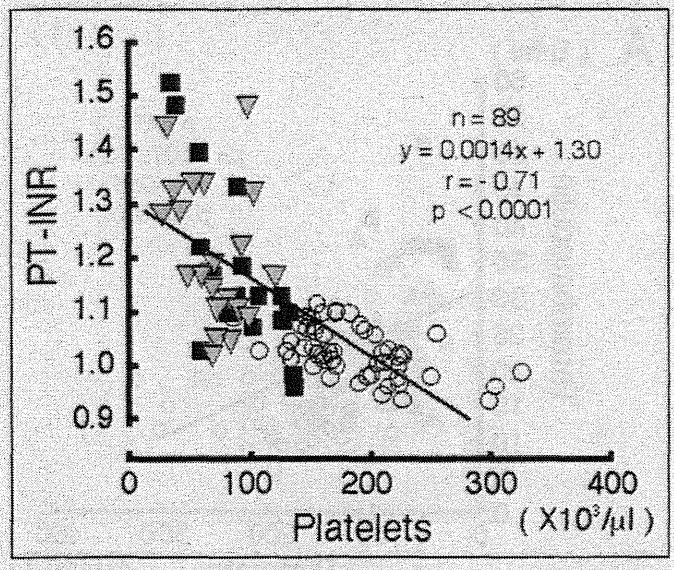

Figure 2. Scattergram showing the correlation between prothrombin time–international normalized ratio (PT-INR) and the platelet count ($r = -.71$, $P < .0001$). The line represents the linear regression of PT-INR versus platelet count. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

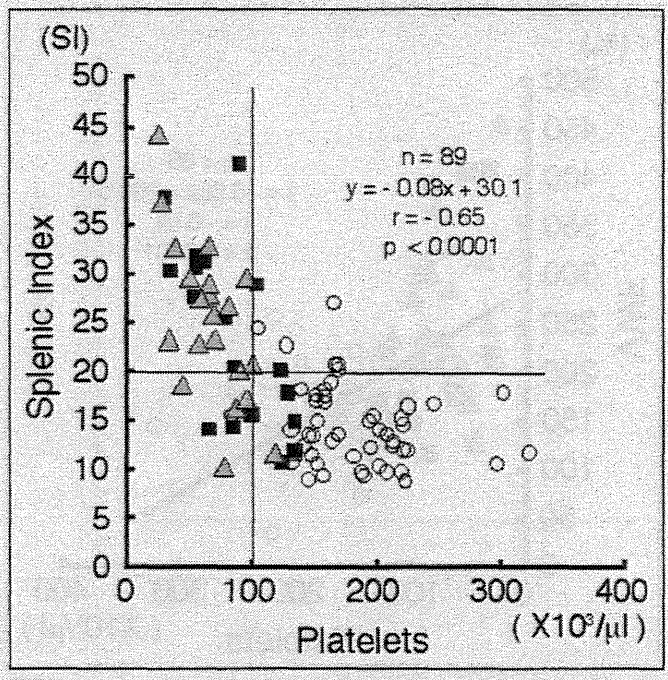

Figure 3. Scattergram showing the correlation between the platelet count and the splenic index (SI; $r = -.65$, $P < .0001$). The horizontal and vertical lines denote the cutoff values used to define splenomegaly, with SI values higher than 20 and thrombocytopenia with a platelet count less than 100×10^3 cells/μL, respectively. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

Thrombopoietin

The TPO concentrations were 42.5 ± 33.1 pg/mL among patients with CH, 39.9 ± 37.8 pg/mL among patients with LC, and 70.3 ± 68.7 pg/mL among patients with LC + HCC, respectively; no significant differences were observed among the stages (Table 1). No correlation between the platelet count and the TPO concentration was seen ($r = -.23$, $P < .0475$).

von Willebrand factor Antigen

The vWF antigen values were 153.4% ± 52.6% among patients with CH, 208.7% ± 83.1% among patients with LC, and 243.6% ± 65.3% among patients with LC + HCC. These values were significantly different ($P < .01$; Table 1), suggesting that vWF antigen increases with the progression of the stage of chronic HCV infection. A negative correlation was observed between the platelet count and the vWF antigen value ($r = -.54$, $P < .0001$; Figure 4).

Thrombomodulin

Thrombomodulin is often used as a marker of endothelial cell damage. As expected, the TM values were 16.0 ± 5.7 U/mL among patients with CH, 22.9 ± 8.8 U/mL among patients with LC, and 25.8 ± 7.5 U/mL among patients with LC + HCC. These values were significantly different ($P < .01$; Table 1). A negative correlation was observed between the platelet count and the TM value ($r = -.51$, $P < .0001$; Figure 5A), and a positive correlation was observed between the vWF antigen value and the TM value ($r = .48$, $P < .0001$; Figure 5B).

ADAMTS13

A decrease in ADAMTS13 activity is known to result in an increase in ultra-large vWF multimers, which is associated with a low platelet count. The ADAMTS13 activities were 97.2% ± 28.8% among patients with CH, 123.0% ± 43.2% among patients with LC, and 102.1% ± 27.5% among patients with LC + HCC. No significant differences were observed among the stages (Table 1). Neither a correlation between ADAMTS13 activity and the platelet count ($r = -.22$, $P < .0825$; Figure 6) nor a correlation between ADAMTS13 activity and the VWF antigen value was observed ($r = -.02$, $P < .8746$).

Multiple Regression Analysis

With the parameters measured in this study, we performed a multiple regression analysis using thrombocytopenia as the target index. As a result, splenomegaly, PT-INR, and the vWF antigen value were extracted as factors that were significantly responsible for thrombocytopenia (Table 2, panel A). Since splenomegaly is related to the impairment of liver function,

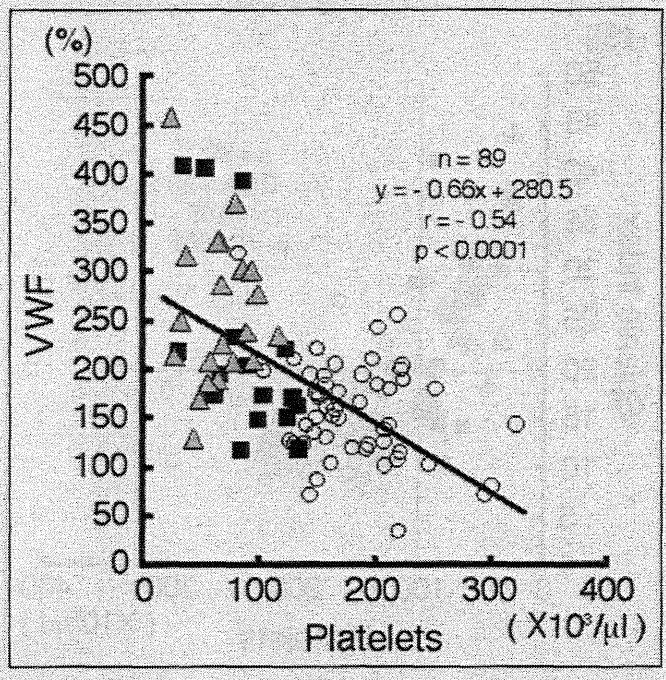

Figure 4. Scattergram showing the correlation between the von Willebrand factor (vWF) antigen value and the platelet count ($r = -.54$, $P < .0001$). The line represents the linear regression of vWF antigen versus the platelet count. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

with which the PT level is assumed to be associated, we also performed an analysis without PT-INR, revealing splenomegaly and the vWF antigen value as significant factors (Table 2, panel B). We further performed a stratified analysis, in which the patients were subdivided into 4 groups, based on 2 parameters: splenomegaly (+, SI > 20) or (−) and thrombocytopenia (+, platelet count < 100×10^3 cells/μL) or (−). In the splenomegaly (+) group, significant differences in spleen size (SI), liver function, and the vWF antigen value were observed between the thrombocytopenia (+) group and (−) group (Table 3). Taking into consideration the fact that impaired liver function is related to splenomegaly and that these 2 factors may be evaluated as one in this group, splenomegaly appears to be the major determinant of thrombocytopenia. However, among the 55 cases without splenomegaly, a significant increase in the vWF antigen value and PT-INR was observed in the thrombocytopenia (+) group compared with the (−) group, while no difference in spleen size or platelet count was observed. Thus, in the group of patients without splenomegaly, some factors related to the vWF antigen increase or liver function impairment may play a role in inducing thrombocytopenia.

Other Markers

Fibrinolysis markers such as D-dimer and PAI-1 were evaluated in reference to thrombocytopenia. However, no correlation was

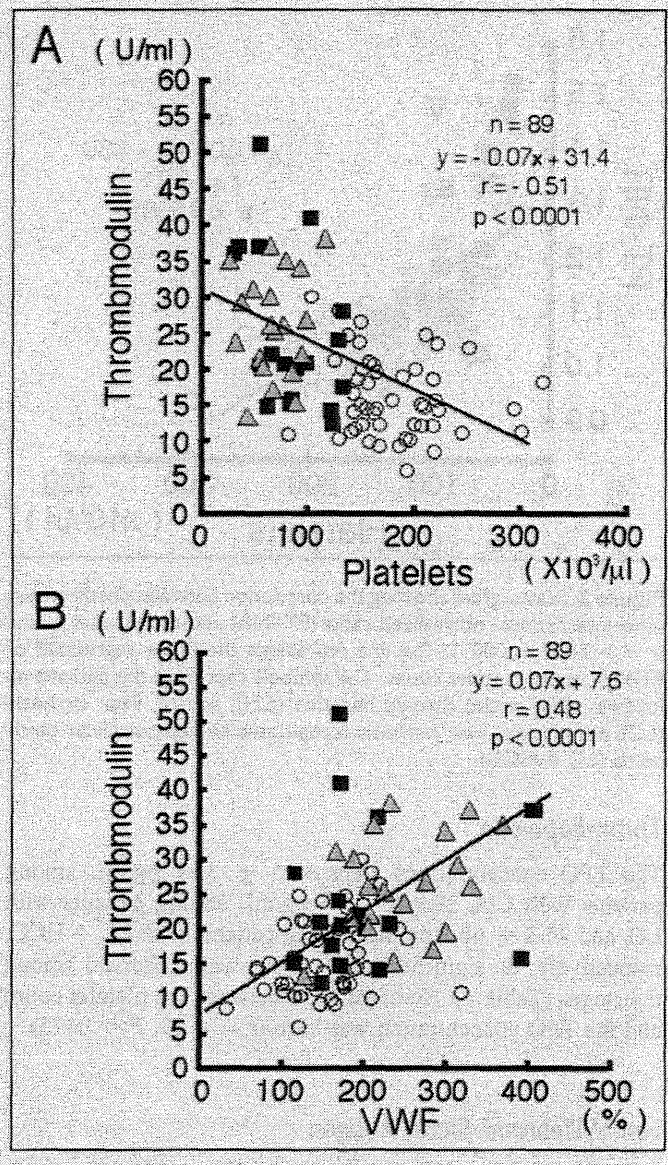

Figure 5. Scattergram showing the correlation between thrombomodulin (TM) and the platelet count ($r = -.51$, $P < .0001$) (A) and between TM and the von Willebrand factor (vWF) antigen value ($r = .48$, $P < .0001$) (B). The line represents the linear regression. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

observed between these markers and the platelet count in patients with chronic HCV infection. Furthermore, no correlation was observed between the platelet count and the CRP level, which is often used a marker of systemic inflammation.

Discussion

Thrombocytopenia in chronic HCV infection may be caused by platelet destruction/sequestration, the decreased production of platelets, or platelet consumption. In this study, we sought to

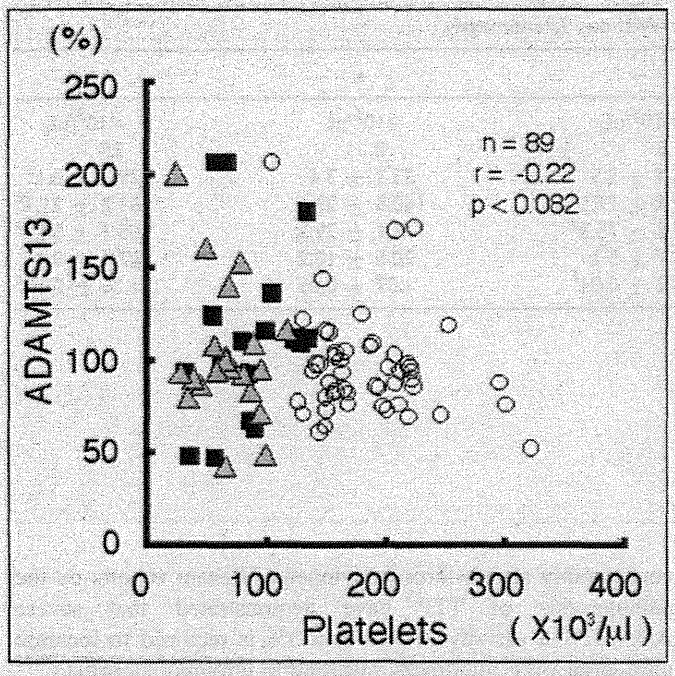

Figure 6. Scattergram showing the correlation between ADAMTS13 activity and the platelet count ($r = -.22$, $P < .0825$). The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

evaluate the roles of various factors that may contribute to thrombocytopenia.

Platelet destruction/sequestration by splenomegaly induced by liver fibrosis is a major cause of thrombocytopenia. In this study, examining 89 patients infected with HCV, splenomegaly, impaired liver function as represented by PT, and the vWF antigen value were correlated well with thrombocytopenia, and a multiple regression analysis also extracted these 3 parameters as explanatory variables for thrombocytopenia, confirming the results of previous reports.[4,14-16] Since splenomegaly and PT are partially dependent, we removed PT from the analysis to extract other underlying factors; only splenomegaly and the vWF antigen value were identified as major determinants of thrombocytopenia, with splenomegaly exhibiting the stronger dependence. On the other hand, a splenectomy or the shunting of the portal veins does not necessarily correct the low platelet count,[6,21] suggesting that some mechanism other than splenomegaly is responsible for thrombocytopenia in liver fibrosis induced by HCV infection. In our stratified analysis of 55 cases without splenomegaly (SI < 20), significant differences in the vWF antigen value and PT were observed between the thrombocytopenia (+) group and the thrombocytopenia (−) group, although no significant difference in spleen size was noted. These findings suggest that even among cases without splenomegaly, thrombocytopenia is induced by some other factors that may be related to an elevation in the vWF antigen level or the impairment of liver function.

Table 2. Multiple Regression Analysis of Variables Associated With Thrombocytopenia

A. Analysis with PT, splenic index, and vWF antigen		
Variable	*t*	*P*
PT-INR	4.662	.000013
Splenic index (SI)	3.079	.002905
vWF antigen, %	2.410	.018406
B. Analysis with splenic index and vWF antigen		
Splenic index (SI)	6.413	<.00001
vWF antigen, %	3.484	.00078

Abbreviation: PT-INR, prothrombin time–international normalized ratio; vWF, von Willebrand factor.

The impaired production of platelets, that is, impaired thrombopoiesis, may be partially responsible for thrombocytopenia in patients infected with HCV. Thrombopoietin is a major cytokine that stimulates the proliferation and differentiation of the megakaryocytic lineage, with resultant platelet production.[22,23] Since TPO is produced by the liver, impaired liver function in chronic HCV infection may lead to a low level of TPO in the blood, which cannot maintain normal thrombopoiesis in the bone marrow and the peripheral platelet count.[24] In agreement with this hypothesis, a negative correlation between the blood TPO level and the progression of the stage of liver disease has been reported in patients with HCV infection.[25] On the other hand, a previous study hypothesized that the total blood TPO is maintained at a certain level, irrespective of liver function, and that the blood TPO level is inversely related to the platelet count since TPO binding to its receptors on platelets and megakaryocytes tends to lower the blood TPO level.[26] Based on this hypothesis, it follows that the blood TPO is elevated in proportion to the severity of thrombocytopenia in patients with chronic HCV infection. In the present study, we found that no correlation existed between the platelet count and the blood TPO level, although the blood TPO level tended to be elevated in the LC + HCC group. A multiple regression analysis did not recognize TPO as an explanatory factor, and it is likely that TPO contributes minimally to thrombocytopenia in patients with chronic HCV infection. Consistently, recent reports have proposed that TPO produced by stromal cells in the bone marrow acts locally on megakaryocytes[27,28] and that the blood TPO level does not reflect thrombopoiesis in the bone marrow.[29]

Other hypotheses have also been presented in relation to vWF-induced platelet consumption, which accounts for the thrombocytopenia in patients infected with HCV. von Willebrand factor associates with Glycoprotein IB (GPIB) molecules on the platelet membrane and leads to platelet adhesion/aggregation at sites of vascular damage. If the blood vWF level is increased in patients with liver fibrosis, its interaction with platelets may lead to the increased consumption of platelets, resulting in thrombocytopenia.[14-16,30] von Willebrand factor antigen has been reported to increase significantly with the progression of the stage of liver fibrosis,[30] and vWF production

Table 3. Stratified Analysis of Patients with Thrombocytopenia With or Without Splenomegaly

Splenomegaly	−	−	+	+
Platelet	$>10^5/\mu L$	$<10^5/\mu L$	$>10^5/\mu L$	$<10^5/\mu L$
n	47	8	8	25
Splenic index (SI)	13.5 ± 2.9	15.4 ± 2.5	23.3 ± 3.4	29.3 ± 6.1[a]
Platelet ($\times 10^3/\mu L$)	182.5 ± 47.1	79.4 ± 17.5[b]	140.5 ± 29.1	61.2 ± 21.7[c]
von Willebrand factor, %	152.7 ± 48.2	202.8 ± 75.3[d]	179.1 ± 29.2	268.7 ± 88.6[e]
Thrombomodulin, U/mL	18.0 ± 9.9	18.7 ± 5.2	20.5 ± 10.2	27.4 ± 9.1
PT-INR	1.03 ± 0.06	1.12 ± 0.04[f]	1.07 ± 0.05	1.26 ± 0.15[c]

Abbreviation: PT-INR, prothrombin time–international normalized ratio.
[a] $P < .05$ versus splenomegaly (+) plus platelet > $10^5/\mu L$.
[b] $P < .01$ versus splenomegaly (−) plus platelet > $10^5/\mu L$.
[c] $P < .01$ versus splenomegaly (+) plus platelet > $10^5/\mu L$.
[d] $P < .05$ versus splenomegaly (−) plus platelet > $10^5/\mu L$.
[e] $P < .01$ versus splenomegaly (+) plus platelet > $10^5/\mu L$.
[f] $P < .01$ versus splenomegaly (−) plus platelet > $10^5/\mu L$.

has been postulated to be facilitated by the remodeling of the liver tissue or endotoxic damage to the hepatocytes[31] or extrahepatic organs, such as the spleen.[32] Recent reports on vWF regulation in patients with chronic HCV infection have focused on ADAMTS13 activity, which cleaves the vWF multimers. An elevated vWF antigen level in patients with chronic HCV infection may reflect a proportional decrease in ADAMTS13 activity and the existence of ultra-large vWF multimers, which are apt to react with platelets.[33] possibly leading to thrombocytopenia. In accordance with this notion, a recent report has demonstrated a correlation between the platelet count and ADAMTS13 activity in patients with advanced stages of liver fibrosis, including HCV infection.[17]

Contrary to our expectation, we were unable to observe a significant correlation between ADAMTS13 antigen/activity (data not shown) and the platelet count in this study, and no significant differences in these ADAMTS13-related parameters were observed among the stages of liver fibrosis. The discrepancy between our study and previous reports, particularly that of Uemura,[17] appears to be attributable to the overall severity of liver fibrosis in patients evaluated in each study. The report of Uemura et al deals with a number of patients with considerably advanced stages of liver fibrosis, such as those complicated with ascites. The patient profile in terms of Child's classification corresponded to 33 cases of CH, 35 cases of LC Child A, 33 cases of Child B, and 41 cases of Child C, with mean ADAMTS13 activities of 87%, 79%, 63%, and 31%, respectively. A clear difference in the ADAMTS13 activities was observed among the stages of liver fibrosis, with the lowest level observed with Child C. On the other hand, the patients in our study all attended our outpatient clinic on a regular basis, and the overall severity of liver fibrosis was far less than that of the series reported by Uemura, which was comprised of 50 cases of CH, 23 cases of LC Child A, 13 cases of Child B, and 3 cases of Child C; the ADAMTS13 activities were 95.9%, 119.5%, 92%, and 81%, respectively. Of note, the ADAMTS13 activities of even our Child B and C groups were fairly well retained (81%-92%), although these patients exhibited considerably severe thrombocytopenia. Recent reports on the pathogenesis of TTP have demonstrated that severe ADAMTS13 activity of less than 3% is required to increase ultra-large vWF multimers, resulting in thrombocytopenia.[34-36] It is also now known that a simple deficiency in ADAMTS13 does not lead to overt TTP.[37] Furthermore, hepatic stellate cells have been reported to possibly be a key factor in the reduction of plasma ADAMTS13 activities in rats with liver injury.[38] Taken together with the findings of these previous reports, our findings that thrombocytopenia occurs in the apparent absence of clear changes in ADAMTS13 activity suggests that ADAMTS13 changes may not be heavily involved in thrombocytopenia during chronic HCV infection.

Stellate cells appear to play an important role in liver fibrosis, and it is well known that the number of hepatic stellate cells is increased in cirrhotic liver in humans as well as rats. Furthermore, the current study revealed a relation between the increased production of ADAMTS13 and the enhanced plasma ADAMTS13 activity in a rat model of steatohepatitis during the process of liver fibrosis, where hepatic stellate cells are known to proliferate, suggesting that hepatic stellate cells in the liver play a significant role in the regulation of plasma ADAMTS13 activity.[39] Thus, it is speculated that stellate cells remain functional until the very last stage of liver fibrosis,[25] and the level of ADAMTS13, which is produced by stellate cells, may be maintained until the most advanced stage of liver cirrhosis,[40] which agrees well with our findings and those of a previous report[30] in which little difference in the ADAMTS13 level was noted among the different stages of liver fibrosis. The regulatory mechanism responsible for ADAMTS13 production by hepatic stellate cells in advanced cirrhosis and the inactivation of ADAMTS13 in humans requires further elucidation.

The vWF antigen value increased significantly with the progression of the stage of liver fibrosis in this study, in agreement with the results of a previous report.[30] Since the vWF value was not correlated with that of ADAMTS13, the increase in the vWF antigen value is likely due to its release from activated endothelial cells. von Willebrand factor antigen is negatively

correlated with the platelet count; in the multiple regression analysis, it was extracted as an explanatory variable for thrombocytopenia, although the P value was significance less than that for splenomegaly. Thus, an elevation in the vWF antigen value may be partly responsible for the thrombocytopenia in patients with chronic HCV infection. However, the reference value for vWF antigen is considerably wide among healthy individuals, and further studies are needed to determine whether the difference in the vWF antigen value according to the stage of fibrosis is related to thrombocytopenia and to elucidate the possible mechanism.

Thrombomodulin is expressed on vascular endothelial cells and acts to regulate coagulation pathways by interacting with thrombin, producing activated protein C. Since TM is cleaved and released into the circulation during inflammatory processes, it is used as a marker of endothelial cell damage. Of particular interest is that this marker can be used to predict endothelial damage in the liver, independent of systemic circulation.[41] In this study, we found that the TM level increased with the progression of the stage of liver fibrosis, in the absence of an elevation in the CRP level, representing systemic inflammation. In chronic HCV infection, inflammatory processes in the liver are assumed to play a role in fibrotic changes, and our findings suggest that TM can be a good marker in predicting endothelial damage, that is, the process of inflammation and fibrosis in the liver. Although a negative correlation was observed between TM and the platelet count, this factor was not extracted as an explanatory variable in the multiple regression analysis. We found a good correlation between the vWF antigen value and TM ($r = .48$, $P < .0001$; Figure 4B), suggesting that both parameters may represent endothelial dysfunction induced by inflammatory changes in the liver. Thrombomodulin might not have been extracted as an explanatory variable in the multiple regression analysis as a result of its close association with vWF antigen. Our notion is in good agreement with a previous report that in liver damage induced by HBV and HCV infection in children, vWF antigen and TM can predict endothelial cell function.[42] Taken together, our findings, along with those of the previous report, suggest that TM can serve as a marker for inflammatory changes in the liver in patients with chronic HCV infection. Furthermore, thrombocytopenia in this disorder may be related to endothelial dysfunction revealed by its activation, that is, vWF release, and its damage, that is TM cleavage. This hypothesis seems consistent with previous results indicating that TM and vWF change in parallel as endothelial dysfunction markers.[43]

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Research Article

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Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C

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Background & Aims: Assessment of the risk of hepatocellular carcinoma (HCC) development is essential for formulating personalized surveillance or antiviral treatment plan for chronic hepatitis C. We aimed to build a simple model for the identification of patients at high risk of developing HCC.

Methods: Chronic hepatitis C patients followed for at least 5 years (n = 1003) were analyzed by data mining to build a predictive model for HCC development. The model was externally validated using a cohort of 1072 patients (472 with sustained virological response (SVR) and 600 with nonSVR to PEG-interferon plus ribavirin therapy).

Results: On the basis of factors such as age, platelet, albumin, and aspartate aminotransferase, the HCC risk prediction model identified subgroups with high-, intermediate-, and low-risk of HCC with a 5-year HCC development rate of 20.9%, 6.3–7.3%, and 0–1.5%, respectively. The reproducibility of the model was confirmed through external validation (r^2 = 0.981). The 10-year HCC development rate was also significantly higher in the high- and intermediate-risk group than in the low-risk group (24.5% *vs.* 4.8%; p <0.0001). In the high-and intermediate-risk group, the incidence of HCC development was significantly reduced in patients with SVR compared to those with nonSVR (5-year rate, 9.5% *vs.* 4.5%; p = 0.040).

Conclusions: The HCC risk prediction model uses simple and readily available factors and identifies patients at a high risk of HCC development. The model allows physicians to identify patients requiring HCC surveillance and those who benefit from IFN therapy to prevent HCC.

Keywords: Decision tree; Prediction; Pegylated interferon; Ribavirin; Risk.
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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide [1] and its incidence is increasing in many countries [2]. Chronic viral hepatitis is responsible for 80% of all HCC cases [2]. The need to conduct HCC surveillance should be determined according to the risk of HCC development because this surveillance is cost-effective only in populations with an annualized cancer development rate of ⩾1.5% [3]. The annualized rate of developing HCC from type C liver cirrhosis is 2–8% [4–6], indicating that this population with type C liver cirrhosis needs surveillance. However, the annualized rate of HCC development is <1.5% in patients with chronic hepatitis C but without cirrhosis and the benefit of surveillance for all patients with chronic hepatitis has not yet been established [3]. HCC surveillance may be needed for patients with advanced fibrosis because the risk of HCC development increases in parallel with the progression of liver fibrosis [7,8]. Liver biopsy is the most accurate means of diagnosing fibrosis, but a single liver biopsy cannot indicate long-term prognosis because liver fibrosis progresses over time. Serial liver biopsies are not feasible because of the procedure's invasiveness. Moreover, factors other than fibrosis, such as advanced age, obesity, sex, lower albumin, and low platelet counts, also contribute to the development of HCC from chronic hepatitis C [8–11]. Therefore, these factors must be considered while assessing the risk of HCC development.

A meta-analysis of controlled trials [12] has shown that interferon (IFN) therapy reduced the rate of HCC development in patients with type C liver cirrhosis. However, there was a marked heterogeneity in the magnitude of the prevention effect

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of IFN on HCC development among the studies, probably due to the large differences in the baseline rate of HCC development among the different trials [12]. Whether the incidence of HCC development could be reduced in all patients with chronic hepatitis C, especially in those without liver cirrhosis, remains to be elucidated.

Data mining analysis, unlike conventional statistical analysis, is performed in an exploratory manner without considering a predefined hypothesis. Decision tree analysis, the major component of data mining analysis, is used to extract relevant factors from among various factors. These relevant factors are then combined in an orderly sequence to identify rules for predicting the incidence of the target outcome [13]. Data mining analysis has been used to define prognostic factors in various diseases [14–20]. In the field of hepatic diseases, data mining analysis has proven to be a useful tool for predicting early response [21], sustained virological response (SVR) [22–25], relapse [26], and adverse events [27] in patients with chronic hepatitis C treated with pegylated interferon (PEG-IFN) plus ribavirin (RBV). The findings of data mining analysis are expressed as flowcharts and are therefore easily understood [28] and readily available for clinical use, even by physicians without a detailed understanding of statistics.

In the present study, data mining analysis was used to identify risk factors for HCC development in a cohort of patients with chronic hepatitis C who had been followed for at least 5 years. An HCC risk prediction model was constructed on the basis of simple and generally available tests because the goal was to make the model easy to use in the clinic. The suitability, reproducibility, and generalizability of the results were validated using the data of an external cohort that was independent of the model derivation cohort.

Cancer

Materials and methods

Patients

The model derivation cohort consisted of 1003 chronic hepatitis C patients without cirrhosis who had a non-sustained virological response (nonSVR) to previous IFN administered at the Musashino Red Cross Hospital and were followed for at least 5 years. Patients who had SVR or those who were followed for less than 5 years were not included. An analytical database on age, body mass index, albumin, aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, γ-glutamyltransferase (GGT) levels, total bilirubin levels, total cholesterol levels, hemoglobin levels, and platelet count at the start of the observation was created. Histological data such as fibrosis stage, activity grade, or degree of steatosis was not included in the database because the goal of the present study was to make the model on the basis of simple and generally available tests. The patients who developed HCC more than 5 years after the start of the observation were considered not to have developed HCC by the 5-year point because the model was intended to predict HCC development within 5 years. The 1072 chronic hepatitis C patients included in the external validation cohort were treated with PEG-IFN and RBV at the University of Yamanashi, Tokyo Medical and Dental University, Osaka University, Osaka City University, Nagoya City University, or Toranomon Hospital and followed for at least 5 years. Among them, 600 had nonSVR and 472 had SVR. Data from nonSVR patients in this external cohort were used for external validation of the HCC prediction model. To assess the preventive effect of PEG-IFN plus RBV therapy on HCC development, the cumulative HCC development rate was compared between SVR and nonSVR patients in the external validation cohort after stratification by the risk of HCC development as determined by data mining analysis. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

HCC surveillance and diagnosis

HCC surveillance was conducted by performing abdominal ultrasonography every 4–6 months. Contrast-enhanced computer tomography, magnetic resonance imaging, or angiography were performed when abdominal ultrasonography suggested a new lesion suspicious for HCC. Classical HCC was diagnosed for tumors showing vascular enhancement with washout on at least two types of diagnostic imaging. Tumor biopsy was used to diagnose tumors with non-classical imaging findings.

Statistical analysis

The IBM-SPSS Modeler 13 (IBM SPSS Inc., Chicago, IL, USA) was used for decision tree analysis. The statistical methods used have been described previously [21,22,24–27]. In brief, the software searched the analytical database for the factor that most effectively predicted HCC development and for its cutoff value. The patients were divided into two groups according to that predictor. Each divided group was repeatedly assessed and divided according to this 2-choice branching method. Branching was stopped when the number of patients decreased to ≤20 to avoid over fitting. Finally, an HCC risk prediction model was created through this analysis. The model classified patients into subgroups with different HCC development rates in a flowchart form. For model validation, nonSVR patients from an external cohort were individually fitted into the model and classified into the subgroups and the HCC development rates of those subgroups were then calculated. The suitability and reproducibility of the model were validated by comparing the subgroup HCC development rates of the model derivation group to those of the validation group.

On univariate analysis, Student's *t*-test was used for continuous variables and Fisher's exact test was used for categorical data. Logistic regression was used for multivariate analysis. A log-rank test for Kaplan–Meier analysis was used to statistically test HCC development rates over time. *p*-Values of <0.05 were considered significant. SPSS Statistics 18 (IBM SPSS Inc.) was used for these analyses.

Results

Univariate and multivariate analysis of factors associated with HCC development

The baseline characteristics of patients are shown in Table 1. The 5-year HCC development rate in the model derivation group was 6.2%, which did not differ significantly from the rate of 6.0% in the nonSVR group of the external cohort, but the rate of 2.0% in the SVR group of the external cohort was significantly lower than that in the model derivation group ($p = 0.0003$) and the nonSVR group of the external cohort ($p = 0.0012$). On univariate analysis, the factors found to be associated with HCC development in the model derivation cohort were age, AST levels, albumin levels, total cholesterol levels, and platelet count. On multivariate analysis, age (odds ratio 1.086), albumin levels (odds ratio 0.248), and platelet count (odds ratio 0.842) were significant predictors of HCC development (Table 2).

HCC risk prediction model by data mining analysis

The results of decision tree analysis are presented in Fig. 1. Age was selected as the first predictor. The 5-year HCC development rate was 3.4% in younger patients (<60 years) and 8.6% in older patients (≥60 years). The second predictor for younger patients (<60 years) was platelet count. The HCC development rate was 6.9% in patients with a lower platelet count ($<150 \times 10^9$/L) and 0.8% in patients with a higher count ($\geq 150 \times 10^9$/L). The second predictor for older patients (≥60 years) was also platelet count. The HCC development rate was 13.1% in patients with a lower platelet count ($<150 \times 10^9$/L) and 1.8% in patients with a higher count ($\geq 150 \times 10^9$/L). The third predictor was albumin levels,

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Table 1. Baseline characteristics of patients for model deviation and external validation.

	Model derivation (n = 1003)	External cohort, non-SVR (n = 600)	External cohort, SVR (n = 472)
Sex: Male/Female*	463 (46%)/540 (54%)	306 (51%)/294 (49%)	299 (63%)/173 (37%)
Age (yr)	57.3 (11.1)	55.9 (9.6)	51.4 (10.6)
Body mass index (kg/m^2)	23.5 (3.2)	23.4 (3.3)	23.3 (3.1)
Albumin (g/dl)	4.1 (0.3)	4.0 (0.4)	4.0 (0.3)
AST (IU/L)	64.2 (36.5)	67.3 (43.8)	62.5 (48.3)
ALT (IU/L)	80.6 (55.1)	81.2 (62.3)	88.6 (82.1)
GGT (IU/L)	59.3 (50.5)	67.6 (65.1)	55.7 (71.2)
Total cholesterol (mg/dl)	172.1 (31.5)	168.2 (31.0)	174.3 (33.7)
Platelet (10^9/L)	154.0 (53.0)	153.7 (53.2)	176.6 (49.7)
Hemoglobin (g/dl)	13.3 (1.5)	14.2 (1.5)	14.4 (1.4)
HCC development within 5 years: n (%)*	62 (6.2%)	36 (6.0%)	10 (2.0%)

Data expressed as mean (standard deviation) unless otherwise indicated.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.
*Data expressed as number of patients (percentage).

whose cutoff value was 3.75 g/dl in patients with a higher platelet count ($\geq 150 \times 10^9$/L). The HCC development rate was 6.3% when albumin levels were lower (<3.75 g/dl) and 1.5% when levels were higher ($\geq$3.75 g/dl). The cutoff value for albumin levels was 4.0 g/dl in patients with a lower platelet count (<150×10^9/L). The HCC development rate was 20.9% when albumin levels were lower (<4.0 g/dl) and 6.4% when levels were higher ($\geq$4.0 g/dl). The fourth and final predictor was AST levels. The HCC development rate was 7.3% when AST levels were at least 40 IU/L and 0% when the levels were <40 IU/L. On the basis of this analysis, seven subgroups with a 5-year HCC development rate of 0–20.9% were identified. The area under the receiver operating characteristic curve according to the HCC risk prediction model was 0.817.

External validation of the HCC risk prediction model with an independent external cohort

Six hundred nonSVR patients from an external cohort were fitted into the HCC risk prediction model and classified into the seven subgroups. The 5-year HCC development rate of these subgroups was 0–17.9%. The HCC development rate in the individual subgroups of the model derivation group was closely correlated to that in the corresponding subgroups of the external validation group (Fig. 2; correlation coefficient $r^2 = 0.981$). The HCC development rate in the subgroup of patients with the highest risk of HCC development (high-risk group) according to the model older age ($\geq$60 years) with a lower platelet count (<150×10^9/L) and lower albumin levels (<4.0 g/dl) was 20.9% in the model derivation group and 17.9% in the external validation group. The intermediate-risk group or the patients with an HCC development rate of at least 5% consisted of the following three subgroups: (1) older age ($\geq$60 years), lower platelet count (<150×10^9/L), higher albumin levels ($\geq$4.0 g/dl), and higher AST levels ($\geq$40 IU/L); (2) older age ($\geq$60 years), higher platelet count ($\geq 150 \times 10^9$/L), and lower albumin levels (<3.75 g/dl); and (3) younger age (<60 years) and lower platelet count (<150×10^9/L). In these intermediate-risk groups, the 5-year HCC development rate was 6.3–7.3% in the model derivation group and 5.3–7.9% in the external validation group. The low-risk group consisted of the following three subgroups: (1) younger age (<60 years) and higher platelet count ($\geq 150 \times 10^9$/L); (2) older age ($\geq$60 years), lower platelet count (<150×10^9/L), higher albumin levels ($\geq$4.0 g/dl), and lower AST levels (<40 IU/L); and (3) older age ($\geq$60 years), higher platelet count ($\geq 150 \times 10^9$/L), and higher albumin levels ($\geq$3.75 g/dl). In these low-risk groups, the 5-year HCC development rate was 0–1.5% in the model derivation group and 0–2.9% in the external validation group.

Table 2. Multivariable analysis of factors associated with subsequent development of HCC within 5 years.

	Odds ratio	95% CI	*p* value
Age	1.086	1.029-1.146	0.003
Albumin	0.248	0.100-0.613	0.003
Platelet	0.842	0.769-0.921	<0.0001

CI, confidence interval.

Predictability of the HCC risk prediction model on HCC development rate beyond 5 years

Cumulative HCC development rates in the high-, intermediate-, and low-risk groups were compared over time using the Kaplan–Meier method. The 10-year rates were 28.9% in the high-risk group, 22.9% in the intermediate-risk group, and 4.8% in the low-risk group (Fig. 3A). The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups had a significantly higher cumulative HCC development rate than the low-risk group beyond 5 years (Fig. 3B; 5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; p <0.0001).

Effect of response to PEG-IFN plus RBV therapy in the reduction of HCC development: analysis stratified by the HCC risk prediction model

The 600 nonSVR patients and 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and

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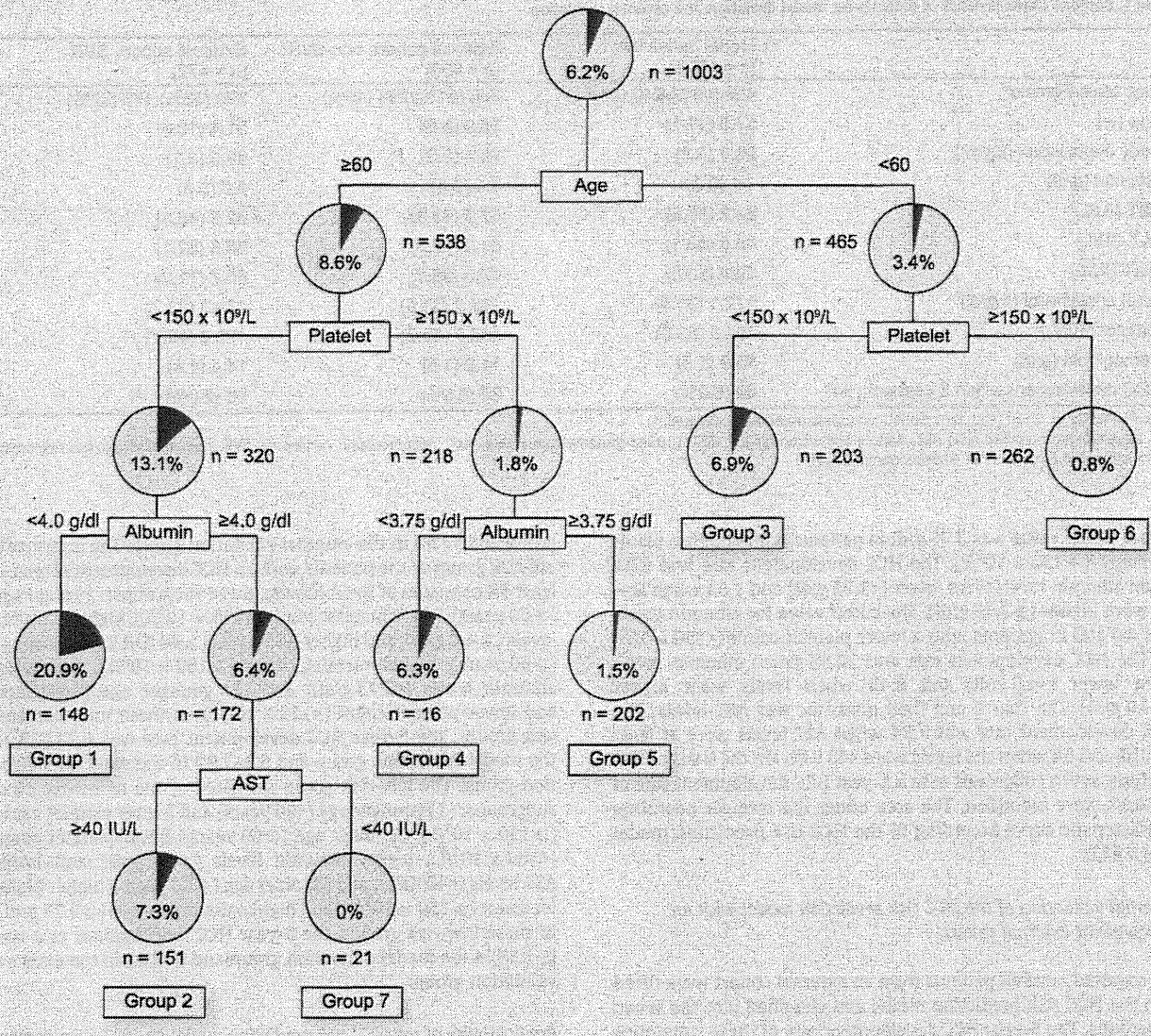

Fig. 1. The decision tree model of HCC development within 5 years. Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups of patients differentiated by analysis are numbered from 1 to 7.

classified into the high-and intermediate-risk group or the low-risk group, as defined above. The HCC development rate was significantly lower in SVR patients than in nonSVR patients in the high-and intermediate-risk group (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$, log-rank test). In the low-risk group, the 5-year rate was 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates were low and not significantly different ($p = 0.331$, log-rank test) (Fig. 4).

Discussion

An awareness of the risk of HCC development in the context of routine care for chronic hepatitis C is essential for formulating an HCC surveillance plan personalized for individual patients. The risk of developing HCC from chronic hepatitis is lower than that from cirrhosis [7]; therefore, across-the-board surveillance for chronic hepatitis C is not recommended [3]. A method to easily determine this risk, without performing serial liver biopsies, would be extremely significant clinically. In the present study, an HCC risk prediction model that included the factors such as age, platelet count, albumin levels, and AST levels was constructed. The model was found to have excellent reproducibility when validated with an external cohort. This model could identify subgroups of chronic hepatitis C patients at high risk of HCC development; the 5-year HCC development rate for the high- and intermediate-risk groups was 11.6%, yielding an annual incidence of 2.3%. This HCC risk prediction model requires only

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