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• CLINICAL RESEARCH •

Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease

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Abstract

AIM: Nonalcoholic steatohepatitis (NASH) is a severe form of nonalcoholic fatty liver disease (NAFLD), and progresses to the end stage of liver disease. Biochemical markers of liver fibrosis are strongly associated with the degree of histological liver fibrosis in patients with chronic liver disease. However, data are few on the usefulness of markers in NAFLD patients. The aim of this study was to identify better noninvasive predictors of hepatic fibrosis, with special focus on markers of liver fibrosis, type VI collagen 7S domain and hyaluronic acid.

METHODS: One hundred and twelve patients with histologically proven NAFLD were studied.

RESULTS: The histological stage of NAFLD correlated with several clinical and biochemical variables, the extent of hepatic fibrosis and the markers of liver fibrosis were relatively strong associated. The best cutoff values to detect NASH were assessed by using receiver operating characteristic analysis: type VI collagen 7S domain $\geqslant$5.0 ng/mL, hyaluronic acid $\geqslant$43 ng/mL. Both markers had a high positive predictive value: type VI collagen 7S domain, 86% and hyaluronic acid, 92%. Diagnostic accuracies of these markers were evaluated to detect severe fibrosis. Both markers showed high negative predictive values: type VI collagen 7S domain ($\geqslant$5.0 ng/mL), 84% and hyaluronic acid ($\geqslant$50 ng/mL), 78%, and were significantly and independently associated with the presence of NASH or severe fibrosis by logistic regression analysis.

CONCLUSION: Both markers of liver fibrosis are useful in discriminating NASH from fatty liver alone or patients with severe fibrosis from patients with non-severe fibrosis.

Key words: Liver fibrosis; Nonalcoholic fatty liver disease; Collagen type IV; Hyaluronic acid

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INTRODUCTION

The prevalence of overweight persons is increasing in developed and developing countries[1,2]. Fatty metamorphosis in the liver is common in these obese subjects and nonalcoholic fatty liver disease (NAFLD) has become widespread with the increasing prevalence of obesity[3-6]. In Japan, the prevalence of NAFLD has been recently increasing[7], which may be attributable to lifestyle change including physical inactivity and an increase in daily fat consumption.

Most patients with NAFLD have a benign clinical course, but some patients progress to advanced liver disease, liver cirrhosis or even hepatocellular carcinoma[3,8-10]. Nonalcoholic steatohepatitis (NASH) is a severe form of NAFLD, and is strongly associated with female gender, older age, obesity and type 2 diabetes mellitus[3,4,11-15]. The definitive diagnosis of NASH requires liver biopsy, but it is an invasive procedure that may cause undesirable complications. Clinical indications for liver biopsy should be limited in patients with NAFLD who are likely to have NASH or significant fibrosis. Liver fibrotic change is an important pathological finding to stage NAFLD or to determine the prognosis of NAFLD[16,17]. NAFLD patients with severe fibrosis have a poor prognosis[18].

Many clinical variables have been proposed as predictors of severe fibrosis in patients with NAFLD: old age, type 2 diabetes mellitus, obesity, serum transaminase levels, peripheral platelet counts, *etc*.[19-22]. Biochemical markers of liver fibrosis are strongly associated with the degree of histological fibrosis in patients with chronic viral hepatitis[23-25]. However, data are few on the usefulness of markers of liver fibrosis to evaluate the degree of liver fibrosis among patients with NAFLD or for distinguishing NASH from benign NAFLD[22].

The aim of the present study was to identify better noninvasive predictors of liver fibrosis, with special focus on the markers of liver fibrosis.

MATERIALS AND METHODS

Patients

From January 1993 to August 2003, NAFLD was diagnosed in 112 patients who visited our hospital or affiliated hospitals. The patients included 36 men and 76 women aged between 19 and 78 years, 87% were 40 years of age or older. All denied regular alcohol use and definitions of nonalcoholic use were 30 g/d or less for men and 20 g/d or less for women. No patient had conditions related to secondary NAFLD: regular use of drugs known to produce steatosis (corticosteroids, tamoxifen, amiodarone), previous gastrointestinal surgery, total parenteral nutrition, *etc*.[3]. Other liver diseases were appropriately excluded:

chronic viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, metabolic liver diseases. For all patients, the body mass index (BMI; kg/m^2) was calculated.

Methods

NAFLD was diagnosed histologically. All liver biopsy samples were examined by two of us (HS, KK) who were unaware of the clinical and biochemical conditions of each patient. Specimens were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin and Azan-Mallory. Fatty change was defined as 10% or more fatty metamorphosis in the hepatocytes. Histological criteria of NASH were based on steatosis ($\geqslant$10% of hepatocytes affected) and two of the following three: lobular inflammation, ballooning degeneration, and pericellular fibrosis. Different histological parameters were evaluated including steatosis, pericellular fibrosis, portal/septal fibrosis, portal inflammation, periportal necrosis, lobular inflammation, ballooning, Mallory body. Pericellular fibrosis was graded on a scale of absent, mild, moderate, severe fibrosis. Portal/septal fibrosis was graded according to the histological criteria for chronic viral hepatitis: 0, none; 1, portal expanding or periportal fibrosis; 2, partial bridging fibrosis; 3, diffuse bridging fibrosis with lobular remodeling, 4, cirrhosis. Histological staging was done according to Brunt *et al*[16,17] with slight modifications: stage 0, no fibrosis (fatty liver alone or fatty liver plus lobular inflammation); stage 1, perisuinasoidal fibrosis without portal fibrosis; stage 2, perisuinasoidal fibrosis plus portal fibrosis; stage 3, presence of bridging fibrosis; stage 4, cirrhosis. Written informed consent was obtained from all patients, and the study was conducted in conformance with the Helsinki Declaration.

Laboratory tests included peripheral blood cell counts, and measurements of albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), fasting glucose, fasting insulin, total cholesterol and triglyceride levels, glycosylated hemoglobin A1c (HbA1c), free fatty acid (FFA), ferritin, immunoglobulin G (IgG), IgA, IgM, type VI collagen 7S domain, hyaluronic acid , hepatitis B surface antigen, antibody to hepatitis C virus. Insulin resistance was calculated by homeostasis model assessment (HOMA-R: fasting glucose (mg/dL) ×fasting insulin (μU/mL) ÷405). Standard liver tests were performed on a multichannel autoanalyzer. Hepatitis B surface antigen was measured by a commercially available enzyme immunoassay (Enzygnost, Berling, Germany). Antibody to hepatitis C virus was tested by a second generation enzyme immunoassay (Ortho Diagnostics, Raritan, NJ).

Type VI collagen 7S domain was measured with a type VI collagen 7S domain RIA kit (Nippon CPC Co., Tokyo, Japan) and serum hyaluronic acid was tested by sandwich binding protein assay by using a commercially available kit (Chugai Co., Tokyo, Japan).

Statistical analysis

The continuous variables were compared using the 2-tailed Student's *t* test. The correlation between these variables was analyzed by Pearson's correlation coefficient or Spearman's correlation coefficient. Categorical variables were compared with Fisher's exact test. The diagnostic values of the clinical variables were assessed by calculating the areas under the receiver operating characteristic (ROC) curves, which were used to assess the best cutoff points to identify the presence of NASH or severe fibrosis. The diagnostic accuracy was calculated by sensitivity, specificity, and positive and negative predictive values (PPV and NPV). Multivariate analysis was tested using logistic regression analysis. The SPSS statistical software (Ver. 11.0) was used for statistical analysis. A P value less than 0.05 was considered statistically significant.

RESULTS

Of the 112 patients with NAFLD, 35 (31.3%) were classified as stage 0, 12 (10.7%) as stage 1, 17 (15.2%) as stage 2, 39 (34.8%) as stage 3 and 9 (8.0%) as stage 4. Seventy patients were diagnosed as NASH, and all of them had liver fibrotic change at stage 1 or at a more severe stage. The remaining 42 patients were diagnosed as having nonalcoholic fatty liver. When the 112 patients were divided into two groups by the severity of fibrosis (mild: stage 0-2 and severe: stage 3 and 4), women were more frequently seen in the severe group ($P = 0.04$), (Table 1).

Correlations were examined between the degree of fibrosis or the stage of NAFLD and the following clinical variables: age, BMI, blood pressure, peripheral platelet counts, serum levels of albumin, total bilirubin, fasting blood glucose, AST, ALT, GGT, ALP, total cholesterol, triglyceride, FFA, IgG, IgA, IgM, type VI collagen 7S domain, hyaluronic acid, ferritin, HbA1c, HOMA-R.

The degree of all three histological criteria of fibrosis and the following quantitative variables were significantly correlated:

Table 1 Correlation between degree of liver fibrosis and clinical and laboratory data (n = 112)

Clinical/laboratory data	Degree of liver fibrosis		
	Portal/Septal	Pericellular	Fibrosis stage
Age (yr)	0.265 (P = 0.005)	0.172 (P = 0.07)	0.302 (P = 0.001)
BMI	0.220 (P = 0.020)	0.236 (P = 0.012)	0.238 (P = 0.011)
Platelet	-0.331 (P<0.001)	-0.133 (P = 0.2)	-0.298 (P = 0.001)
Albumin	-0.295 (P = 0.002)	-0.078 (P = 0.4)	-0.291 (P = 0.002)
AST	0.306 (P = 0.001)	0.384 (P P<0.001)	0.340 (P<0.001)
AST/ALT	0.458 (P<0.001)	0.420 (P P<0.001)	0.438 (P<0.001)
IgG (n=100)	0.261 (P =0.009)	0.141 (P = 0.1)	0.208 (P = 0.038)
IgA (n=94)	0.281 (P<0.006)	0.285 (P = 0.005)	0.291 (P = 0.004)
IgM (n=100)	0.256 (P =0.010)	0.188 (P = 0.06)	0.266 (P = 0.008)
Type IV Ccollagen 7S	0.580 (P<0.001)	0.516 (P<0.001)	0.607 (P<0.001)
Hyaluronic acid	0.543 (P<0.001)	0.387 (P<0.001)	0.553 (P<0.001)
Ferritin (n = 98)	0.205 (P = 0.043)	0.120 (P = 0.2)	0.252 (P = 0.012)
HbA1c (n = 96)	0.309 (P = 0.002)	0.211 (P = 0.039)	0.315 (P = 0.002)
FFA (n = 80)	0.238 (P = 0.033)	0.271 (P = 0.015)	0.276 (P = 0.013)
HOMA-R (n = 82)	0.224 (P = 0.043)	0.164 (P = 0.1)	0.222 (P = 0.045)

age, BMI, platelet counts, albumin, AST, AST/ALT ratio, IgA, type VI collagen 7S domain, hyaluronic acid, HbA1c, FFA. Serum IgG and IgM concentrations, ferritin and HOMA-R were significantly correlated with either the degree of portal/septal fibrosis or fibrosis stage, but were not significantly correlated with the degree of pericellular fibrosis. Among these variables, the markers of liver fibrosis, type VI collagen 7S domain and hyaluronic acid, showed relatively high correlation coefficients. ALT, GGT, ALP, total serum cholesterol, triglyceride, peripheral hemoglobin concentration, systolic blood pressure, diastolic blood pressure, and fasting blood glucose level were not significantly correlated with any degree of the three histological criteria (Table 1).

When the patients having fatty liver alone were compared with the patients having NASH, the BMI, ALT, GGT, IgG, IgA, fasting glucose, ferritin, and HOMA-R were not significantly different, but several clinical variables were significantly different between the two groups, particularly the differences in AST level, AST/ALT ratio, and the markers of liver fibrosis were highly significant (Table 2).

Table 2 Comparison between patients with fatty liver and with NASH (mean±SD)

	Fatty liver alone	NASH	*P* value
Number	42	70	
Age (yr)	45.2±13.6	53.8±14.4	0.002
Gender (female)	22 (52.4%)	54 (77.1%)	0.012
BMI	28.2±4.9	29.6±5.2	NS
Diabetes mellitus (%)	8 (19.0%)	26 (37.1%)	0.044
Platelet count (×10⁴/μL)	23.8±4.8	21.1±6.8	0.036
Prothrombin time (%)	98.5±11.1	91.6±13.6	0.009
Albumin (g/dL)	4.4±0.3	4.3±0.4	0.017
AST (IU/L)	56.7±30.8	87.5±48.1	<0.001
ALT (IU/L)	110.1±68.2	117.1±65.1	NS
AST/ALT ratio	0.56±0.2	0.82±0.4	<0.001
GGT (IU/L)	124.9±96.6	106.2±129.3	NS
IgG (mg/dL)	1 377.1±366.7	1 526.9±423.3	NS
IgA (mg/dL)	313.1±191.3	356.9±134.1	NS
IgM (mg/dL)	124.2±47.9	162.8±82.4	0.004
Type IV collagen 7S (ng/mL)	4.1±0.9	6.0±2.0	<0.001
Hyaluronic acid (ng/mL)	29.0±20.0	103.4±116.8	<0.001
Fasting glucose (mg/dL)	107.8±34.4	120.2±51.8	NS
HbA1c (%)	5.4±1.0	6.2±1.7	0.005
Ferritin (ng/mL)	151.7±107.0	351.5±455.7	0.001
FFA (mEq/L)	0.6±0.2	0.7±0.3	0.022
HOMA-R	5.1±6.3	6.0±6.6	NS

When the patients having stage 0-2 fibrosis were compared with the patients having stage 3 and 4 fibrosis, the BMI, ALT level, any subclass of immunoglobulins, fasting glucose and HOMA-R were not significantly different. The frequency of diabetes mellitus was not significantly different between these groups, but the difference was significant between patients having fatty liver alone and patients having NASH (Table 3).

Table 3 Comparison between NAFLD patients with stage 0-2 fibrosis and those with stage 3 and 4 fibrosis (mean±SD)

	Stages 0-2 fibrosis	Stages 3,4 fibrosis	*P* value
Number	64	48	
Age (yr)	47.8±14.5	54.3±14.0	0.02
Gender (female)	38 (59.4%)	38 (79.2%)	0.04
BMI	28.3±4.8	30.1±5.4	NS
Diabetes mellitus (%)	15 (23.4%)	19 (39.6%)	NS
Platelet count (×10⁴/μL)	23.8±5.1	20.3±7.1	0.004
Prothrombin time (%)	96.9±11.1	90.1±14.7	0.018
Albumin (g/dL)	4.4±0.3	4.3±0.4	0.03
AST (IU/L)	67.0±37.5	87.9±51.2	0.02
ALT (IU/L)	113.1±65.2	116.3±67.9	NS
AST/ALT ratio	0.66±0.4	0.82±0.3	0.015
GGT (IU/L)	133.5±141.6	85.7±66.6	0.019
IgG (mg/dL)	1 408.1±367.0	1 555.5±446.4	NS
IgA (mg/dL)	316.3±172.3	371.0±133.5	NS
IgM (mg/dL)	137.2±79.1	163.8±64.9	NS
Type IV collagen 7S (ng/mL)	4.6±1.5	6.3±2.0	<0.001
Hyaluronic acid (ng/mL)	50.4±74.3	108.8±118.7	0.004
Fasting glucose (mg/dL)	115.1±49.4	116.6±43.1	NS
HbA1c (%)	5.6±1.2	6.4±1.9	0.01
Ferritin (ng/mL)	253.8±52.6	320.1±373.0	NS
FFA (mEq/L)	0.6±0.3	0.8±0.3	0.007
HOMA-R	5.7±5.8	5.8±4.6	NS

Relatively high correlation coefficients were seen between the degree of hepatic fibrosis and the markers of fibrosis. We therefore examined the diagnostic accuracy of the markers of fibrosis for NASH and severe fibrosis. To detect NASH, the area under the curves for type VI collagen 7S domain and hyaluronic acid were 0.828 and 0.797, respectively, by ROC analysis (Table 4). The best cutoff values to detect NASH were also assessed using the ROC analysis, and sensitivity, specificity, PPV, and NPV were calculated. Both markers had high PPV: type VI collagen 7S domain, 86.0% and hyaluronic acid, 92.0%. A combination of these markers was also useful to discriminate NASH from fatty liver alone. If both markers combined were positive, the PPV was high (97.1%) (Table 4). To detect severe

Table 4 Diagnostic accuracy of the markers of liver fibrosis for NASH

	ROC analysis		Cutoff value	Accuracy			
	AUC (95% CI)	*P* value		Se (%)	Sp (%)	PPV (%)	NPV (%)
(a) Type IV collagen 7S	0.828 (0.754-0.902)	0.000	≥5.0 ng/mL	70.0	81.0	86.0	61.8
(b) Hyaluronic acid	0.797 (0.716-0.879)	0.000	≥43 ng/mL	65.7	90.5	92.0	61.3
Combination of Markers			a≥5.0 ng/mL or b≥43 ng/mL	87.1	73.8	84.7	77.5
			a≥5.0 ng/mL and b≥43 ng/mL	48.6	97.6	97.1	53.2

Abbreviations: ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

Table 5 Diagnostic accuracy of the markers of liver fibrosis for severe fibrosis

	ROC analysis		Cutoff value	Accuracy			
	AUC (95% CI)	*P* value		Se (%)	Sp (%)	PPV (%)	NPV (%)
(a) Type IV collagen 7S	0.817 (0.736-0.897)	0.000	≥5.0 ng/mL	81.3	71.4	68.4	83.6
(b) Hyaluronic acid	0.797 (0.652-0.845)	0.000	≥50 ng/mL	68.8	82.8	75.0	77.9
Combination of Markers			a≥5.0 ng/mL or b≥50 ng/mL	95.8	62.5	65.7	95.2
			a≥5.0 ng/mL and b≥50 ng/mL	54.2	92.2	83.9	72.8

Abbreviations: ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

fibrosis, the type VI collagen 7S domain cutoff value for the best compromise sensitivity-specificity was 5.0 ng/mL. This value was the same as in detecting NASH. Type VI collagen 7S domain and hyaluronic acid showed relatively high NPVs in detecting severe fibrosis. If both markers were concomitantly negative, severe fibrosis was unlikely to be present, the NPV of either type VI collagen 7S domain ≥5.0 ng/mL or hyaluronic acid ≥50 ng/mL in detecting severe fibrosis was high (95.2%) (Table 5).

Although several clinical variables were associated with the presence of NASH or severe fibrosis by univariate analysis (Tables 2, 3), these factors were too many to be evaluated in logistic regression analysis. We therefore selected eight variables (age, gender, BMI, peripheral platelet counts, AST/ALT ratio, type VI collagen 7S domain, hyaluronic acid, presence of diabetes) for logistic regression analysis. The cutoff values for continuous variables (age, peripheral platelet counts, AST/ALT ratio, type VI collagen 7S domain, hyaluronic acid) were determined based on ROC analysis. The cutoff value for BMI was adopted as ≥30 kg/m^2. The logistic regression analysis indicated that three (AST/ALT ratio ≥0.55, type VI collagen 7S domain ≥5.0 ng/mL, hyaluronic acid ≥43 ng/mL) out of the eight variables were significantly and independently associated with NASH. Odds ratios for the three independent predictors of NASH were AST/ALT ratio 10.1, 95% confidence interval (CI) 2.0-52.3, $P=0.006$; type VI collagen 7S domain 6.9, 95% CI 2.1-23, $P=0.002$; hyaluronic acid 12.7, 95% CI 3.0-54, $P=0.001$. The logistic regression analysis also indicated that two (type VI collagen 7S domain ≥50 ng/mL, hyaluronic acid ≥50 ng/mL) of the eight variables were significantly and independently associated with severe fibrosis. Odds ratios of the two markers of liver fibrosis were type VI collagen 7S domain 10.4, 95% CI 3.2-34, $P=0.000$; hyaluronic acid 5.6, 95% CI 1.7-19, $P=0.005$.

DISCUSSION

In this study, the markers of liver fibrosis (type VI collagen 7S domain and hyaluronic acid) correlated well with the degree of liver fibrosis among patients with NAFLD compared with several clinical variables (age, serum AST level, AST/ALT ratio, BMI, presence of diabetes mellitus, peripheral platelet count, *etc*.) previously reported to be useful to diagnose NASH and severe fibrosis. The performances of these markers in predicting NASH and severe fibrosis were examined by either univariate analysis, ROC analysis, or multiple logistic regression analysis. The likelihood of having NASH could be 97% if patients showed a positive level of serum type VI collagen 7S domain and hyaluronic acid. Also, 96% of the NASH patients had a positive level of either type VI collagen 7S domain or hyaluronic acid.

Most NASH patients were asymptomatic, and had abnormally increased ALT levels[4]. The diagnosis of NASH should be suspected in people who have an asymptomatic increase in liver enzymes, and increased liver echogenicity by ultrasound examination. In Japan, health check-ups usually include ultrasound examination. Persons with ultrasonographic evidence of fatty liver and an increased ALT level unrelated to chronic hepatitis virus infection are frequently found among health check-up participants, and then are referred to hepatologists. Some of them may have NASH and may include cases of severe fibrosis, and others may have fatty liver alone or fat plus inflammation despite of increased ALT levels. The discrimination between NASH and fatty liver alone is difficult without liver biopsy. However, liver biopsy is not always justified in those cases. Clinicians need a sensible reason to obtain agreement for liver biopsy from reluctant patients.

The degree of liver fibrosis is important to evaluate the prognosis in patients with NAFLD. Inter- and intra-observer discrepancies in assessing the extent of liver fibrosis were small compared with assessing the extent of inflammation[26]. Prediction of liver fibrosis is challenging, and many investigations have been made of patients with chronic hepatitis C[23-25,27-29]. However, such investigations have been limited to patients with NAFLD[19-22]. Angulo *et al*[19] reported that age, presence of diabetes, AST/ALT ratio were the predictors of severe fibrosis in patients with NASH. However, these clinical predictors were not useful to discriminate NASH from fatty liver alone or patients with severe fibrosis from those with non-severe fibrosis in this study.

The usefulness of markers of liver fibrosis (type VI collagen 7S domain or hyaluronic acid) has been assessed for patients with chronic viral hepatitis[23-25]. However, studies are few on the clinical usefulness of markers of liver fibrosis to predict severe fibrosis in patients with NAFLD[22]. The serum level of type VI collagen 7S domain type, but not hyaluronic acid, was reported to be significantly different between NASH patients with severe fibrosis and those with mild to moderate fibrosis. Platelet count and AST/ALT ratio might be more useful predictors of the presence of severe fibrosis compared with markers of liver fibrosis[22]. However, the fibrotic markers were more useful predictors of NASH or severe fibrosis in this study.

Our study showed discrepancies with other previous studies[19-22] in the assessment of useful predictors of NASH or severe fibrosis in patients with NAFLD. The discrepancies may be attributed to a difference in percentage of patients with severe fibrosis. The percentage of patients with liver cirrhosis in this study was 8% (9/112), but in previous studies the percentages of patients with liver cirrhosis among NASH patients were 17[19] and 21%[22]. Only 10% of patients in this study showed AST/ALT ratio>1, which was a useful predictor in previous studies[19]. We experienced patients with cryptogenic liver cirrhosis who were suspected of having sequelae of NASH, but they were excluded from this study because of inadequate fatty change (<10%).

In conclusion, the biochemical markers of liver fibrosis, type 4 collagen 7S domain and hyaluronic acid, are useful predictors to evaluate the degree of hepatic fibrosis in patients with NAFLD. These markers are also useful to discriminate NASH from fatty liver alone or patients with severe fibrosis from patients with non-severe fibrosis.

REFERENCES

1 **Kuczmarski RJ**, Carroll MD, Flegal KM, Troiano RP. Varying body mass index cutoff points to describe overweight prevalence among U.S. adults: NHANES III (1988 to 1994). *Obes Res* 1997; **5**: 542-548
2 **Kopelman PG**. Obesity as a medical problem. *Nature* 2000; **404**: 635-643
3 **Falck-Ytter Y**, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 2001; **21**: 17-26
4 **Day CP**. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? *Gut* 2002; **50**: 585-588
5 **Shen L**, Fan JG, Shao Y, Zeng MD, Wang JR, Luo GH, Li JQ, Chen SY. Prevalence of nonalcoholic fatty liver among administrative officers in Shanghai: an epidemiological survey. *World J Gastroenterol* 2003; **9**: 1106-1110
6 **Chitturi S**, Farrell GC, George J. Non-alcoholic steatohepatitis in the Asia-Pacific region: future shock? *J Gastroenterol Hepatol* 2004; **19**: 368-374
7 **Kojima S**, Watanabe N, Numata M, Ogawa T, Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* 2003; **38**: 954-961
8 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140
9 **Hui JM**, Kench JG, Chitturi S, Sud A, Farrell GC, Byth K, Hall P, Khan M, George J. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 2003; **38**: 420-427
10 **Sakugawa H**, Nakasone H, Nakayoshi T, Kawakami Y, Yamashiro T, Maeshiro T, Kobashigawa K, Kinjo F, Saito A. Clinical characteristics of patients with cryptogenic liver cirrhosis in Okinawa, Japan. *Hepatogastroenterology* 2003; **50**: 2005-2008
11 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438
12 **Sheth SG**, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. *Ann Intern Med* 1997;**126**: 137-145
13 **James OF**, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol* 1998; **29**: 495-501
14 **Bacon BR**, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology* 1994; **107**: 1103-1109
15 **Harrison SA**, Kadakia S, Lang KA, Schenker S. Nonalcoholic steatohepatitis: what we know in the new millennium. *Am J Gastroenterol* 2002; **97**: 2714-2724
16 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474
17 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
18 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419
19 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362
20 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123
21 **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
22 **Shimada M**, Hashimoto E, Kaneda H, Noguchi S, Hayashi N. Nonalcoholic steatohepatitis: risk factors for liver fibrosis. *Hepatol Res* 2002; **24**: 429-438
23 **Guechot J**, Poupon RE, Giral P, Balkau B, Giboudeau J, Poupon R. Relationship between procollagen III aminoterminal propeptide and hyaluronan serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis C. *J Hepatol* 1994; **20**: 388-393
24 **Murawaki Y**, Ikuta Y, Koda M, Kawasaki H. Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. *Hepatology* 1994; **20**: 780-787
25 **Takamatsu S,** Nakabayashi H, Okamoto Y, Nakano H. Noninvasive determination of liver collagen content in chronic hepatitis. Multivariate regression modeling with blood chemical parameters as variables. *J Gastroenterol* 1997; **32**: 355-360
26 **Younossi ZM**, Gramlich T, Liu YC, Matteoni C, Petrelli M, Goldblum J, Rybicki L, McCullough AJ. Nonalcoholic fatty liver disease: assessment of variability in pathologic interpretations. *Mod Pathol* 1998; **11**: 560-565
27 **Imbert-Bismut F**, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069-1075
28 **Forns X**, Ampurdaners S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, Bruguera M, Shanchez-Tapias JM, Rodes J. Identification of chronic hepatitis C without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992
29 **Wai CT**, Greenson JK, Fontana RJ, Kalbleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526

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