the conditions under which splenectomy can be performed to prevent the development of thrombocytopenia following LTx should be carefully defined. Analyzing the association between the extent of splenomegaly and thrombocytopenia in LTx recipients would provide important information in this respect.

In this study, we report an analysis of the relationship between preoperative spleen volume (SV) and blood cytopenia in 45 patients who underwent LTx at the Hiroshima University Hospital.

PATIENTS AND METHODS

Patients

Between January 2002 and May 2007, 83 LTx on 81 patients were underwent at the University of Hiroshima. Of these, 36 patients were excluded from the study because of death within 1 year (n = 13), fulminant hepatitis as the primary disease (n = 7), retransplantation (n = 2), splenectomy that had already been performed at LTx (n = 2), or insufficient clinical examinations (n = 12). The remaining 45 patients who had undergone LTx because of liver cirrhosis were analyzed. The profiles of these patients are shown in Table 1. Computed tomography was performed preoperatively and at 1 and 6 months after LTx. The hemoglobin (Hgb) levels and the serial white blood cell and PLT counts were obtained from the medical charts of the LTx recipients. The SV was measured from computed tomography images obtained with a workstation (Virtual Place Advance 300, AZE, Ltd.). The body surface area (BSA) was calculated as follows with the equation of Whitington et al.[14]:

$$BSA\ (m^2) = Body\ weight\ (kg)^{0.425} \times Body\ height\ (cm)^{0.725} \times 0.007184$$

In this study, the SV/BSA value was used as a parameter for assessing splenomegaly.

Statistical Analysis

The postoperative data were compared with an unpaired Student t test. The correlations between variables were assessed with the Spearman rank order correlation coefficient, and a P value < 0.05 was considered statistically significant. The data are expressed as mean ± standard deviation.

RESULTS

The extent of thrombocytopenia and splenomegaly prior to LTx varied in the 45 patients. This might reflect various degrees of liver cirrhosis. The PLT count ranged from $2.6 \times 10^4/mm^3$ to $18.0 \times 10^4/mm^3$, and the SV ranged from 98 to 1299 mL. The average PLT count and SV of the 45 patients before and after LTx are shown in Table 2. The PLT count was observed to increase significantly 1 month after LTx. However, no further increase was observed thereafter. In contrast, the SV values demonstrated a downward trend until 1 month after LTx and plateaued thereafter.

Because both the PLT count and SV stabilized at 1 month after LTx, we investigated the correlation be-

TABLE 1. Perioperative Clinical Characteristics of Liver Transplant Recipients

Number of patients	45
Gender (male/female)	26/19
Recipient age (years, mean ± SD)	54.5 ± 6.3
Donor age (years, mean ± SD)	34.4 ± 12.9
MELD score (mean ± SD)	13.8 ± 7.5
Blood loss (mL, mean ± SD)	4245 ± 3806
Graft weight/standard liver volume (%, mean ± SD)	51.0 ± 10.9
Spleen volume (cm^3, mean ± SD)	516 ± 304
SV/BSA (mean ± SD)	306.5 ± 177.6
Portal venous pressure (mm Hg, mean ± SD)	
Initial	22.9 ± 6.3
Closure	17.1 ± 6.3
WBC count (mean ± SD)	3901 ± 2097
Hgb (mean ± SD)	10.0 ± 1.4
T-Bil (mg/dL, mean ± SD)	6.0 ± 9.4
ALT (IU/L, mean ± SD)	40.8 ± 28.2
Platelet count ($\times 10^4/mm^3$, mean ± SD)	6.6 ± 3.0
Etiology of liver disease	
Alcoholic	4
Primary biliary cirrhosis	1
Autoimmune liver disease	3
Chronic hepatitis B	15
Chronic hepatitis C	18
Hepatocellular carcinoma	27
Other	3

Abbreviations: ALT, alanine aminotransferase; Hgb, hemoglobin; MELD, Model for End-Stage Liver Disease; SD, standard deviation; SV/BSA, spleen volume to body surface area ratio; T-Bil, total bilirubin; WBC, white blood cell.

TABLE 2. Changes in the Spleen Volume and Platelet Count After Liver Transplantation

Spleen volume (cm^3, mean ± SD)	
Before LTx	516 ± 304
1 month after LTx	421 ± 220
6 months after LTx	417 ± 212
Platelet count ($\times 10^4/mm^3$, mean ± SD)	
Before LTx	6.6 ± 3.0
1 month after LTx	12.4 ± 6.0
6 months after LTx	12.3 ± 5.7

Abbreviations: LTx, liver transplant; SD, standard deviation.

TABLE 3. Correlation Between Postoperative Thrombocytopenia and Clinical Variables

	Correlation Coefficient	*P* Value
Recipient age	0.20	0.180
Donor age	−0.09	0.574
MELD score	−0.05	0.745
Blood loss	−0.15	0.333
Graft weight/standard liver volume	0.38	0.010
Portal venous pressure		
Initial	−0.18	0.260
Closure	−0.26	0.101
Pre-LTx WBC count	0.37	0.012
Pre-LTx hemoglobin	−0.10	0.500
Pre-LTx PLT count	0.61	0.00001
Pre-LTx T-Bil	−0.24	0.111
Pre-LTx ALT	−0.13	0.400
SV/BSA	−0.67	0.000006

Abbreviations: ALT, alanine aminotransferase; LTx, liver transplant; MELD, Model for End-Stage Liver Disease; PLT, platelet; SV/BSA, spleen volume to body surface area ratio; T-Bil, total bilirubin; WBC, white blood cell.

tween the thrombocytopenia at 1 month after LTx and the perioperative clinical variables by a simple linear regression analysis. The PLT count at 1 month after LTx was clearly inversely related to the pre-LTx SV/BSA value and positively related to the PLT count prior to LTx (Table 3).

The relationship between the pre-LTx SV/BSA value and the PLT count at 1 month after LTx is shown in Fig. 1A. On the basis of the regression line, thrombocytopenia of less than 10×10^4 PLTs/mm^3 at 1 month after LTx could be expected in patients who demonstrated pre-LTx SV/BSA levels of >400. The patients were divided into 2 groups: those with a pre-LTx SV/BSA value < 400 (SV < 400 group; n = 33) and those with a pre-LTx SV/BSA value ≥ 400 (SV ≥ 400 group; n = 12). No significant differences were observed in the Hgb concentrations between the groups. The PLT count in the SV < 400 group significantly increased immediately after LTx and was maintained until 6 months. In contrast, during the observation period, the PLT count was maintained at a lower level and the SV was maintained at a high level in the SV ≥ 400 group ($P < 0.01$; Fig. 1). Thus, preoperative splenomegaly may influence the SV and PLT count at 1 and 6 months after LTx.

A plot of the PLT count before LTx versus the PLT count 1 month after LTx is shown in Fig. 2A. As indicated by the regression line, thrombocytopenia of $<10 \times 10^4$ PLTs/mm^3 at 1 month after LTx could be expected in patients who demonstrated pre-LTx PLT counts of less than 5×10^4/mm^3. The patients were divided into 2 groups: those in whom the PLT count prior to LTx was greater than 5×10^4/mm^3 (PLT > 50K group; n = 28) and those in whom the PLT count prior to LTx was less than or equal to 5×10^4/mm^3 (PLT ≤ 50K group; n = 17). During the observation period, the white blood cell and PLT counts in the PLT > 50K group were significantly higher than those in the PLT ≤ 50K group ($P < 0.05$ and $P < 0.01$, respectively). Furthermore, the SV in the PLT > 50K group was lower than that in the PLT ≤ 50K group ($P < 0.05$). Among the various immunosuppressants, inhibitors of nucleic acid synthesis such as mycophenolate mofetil and azathioprine possibly worsen thrombocytopenia.[15] In this study, 23, 7, 22, and 8 patients were orally administered mycophenolate mofetil within 6 months after LTx in the SV < 400 group, SV ≥ 400 group, PLT > 50K group, and PLT ≤ 50K group, respectively. The dosage of this immunosuppressant was not significantly different among the groups.

Thus, the pre-LTx values of both the SV/BSA level and the PLT count had a significant impact on the PLT count at 1 month after LTx. We further examined whether these factors mutually influence the PLT count at 1 month after LTx. The patients were categorized as follows: the PLT > 50K, SV < 400 group, which consisted of 26 patients without severe thrombocytopenia (pre-LTx PLT count > 5×10^4/mm^3) and with an SV/BSA value < 400; the PLT > 50K, SV ≥ 400 group, which consisted of 2 patients without severe thrombocytopenia and with an SV/BSA value ≥ 400; the PLT ≤ 50K, SV < 400 group, which consisted of 7 patients with severe thrombocytopenia (pre-LTx PLT count ≤ 5×10^4/mm^3) and an SV/BSA value < 400; and the PLT ≤ 50K, SV ≥ 400 group, which consisted of 10 patients with severe thrombocytopenia and an SV/BSA value ≥ 400 (Fig. 3A). The PLT > 50K, SV < 400 group did not suffer from severe splenomegaly, and their PLT count was 15.2 ± 6.2/mm^3 at 1 month after LTx (data not shown). The number of patients in the PLT > 50K, SV ≥ 400 group was too small for a meaningful analysis. The PLT ≤ 50K, SV ≥ 400 group suffered from splenomegaly, and their PLT count at 1 month after LTx was only 7.0 ± 2.1/mm^3. The PLT ≤ 50K, SV < 400 group did not suffer from splenomegaly, and their PLT count increased to 11.3 ± 3.0/mm^3 at 1 month after LTx. The PLT count in the PLT ≤ 50K, SV < 400 group was observed to be significantly elevated versus the PLT ≤ 50K, SV ≥ 400 group ($P = 0.005$; Fig. 3B). Thus, in LTx recipients without splenomegaly, the PLT count can be expected to increase shortly after the operation.

At our institute, preemptive IFN therapy for HCV-infected recipients has been practiced since 2005. We decided to administer preemptive IFN therapy to 9 HCV-infected recipients within 6 months after LTx. In 8 of the 9 HCV patients, neither pre-LTx splenomegaly (SV/BSA value ≥ 400) nor thrombocytopenia (PLT count ≤ 5×10^4/mm^3) existed. They were able to continuously receive IFN therapy without severe thrombocytopenia. In the remaining HCV patient, pre-LTx splenomegaly and thrombocytopenia coexisted. This particular patient suffered from persistent thrombocy-

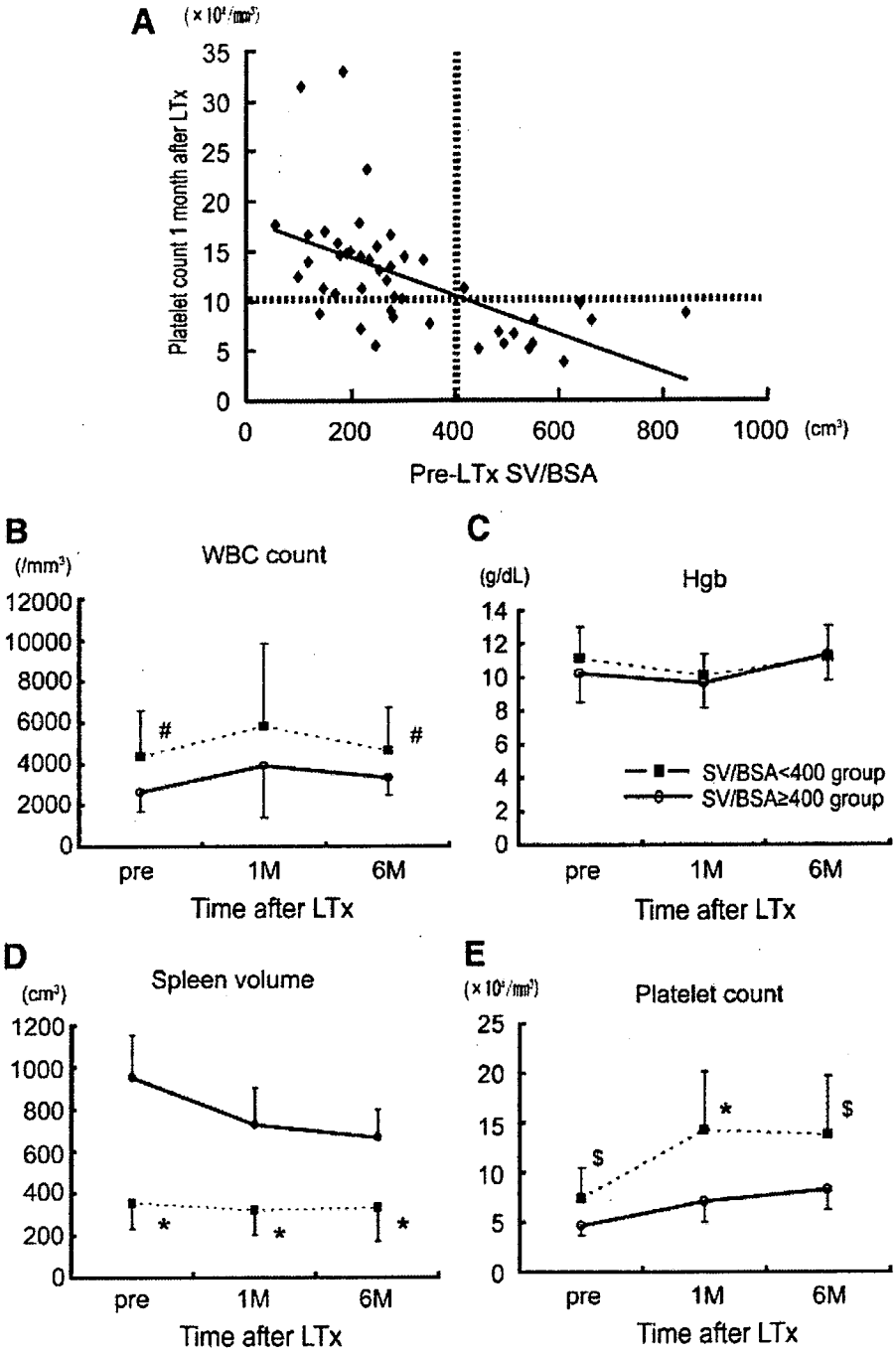

Figure 1. (A) Correlation between the pre-LTx SV/BSA value and PLT count at 1 month after LTx ($r = 0.67$, $P < 0.0001$). A regression line is superimposed on the plot: $y = -0.02x + 18.23$ (x axis: SV/BSA value; y axis: post-LTx PLT count at 1 month). Changes in (B) the WBC count, (C) hemoglobin concentration, (D) spleen volume, and (E) PLT count. The post-LTx values of these variables in the SV < 400 group (broken line with closed squares) and SV ≥ 400 group (thick line with open circles) are shown. There was a significant difference between the groups with respect to the WBC count, PLT count, and spleen volume ($^{\#}P < 0.05$, $^{\$}P < 0.01$, and $^{*}P < 0.001$ for the SV < 400 group versus the SV ≥ 400 group). Abbreviations: BSA, body surface area; Hgb, hemoglobin; LTx, liver transplant; PLT, platelet; SV, spleen volume; WBC, white blood cell.

topenia and eventually underwent splenectomy so that IFN therapy could be commenced only 9 months after LTx.

DISCUSSION

Thrombocytopenia is an extremely common complication in LTx patients. Several causes have been postulated for this reduced concentration of PLTs, including hypersplenism,[16,17] decreased thrombopoietin levels,[18,19] and destruction by anti-PLT antibodies.[20,21] It has also been reported that serum thrombopoietin levels or anti-PLT antibodies levels correlate with the spleen size.[22-24] This fact is consistent with the finding that the spleen size correlates with portal hypertension and the PLT count in patients with cirrhosis.[16] Our data also demonstrate that pre-LTx splenomegaly is associated with the pre-LTx PLT count. Uneventful LTx is expected to improve splenomegaly.[25,26] However, our data show that splenomegaly remained unchanged in

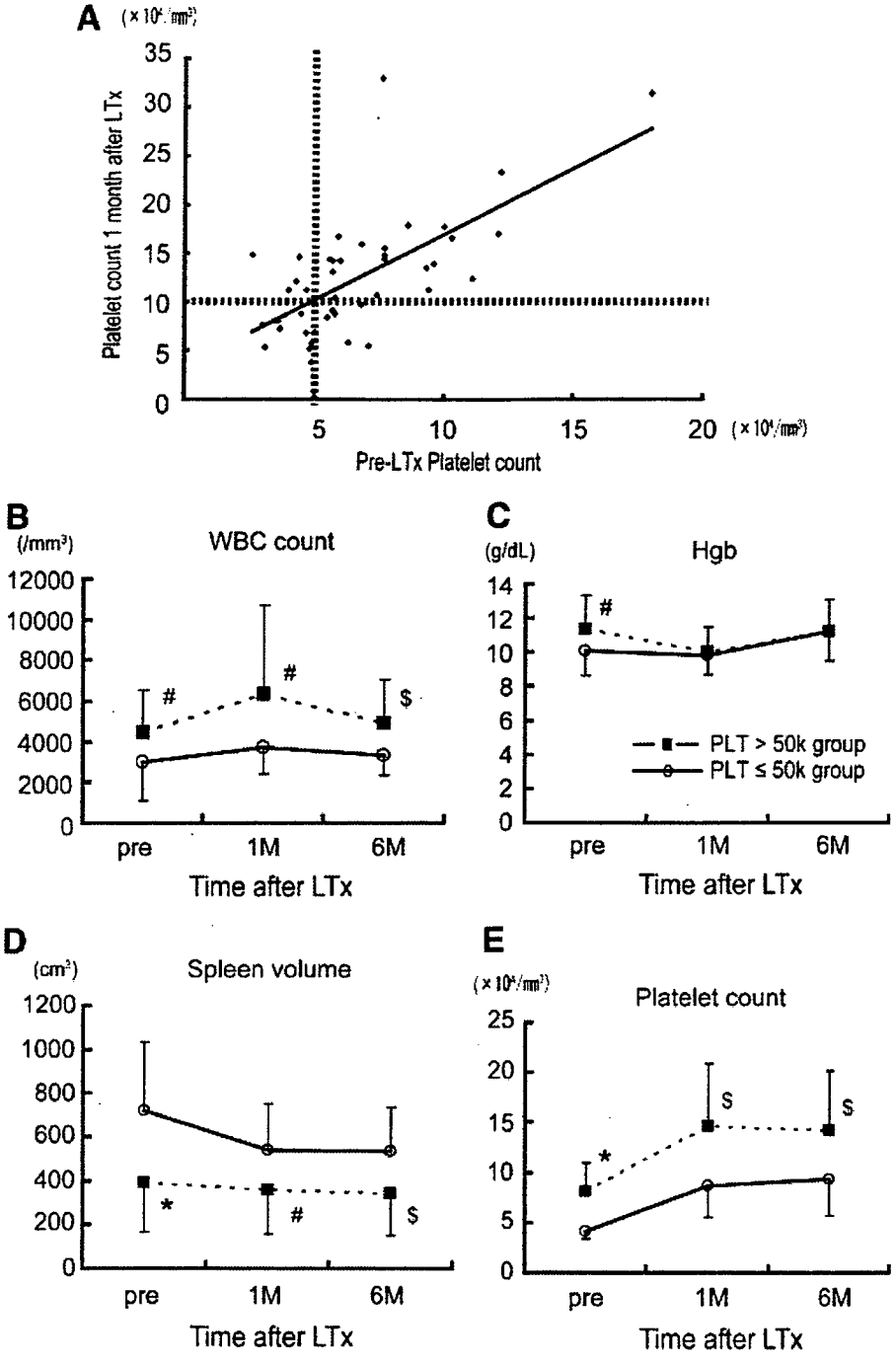

Figure 2. (A) Correlation between the pre-LTx PLT count and PLT count at 1 month after LTx ($r = 0.61$, $P < 0.0001$). A regression line is superimposed on the plot: $y = 1.35x + 3.48$ (x axis: pre-LTx PLT count; y axis: post-LTx PLT count at 1 month). Changes in (B) the WBC count, (C) hemoglobin concentrations, (D) spleen volume, and (E) PLT count. The values of these variables after LTx in the PLT > 50K group (broken line with closed squares) and PLT ≤ 50K group (thick line with open circles) are shown. There was a significant difference between the groups with respect to the WBC count, hemoglobin concentration, PLT count, and spleen volume ($^{\#}P < 0.05$, $^{\$}P < 0.01$, and $^{*}P < 0.001$ for the PLT > 50K group versus the PLT ≤ 50K group). Abbreviations: Hgb, hemoglobin; LTx, liver transplant; PLT, platelet; WBC, white blood cell.

LTx recipients whose pre-LTx SV/BSA level exceeded 400. Among the various perioperative clinical factors, the SV/BSA level was the most significant determinant of the PLT count after LTx. In the present analysis, the PLT count of patients with pre-LTx thrombocytopenia (PLT count ≤ $5 \times 10^4/mm^3$) increased significantly after LTx in the group with no pre-LTx splenomegaly (SV/BSA value < 400) versus the group with pre-LTx splenomegaly ($P < 0.01$).

We recently reported that splenectomy should be performed simultaneously with LTx in HCV patients with a PLT count < $6 \times 10^4/mm^3$ in order to complete preemptive IFN therapy at an earlier time point in the postoperative period.[27] Several authors have reported that the only indication for simultaneous splenectomy in LTx is the preoperative PLT count[12,28,29] because thrombocytopenia in the immediate posttransplant period is correlated with a low preoperative PLT count.[30]

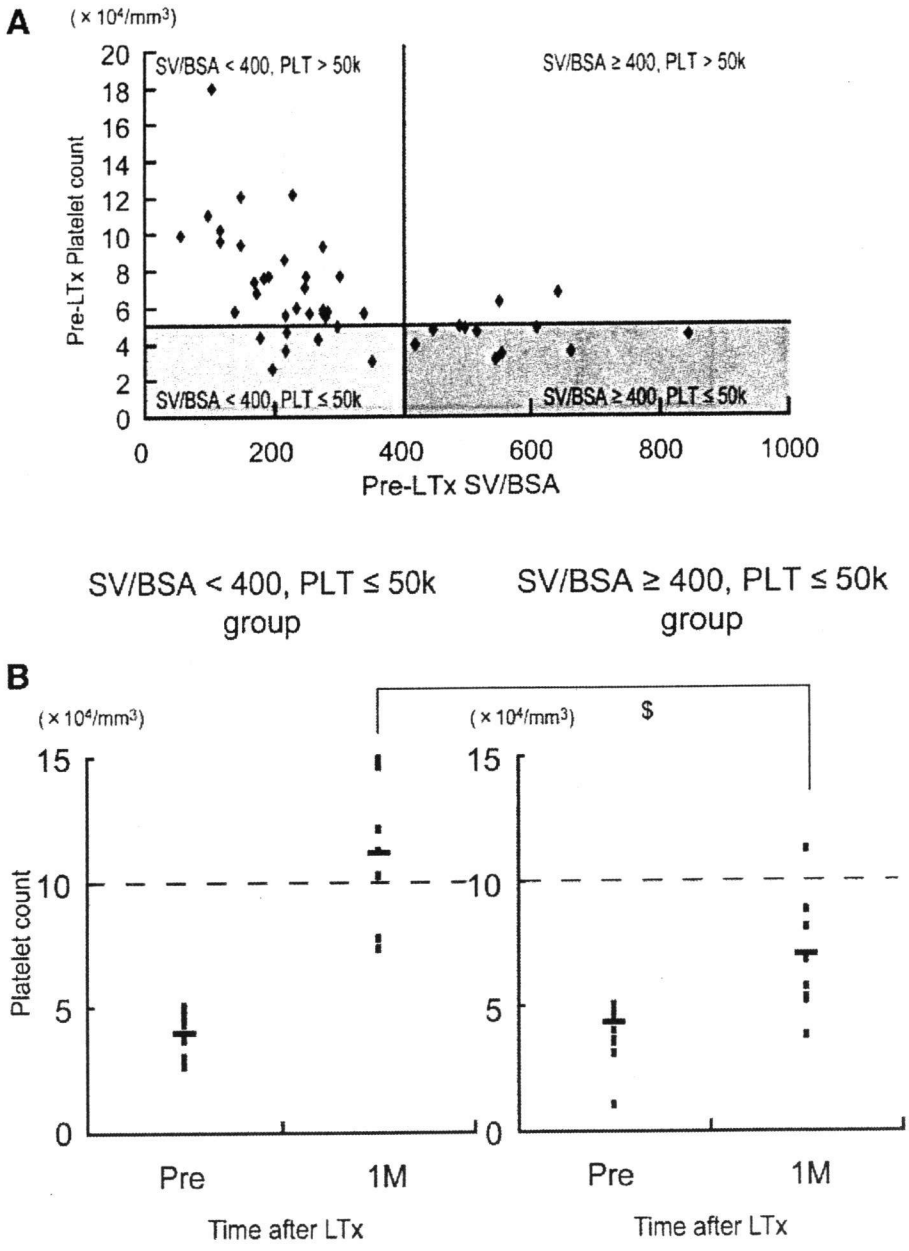

Figure 3. (A) Correlation between the pre-LTx SV/BSA value and pre-LTx PLT count. The patients were categorized as follows: the PLT > 50K, SV < 400 group, which consisted of patients without severe thrombocytopenia (pre-LTx PLT count > 5 × 10^4/mm^3) and without severe splenomegaly (pre-LTx SV/BSA level < 400); the PLT > 50K, SV ≥ 400 group, which consisted of patients without severe thrombocytopenia and with severe splenomegaly (pre-LTx SV/BSA value ≥ 400); the PLT ≤ 50K, SV < 400 group, which consisted of patients with severe thrombocytopenia (pre-LTx PLT count ≤ 5 × 10^4/mm^3) and without severe splenomegaly; and the PLT ≤ 50K, SV ≥ 400 group, which consisted of patients with severe thrombocytopenia and with severe splenomegaly. (B) Changes in the PLT count in the PLT ≤ 50K, SV < 400 group and PLT ≤ 50K, SV ≥ 400 group. The PLT count in the PLT ≤ 50K, SV < 400 group was significantly elevated versus that in the PLT ≤ 50K, SV ≥ 400 group ($^{\$}P < 0.01$). Abbreviations: BSA, body surface area; LTx, liver transplant; PLT, platelet; SV, spleen volume.

Studies have also reported that the routine administration of simultaneous splenectomy and LTx in all HCV patients conditions them for anti-HCV IFN therapy.[31] Although splenectomy strongly affects thrombocytopenia, it might predispose patients to develop portal vein thrombosis or increase the risk of sepsis, which is particularly lethal for immunosuppressed subjects.[32] Thus, caution is advised when recommending splenectomy for patients undergoing LTx. Compared with splenectomy, splenic artery ligation is a technically simpler procedure that can easily be included in a complicated transplant operation.[33] However, the benefit of splenic artery ligation in reducing posttransplant thrombocytopenia is controversial.[34,35] Recently, partial splenic embolization (PSE) has been described as a useful procedure for severe post-LTx thrombocytopenia,[36,37] and PSE could also be an option for pre-LTx.[38] However, several groups have reported complications generally observed after PSE, including splenic infarction, abscess formation, reduced immunity-related septic complications, and portal thrombosis.[39,40] Thus, the most appropriate methods among the strategies or alternative methods for avoiding persistent thrombocytopenia remain to be elucidated.

In conclusion, the pre-LTx SV/BSA value and PLT count have been correlated with post-LTx thrombocytopenia. If both splenomegaly and thrombocytopenia coexist (PLT count ≤ 5 × 10^4/mm^3 and SV/BSA

value ≥ 400), persistent thrombocytopenia is predictable after LTx.

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Evaluation of Patients with Esophageal Varices After Endoscopic Injection Sclerotherapy Using Multiplanar Reconstruction MDCT Images

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NAIDBS HUD- The purpose of our study was to assess the relationship between hemodynamic changes in portosystemic collaterals and the prognosis of patients with esophageal varices after endoscopic injection sclerotherapy using multiplanar reconstruction (MPR) MDCT images.

RT AIDBS R@MC L DS GNC R- The subjects of this prospective study were 53 patients who underwent endoscopic injection sclerotherapy for esophageal varices. We evaluated the reconstructed MPR images of portosystemic collaterals before and after endoscopic injection sclerotherapy. Patients were divided into three groups based on the rate of change in the diameter of the feeding vessel into complete eradication (group A), narrowing (group B), and no change (group C). We analyzed the relationship between hemodynamic change in portosystemic collaterals and prognosis.

QDRT KS R- The left gastric vein, posterior gastric vein, and left gastric vein plus posterior gastric vein were the main feeding vessels (n = 44 [83%] of patients, n = 5 [9%], and n = 4 [8%], respectively). The proportions of patients of groups A, B, and C were 19% (n = 10), 24% (n = 13), and 57% (n = 30), respectively. The relapse-free rates at 2 years after endoscopic injection sclerotherapy were 100%, 65%, and 52% in groups A, B, and C, respectively ($p < 0.05$). For group C, the relapse-free rate at 2 years after endoscopic injection sclerotherapy of patients with a large-diameter paraesophageal vein (≥ 3 mm, 63%) was significantly higher than in those with a small-diameter paraesophageal vein (< 3 mm, 36%; $p < 0.05$). However, there were no significant differences in the survival rate among the three groups.

BNMB KT RHNM- MPR MDCT images on portosystemic collaterals can accurately predict relapse of esophageal varices after endoscopic injection sclerotherapy.

Keywords: endoscopic injection sclerotherapy, esophageal varices, multiplanar reconstruction image, portosystemic collaterals, recurrence

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Hemorrhage from esophageal or gastric varices is one of the main causes of death in patients with liver cirrhosis. The reported prevalence of esophageal varices in patients with cirrhosis ranges from 80% to 90% [1, 2], and 10–30% of patients with esophageal varices per year develop variceal hemorrhage [3]. Despite substantial improvements in early diagnosis and treatment of variceal hemorrhage, the associated mortality remains high (20–35%) [4–6]. Therefore, proper management of esophageal varices could improve the prognosis of patients with liver cirrhosis. Although several treatment techniques for esophageal varices have been developed, including pharmacologic therapy [7, 8], transjugular intrahepatic portosystemic shunts [9], endoscopic sclerotherapy [10, 11], endoscopic ligation [12, 13], percutaneous transhepatic obliteration [14], and surgery [15], complete eradication of esophageal varices by endoscopic injection sclerotherapy is effective in preventing variceal hemorrhage and recurrence after therapy [16–19]. Because of the close relationship between the effects of endoscopic injection sclerotherapy and changes in hemodynamics in portosystemic collaterals, it is important to carefully assess the hemodynamics before beginning treatment.

The portal venous system is evaluated by invasive methods such as angiography and percutaneous transhepatic portography (PTP) [19]. However, these techniques do not visualize about 20–25% of varices-related portosystemic collaterals seen at endoscopy [20].

MDCT represents a major advancement in the field of diagnostic imaging because it provides a fast table speed and, when slices are combined, permits data collection that is well suited for workstation analysis. Multiplanar

reconstruction (MPR) MDCT images provide rapid assessment of portosystemic collaterals along different planes without losing information about the surrounding structures. MDCT can achieve rapid acquisition and higher longitudinal resolution than single-detector CT [21]. The use of MPR significantly improves the images of portosystemic collaterals and the sites of confluence compared with those obtained by axial CT [22, 23].

Noninvasive MDCT with MPR before endoscopic injection sclerotherapy for patients with esophageal varices provides detailed information on the hemodynamics of portosystemic collaterals [23]. Thus, MPR imaging potentially could be an important tool for evaluation of esophageal varices before other invasive imaging techniques, such as angiography and PTP, and thus the management and outcome of endoscopic injection sclerotherapy.

The aim of the present study was to evaluate the utility of MDCT with MPR for visualizing the portal venous system and to measure the long-term effect of endoscopic injection sclerotherapy on portosystemic collaterals, including the rate of relapse and overall prognosis.

Subjects and Methods

Patients

This study was approved by the institutional review board and was based on the Declaration of Helsinki as declared by the World Health Organization; all subjects gave informed consent. All patients were prospectively enrolled in this study and underwent endoscopy and MDCT. The endoscopy was performed by a single endoscopist in the presence of another endoscopist. The final assessment of the endoscopic findings was determined by agreement between the two endoscopists. MDCT findings were interpreted by two radiologists blinded to the clinical and endoscopic findings. The interobserver agreement between the radiologists and endoscopists was determined. The endoscopic findings of esophageal varices were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [24]. The form (F) of esophageal varices was classified as complete eradication after treatment (F0), small straight (F1), enlarged tortuous (F2), and large coiled-shaped (F3). The red color sign (RC) was also used in the present study based the criteria of the Japanese Society for Portal Hypertension and Esophageal Varices [24]. RC was defined as endoscopically detected dark-red-colored spots on the mucosa of the lower end of the esophagus. To evaluate the risk of hemorrhage and provide a rough estimate of intravascular pressure within the esophageal varices, RC was classified into four grades: RC0, no mucosal coloring; RC1, a few localized red-colored spots: RC2, between RC1 and RC3; and RC3, several mucosal red-colored spots throughout the circumference of the lower end of the esophagus.

Seventy-two consecutive patients with esophageal varices underwent endoscopic therapy at Hiroshima University Hospital between January 2002 and December 2006. The inclusion criteria were as follows: esophageal varices evaluated as F2 or F3 or RC on endoscopy according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [24], Child-Pugh classification of grade A or B, performance status of grade 0 or 1, absence of tumor thrombus in portal vein trunk, and absence of refractory ascites. Of those patients, five were excluded because they preferred to undergo endoscopic ligation for esophageal varices rather than endoscopic injection sclerotherapy. We also excluded two patients who had a tumor thrombus in the portal vein trunk. Two other patients who had refractory ascites also were excluded. Three patients were excluded because they refused to enroll in the study and sign a consent form. Therefore, 60 patients were included in this study. We defined relapse of esophageal varices as the primary end point. Seven patients who showed lack of complete eradication of esophageal varices on endoscopy were also excluded because relapse could not be recognized in these patients without endoscopically confirmed eradication of esophageal varices. After exclusion of those patients, data of the remaining 53 patients were analyzed for this study.

Endoscopic injection sclerotherapy resulted in evaluation of F0 on endoscopy in all 53 patients. The endoscopic findings of esophageal varices were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [24]. Table 1 lists the clinical characteristics of patients. They consisted of 42 men and 11 women with an age range of 30–84 years (mean age [± SD], 60 ± 11 years). The cause of liver cirrhosis was hepatitis B virus infection (n = 9), hepatitis C virus infection (n = 34), alcohol abuse (n = 4), and other causes (n = 6). The severity of liver dysfunction before treatment was evaluated according to Child's classification as A in 18 patients and B in 35 patients. Endoscopic findings of esophageal varices before treatment were evaluated as F1 in nine patients, F2 in 30 patients, and F3 in 14 patients as well as RC0 in 10 patients, RC1 in 11 patients, and RC2 in 32 patients.

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Characteristic	Value
Sex	
M	42
F	11
Age range (y) (mean ± SD)	30–84 (60 ±11)
Cause	
Hepatitis B virus	9
Hepatitis C virus	34
Alcohol	4
Other	6
Child-Pugh classification	
A	18
B	35
Variceal size	
F1	9
F2	30
F3	14
Red color sign	
RC0	10
RC1	11
RC2	32
RC3	0

Note—The red color sign (RC) was defined as endoscopically detected dark-red-colored spots on the mucosa of the lower end of the esophagus. To evaluate the risk of hemorrhage and provide a rough estimate of intravascular pressure within the esophageal varices, RC was classified into four grades: RC0, no mucosal coloring; RC1, a few localized red-colored spots; RC2, between RC1 and RC3; RC3, several mucosal red-colored spots throughout the circumference of the lower end of the esophagus. The form (F) of esophageal varices was classified as small straight (F1), enlarged tortuous (F2), and large coiled-shaped (F3) according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [24].

Endoscopic Injection Sclerotherapy Procedure

The concept of our endoscopic injection sclerotherapy technique is embolization of feeding vessels of esophageal varices within portosystemic collaterals by injecting a sclerosing agent. Before endoscopic injection sclerotherapy, each patient was premedicated with an intramuscular injection of 0.5% atropine sulfate, 15–30 mg of pentazocine, and 7.5 mg of timepidium bromide. Lidocaine jelly or spray was applied to the pharyngeal area as a topical anesthetic. A balloon, referred to as the oral side balloon in this study, was attached to the tip of an endoscope (GIF-XQ 240, Olympus) and inflated as the contrast medium (iopamidol)

was injected to prevent the sclerosant (5% ethanolamine oleate) from flowing out of the varices into the systemic circulation. After the start of injection of the sclerosant into the varices, the flow of the sclerosant was monitored using x-ray fluoroscopy. The injection of the sclerosant was stopped just as it filled the portosystemic collaterals. However, embolization of the feeder could not be achieved when variceal puncture or accidental retraction of the needle from the varices occurred during injection of the sclerosant. Puncture needles ranged in size from 23 to 25 gauge according to the size of the pore for the biopsy forceps of the endoscope. This treatment was repeated with an interval of 1–2 weeks, and 2–4 sessions were needed to complete one series of treatment and eliminate all varices. Additional treatment with aethoxysclerol was applied at F0 on the basis of the endoscopic findings.

CT Examination

CT was first performed unenhanced to define the liver location, followed by injection of the contrast medium. For the latter, 100 mL of iopamidol 300 (Iopamiron 300, Schering) heated to 37°C was injected using a power injector (Auto Enhance A-250, Nemoto-Kyorindo), at a rate of 4.0 mL/s through a 22-gauge IV catheter inserted into an antecubital vein. Four sets of images were acquired in a craniocaudal direction at 20, 40, 65, and 180 seconds after initiation of contrast medium injection. The first and second acquisitions were used for hepatic artery phase images, the third acquisition for portal venous phase images, and the fourth acquisition for hepatic venous phase images. The third set of images was obtained during 20-second breath-holding, whereas those of other acquisitions were achieved during 10-second breath-holding. This protocol is used routinely in all patients with chronic liver diseases at our institution, and the data of the third acquisition are used for construction of 3D images of the portosystemic collaterals. All scanning was performed using a LightSpeed QX/i CT scanner (GE Healthcare). Specific scanning parameters vary among various scanners and are selected for imaging the details of vascular anatomy. We used the high-quality scanning mode, 1.25-mm slice thickness, and reconstruction intervals of 0.625-mm for portal venous phase images. MDCT was performed with Virtual Place Advance (AZE Ltd.) [25]. There are currently three reformatting techniques available. MPR was used for image reconstruction in this study. In every patient, CT was performed within 1 month before endoscopic injection sclerotherapy and after the final session of endoscopic injection sclerotherapy (median, 29 days; range, 25–34 days).

Evaluation of Portosystemic Collaterals

Portosystemic collaterals were independently assessed on MPR MDCT images before and after endoscopic injection sclerotherapy for esophageal varices by two radiologists (one with 17 and the other with 35 years of experience) who were blinded to the clinical and endoscopic results of endoscopic injection sclerotherapy. The diameter of the main portosystemic collateral vessel, such as the left gastric vein, posterior gastric vein, and paraesophageal vein, before and after endoscopic injection sclerotherapy was measured. The thickest portion of the vessel was measured in all cases. For assessment of changes of the feeding vessel after endoscopic injection sclerotherapy, we used the rate of reduction, which was calculated using the following formula: rate of reduction of the diameter of the feeding vessel (%) = [(diameter of feeding vessel before endoscopic injection sclerotherapy − diameter of feeding vessel after endoscopic injection sclerotherapy) / diameter of feeding vessel before endoscopic injection sclerotherapy] × 100.

Patients were divided into three groups according to the rate of reduction of the diameter of the feeding vessel. Patients with a reduction rate of ≥ 80% were classified as group A (complete eradication group), those with a rate of < 80% but > 40% were classified as group B (narrowing group), and those with a rate of ≤ 40% were classified as group C (no change group). Patients who showed no enhancement of the feeding vessel on MDCT were defined as group A. Moreover, on the basis of the diameter of the paraesophageal vein, which is the draining vessel of esophageal varices, patients were divided into two groups; the large paraesophageal vein group (≥ 3 mm) and the small paraesophageal vein group (< 3 mm). The cutoff diameter of 3 mm represents the median value of the paraesophageal vein. Patients with a paraesophageal vein that was too narrow to be recognized on MDCT were classified in the small paraesophageal vein group.

Follow-Up Study

Relapse after endoscopic injection sclerotherapy was assessed by endoscopy. Follow-up endoscopy was performed at 6-month intervals after treatment. Esophageal varices were evaluated independently at endoscopy by two endoscopists (one with 10 and the other with 15 years of experience). The endoscopic findings of esophageal varices were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [24]. A final decision regarding the endoscopic finding was reached by consensus. The appearance of RC1, F1, or bleeding on follow-up endoscopy was regarded as a relapse of esophageal varices. In this prospective study, we defined relapse of esophageal varices as the primary end point and survival as the secondary end point. Data were analyzed in October 2007. The relationship between hemodynamic changes in portosystemic collaterals and prognosis of endoscopic injection sclerotherapy for esophageal varices was analyzed by the results of MPR MDCT images.

Statistical Analysis

All data of portosystemic collaterals are expressed as mean ± SD or median values. The cumulative relapse-free rate and cumulative survival rate among groups by rate of reduction after treatment were determined using the Kaplan-Meier method and statistical software (JMP, version 5, SAS Institute Japan). Significance was tested using the generalized Wilcoxon's test and Student's *t* test. A p value of less than 0.05 was regarded as significant.

Results

Portosystemic Collaterals Before Endoscopic Injection Sclerotherapy

Portosystemic collaterals were recognized on MPR MDCT images of all patients with esophageal varices. Table 2 summarizes the portosystemic collaterals evaluated on MPR MDCT images. The left gastric vein, posterior gastric vein, and left gastric vein plus posterior gastric vein were the main feeding vessels for esophageal varices in 83%, 9%, and 8% of patients, respectively. The largest mean diameter of the main feeding vessel was for the left gastric vein followed by the

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Portosystemic Collateral	No. (%)	Diameter (mm)
Left gastric vein	44 (83)	6.6 ± 2.4 (3.3–15)
Posterior gastric vein	5 (9)	4.1 ± 1.7 (2.7–7.5)
Left gastric vein and posterior gastric vein	4 (8)	6.0 ± 2.3 (2.7–10.3)
Paraesophageal vein	46 (87)	3.8 ± 1.8 (1.8–8.8)
Gastrorenal shunt	43 (81)	3.7 ± 1.4 (1.7–5.2)

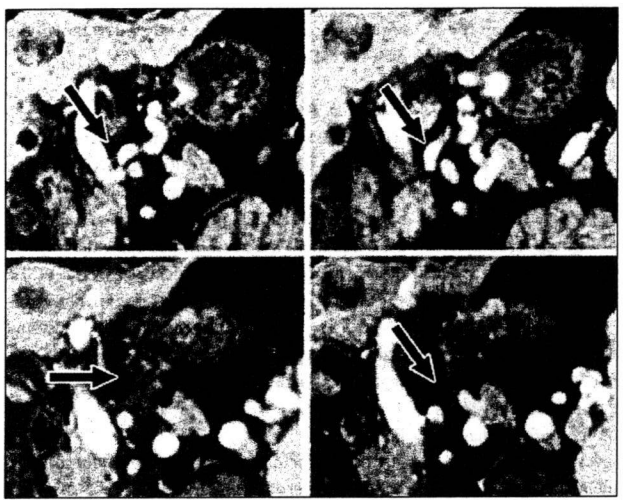
Note—Data for diameter are given as mean ± SD with range in parentheses.

posterior gastric vein. The left gastric vein was recognized as the portosystemic collateral for esophageal varices in 91% of the patients (83% of the patients with left gastric vein alone and 8% with left gastric vein plus posterior gastric vein) (Table 2). Furthermore, paraesophageal vein and gastrorenal shunts were found in 87% and 81% of patients, respectively, each with a mean of diameter of 4 mm.

With regard to the relationship between endoscopic findings and portosystemic collaterals, the mean diameter of portosystemic collaterals measured on MPR images was 5.7, 6.4, and 6.6 mm for F1, F2, and F3 esophageal varices, respectively. The portosystemic collaterals tended to be thicker, with higher levels of esophageal varices development. The gastrorenal shunt was also identified as the portosystemic collateral vein, with a median diameter of 3.7 mm. However, there were no differences in relapse-free rates related to the presence or absence or size of the gastrorenal shunt.

Effect of Endoscopic Injection Sclerotherapy on Portosystemic Collaterals

Analysis of the rate of diameter change of portosystemic collaterals after endoscopic injection sclerotherapy allowed classification of patients into groups A (n = 10), B (n = 13), and C (n = 30). Figure 1 shows typical MPR images of portosystemic collaterals of representative patients of the three groups. Figure 1A shows typical MPR images of portosystemic collaterals of a patient from group A, with complete eradication of the left gastric vein after endoscopic injection sclerotherapy. The diameter of the left gastric vein before and after endoscopic injection sclerotherapy was 5 and 0 mm, respectively, with a rate of left gastric vein diameter reduction of 100%. Figure 1B shows typical MPR images of portosystemic collaterals of a patient from group B, with narrowing of the left gastric vein after endoscopic injection sclerotherapy. The diameter of the left gastric vein before and after endoscopic injection sclerotherapy was 5.5 and 3.0 mm, respectively, with a rate of left gastric vein diameter reduction of 46%. Figure 1C shows typical MPR images of portosystemic collaterals of a patient from group C, with no change in the diameter of the left gastric vein after endoscopic injection sclerotherapy. The diameter of the left gastric vein before and after endoscopic injection sclerotherapy was 10 and 10 mm, respectively, with a rate of left gastric vein diameter reduction of 0%.



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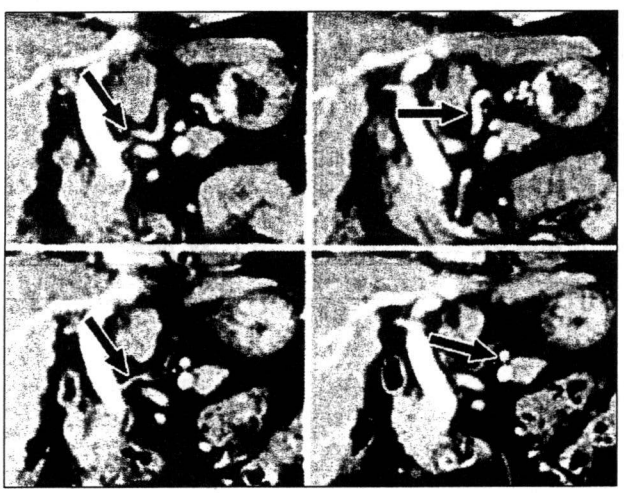

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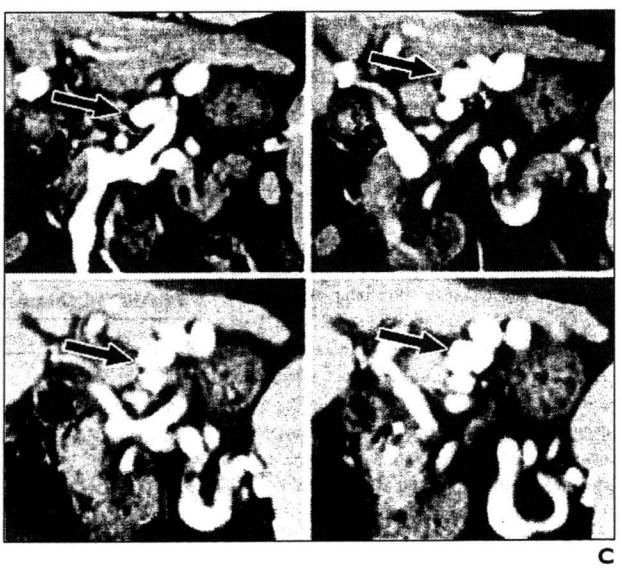

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Fig. 1—Patients with esophageal varices who underwent endoscopic injection sclerotherapy.
A–C, Typical multiplanar reconstruction MDCT images of portosystemic collaterals in representative patients of groups A (**A**), B (**B**), and C (**C**) show left gastric vein (*arrow*). See text for definition of each group. Images on top row are before endoscopic injection sclerotherapy and images on bottom row are after endoscopic injection sclerotherapy.

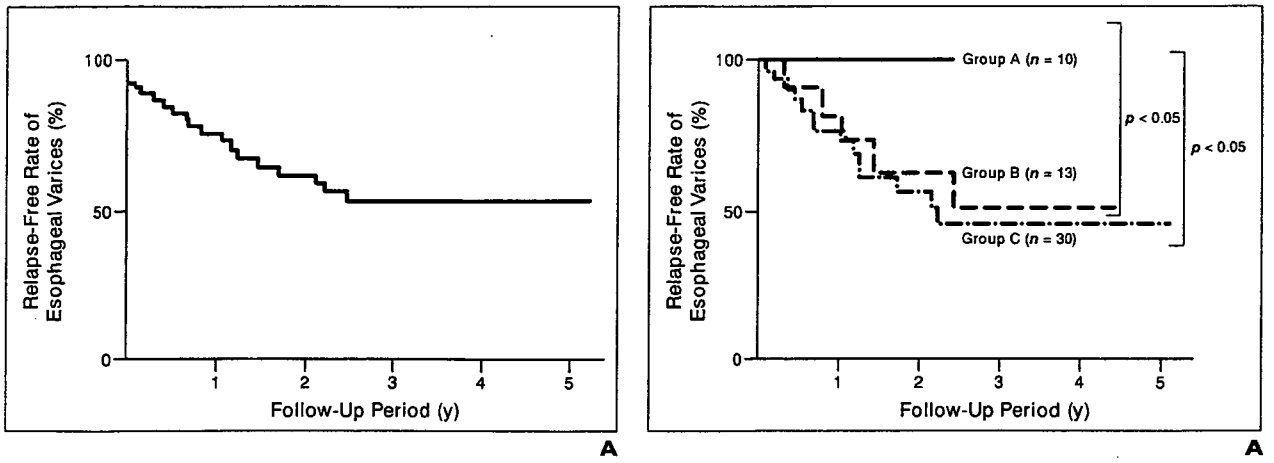


Fig. 2—Cumulative relapse-free rates of esophageal varices after sclerotherapy.
A, Graph shows cumulative relapse-free rates of esophageal varices after sclerotherapy for all patients.
B, Graph shows cumulative relapse-free rates of esophageal varices according to rate of reduction of portosystemic circulation after sclerotherapy.

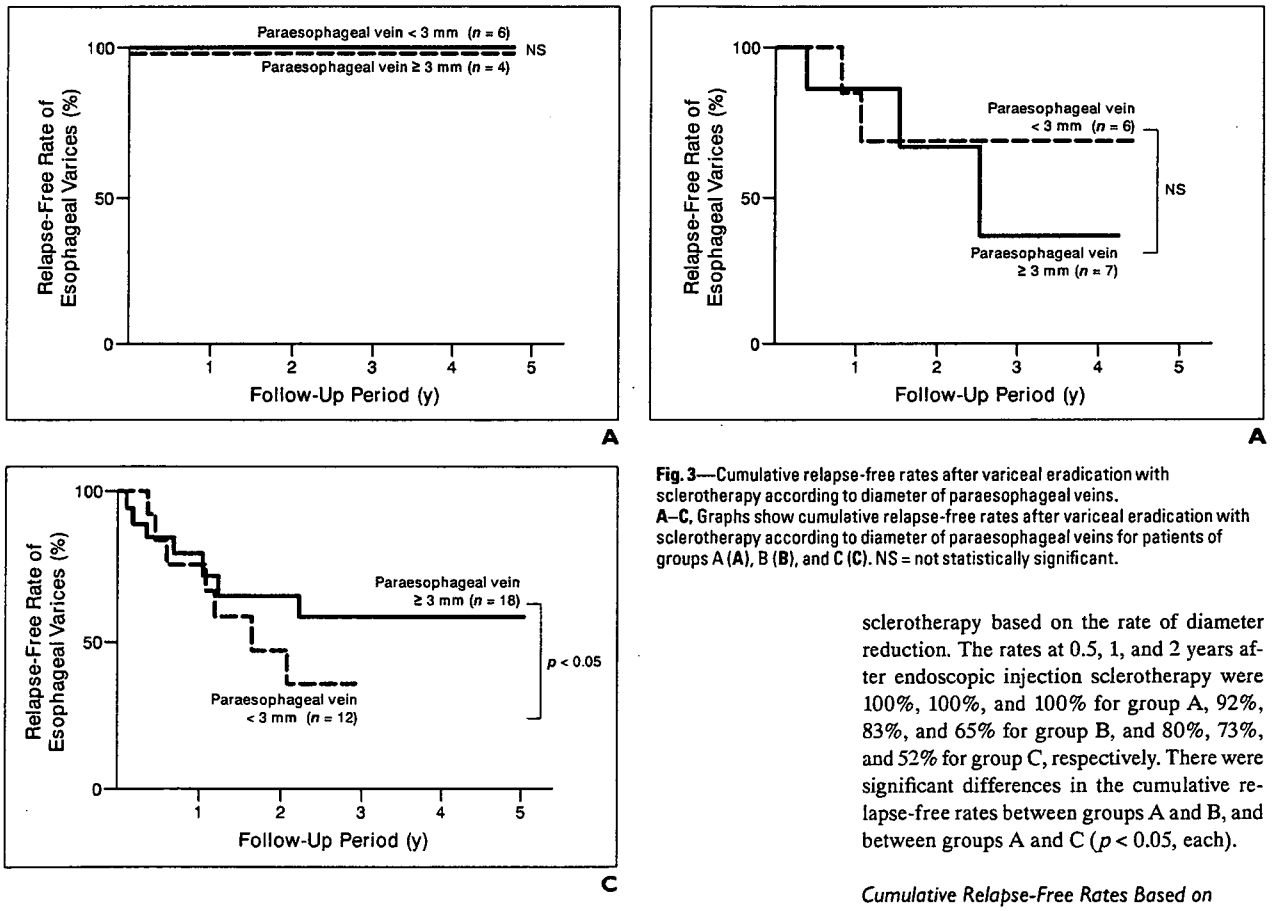


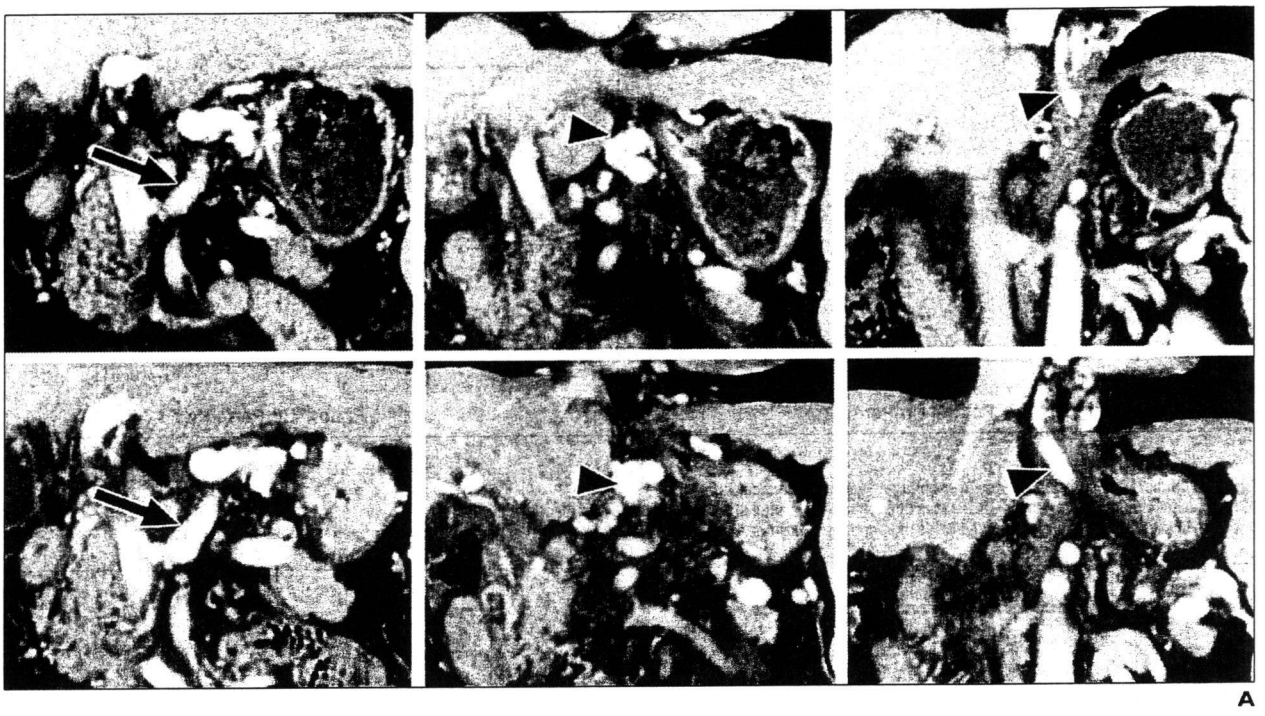
Fig. 3—Cumulative relapse-free rates after variceal eradication with sclerotherapy according to diameter of paraesophageal veins.
A–C, Graphs show cumulative relapse-free rates after variceal eradication with sclerotherapy according to diameter of paraesophageal veins for patients of groups A (**A**), B (**B**), and C (**C**). NS = not statistically significant.

Cumulative Relapse-Free Rates

For all patients, the cumulative relapse-free rates after endoscopic injection sclerotherapy were 87%, 81%, and 61% at 0.5, 1, and 2 years after treatment, respectively (Fig. 2A). The median follow-up period was 22 months. Figure 2B shows the cumulative relapse-free rates after endoscopic injection sclerotherapy based on the rate of diameter reduction. The rates at 0.5, 1, and 2 years after endoscopic injection sclerotherapy were 100%, 100%, and 100% for group A, 92%, 83%, and 65% for group B, and 80%, 73%, and 52% for group C, respectively. There were significant differences in the cumulative relapse-free rates between groups A and B, and between groups A and C ($p < 0.05$, each).

Cumulative Relapse-Free Rates Based on Paraesophageal Vein Diameter

Patients of groups A, B, and C were also divided into large and small paraesophageal vein groups using a cutoff diameter of 3 mm.



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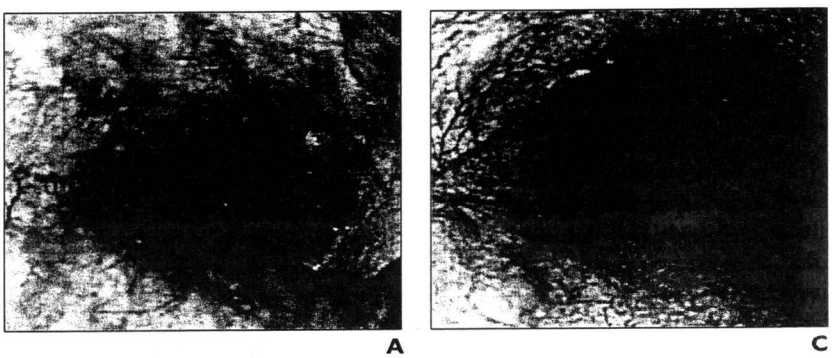


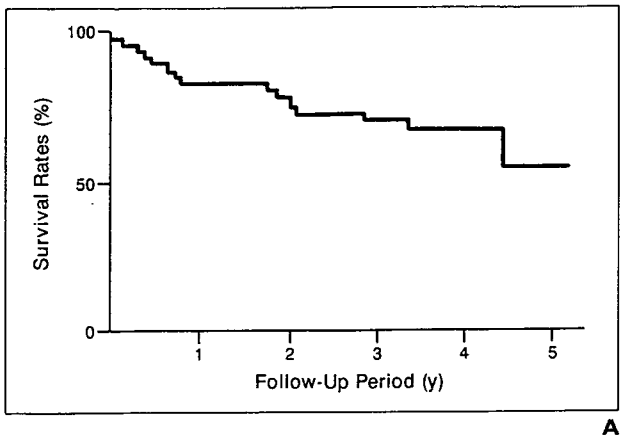
A C

Fig. 4—56-year-old man with Child classification B hepatitis C virus–related liver cirrhosis and esophageal varices.
A, Multiplanar reconstruction MDCT images of portosystemic collaterals of this patient (from group C) show large left gastric vein (*arrow*) and large paraesophageal vein (*arrowhead*). Images on top row are before endoscopic injection sclerotherapy and images on bottom row are after endoscopic injection sclerotherapy.
B and **C,** Endoscopic findings in same patient show esophageal varices classified as F2RC1 before endoscopic injection sclerotherapy (**B**) and F0RC0 1 year after endoscopic injection sclerotherapy (**C**).

There were no significant differences in the cumulative relapse-free rates between the large paraesophageal vein group and the small paraesophageal vein group after endoscopic injection sclerotherapy for groups A and B (Figs. 3A and 3B). However, for group C, the cumulative relapse-free rates at 0.5, 1, and 2 years after endoscopic injection sclerotherapy were 83%, 78%, and 63% for the large paraesophageal vein group and 75%, 67%, and 36% for the small paraesophageal vein group, respectively (Fig. 3C). Thus, the diameter of the paraesophageal vein significantly influenced the cumulative relapse-free rates only in those patients who showed < 40% reduction in the diameter of feeding vessels after endoscopic injection sclerotherapy ($p < 0.05$). Figure 4 shows the MDCT and endoscopic findings of a representative patient from group C with a large paraesophageal vein. Figure 4 contains images of both before and after endoscopic injection sclerotherapy. Although insufficient embolization of the feeding vessels was evident after endoscopic injection sclerotherapy on the MPR MDCT images, esophageal varices relapse was not recognized on endoscopy 1 year after endoscopic injection sclerotherapy.

Survival Rates

The cumulative survival rates after endoscopic injection sclerotherapy were 91%, 83%, and 78% at 0.5, 1, and 2 years after endoscopic injection sclerotherapy, respectively (Fig. 5A). In this study, the main cause of death was hepatocellular carcinoma (HCC) (60%). The cumulative survival rates after endoscopic injection sclerotherapy according to the rate of reduction of the diameter of feeding vessels (groups A–C) are shown in Figure 5B. There were no significant differences among the three groups with regard to the cumulative survival rates after endoscopic injection sclerotherapy. On the other hand, the cumulative survival rates after endoscopic injection sclerotherapy of patients with HCC (90%, 74%, and 62% at 0.5, 1, and 2 years, respectively) were significantly lower than those of patients without HCC (96%, 96%, and 96%, respectively; $p < 0.05$) (Fig. 5C).



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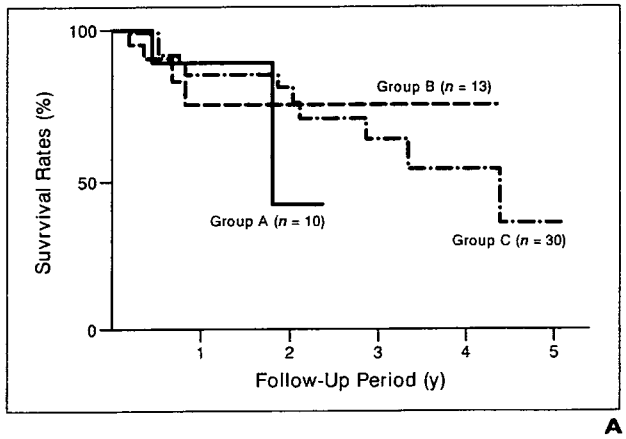

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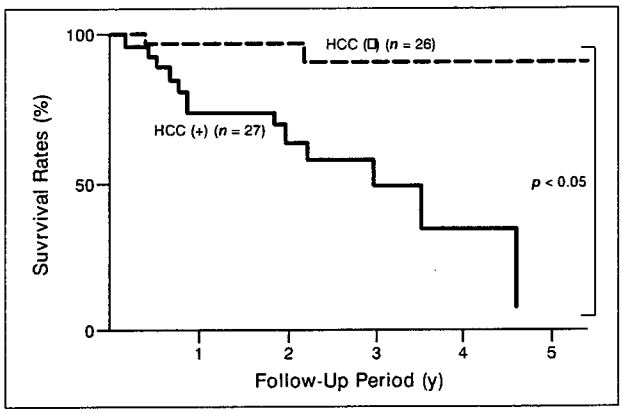

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Fig. 5—Cumulative survival rates of patients with esophageal varices after sclerotherapy.
A–C, Graphs show cumulative survival rates of patients with esophageal varices after sclerotherapy for all patients (**A**), patients of groups A, B, and C (**B**), and patients with and without hepatocellular carcinoma (HCC)(**C**). HCC (+) = patients with HCC, HCC (–) = patients without HCC.

Discussion

Esophageal varices, which are present in most patients with liver cirrhosis or portal hypertension, are located within the wall of the lower esophagus, whereas paraesophageal veins are situated outside the wall of the esophagus. These vessels are supplied primarily by the left gastric vein, which divides into anterior and posterior branches. The anterior branch supplies the esophageal varices, and the posterior branch forms the paraesophageal vein [23]. Endoscopic therapy, such as endoscopic injection sclerotherapy and endoscopic ligation, is now a well-accepted procedure for the control and prevention of bleeding from esophageal varices. Whereas endoscopic injection sclerotherapy is performed by intravariceal injection of 5% ethanolamine oleate with iopamidol, endoscopic ligation is performed using a ligation ring, as described by Van Stiegmann and Goff [26]. Endoscopic ligation obliterates mucosal and submucosal varices but not the perforating veins or feeding veins. Furthermore, the procedure does not alter portosystemic hemodynamics. It is reported that endoscopic injection sclerotherapy results in complete eradication of esophageal varices and minimizes the likelihood of recurrence and variceal hemorrhage after therapy [27]. In this regard, it is important to achieve not only endoscopic eradication of esophageal varices but also embolization of the feeding vessels supplied by the portal venous system. In this context, the observed differences in the effects of endoscopic injection sclerotherapy depend on anatomic variability in the portal venous system [28]. Therefore, detailed evaluation of portosystemic collaterals is important before endoscopic injection sclerotherapy.

Conventional CT (i.e., nonhelical CT and single-detector helical CT) provides less information about vascular anatomy of the lower esophagus and upper stomach in patients with esophageal varices compared with axial images or reconstruction images using MDCT. Angiography and PTP are considered the leading techniques in evaluation of vascular anatomy of the lower esophagus and upper stomach in patients with portal hypertension. However, these techniques are more invasive and time-consuming than CT. Recently, MDCT has become more useful for examining the whole portosystemic shunt because of advances in biotechnology and software development. MPR images using MDCT allow rapid assessment of portosystemic collaterals along different planes without losing information about the surrounding structures [22, 23]. Therefore, we evaluated portosystemic collaterals using MPR MDCT images and investigated the relationships between changes in dynamics after endoscopic injection sclerotherapy and clinical course.

In the treatment of esophageal varices by endoscopic injection sclerotherapy, there is a close relationship between the degree of eradication of the feeding vessel and recurrence of esophageal varices. Previous reports showed that the relapse rate in patients who underwent adequate embolization of the feeding vessel to the varices was significantly lower than the rates in those with inadequate embolization [11, 19, 29]. The results of our prospective study using MPR MDCT images emphasize the importance of sufficient eradication of the feeding vessels.

The relapse rate of esophageal varices is higher in patients with inadequate eradication of the feeding vessels than in those with adequate eradication. However, in patients with insufficient embolization of the feeding vessel, such as patients from group C in the present study, the relapse rate of esophageal varices in patients with a large-diameter

paraesophageal vein was significantly lower than that for patients with a small-diameter paraesophageal vein (Fig. 3).

Because of these results, we think that the presence of a large paraesophageal vein enhances vein drainage because flow is from the left gastric vein to the paraesophageal vein, thus making relapse of esophageal varices a less likely event in patients with complete eradication of esophageal varices alone. For esophageal varices with a large paraesophageal vein, even obliteration of esophageal varices on the esophageal mucosa alone, without obliteration of the feeding vessel, might provide a favorable esophageal varices relapse-free rate. In addition, endoscopic ligation, which does not influence the feeding vessel, might result in the same outcome for these esophageal varices. On the other hand, the development of other portosystemic collaterals, such as a gastrorenal shunt, was unrelated to relapse of esophageal varices because those shunts did not always communicate with the left gastric vein and did not enhance drainage of esophageal varices.

We found a close relationship between the outcome of endoscopic injection sclerotherapy for esophageal varices and variability of portosystemic collaterals. MPR MDCT images of portosystemic collaterals before endoscopic injection sclerotherapy are useful for predicting the outcome and might provide useful information for selecting the treatment technique for esophageal varices, such as endoscopic injection sclerotherapy or endoscopic ligation. This issue should be further investigated.

Our results also showed that embolization of the feeding vessel in endoscopic injection sclerotherapy does not always result in improvement of survival rates (Fig. 5). The main cause of death was HCC, and death due to cancer amounted to 60% of deaths in total. In fact, no patients died after rupture of esophageal varices in our study. Because most patients with esophageal varices have liver cirrhosis or HCC, survival depends on the severity of chronic liver disease or the stage of malignancy. Although endoscopic injection sclerotherapy cannot improve prognosis of patients with esophageal varices, we consider the procedure a method that prevents variceal hemorrhage and variceal hemorrhage-related death.

Although MPR MDCT images are of high quality, this technology has several limitations. MDCT requires skilled techniques to obtain adequate source data after bolus IV injection of the contrast material. Enhancement of the portal vein depends considerably on the patient's physique. A large number of source images may be produced, requiring an expensive high-power workstation to handle these data sets. The pulsation artifacts of the heart and aorta also affect visualization of the esophageal varices on MDCT and may reduce image quality. Furthermore, MDCT is not suitable for patients with renal dysfunction [30]. In this regard, a number of studies have evaluated portosystemic collaterals by using endoscopic sonography. This technique is more useful for estimation of the paraesophageal vein and perforating veins around the esophagus measuring < 2 mm in diameter and blood flow than that of MPR images [13, 27, 31]. The combination of MPR MDCT images and endoscopic sonography before endoscopic injection sclerotherapy may provide more useful information. Despite these limitations, evaluation of portosystemic collaterals by MPR images provides important information regarding the prediction of relapse of esophageal varices.

In conclusion, MPR imaging using MDCT provides excellent visualization of portosystemic collateral circulation. Accurate evaluation using MPR MDCT images should help predict esophageal varices relapse after endoscopic injection sclerotherapy.

Acknowledgments

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Differential MicroRNA Expression Between Hepatitis B and Hepatitis C Leading Disease Progression to Hepatocellular Carcinoma

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MicroRNA (miRNA) plays an important role in the pathology of various diseases, including infection and cancer. Using real-time polymerase chain reaction, we measured the expression of 188 miRNAs in liver tissues obtained from 12 patients with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) and 14 patients with hepatitis C virus (HCV)-related HCC, including background liver tissues and normal liver tissues obtained from nine patients. Global gene expression in the same tissues was analyzed via complementary DNA microarray to examine whether the differentially expressed miRNAs could regulate their target genes. Detailed analysis of the differentially expressed miRNA revealed two types of miRNA, one associated with HBV and HCV infections (n = 19), the other with the stage of liver disease (n = 31). Pathway analysis of targeted genes using infection-associated miRNAs revealed that the pathways related to cell death, DNA damage, recombination, and signal transduction were activated in HBV-infected liver, and those related to immune response, antigen presentation, cell cycle, proteasome, and lipid metabolism were activated in HCV-infected liver. The differences in the expression of infection-associated miRNAs in the liver correlated significantly with those observed in Huh7.5 cells in which infectious HBV or HCV clones replicated. Out of the 31 miRNAs associated with disease state, 17 were down-regulated in HCC, which up-regulated cancer-associated pathways such as cell cycle, adhesion, proteolysis, transcription, and translation; 6 miRNAs were up-regulated in HCC, which down-regulated anti-tumor immune response. *Conclusion:* miRNAs are important mediators of HBV and HCV infection as well as liver disease progression, and therefore could be potential therapeutic target molecules. (HEPATOLOGY 2009;49:1098-1112.)

MicroRNA (miRNA) is an endogenous, small, single-strand, noncording RNA consisting of 20 to 25 bases and regulates gene expression of various cell types. It plays an important role in various biological processes, including organ development and differentiation as well as cellular death and proliferation, and is also involved in various diseases such as infection and cancer.[1-3]

miRNAs are produced as follows. A primary miRNA with a hairpin loop structure is cleaved into a precursor miRNA and transported out of the nuclei with a carrier protein (Exportin-5). The precursor miRNA is then processed by Dicer and converted into an active single-strand RNA in the cytoplasm. The miRNA binds to a target messenger RNA in a sequence-dependent manner and induces degradation of the target messenger RNA and translational inhibition. One miRNA regulates the expression of multiple target genes; bioinformatics analyses have suggested that the expression of more than 30% of human genes is regulated by miRNAs.[4-7]

Abbreviations: cDNA, complementary DNA; CH, chronic hepatitis; CH-B, chronic hepatitis B; CH-C, chronic hepatitis C; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCC-B, hepatitis B-related hepatocellular carcinoma; HCC-C, hepatitis C-related hepatocellular carcinoma; HCV, hepatitis C virus; miRNA, microRNA; RTD-PCR, real-time detection polymerase chain reaction; SVM, support vector machine.

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Table 1. Characteristics of Patients Used for Analysis of miRNA and Microarray Samples

Patient No.	Virus	Age	Sex	ALT	Histology of Activity	Background Liver Fibrosis	Histological Grade of HCC	Tumor Size (mm)	TNM Staging	HCV-RNA (KIU/mL)	HBV-DNA (LEG/mL)
1	HBV	57	M	16	2	4	Moderate	20	II	–	3.4
2	HBV	51	M	57	1	2	Moderate	48	II	–	< 2.6
3	HBV	61	M	17	1	4	Well	16	II	–	< 3.7
4	HBV	47	M	19	1	4	Moderate	15	I	–	< 3.7
5	HBV	72	M	19	1	1	Well	25	II	–	NA
6	HBV	73	M	62	1	3	Moderate	45	III	–	5.7
7	HBV	42	M	36	1	4	Moderate	18	I	–	< 3.7
8	HBV	63	M	13	1	2	Moderate	15	I	–	2.8
9	HBV	68	F	54	1	2	Well	56	II	–	4.1
10	HBV	70	M	13	0	2	Well	40	II	–	< 3.7
11	HBV	58	M	29	1	4	Moderate	35	IVA*	–	3.3
12	HBV	72	M	22	1	4	Moderate	18	I	–	6
13	HCV	66	F	33	2	4	Well	25	II	423	–
14	HCV	67	M	89	1	4	Well	30	II	> 850	–
15	HCV	64	M	31	1	4	Moderate	75	III	< 5 (+)	–
16	HCV	68	M	30	0	4	Well	23	II	> 850	–
17	HCV	46	M	98	2	3	Moderate	20	I	> 850	–
18	HCV	68	F	32	2	4	Moderate	25	III	< 5 (+)	–
19	HCV	66	F	46	2	4	Well	25	II	> 850	–
20	HCV	47	M	246	1	3	Moderate	20	I	262	–
21	HCV	75	M	27	1	3	Moderate	19	II	85.1	–
22	HCV	77	M	21	0	1	Moderate	20	II	< 5 (−)	–
23	HCV	66	M	46	2	2	Well	60	II	50.3	–
24	HCV	65	M	89	1	1	Poorly	25	III	850	–
25	HCV	53	M	54	0	1	Moderate	28	II	< 5 (−)	–
26	HCV	75	F	212	1	4	Well	19	I	580	–
27	–	51	F	18	0	0	–	–	–	–	–
28	–	78	F	13	0	0	–	–	–	–	–
29	–	75	M	20	0	0	–	–	–	–	–
30	–	34	M	12	0	0	–	–	–	–	–
31	–	64	M	30	0	0	–	–	–	–	–
32	–	78	M	9	0	0	–	–	–	–	–
33	–	53	M	19	0	0	–	–	–	–	–
34	–	64	F	12	0	0	–	–	–	–	–
35	–	60	F	20	0	0	–	–	–	–	–

HCV RNA was assayed via Amplicor Monitor Test (KIU/mL); HBV DNA was assayed via transcription-mediated amplification (LEG/mL).

Abbreviations: ALT, alanine aminotransferase; F, female; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, male; TNM, tumor-node–metastasis.

*Vascular invasion (+).

Infection of the human liver with hepatitis B virus (HBV) and hepatitis C virus (HCV) induces the development of chronic hepatitis (CH), cirrhosis, and in some instances hepatocellular carcinoma (HCC).[8] The virological features of these two distinct viruses are completely different; however, the viruses infect the liver and cause CH, which is not distinguished by histological examination or clinical manifestations. We previously reported that gene expression profiles in chronic hepatitis B (CH-B) and chronic hepatitis C (CH-C) are different. Proapoptotic and DNA repair responses were predominant in CH-B, and inflammatory and antiapoptotic phenotypes were predominant in CH-C. However, factors inducing these differences in gene expression remain to be elucidated.[9,10]

We examined miRNA expression in liver tissue with HBV-related liver disease (CH-B and HCC-B) and HCV-related liver disease (CH-C and HCC-C) and in normal liver tissue via real-time detection polymerase chain reaction (RTD-PCR). We also performed global analysis of messenger RNA expression in these tissues using complementary DNA (cDNA) microarray. These analyses allowed us to find characteristic miRNAs associated with HBV or HCV infection as well as the progression of liver disease.

Patients and Methods

Patients. The study subjects included 12 patients with CH-B complicated by HCC and 14 patients with

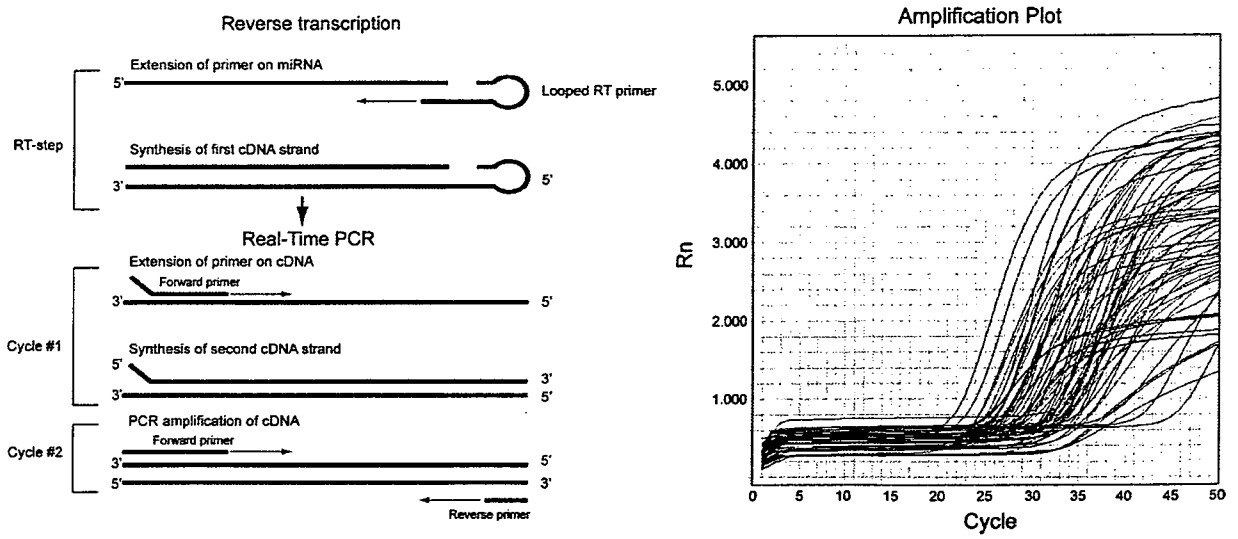

Fig. 1. (A) miRNA-specific RTD-PCR using sheet hairpin primers. (B) miRNA amplification curves by RTD-PCR.

CH-C complicated by HCC. Gene expression analysis was approved by the ethics committee of the Graduate School of Medicine, Kanazawa University Hospital, Japan, between 1999 and 2004. In addition, nine normal liver tissue samples obtained during surgery for metastatic liver cancer were used as control samples. Surgically removed liver tissues were stored in liquid nitrogen until analysis. Histological classification of HCC and histological evaluation of hepatitis in noncancerous regions for each patient are shown in Table 1. HCV viremia in two patients with CH-C was persistently cleared by interferon therapy before HCC development. There were no significant differences in the histological findings of HCC and noncancerous regions, as well as in sex, age, and hepatic function between the HBV and HCV infection groups.

Quantitative RTD-PCR. Approximately 1 mg of each liver tissue sample stored in liquid nitrogen was ground with a homogenizer while still frozen, and total RNA containing miRNA was isolated according to the protocol of the mirVana miRNA Isolation kit (Ambion, Austin, TX) and stored at −80°C until analysis. miRNA expression levels were quantitated using the TaqMan MicroRNA Assays Human Panel Early Access kit (Applied Biosystems, Foster City, CA). cDNA was prepared via reverse transcription using 10 ng each of the isolated total RNA and 3 μL each of the reverse transcription primers with specific loop structures. Reverse transcription was performed using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's protocol. Then, a mixture of 6.67 μL of nuclease-free water, 10 μL of TaqMan 2 × Universal PCR Master Mix (No AmpErase UNG; Applied Biosystems), and 2 μL of TaqMan MicroRNA Assay Mix, which was included in the kit, was prepared for each sample on a 384-well plate; 1.33 μL of the reverse transcription product was added to the mixture, and amplification reaction was performed on an ABI PRISM 7900HT (Applied Biosystems). Expression levels of 188 miRNAs in each sample were quantitated.

Analysis of RTD-PCR Data. The measured 188 miRNAs included RNU6B, which is commonly used as a control for miRNA. β-Actin and glyceraldehyde 3-phosphate dehydrogenase were also measured simultaneously for correcting RNA amount. The mean Ct values and standard deviations of each miRNA were calculated from expression data of all patients obtained by RTD-PCR. miRNA with the lowest expression variation was used as the internal control. Ct values of each miRNA were then corrected by the Ct value of the internal control to yield −ΔCt values defined as relative miRNA expression levels and used for analyses. Statistical analyses and hierarchical cluster analyses of expression data were performed using BRB ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.html). Relative miRNA expression levels were further normalized using the median over the all patients so that the normalized expression levels of each patient have a median log ratio of 0. A class prediction method was used for classifying two patient groups based on the supervised learning method, and a binary tree classification method was used for classifying three or more patient groups with a statistical algorithm of the support vector machine (SVM). Class prediction was performed using SVM incorporating genes differentially expressed at a univariate parametric significance level of $P = 0.01$. The prediction rate was estimated via cross-validation and the bootstrap method for small sample data.[11] (It is worth

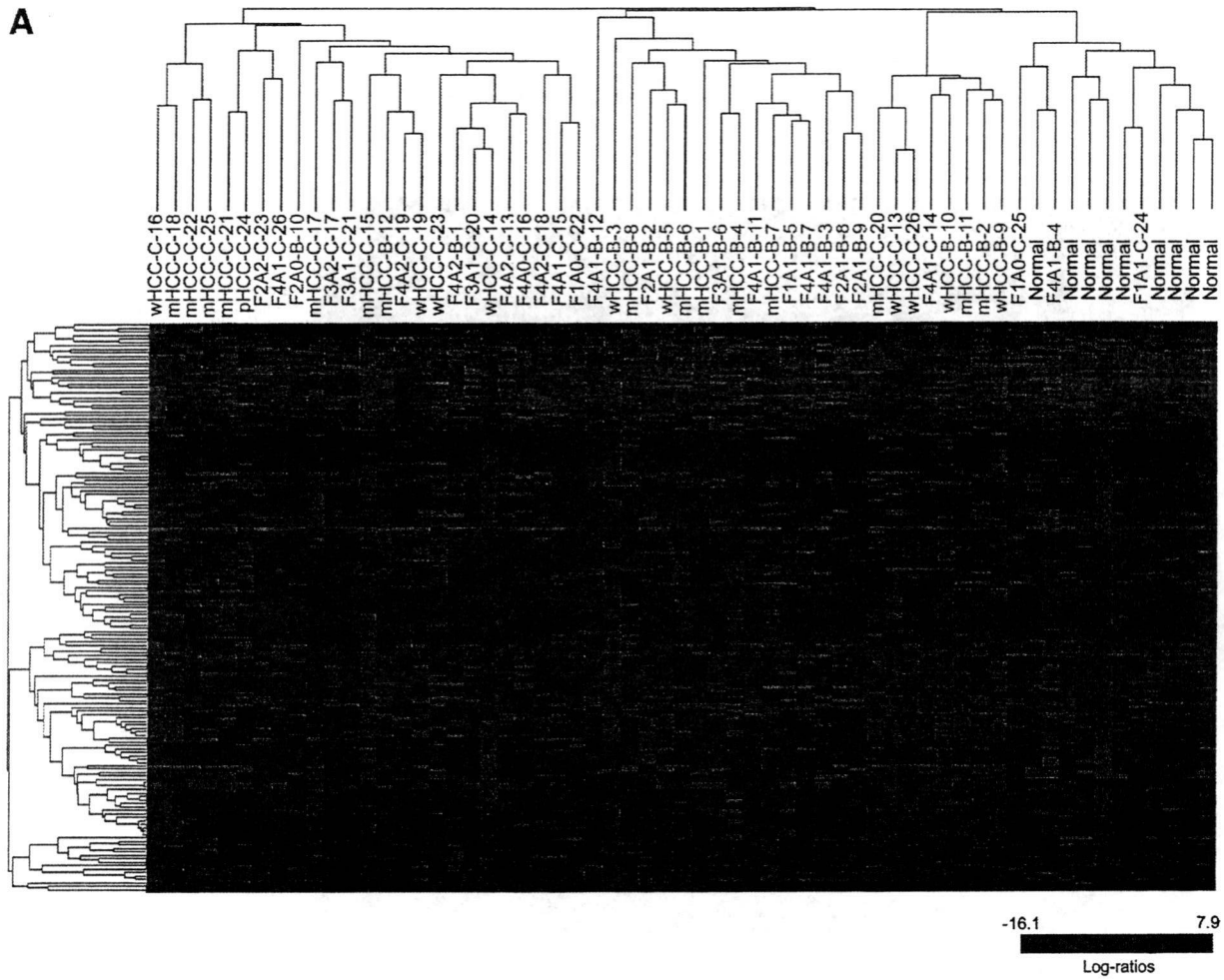

Fig. 2. (A) Hierarchical cluster analysis using total miRNA. Chronic hepatitis is indicated by histological stage and grade (F, fibrosis; A, activity) and type of infecting virus (B or C). HCC is indicated by histological grade (w, well differentiated; m, moderately differentiated; p, poorly differentiated) and type of infecting virus (B or C), with the patient number added at the end. (B) Relationship between five classes divided by binary tree classification. Expression profiles were first classified into normal liver and non-normal liver groups (node 1), then into HBV and HCV groups (node 2). The HBV group was further divided into HCC-B and CH-B (node 3), and the HCV group into HCC-C and CH-C (node 4).