INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Predictive Value of the Fibrosis Scores in Patients with Chronic Hepatitis C Associated with Liver Fibrosis and Metabolic Syndrome

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Abstract

Objective We evaluated patients with chronic hepatitis C (CHC) and compared the clinical and pathological features of steatosis and metabolic syndrome to identify the risk factors for CHC with severe fibrosis.

Methods One hundred seventy-one patients with biopsy-confirmed CHC were included in the study: 90 males and 81 females, age 56.2±12.8 years; 46 with obesity (BMI≥25 kg/m^2); 51 with hypertension; 36 with type 2 diabetes mellitus; and 20 with hypertriglyceridemia.

Results Steatosis was detected in 79 patients (46%); 92 patients (54%) showed no steatosis. Seventy-four patients (43%) showed mild fibrosis and 97 patients (56%) showed severe fibrosis. The variables that were significantly associated with steatosis were obesity [odds ratio 2.160 (1.010-4.727), p=0.046] and type 2 diabetes [odds ratio 3.667 (1.559-8.430), p=0.027]. The variables that were significantly associated with severe fibrosis were older age [odds ratio 2.675 (1.309-5.464), p=0.007], obesity [odds ratio 2.156 (1.006-4.619), p=0.048] and type 2 diabetes [odds ratio 8.739 (2.845-26.846), p=0.0002]. Nagasaki (N) score (the total number of specific risk factors, namely an older age, obesity, and type 2 diabetes) was higher in the severe fibrosis group than in the mild fibrosis group (mild fibrosis: severe fibrosis=1.48±1.14 vs.2.66±94, p<0.001).

Conclusion Metabolic syndrome factors, including obesity and diabetes, play a critical role in the pathogenesis of fibrosis in CHC. The N score was therefore found to be a significant predictor of severe fibrosis in CHC.

Key words: chronic hepatitis C, steatosis, liver fibrosis, type 2 diabetes mellitus, obesity

(Intern Med 50: 1137-1141, 2011)
(DOI: 10.2169/internalmedicine.50.4447)

Introduction

Hepatitis C virus (HCV) infection is one of the most common causes of chronic liver disease, affecting 2 million persons in Japan. It can lead to end-stage liver disease and hepatocellular carcinoma. Chronic HCV infection is associated with metabolic abnormalities, including insulin resistance (1-3). We recently reported that insulin resistance is associated with interferon signalling, which plays an important role in the clearance of chronic hepatitis C (CHC) during interferon therapy (4, 5). Previous studies have demonstrated the association between metabolic syndrome and hepatic fibrosis in patients with hepatitis C (6-11). Steatosis is a frequent histological finding in chronic hepatitis C virus infection, one that affects disease progression and occurrence of hepatocellular carcinoma (7, 12-15). Hepatic steatosis is associated with metabolic syndrome and non-alcoholic steatohepatitis (NASH). We previously reported that risk factors for severe fibrosis in patients with NASH were metabolic syndrome, hypertension, and, in particular, diabetes mellitus (16). In this study, we evaluated patients with chronic hepatitis C (CHC) and compared the clinical and pathological features of metabolic syndrome to identify the risk factors for CHC with severe fibrosis.

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Received for publication August 30, 2010; Accepted for publication February 20, 2011
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Table 1. Clinical Data of the Patient Population

age	56.2 ± 12.8
Gender	male: female=90:81
HCV genotype (1:2)	123: 48
HCV viral load (Fmol/L)	5325 ± 5297
BMI (kg/m^2)	22.8 ± 2.9
BMI ≥ 25 (kg/m^2)	49 cases (29%)
Platelet (×10^4/mm^3)	15.9 ± 5.8
AST (IU/L)	72 ± 100
ALT (IU/L)	93 ± 110
Type 2 diabetes mellitus	36 cases (21%)
Hypertension	51 cases (30%)
Hyperlipidemia	20 cases (11%)

Table 2. Comparison between the Non-steatosis Group and Steatosis Group

	Steatosis n=79	Non-steatosis n=92	p value
Age (≥60 years old)	38 (48%)	45 (48%)	0.916*
Gender (male)	48 (60%)	42 (45%)	0.049*
HCV genotype 1	55 (69%)	68 (73%)	0.533*
HCV viral load (Fmol/L)	4611 ± 4872	5968 ± 5514	0.109
Platelet (×10^4/ mm^3)	15.4 ± 5.7	16.2 ± 5.7	0.324
ALT (IU/L)	106 ± 139	82 ± 77	0.173
Obesity	30(38%)	19 (20%)	0.013*
Diabetes mellitus	24 (36%)	11(12%)	0.001*
Hypertension	33 (42%)	18 (20%)	0.002*
Hypertriglycerides	13 (17%)	7 (7%)	0.070*

* χ^2 test

Methods

This retrospective study included 171 consecutive patients with biopsy-confirmed CHC who were assessed between 1996 and 2008 in Nagasaki Universities and associated hospitals. Inclusion criteria were an increased serum aminotransferase level for at least 6 months; serum anti-HCV (ELIZA; third generation); positive HCV RNA (PCR); negative serum HBs; and no other cause of liver disease, such as alcohol intake >30d/d or autoimmune or metabolic disorders (genetic hemochromatosis). Obesity was defined as a body mass index (BMI) >25 according to the World Health Organization criteria (17). Type 2 diabetes was diagnosed according to International Diabetes Federation criteria (18) (fasting glucose >110 mg/dL, or previously diagnosed type 2 diabetes). Hypertension was also diagnosed according to International Diabetes Federation criteria (18) (systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg). Hypertriglyceridemia was diagnosed if there was documented use of anti-hypertriglyceride medications, or if fasting triglyceride levels were >150 mg/dL. All liver biopsy tissue specimens were examined using Hematoxylin-Eosin, Azan-Mallory, and silver reticulum staining. The specimens were assessed by one reviewer blinded to patient clinical and biochemical data. The diagnosis of each case was independently and histologically confirmed by liver pathologists according to the Japanese chronic hepatitis classification (New Inuyama classification) (19). Fatty change in >5% of all areas was defined as steatosis. The patients were divided into two groups based on their degree of inflammation defined as mild activity and severe activity. Fibrosis staging was scored using a 5-grade scale: F0 indicated no fibrosis, F1 was defined as fibrous portal expansion, F2 was bridging fibrosis, F3 was bridging fibrosis with lobular distortion, and F4 indicated the presence of cirrhosis. The mild fibrosis group was defined as having a score of F0-2 and severe fibrosis was defined as a score of F3 or F4.

We defined the Nagasaki (N) score as the total number of risk factors for severe fibrosis. The risk factors were age (≥ 60 years old), obesity, and type 2 diabetes. In the N score, we doubled the presence of diabetes as a factor because the estimation value of DM was 2 times higher than that of the other factors (older age and obesity) in our logistic regression formula. Therefore, we defined N score as follows (20):

N score= age (≥60 years old) +Obesity (BMI≥25) +2× diabetes mellitus

The N score was compared with other non-invasive predictors of fibrosis stage, such as the AST to platelet ratio index (APRI), and the platelet APRI was defined as follows:

APRI=AST level (/ULN)/ platelet count (10^9/L) ×100.

Statistical significance was determined by SPSS analytical software (IBM, Armonk, NY). We used Student's t-test and the chi-square test to perform analyses. A multivariate analysis was performed using binary logistic regression analysis.

Results

Among the 171 patients, 90 males and 81 females were examined. The median body mass index (BMI) was 22.8± 2.9 kg/m^2. Type 2 diabetes was diagnosed in 21% of patients, hypertension in 30%, and hyperlipidemia in 11% (Table 1).

Histological findings

Steatosis was detected in 79 patients (46%). We found mild activity in 149 patients and severe activity in 22 patients. We found no fibrosis in 18 patients (11%) (F0); mild fibrosis in 56 patients (33%) (F1); moderate fibrosis in 33 patients (19%) (F2); bridging fibrosis in 30 patients (17%) (F3); and cirrhosis in 34 patients (20%) (F4).

Steatosis

The two steatosis groups are shown in Table 2. Males were significantly more common in the non-steatosis group than in the steatosis group (60% vs. 45%, p=0.049). The patients with obesity (38% vs. 20%, p=0.013), type 2 diabetes

Table 3. Multivariate Logistic Regression Analysis of the Association of Steatosis with the Risk of Fibrosis

	odds ratio	p value
Gender (male)	1.462 (0.765~2.888)	0.263
Obesity (BMI≥ 25 kg/m^2)	2.160 (1.010~4.227)	0.046
Type 2 diabetes	3.667 (1.559~8.430)	0.027
Hypertension	2.318 (1.151~4.864)	0.002

Table 4. Comparison between Mild Fibrosis and Severe Fibrosis

	Severe fibrosis n=97	Mild fibrosis n=74	p value*
Age (≥60 years old)	56 (59%)	23(32%)	0.001*
gender (male)	51(52%)	39 (52%)	0.987*
HCV genotype 1	73 (75%)	50 (67%)	0.348 *
HCV viral load	5924 ± 5490	4864 ± 5035	0.214
Platelet (×10^4/ mm^3)	13.5 ± 4.7	19.0 ± 5.4	<0.001
ALT	104.2 ± 129.9	79.5 ± 77.7	0.126
obesity	34 (31%)	15 (20%)	0.034*
Type 2 diabetes	32 (33%)	4 (5%)	<0.001*
Hypertension	35 (38%)	16 (18%)	0.041 *
Hypertriglyceride	11 (11%)	9 (12%)	0.868 *

χ^2 test

(36% vs. 12%, p=0.001), and hypertension (42% vs. 20%, p=0.002) were significantly more likely to be in the non-steatosis group than the steatosis group. There were no significant differences between the other clinical features (age, incidence of the HCV genotype, HCV viral load, ALT, platelet count, hypertriglycemia). In a multivariate logistic regression analysis, obesity, type 2 diabetes, and hypertension were independent predictors for steatosis [odds ratio 2.160 (1.010-4.227), p=0.046, odds ratio 3.667 (1.559-8.430), p=0.027, odds ratio 2.318 (1.151-4.864), p=0.002)] (Table 3).

The incidence of severe activity was not significantly different between the two groups (non-steatosis group: steatosis group= 9.7%: 16.4%, p=0.2849).

Severe fibrosis risk factors

The risk factors of the two fibrosis groups are shown in Table 4. The prevalence of older patients (≥60 years old) in the severe fibrosis group was significantly greater than that of younger patients (32% vs. 59%, p=0.001). The prevalence of obesity, type 2 diabetes, and hypertension was significantly higher in the severe fibrosis group than in the mild fibrosis group (31% vs. 20%, p=0.034, 33% vs. 5%, p <0.001, 38% vs. 18%, p=0.041, respectively). The platelet count was significantly lower in the severe fibrosis group than in the mild fibrosis group (13.5±4.7 vs. 19.0±5.4, < 0.001). There were no significant differences between the other clinical features (age, incidence of the HCV genotype, HCV viral load, ALT, hypertriglycemia). In a multivariate logistic regression analysis, older age, type 2 diabetes, and obesity were independent predictors for severe fibrosis [coefficient 0.984 odds ratio 2.675 (1.309-5.464), p=0.007, coefficient 2.168 odds ratio 8.739 (2.845-26.846), p=0.0002, coefficient 0.768 odds ratio 2.156 (1.006-4.619), p=0.048)] (Table 5).

Table 5. A Multivariate Logistic Regression Analysis of the Association of Severe Fibrosis with Various Risk Factors

	Odds ratio	p value
Age (60 years old)	2.675 (1.309～5.464)	0.007
Obesity	2.156 (1.006～4.619)	0.048
Type 2 DM	8.739 (2.845～26.846)	0.0002
Hypertension	1.087 (0.487～2.426)	0.8394

The fibrosis stage was significantly worse in the steatosis group than in the non-steatosis group (1.78±1.28 vs. 2.34± 1.29, p=0.005).

Predictive score of severe fibrosis

The N score is the total number of risk factors, including: older age (≥60 years old), obesity, and type 2 diabetes. N score were significantly higher in the severe fibrosis group than in the mild fibrosis group (1.48±1.14 vs. 2.66±94, p< 0.001).

We found that 17 of 53 patients (32%) with an N score of 0 had severe fibrosis, 31 of 63 (49%) with an N score of 1, 25 of 29 (86%) with an N score of 2, 21 of 23 (91%) patients with an N score of 3, and 3 of 3 (100%) patients with an N score of 4 had severe fibrosis.

The ROC curve (Fig. 1) shows the respective sensitivities and specificities for any combination of 1 to 4 of the N score. An N score of 2 provides the best combination of sensitivity (0.50) and specificity (0.91) for predicting severe fibrosis.

Compared with other non-invasive predictors of significant fibrosis, the sensitivity and specificity of an APRI of 1.5 were 46% and 93%, and those for a platelet count of 12×10^4/mm^3 were 44% and 91%.

Discussion

In this study, we analyzed the correlation between metabolic syndrome and pathological findings in CHC patients, and identified the clinical risk factors for severe fibrosis.

Obesity, type 2 diabetes, and hypertension were significant risk factors for severe steatsosis and fibrosis. These comorbidities of metabolic syndrome affected steatosis and the progression of severe fibrosis in the liver. Previous data suggest a strong association between the presence of steatosis and severe fibrosis in CHC (7, 12, 13). In this study, patients in the steatosis group had more severe fibrosis than

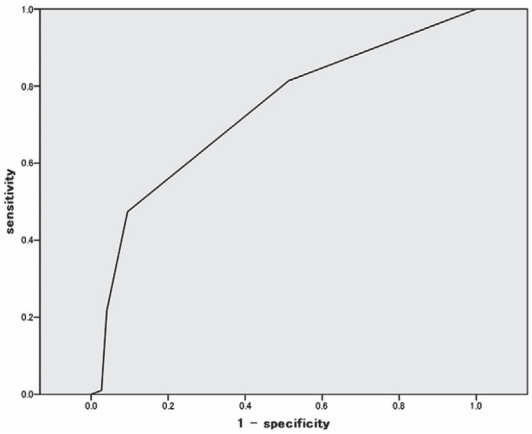

Figure 1. **ROC curve for the Nagasaki (N) score. A cut-off N score of 2 gives the sensitivity (0.50) and specificity (0.91). The area under the ROC curve is 0.732.**

Table 6. **The Incidence of Severe Fibrosis in Patients with Different N Scores**

	Severe fibrosis
N Score 0	17/53 (32%)
N Score 1	31/63 (49%)
N Score 2	25/29 (86%)
N Score 3	21/23 (91%)
N Score 4	3/3 (100%)

those in the non-steatosis group. It remains controversial whether or not hepatic steatosis may accelerate fibrosis by stimulating the activity of CHC. In this study, the ALT levels and the incidence of severe inflammation in the steatosis group were not significantly higher than in the non-steatosis group. Therefore, hepatic steatosis may not promote liver fibrosis by liver cell injury.

Evidence indicates that hepatic steatosis, which is affected by metabolic syndrome, may accelerate the progression of fibrosis in patients with CHC. The findings are consistent with those from other reports that demonstrate an association between effective weight loss and reductions in steatosis, ALT levels, and fibrosis stage in patients with CHC (21).

HCV core protein increases reactive oxygen species (ROS) and lipid peroxidation, leading to liver damage and fibrosis (22, 23). Core protein also reduces microsomal triglyceride transport protein function, leading to hepatic steatosis. Infection with the HCV virus affects liver steatosis as well as fibrosis (24). Thus, both host and viral factors induce steatosis and play a role in severe fibrosis in chronic hepatitis C.

We found a significant correlation between the severity of hepatic fibrosis and the comorbidites of metabolic syndrome, including obesity, diabetes mellitus, and hypertension. Our previous study showed that metabolic syndrome, including diabetes mellitus and hypertension, was a risk factor for severe fibrosis in patients with NASH (16). These risk factors in patients with NASH were similar to those in CHC patients, suggesting that the mechanism underlying the liver fibrosis in CHC patients resembles that of NASH.

Type 2 diabetes and obesity are correlated with insulin resistance. We previously reported that the development of liver fibrosis is associated with insulin resistance in CHC patients (25). Outcomes from the present study show the adverse effects of insulin resistance on liver fibrosis in CHC patients.

Previous data also have shown that obesity and diabetes mellitus are associated with progression of fibrosis in CHC (6, 8, 9, 26). Few prior studies have been conducted in Asian patients. The present study shows that metabolic syndrome, including obesity and diabetes, also predict severe fibrosis in Asian patients.

Taken together, although steatosis, fibrosis and metabolic syndrome seem to be associated with each other, our cross-sectional study did not identify any associations between these factors. Further studies will be necessary to confirm whether these conditions are associated, or whether they act as independent risk factors.

We defined the N score as the total number of risk factors for severe fibrosis. The risk factors were age ($\geq$60 years old), obesity, and type 2 diabetes. In the N score, we doubled the presence of diabetes as a factor because the estimation value of DM was 2 times that of other factors (older age and obesity) in our logistic regression formula (risk factor). The N score was significantly higher in the severe fibrosis group than in the mild fibrosis group. About 90% of the patients in the severe fibrosis group had an N score $\geq$2. An N score $\geq$2 indicates a high risk for severe fibrosis. Our results suggest that half of the patients in the severe fibrosis group also had metabolic disorders, including diabetes and obesity. Conversely, there was no association between metabolic syndrome and other factors.

While the specificity of the N score (0.91) is very good, the sensitivity (0.5) is not sufficient. This suggests that there are two or more mechanisms underlying the progression of fibrosis, and metabolic syndrome represents one of them.

Compared to other non-invasive markers, the sensitivity and specificity of the N score was equal to the platelet count and APRI. The N score is a simple score to calculate, and it adds together the three risk factors. Therefore, determining the N score is considered to be an easy way to predict the presence of severe liver fibrosis in CHC patients.

The present study has limitations. We cannot perform a validation due to the fact that our sample size was so small. Now, we are planning to perform a validation set to confirm the value of N score.

In conclusion, older age, obesity and type 2 diabetes are significant predictors of severe fibrosis in Japanese CHC patients. The total number of these risk factors in patients could be a useful marker for predicting severe fibrosis in pa-

tients with CHC.

The authors state that they have no Conflict of Interest (COI).

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