis (NASH) in patients with serum insulin levels of 10–15 μU/mL and ≥15 μU/mL relative to those with insulin levels of <10 μU/mL. †The odds ratio of NASH in patients with serum ferritin levels of ≥200 ng/mL (female) or ≥300 ng/mL (male) relative to those with serum ferritin levels of <200 ng/mL (female) or <300 ng/mL (male) and with serum IV collagen 7S levels of ≥5 μU/mL relative to those with serum IV collagen 7S levels of <5 μU/mL.

percentages of NASH patients with insulin levels of <5, 5–10, 10–15, 15–20 and ≥20 μU/mL were 10.0, 32.7, 53.2, 75.0 and 81.0%, respectively. This result showed a higher percentage of NASH patients not only among patients with serum insulin levels greater than 10 μU/mL, which was the traditional cut-off value for estimating the NAFIC score, but also in those with serum levels greater than 15 μU/mL. Indeed, the odds ratio of NASH in patients with serum insulin levels of 10–15 μU/mL relative to those with insulin levels of <10 μU/mL was 2.81, whereas the odds ratio of NASH in patients with serum insulin levels of ≥15 μU/mL relative to those with serum insulin levels of <10 μU/mL was 8.78 (Table 4). Taking into consideration these findings with our results of the odds ratio of NASH estimated by serum ferritin and IV collagen 7S levels (Table 4), we now advocate use of the modified NAFIC scoring system, as follows: serum ferritin ≥200 ng/mL (female) or ≥300 ng/mL (male), 1 point; serum type IV collagen 7s ≥5.0 ng/mL, 2 points; serum fasting insulin 10–15 μU/mL, 1 point and ≥15 μU/mL, 2 points (Table 1).

Prediction of NASH by the Modified NAFIC Score

The modified NAFIC scores ranging from 0 to 5 were calculated. The percentages of patients in the estimation group with modified NAFIC scores of 0, 1, 2, 3, 4 and 5 were 27.9, 23.1, 22.4, 10.2, 9.5 and 6.8%, respectively. The scores were significantly higher in the NASH group (2.51 ± 1.54) than in the non-NASH group (0.89 ± 1.03, $P < 0.0001$). Whereas the percentages of patients in the non-NASH group with modified NAFIC scores of 0, 1, 2, 3, 4 and 5 were 45.2, 31.5, 15.1, 5.5, 2.7 and 0%, respectively, the corresponding percentages in the NASH group were 10.8, 14.9, 29.7, 14.9, 16.2 and 13.5% (Figure 1b). ROC curve analysis showed an AUC for the modified NAFIC scoring system of 0.801. From the result of the Youden index derived from the ROC curve, the most appropriate cut-off value of the modified NAFIC score for the diagnosis of NASH was determined as 2. A 2 × 2 cross tabulation of the numbers of patients with and without NASH arranged in groups with modified NAFIC scores of <2 or ≥2 is shown in Table 3. At the cut-off value of the modified NAFIC score of 2, the sensitivity, specificity, PPV and NPV for the diagnosis of NASH were 74.3, 76.7, 76.4 and 74.7%, respectively. These results suggested that the sensitivity and NPV for the diagnosis of NASH increased with the use of the modified NAFIC score.

Validation Results

The clinical and biochemical characteristics of the patients in the validation group are shown in Table 2. Of the 355 patients in this group, 164 (46.2%) were histologically diagnosed as having NASH, and the remaining 191 (53.8%) as not having NASH. The percentage of females, diabetes, BMI, and serum levels of AST, ALT, AAR, IV collagen 7S and insulin were significantly higher in the NASH group than in the non-NASH group. The percentages of patients in the validation group with NAFIC scores of 0, 1, 2, 3 and 4 were 23.1, 42.0, 19.2, 10.7 and 5.1%, respectively. The scores were significantly higher in the NASH group (1.76 ± 1.12) than in the non NASH group (0.96 ± 0.93, $P < 0.0001$). Whereas the percentages of patients in the non-NASH group with NAFIC scores of 0, 1, 2, 3 and 4 were 35.1, 42.4, 15.7, 5.2 and 1.6%, respectively, the corresponding percentages in the NASH group were 9.1, 41.5, 23.2, 17.1 and 9.1%. ROC curve analysis showed an AUC for this scoring system of 0.701. At the cut-off value of the NAFIC score of 2, the sensitivity, specificity, PPV and NPV for the diagnosis of NASH were 49.4, 77.5, 65.3 and 64.1%, respectively.

When the modified NAFIC scores ranging from 0 to 5 were calculated, the percentages of patients in the validation group with modified NAFIC scores of 0, 1, 2, 3, 4 and 5 were 23.1, 23.1, 26.8, 16.1, 7.9 and 3.1%, respectively. The scores in the NASH group (2.27 ± 1.33) were significantly higher than those in the non-NASH group (1.24 ± 1.19, $P < 0.0001$). Whereas the percentages of patients in the non-NASH group with modified NAFIC scores of 0, 1, 2, 3, 4 and 5 were 35.1, 26.7, 21.5, 13.1, 3.1 and 0.5%, respectively, the corresponding percentages in the NASH group were 9.1, 18.9, 32.9, 19.5, 13.4 and 6.1%. ROC curve analysis showed an AUC for the modified NAFIC scoring system of 0.714. At the cut-off value of the modified NAFIC score of 2, the sensitivity, specificity, PPV and NPV for the diagnosis of NASH were 72.0, 61.8, 61.8 and 72.0%, respectively. These results in the validation group suggest that the modified NAFIC score had a higher sensitivity and NPV for the diagnosis of NASH, as compared with the original NAFIC score.

DISCUSSION

The prevalence of NAFLD, including NASH, has been increasing with the growing epidemics of obesity and diabetes[3]. Thus, development of a rapid and non-invasive method for the detection of NASH in NAFLD patients is of major clinical interest. In the present study, we first estimated the usefulness

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

of the NAFIC scoring system for distinguishing between NASH and SS, and reconfirmed the usefulness of this scoring system for the diagnosis of NASH. This was borne out by the higher AUC for this scoring system than that for the BARD scoring system, which is also an easily calculated composite scoring system[10]. Thus, the NAFIC score could predict the presence of NASH in Japanese NAFLD patients with sufficient accuracy and simplicity, as previously reported[7]. However, one of the problems with the NAFIC scoring system is that its sensitivity and NPV for diagnosing NASH are slightly low. Since in clinical practice, this scoring system is important as a screening tool, we sought a method to increase the sensitivity and NPV of the scoring system for the diagnosis of NASH, and reduce the number of false-negative cases. As reduced survival and higher mortality from cardiovascular and liver-related causes have been reported among NASH patients in comparison with a reference population[14], it is important not to miss the presence of NASH in patients with NAFLD. Here, we report the usefulness of the modified NAFIC scoring system, modified by changing the weightage assigned to the serum insulin levels, to obtain a reduced number of false-negative cases.

Hyperinsulinemia and increased insulin resistance could have important roles in the pathogenesis of NASH in both Western and Asian countries[15–18], and improvement of the insulin sensitivity has been reported to reverse some of the clinical manifestations and histological changes in NASH[19,20]. We have also shown the possible important role of hyperinsulinemia in the pathogenesis of NASH in humans[8]. Furthermore, we recently showed that long-term high-fat (HF) diet loading was sufficient to induce NASH in C57bl/6J mice, and that the amelioration of obesity and hyperinsulinemia, by switching from the HF diet to a standard chow diet, protected against the development of NASH in the same mice without any changes of the blood glucose levels[21]. Therefore, the clinical usefulness of the modified NAFIC scoring system, modified by changing the weightage assigned to the serum insulin level, was investigated. Our results showed that the percentage of NASH patients increased with increasing serum insulin levels (Figure 2). Furthermore, the percentage of NASH patients increased not only among patients with serum insulin levels greater than 10 μU/mL, but also in those with serum levels greater than 15 μU/mL. Therefore, we placed emphasis on serum insulin levels of more than 15 μU/mL for predicting the presence of NASH, and modified the NAFIC scoring system by assigning 1 point to a fasting serum insulin level of 10–15 μU/mL, and 2 points for that of ≥15 μU/mL. This modification was realistic judging from the comparison of the odds ratio of the serum insulin level relative to that of the serum ferritin and serum IV collagen 7S levels. Thus, we now advocate the use of the modified NAFIC scoring system for the diagnosis of NASH, as follows: serum ferritin ≥200 ng/mL (female) or ≥300 ng/mL (male), 1 point; serum type IV collagen 7s ≥5.0 ng/mL, 2 points; serum fasting insulin 10–15 μU/mL, 1 point and ≥15 μU/mL, 2 points.

As expected, the modified score showed a higher sensitivity and NPV for the diagnosis of NASH in both the estimation group and the validation group. However, one problem with the modified NAFIC score was that the AUC of the modified NAFIC score was only slightly higher than that of the original NAFIC score, associated with the slight decrease of the specificity of the modified NAFIC score as compared with that of the original NAFIC score. We speculated that the slight decrease of specificity was the reason for the slight increase of the number of false-positive cases associated with the allocation of 2 points for fasting insulin ≥15 μU/mL. However, we consider that the modified score would be very useful as a diagnostic screening tool for NASH, based on its high diagnostic sensitivity.

One of the limitations of the present study was its retrospective design. Another was that the patients were recruited from hepatology centers in Japan with a particular interest in the study of NAFLD, which could have introduced some referral bias. Patient selection bias could also have existed, because liver biopsy might have been considered only for NAFLD patients who were likely to have NASH. The findings might thus not entirely represent those of NAFLD patients in the general population. Because of these limitations, the results would need to be validated in independent populations by other investigators. Furthermore, it is not yet clear whether this scoring system is also applicable to non-Japanese patients. Although it has been suggested that the NAFIC scoring system is a simple and reasonably accurate tool for the prediction of NASH in Japanese NAFLD patients as compared with other scoring systems[7], there are also some problems with it that need to be addressed. One pertains to the items included for calculation of the NAFIC score. In Japan, serum type IV collagen 7s is now widely used for assessing the extent of hepatic fibrosis in patients with chronic liver disease, including NASH[22,23], because the test is covered by public health insurance, but there is little evidence of assessment of this parameter in NAFLD patients in Western countries. Another limitation is the differences in the serum insulin levels between Japanese and Western patients related to ethnic differences in the relevant pathophysiological mechanisms, such as the insulin secretion capacity and insulin resistance. For example, the HAIR criteria (presence of hypertension, serum ALT >40 IU/L and insulin resistance index [log fasting insulin (μU/mL) + log fasting plasma glucose (mg/dL) >5], with the presence of any two being considered to be indicative NASH) have been used as a highly sensitive and specific tool for the diagnosis of NASH in Australia[24]; however, none of the patients in our cohort had an insulin resistance index of >5 (data not shown). Also, it is suggested that there were differences in genetic and environmental factors related to susceptibility to hepatic steatosis between Japanese and Western patients related to ethnic differences[25]. Therefore, one of the challenges for the future is to estimate the usefulness of this modified scoring system for the prediction of NASH in other ethnicities.

In conclusion, we evaluated the usefulness of the modified NAFIC score, modified by changing the weightage assigned to

 © 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

the serum insulin levels, in Japanese NAFLD patients who had had liver biopsies. The modified NAFIC scoring system showed a higher sensitivity and NPV for the diagnosis of NASH than the conventional scoring system in both the estimation group and the validation group. These results show that the modified NAFIC scoring system could be a clinically useful diagnostic screening tool for NASH.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (B) 21390282 and (B) 24390235 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, a Medical Award from the Japan Medical Association, a Grant-in-Aid from the Uehara Memorial Foundation, a Grant-in-Aid from the Daiichi-Sankyo Foundation of Life Science, a Grant-in-Aid from the Naito Foundation (to YT), and a Grant-in-aid for Front Runner of Future Diabetes Research (to AN). The authors have no conflicts of interests to declare.

REFERENCES

1. Bellentani S, Scaglioni F, Marino M, *et al.* Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; 28: 155–161.
2. Eguchi Y, Hyogo H, Ono M, *et al.* Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2012; 47: 586–595.
3. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; 51: 1820–1832.
4. Musso G, Gambino R, Cassader M, *et al.* Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; 43: 617–649.
5. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007; 46: 582–589.
6. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. *Hepatology* 2009; 49: 306–317.
7. Sumida Y, Yoneda M, Hyogo H, *et al.* A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; 46: 257–268.
8. Nakamura A, Yoneda M, Fujita K, *et al.* Impact of glucose tolerance on the severity of non-alcoholic steatohepatitis. *J Diabetes Invest* 2011; 2: 483–489.
9. Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; 123: 1705–1725.
10. Harrison SA, Oliver D, Arnold HL, *et al.* Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; 57: 1441–1447.
11. The Committee of Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Invest* 2010; 1: 212–228.
12. Matteoni CA, Younossi ZM, Gramlich T, *et al.* Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; 116: 1413–1419.
13. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; 37: 1202–1219.
14. Ekstedt M, Franzén LE, Mathiesen UL, *et al.* Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; 44: 865–873.
15. Chitturi S, Abeygunasekera S, Farrell GC, *et al.* NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; 35: 373–379.
16. Pagano G, Pacini G, Musso G, *et al.* Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 2002; 35: 367–372.
17. Marchesini G, Bugianesi E, Forlani G, *et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; 37: 917–923.
18. Ono M, Saibara T. Clinical features of nonalcoholic steatohepatitis in Japan: evidence from the literature. *J Gastroenterol* 2006; 41: 725–732.
19. Mathurin P, Hollebecque A, Arnalsteen L, *et al.* Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. *Gastroenterology* 2009; 137: 532–540.
20. Musso G, Cassader M, Rosina F, *et al.* Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* 2012; 55: 885–904.
21. Nakamura A, Tajima K, Zolzaya K, *et al.* Protection from non-alcoholic steatohepatitis and liver tumourigenesis in high fat-fed insulin receptor substrate-1-knockout mice despite insulin resistance. *Diabetologia* 2012; 55: 3382–3391.
22. Yoneda M, Mawatari H, Fujita K, *et al.* Type IV collagen 7s domain is an independent clinical marker of the severity of fibrosis in patients with nonalcoholic steatohepatitis before the cirrhotic stage. *J Gastroenterol* 2007; 42: 375–381.
23. Shimada M, Kawahara H, Ozaki K, *et al.* Usefulness of a combined evaluation of the serum adiponectin level,

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd

HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. *Am J Gastroenterol* 2007; 102: 1931–1938.
24. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; 121: 91–100.
25. Fujii H, Enomoto M, Fukushima W, *et al.* Applicability of BARD score to Japanese patients with NAFLD. *Gut* 2009; 58: 1566–1567.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | The breakdown of antidiabetic treatment.

© 2013 Asian Association for the Study of Diabetes and Wiley Publishing Asia Pty Ltd