

nostic imaging offers diverse modalities, including non-invasive evaluations [5–14]. Many types of imaging softwares using multiplanar reconstruction (MPR) have become available for the diagnosis and/or treatment guidance for liver cancers [15–18].

Ultrasound (US) fusion imaging (Real-Time Virtual Sonography, HITACHI ALOKA Medical Systems, Tokyo, Japan; Fusion, GE Healthcare, Chalfont St. Giles, UK; Smart Fusion, Toshiba Medical Systems, Tokyo, Japan) is a new system using MPR, and fusion imaging of B-mode US and CT/MRI can be displayed simultaneously and in real time according to the angle of the transducer in the magnetic field [19–22]. Fusion imaging can help us understand the 3D relationship between the liver vasculature and tumors. However, this system only operates with high-end US machines, and the fusion process is somewhat complex.

The volume analyzer SYNAPSE VINCENT® (Fujifilm Medical Co., Tokyo, Japan) is a 3D image analysis system that enables quick and easy access to high-definition 3D images of organs and vessels using previously captured CT or MRI, while also providing highly practical analysis functions at the workstation [23]. In particular, this can also generate virtual sonographic images using MPR with a quick and easy operation. In this study, we evaluated the usefulness of SYNAPSE VINCENT, a virtual US imaging device, as a tool to assist US beginners.

## Materials and Methods

A prospective blinded pilot study was conducted involving patients with liver lesions. Two medical doctors and 2 sonographers with less than 5 years of experience performed US examinations. The primary objective was to compare the liver lesion detecting time on US and the success rate for detecting liver lesions with or without using the virtual US imaging device SYNAPSE VINCENT before US examination.

### Equipment

The local area network system is connected to a computer with SYNAPSE (Fujifilm Medical Co.), a medical imaging and information management system, at the Takamatsu Red Cross Hospital. VINCENT is an application of imaging analyses using SYNAPSE and can display 2D MPR images as virtual sonography corresponding to the angle in the plane of 3D volume image data. This angle of the plane can be operated quickly and freely at the workstation for scanning in epigastric, subcostal, and intercostal positions.

B-mode sonographic scans were obtained using LOGIQ E9 (GE Healthcare) with a 2- to 5-MHz convex probe (C1-5D) and a 4- to 9-MHz linear probe (9LD), an Ascendas (HITACHI ALOKA Medical Systems) with a 1- to 5-MHz convex probe (EUP C715)

and a 3- to 7-MHz linear probe (EUP L52), or a Xario XG (Toshiba Medical Systems) with a 3- to 6-MHz convex probe (PVT-375BT).

CT was performed using a 64-slice multidetector-row CT scanner (Aquilion 64, Toshiba Medical Systems) with the following scan parameters: reconstructed slice thickness = 1 mm; rotation time = 0.5 s; helical pitch = 23.0; pitch factor = 0.791; X-ray tube parameters = 120 kV, 300–400 mA. Triple-phase contrast-enhanced CT was performed at 40, 70, and 180 s after initiating the injection of contrast media to obtain hepatic arterial, portal venous, and equilibrium phase images, respectively. A total of 100 ml of nonionic contrast material containing 300 mg of iodine per milliliter (Iopamidol, Bayer Yakuhin, Osaka, Japan) was injected intravenously at a rate of 3 ml/s using an automatic power injector.

### Evaluation

Patients with liver tumors who have previously been diagnosed by dynamic CT or MRI were selected for this study. US beginners were permitted to obtain imaging information of some patients using VINCENT, whereas information on previous imaging results of other patients was withheld.

The liver was examined first using a subcostal approach in sagittal and paraxial planes. As a rule, the right hepatic lobe was also examined with a lateral approach through the intercostal space. Sonographic reports and images were reviewed in conjunction with CT/MRI to determine whether a determinate lesion shown on CT/MRI could be detected sonographically and to confirm lesion correspondence. Thereby, the detection rates and duration of detecting liver lesions on US were evaluated.

### Statistical Analysis

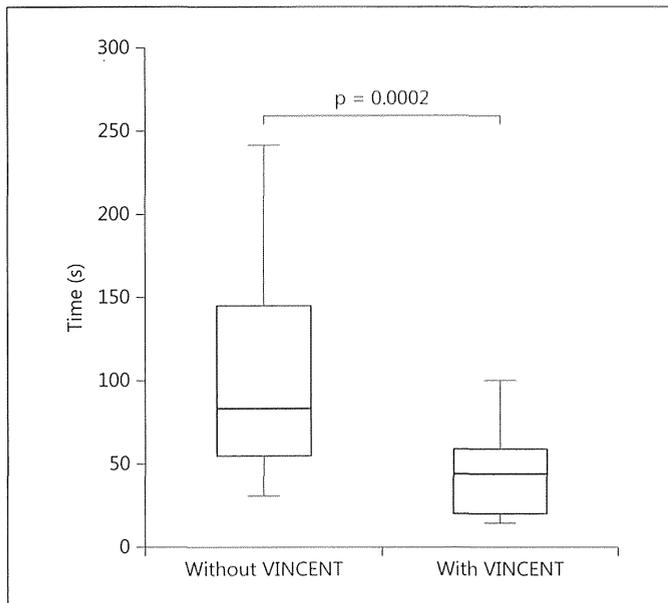
All values are expressed as the mean  $\pm$  standard deviation (SD). Comparisons between the two groups were analyzed using Student's t test and Fisher's exact test.  $p < 0.05$  was considered significant. Statistical analyses were performed using Microsoft Excel 2013 for Windows.

## Results

This pilot study involved 32 patients undergoing routine US examinations. All patients with the following 42 liver lesions were included: liver cyst ( $n = 24$ ), hemangioma ( $n = 8$ ), hepatocellular carcinoma ( $n = 6$ ), and liver metastasis ( $n = 4$ ). The maximal diameter of these lesions ranged from 0.3 to 1.5 cm (mean  $\pm$  SD,  $0.8 \pm 0.4$ ) on CT.

The average time for detecting liver lesions on US was 47.8 s (range, 7–113) with VINCENT and 112.9 s (range, 14–313) without VINCENT before US examination. There were significant differences in the duration of US examination with and without VINCENT ( $p = 0.0002$ , Student's t test; fig. 1).

The rates for accurately detecting liver lesions were 100% (21/21) and 76.2% (16/21) in US beginners with

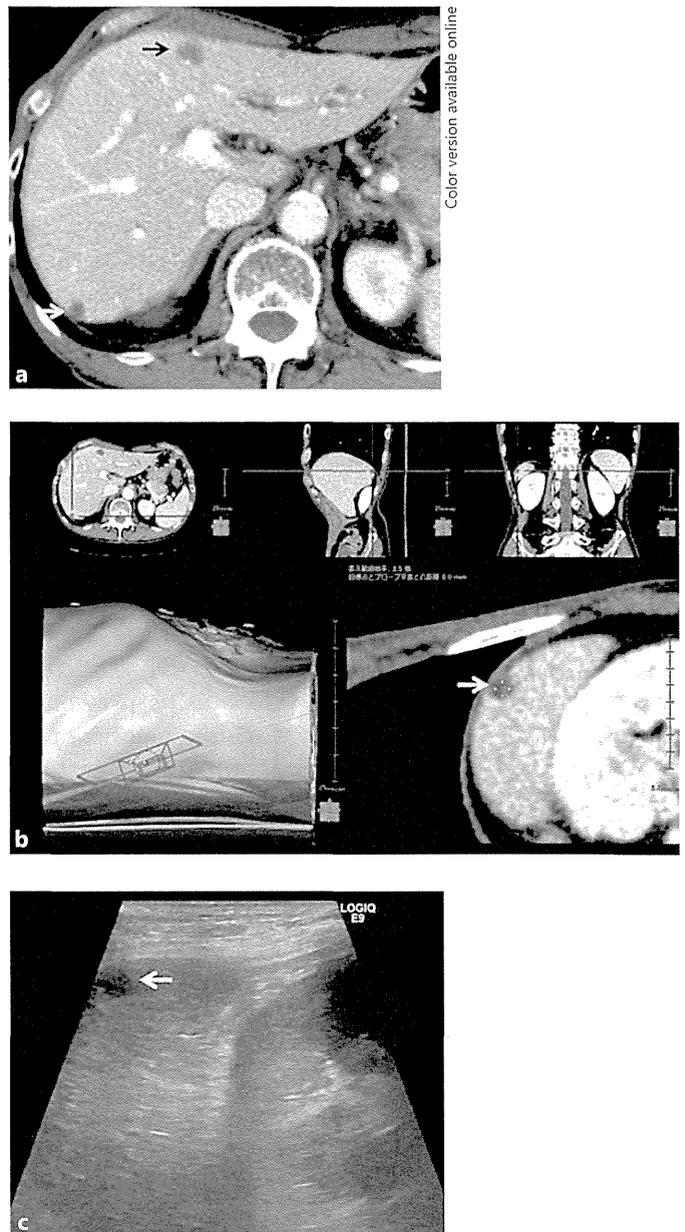


**Fig. 1.** The time needed to detect the liver lesions with and without VINCENT.

and without VINCENT, respectively. Significantly higher detection rates were found in the US beginners who used VINCENT compared to those who did not use VINCENT ( $p = 0.047$ , Fisher's exact test).

## Discussion

Preparation is a necessary ingredient for success, and even more so for beginners. This study demonstrated that pre-check imaging by SYNAPSE VINCENT could contribute to the successful detection of liver lesions and could be time-saving on US examination for beginners. In general, axial imaging on CT is common in clinical use, whereas US shows cross-sectional images with various angles. In particular, an intercostal view of the liver on US provides quite a different image from usual CT images because the intercostal view is in a diagonal direction against the body trunk. Therefore, it is often difficult for US beginners to understand the 3D anatomy of the liver and display available images with an intercostal view. Moreover, US images may differ from a familiar view because the shape of the liver changes after surgical liver resection. This could also lead to wasting time on US examination. However, SYNAPSE VINCENT has the potential to resolve these problems. VINCENT can



**Fig. 2.** Imaging of a 66-year-old man with liver cysts. **a** Transverse portal phase CT shows two cysts (arrows) in segment III and VI of the liver. **b** Screen shot shows setting windows for VINCENT. Upper images show the transverse, sagittal, and coronal views for the cyst in segment VI. The lower left image shows that the transducer angle in the plane of the body trunk indicates images obtained from the subcostal view. The lower right image displays the virtual US image and the cyst as low-density area. **c** B-mode US image corresponds to the virtual US image. The cyst is shown as low-echoic area (arrow). Our US beginners missed this cyst without using VINCENT because the rib bone hid it.

simulate US examinations by virtual US imaging at a workstation [23]. Preparation using VINCENT could promote an efficient and successful US examination (fig. 2).

While the basic technical development depends on the frequency of US examination, successful experience would stimulate a US beginner's growth. This study gives powerful support for the effectiveness of training in early-phase US skill acquisition with the use of VINCENT. To perform US examination with competence, not only the sonographer, but also the medical doctor must have a good understanding of the abdominal anatomy. The use of various models and stimulators will help to understand the abdominal anatomy and shorten the learning curve. Therefore, a training program with the use of virtual US imaging would shorten the learning curve for US beginners.

However, virtual US devices such as VINCENT should provide reference images at a workstation. Virtual US images do not completely correspond to US images. This imaging incompatibility could be attributed to

variations in the depth of breath holding on CT and US examination [20]. In addition, the liver is also rotated to varying degrees from the time when CT was previously captured.

The principal limitation of this study is that it could suffer from selection bias because patients with liver lesions were enrolled according to the senior doctors' subjective selections. Second, it is a preliminary study with a relatively small number of patients. Further studies of this technique with a larger number of patients are warranted.

In summary, SYNAPSE VINCENT could display virtual US images clearly and smoothly. Before US examination, a reference with VINCENT could contribute to the successful detection of liver lesions and could be time-saving for US beginners.

### Disclosure Statement

The authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

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Original Article

# Influence of splenectomy in patients with liver cirrhosis and hypersplenism

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**Aim:** Splenectomy improves hypersplenic thrombocytopenia in cirrhotic patients with hypersplenism. However, the long-term influence of splenectomy has not been clarified. We examined whether splenectomy improved liver fibrosis and caused immunological changes.

**Methods:** We collected liver and spleen specimens and peripheral blood (PB) from 26 patients with hepatitis C virus-related liver cirrhosis. An immunohistochemical examination of CD4, CD8, forkhead box P3, granzyme B and transforming growth factor- $\beta$ 1, and Masson-trichrome stain were performed in spleen and liver tissues and in seven cases of follow-up liver biopsy sections obtained after splenectomy. We obtained PB before and at various intervals after splenectomy. We also examined the ratio of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes in PB using flow cytometry.

**Results:** We observed improvements in liver fibrosis in four biopsy specimens obtained after splenectomy, in which

fibrotic areas significantly decreased from 19.5% to 8.2% ( $P < 0.05$ ). Increases were also observed in the ratio of CD8<sup>+</sup> cells in PB after splenectomy, which resulted in a significant decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio ( $P < 0.001$ ). The carcinogenic rate in patients with a CD4<sup>+</sup> : CD8<sup>+</sup> ratio that decreased by more than 0.5 at 1 month after splenectomy was significantly lower than that in patients with a ratio that decreased by less than 0.5 ( $P < 0.05$ ).

**Conclusion:** Splenectomy may improve liver fibrosis and cause beneficial immunological changes in cirrhotic patients with hepatitis. Improvements in antitumor mechanisms can be also expected.

**Key words:** CD4<sup>+</sup> cytotoxic T lymphocytes, CD8<sup>+</sup> cytotoxic T lymphocytes, liver cirrhosis, liver fibrosis, splenectomy

## INTRODUCTION

SPLENECTOMY IS A common treatment used to improve hypersplenic thrombocytopenia in cirrhotic patients with splenomegaly in Japan.<sup>1–7</sup> Splenectomy has recently been applied as another option to cure hepatocellular carcinoma (HCC) and for cirrhotic patients with no potential donor for liver transplantation. Thus, the clinical application of splenectomy has been expanded; however, the immunophysiology of the spleen in cirrhotic patients and the long-term outcome after splenectomy have not been clarified.<sup>8–14</sup> This study was designed to clarify the long-term changes and prediction of HCC development following splenectomy,

with a focus on hepatic fibrosis and immunology. Regarding hepatic fibrosis, Akahoshi *et al.* reported that transforming growth factor (TGF)- $\beta$ 1 derived from the spleen could have an inhibitory role in healing liver cirrhosis by inhibiting the regeneration of the damaged liver<sup>15</sup> and we experimentally confirmed that splenectomy significantly reduced liver fibrosis and decreased TGF- $\beta$ 1 in the serum of a dimethylnitrosamine-induced cirrhotic rat model.<sup>16</sup> However, no studies have yet described a reduction in hepatic fibrosis following splenectomy in humans.

The spleen plays an important role in the immune response; however, the functional aspects of the spleen in cirrhotic patients with hepatitis C virus (HCV) infection are largely unknown.<sup>2,17</sup> Hashimoto *et al.* reported that splenectomy was followed by an increased ratio of interferon (IFN)- $\gamma$  to interleukin (IL)-10 and a reduction in programmed death (PD)-1-expressing CD4<sup>+</sup> T cells in peripheral blood (PB).<sup>7</sup> In order to clarify chronological changes in immunity after splenectomy, we examined liver and spleen tissues and sera to assess CD4<sup>+</sup> and

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CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) and regulatory T (Treg) cells.<sup>18,19</sup> TGF- $\beta$ 1 was also examined as it is a multifunctional cytokine that inhibits the growth of tumor cells<sup>20–23</sup> and liver regeneration by facilitating tissue fibrosis in the liver.<sup>16</sup>

Host immunoreactions against cancer were shown to be closely related to cellular immunity by CD8<sup>+</sup> CTL and Treg cells, produced by T lymphocytes, and CD8<sup>+</sup> CTL in particular.<sup>19</sup> The level of Treg cells, characterized by the expression of forkhead box P3 (FOXP3) transcription factor in the PB and tumor tissues of patients with HCC, was elevated and appeared to be negatively correlated with prognosis.<sup>21,24,25</sup>

In the present study, we examined whether splenectomy could improve liver fibrosis, cause immunological changes, especially in CTL, or be used to predict the risk of carcinogenesis.

## METHODS

### Patients and samples (Table 1)

AT THE DEPARTMENT of Surgery, Kurume University Hospital, 26 patients (Child A, 16 cases; Child

B/C, 10 cases) with HCV-related liver cirrhosis (with HCC, seven cases; without HCC, 19 cases) and hypersplenism underwent splenectomy (splenectomy group). The purpose of splenectomy was to improve hypersplenic thrombocytopenia and introduce IFN for clearance of the HCV virus. Forty-eight patients who underwent hepatectomy due to liver tumors were recruited as controls (control group 1). PB samples from 10 healthy adult volunteers (control group 2) and spleen tissues obtained by splenectomy from seven patients because of trauma (control group 3) were also used as controls. In addition, all patients were HIV negative. Patients received no medical treatment except splenectomy during the study period. All samples were studied after obtaining the appropriate institutional informed consent. We also obtained permission from the ethical review board.

### Liver tissue

A total of 26 pieces from the resected liver specimens of patients with HCV-related liver cirrhosis and hypersplenism who underwent splenectomy were also examined for the immunohistochemical expression of CD4<sup>+</sup>

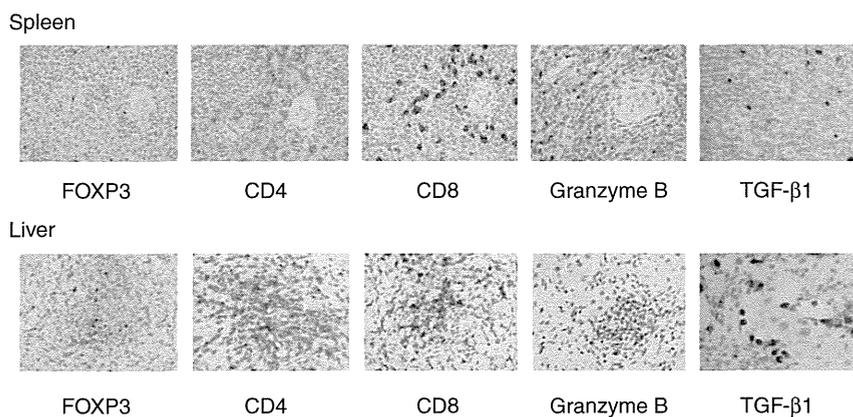
**Table 1** Subject characteristics

Variables	Results
Splenectomy group: splenectomy (26 cases, seven with HCC, 19 without HCC)	
Age, median (range)	60.4 $\pm$ 1.36 (46–75)
Sex (male/female)	12/14
Virus infection (HCV <sup>+</sup> )	26
Fibrosis (F0/F1/F2/F3/F4)	0/0/0/0/26
Child–Pugh classification (A/B/C)	16/8/2
Tumor nodules (presence/absence)	7/19
Weight of the spleen (g)	510.4 $\pm$ 55.6 (125–1065)
Control 1: hepatectomy with HCC (48 cases)	
Age, median (range)	70.5 $\pm$ 1.33 (42–82)
Sex, male/female	29/19
Virus infection (HCV <sup>+</sup> )	40
Fibrosis (F0/F1/F2/F3/F4)	8/10/10/10/10
Tumor nodules (presence/absence)	48/0
Control 2: healthy adult volunteers (10 cases)	
Age, median (range)	40.1 $\pm$ 2.97 (32–57)
Sex (male/female)	3/7
Control 3: splenectomy control (seven cases; trauma)	
Age, median (range)	59.8 $\pm$ 6.27 (36–82)
Sex (male/female)	6/1

Continuous variables are expressed as the mean  $\pm$  standard deviation.

Fibrosis: F0, no fibrosis in the portal tract; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.

HCC, hepatocellular carcinoma; HCV, hepatitis C virus.



**Figure 1** Immunohistochemical staining of spleen and liver specimens with forkhead box P3 (FOXP3), CD4, CD8, granzyme B and transforming growth factor (TGF)- $\beta$ 1 in the spleen and liver.

lymphocytes, CD8<sup>+</sup> lymphocytes, FOXP3, granzyme B and TGF- $\beta$ 1 positive cells (Fig. 1). We classified liver specimens into five stages according to the degree of fibrosis as follows: F0, no fibrosis in the portal tract; F1, portal fibrosis without septa; F2, portal fibrosis with a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. We collected resected liver specimens from 10 cases each of F1, F2, F3 and F4 with HCV-related liver disease. We also collected specimens from eight cases of liver hemangioma of F0 with both negative hepatitis B surface antigen and HCV antibody. Follow-up liver biopsy sections were obtained from the same part of the liver if possible from seven of the 26 patients at various intervals after splenectomy (Table 2). These sections were used for CD4 and CD8 immunostaining and Masson-trichrome staining for the morphometric evaluation of fibrotic areas.

### Spleen tissue

A total of 26 spleens with HCV-related liver cirrhosis and hypersplenism were examined for the immunohis-

tochemical expression of CD4 positive lymphocytes, CD8 positive lymphocytes, FOXP3, granzyme B and TGF- $\beta$ 1 positive cells. We measured the same parameters in spleens from the seven control cases in control group 3 as a non-cirrhotic control (Fig. 1). Spleen and liver tissues were pathologically assessed by two pathologists (Y. N. and M. K.).

### Peripheral blood cells

Peripheral blood samples were serially collected from 26 patients with HCV-related liver cirrhosis and hypersplenism just before and 14 days, 1 month, 3 months, 6 months and 1 year after splenectomy. We examined the ratio of CD4<sup>+</sup> T cells to all lymphocytes, CD8<sup>+</sup> T cells to all lymphocytes, and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in PB samples using flow cytometry. TGF- $\beta$ 1 levels in PB were also measured using enzyme-linked immunoassays in the sera just before and 14 days, 1 month, 3 months, 6 months and 1 year after splenectomy. Patients were excluded from the protocol if IFN or other therapeutics were introduced for the liver disease. Ten healthy adult

**Table 2** Clinical and pathological findings of 7 patients who underwent follow-up liver biopsies

Case	Age	Sex	Activity	Child-Pugh (score)	CD4/8	Follow-up range (days)	Before (%)	After (%)	Rate of change
1	63	M	1	A (5)	1.73	581	6.59	18.31	2.78
2	58	M	2	A (5)	1.22	24	7.38	8.99	1.22
3	58	M	2	B (7)	1.57	333	9.92	12.02	1.21
4	52	M	2	A (5)	1.08	431	16.71	5.10	0.30
5	74	M	2	A (6)	0.63	353	20.02	6.31	0.32
6	53	F	2	A (6)	0.93	248	30.03	13.34	0.44
7	59	M	2	A (5)	0.95	42	11.27	8.05	0.71

Activity: A0, none; A1, portal inflammation only; A2, mild interface hepatitis; A3, moderate interface hepatitis; A4, severe interface hepatitis.

Before, the rate of fibrotic areas before splenectomy; after, the rate of fibrotic areas after splenectomy.

volunteers in control group 2 without a history of liver disease or splenomegaly were also recruited as controls, and samples were collected only once.

### Immunohistochemical analysis

All fresh specimens were fixed by 10% formalin, and paraffin-embedded tissue samples were cut at a thickness of 4  $\mu$ m, examined on a coated slide glass, and labeled with the following antibodies using the Bond-Max autostainer (Leica Microsystems, Newcastle, UK) and DAKO autostainer (DakoCytomation, Glostrup, Denmark): CD4 ( $\times$ 200; Leica Microsystems), CD8 ( $\times$ 200; Leica Microsystems), granzyme B ( $\times$ 50; Leica Microsystems), TGF- $\beta$ 1 ( $\times$ 300; Santa Cruz Biotechnology, Heidelberg, Germany) and FOXP3 ( $\times$ 600; Abcam, Cambridge, MA, USA).

Immunohistochemical examinations with CD4, CD8, granzyme B and TGF- $\beta$ 1 were performed on the same fully automated Bond-Max system using onboard heat-induced antigen retrieval with ER2 for 10 min and the Refine polymer detection system (Leica Microsystems). 3,3'-Diaminobenzidine-tetrachloride (DAB) was used as the chromogen for all immunostaining. FOXP3 immunostaining was carried out using the DAKO autostainer with the ChemMate ENVISION method (DakoCytomation). Briefly, specimens were boiled in a microwave for 30 min in 1 mmol/L ethylenediamine-tetraacetic acid, pH 9.0, and target retrieval solution (DakoCytomation) to recover antigens, and the specimens were then incubated with the antibody at 4°C overnight. After washing in Tris-buffered saline (TBS), slides were incubated with the labeled polymer-horseradish peroxidase secondary antibody for 30 min at room temperature. After washing in TBS, slides were visualized using DAB.

### Detection of immune function using flow cytometry

T-lymphocyte subsets in PB such as CD4, CD8 and CD4/8 were determined by flow cytometry, and the monoclonal antibodies of CD4 and CD8 (labeled CD4-FITC, CD-8-RD1) were purchased from Beckman Coulter (Danvers, MA, USA).

### Result assessment

For assessment criteria for lymphocytes and other positive cell counts, the number of lymphocytes and other positive cells were counted in 20 areas within a specimen under high-power fields ( $\times$ 40 objective,  $\times$ 10 eyepiece). Ten areas of white and red pulp were assessed in

the spleen, and 10 periportal areas and 10 hepatic lobule areas (Fig. 1) were assessed in a non-tumor area of the liver.

Morphometric analysis (computer image analysis) was performed in the following manner on specimens stained with Masson-trichrome. The equipment used to assess morphometry consisted of a light microscope, a three-color charge-coupled device camera, and a high resolution computer image analysis system (WinRoof software package version 6.1; Mitani, Fukui, Japan). The magnified images ( $\times$ 40) of specimens captured by the camera mounted on the microscope were sent to the image analyzing computer. Collagen fibers stained with Masson-trichrome were then selected. In this study, this scanning procedure was repeated 10 times in random areas. The area of fibrosis (AF) was defined as the ratio (%) of the whole area of collagen fibers to that of the liver tissue scanned.

### Statistical analyses

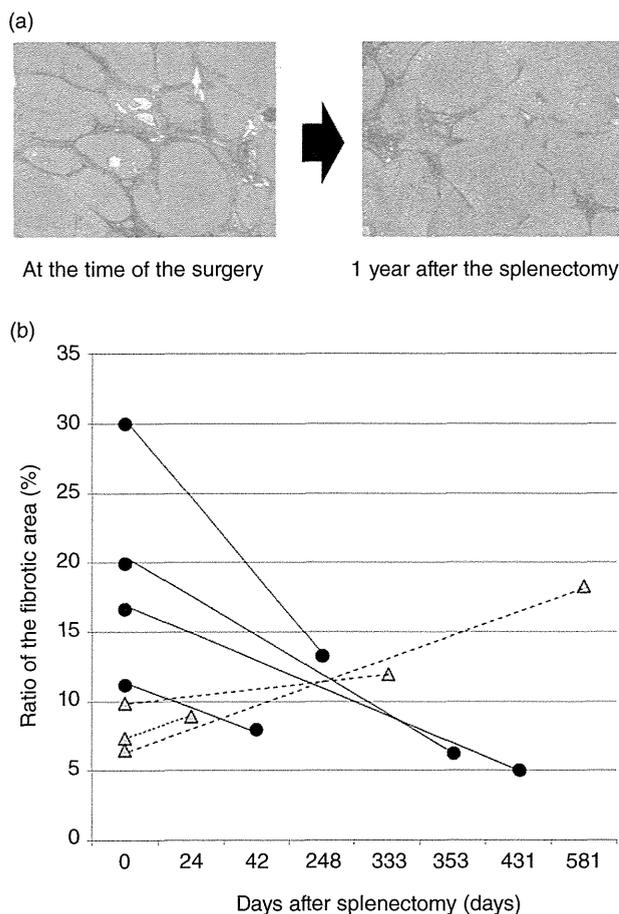
Statistical analysis was performed using Student's *t*-test. A *P*-value of less than 0.05 was considered to be significant.

The follow-up time was calculated as the interval between the date of surgery and intervention of the medical treatment, last follow up or recognition of HCC. Survival rates or failure rates were analyzed with the Kaplan–Meier method using the log–rank test to assess differences between curves. A *P*-value of less than 0.05 was considered to be significant. Statistical calculations were performed using the JMP software package (release 10, SAS Institute, Cary, NC, USA).

## RESULTS

### Liver

IN THE SEVEN follow-up liver biopsy sections (Table 2) available for histological examination, liver fibrosis in the hepatic lobules improved from F4 to F3 in four cases (cases 4–7: average, 268.5  $\pm$  168.6 days; range, 42–431 days) (Fig. 2a). Improvements were not observed in the remaining three cases (cases 1–3: average, 312  $\pm$  279.1 days; range, 24–581 days) (Fig. 2b). There were no statistical differences in the duration between the improvement cases and non-improvement cases (*P* = 0.80). Conducting an evaluation was difficult because only a few specimens were available; however, no significant differences in clinical profiles were observed among the seven patients. In four of these cases (cases 4–7), the ratio significantly



**Figure 2** (a) Improvements in liver fibrosis. Distortions in hepatic lobules improved in the liver biopsy sections of four cases after splenectomy, and fibrotic areas significantly decreased from 19.5% to 8.2% in these sections. (b) Changes in the fibrotic areas of seven patients at various intervals. ●—● shows patients in whom the fibrotic area significantly decreased after splenectomy. △---△ shows patients in whom fibrosis deteriorated.

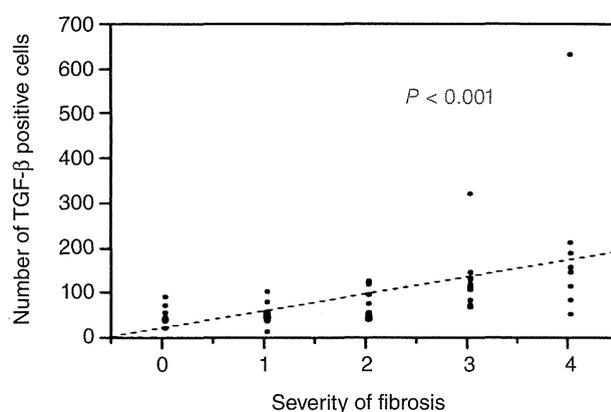
decreased from 19.5% to 8.2% ( $P < 0.05$ ) (Fig. 2b), while the average AF in the remaining three cases (cases 1–3) increased from 8.0% to 13.1% ( $P = 0.15$ ). The four cases of improved fibrosis were all Child–Pugh A, and one of the three cases that showed no improvement was Child–Pugh B. In addition, AF before splenectomy was slightly higher in the improvement cases than in the non-improvement cases, while the  $CD4^+/CD8^+$  ratio before splenectomy was lower in the improvement cases than in the non-improvement cases ( $P < 0.05$ ). Histopathologically,  $CD4^+$  and  $CD8^+$  lymphocytes were mainly seen in the periportal area, and  $CD4^+$  lympho-

cytes were rarely seen in the hepatic lobules. The epithelial cells, fibroblasts, monocytes and macrophages also produced TGF- $\beta 1$ .<sup>4,21,26</sup> However, we picked up and counted the TGF- $\beta 1$  positive cells that were seen in the lymphocytes and found that these cells were distributed diffusely in the hepatic lobules and periportal area. The distribution pattern of Treg and granzyme B was the same as that of  $CD4^+$  and  $CD8^+$  lymphocytes, respectively. No significant differences were observed in the  $CD4^+/CD8^+$  ratio ( $P = 0.21$ ) in liver specimens, regardless of the association of HCC. The  $CD4^+/CD8^+$  ratio ( $P < 0.05$ ) and FOXP3/ $CD4^+$  ratio ( $P < 0.001$ ) significantly increased with the progression of liver fibrosis (from F0 to F4). However, the granzyme B/ $CD8^+$  ratio was approximately constant, and was unrelated to the progression of liver fibrosis ( $P = 0.32$ ).

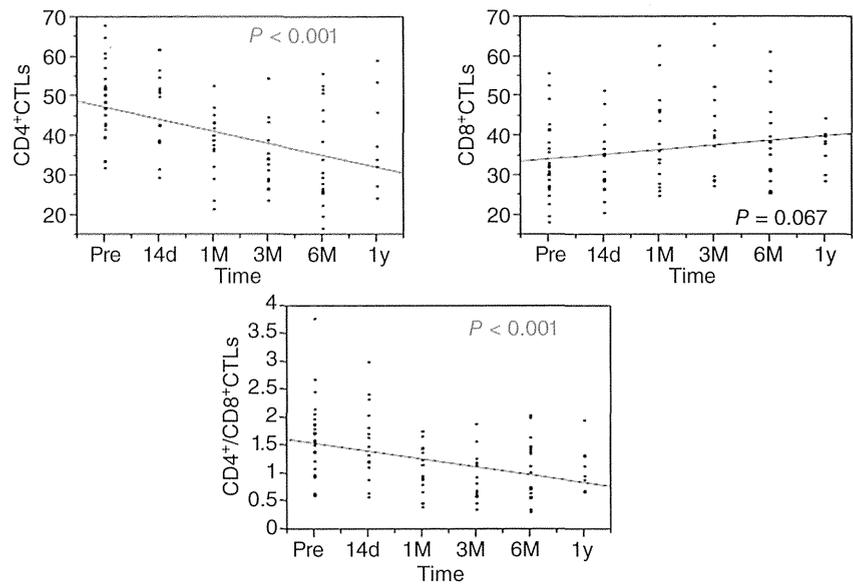
The number of TGF- $\beta 1$  positive cells in livers with HCC was slightly higher than that in livers without ( $P = 0.06$ ), and the number of TGF- $\beta 1$  positive cells also significantly increased with the progression of liver fibrosis ( $P < 0.001$ ) (Fig. 3).

### Spleen

Histopathologically,  $CD4^+$  and  $CD8^+$  lymphocytes were found more in the white pulp than in the red pulp. The results of the clinicopathological analysis showed that the  $CD4^+/CD8^+$  ratio in spleens with HCV-related liver cirrhosis and hypersplenism was higher than that in the spleens of control group 3 ( $P = 0.06$ ). The FOXP3/ $CD4^+$  ratio in control group 3 was higher than that in cases of hypersplenism ( $P < 0.05$ ), and no significant differences



**Figure 3** Correlation between transforming growth factor (TGF)- $\beta 1$  positive cells and fibrosis in the liver. The number of TGF- $\beta 1$  positive cells also significantly increased with the progression of liver fibrosis.



**Figure 4** Changes in peripheral blood after splenectomy. pre, preoperative; d, days; M, months; y, year. The ratio of CD4<sup>+</sup> T cells to all lymphocytes significantly decreased 1 year after splenectomy, while the ratio of CD8<sup>+</sup> T cells to all lymphocytes slightly increased, resulting in a significant decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio.

in the granzyme B/CD8<sup>+</sup> ratio ( $P = 0.82$ ) were observed between the splenectomy group and control group 3 (data not shown).

### Peripheral blood

The ratio of CD4<sup>+</sup> T cells to all lymphocytes and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in PB samples obtained from 26 patients before splenectomy were significantly higher than those from control group 2 ( $P < 0.01$ ,  $P < 0.05$ ). In contrast, the ratio of CD4<sup>+</sup> T cells to all lymphocytes significantly decreased 1 year after splenectomy ( $P < 0.001$ ), while the ratio of CD8<sup>+</sup> T cells to all lymphocytes slightly increased ( $P = 0.07$ ), resulting in a significant decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio ( $P < 0.001$ ) (Fig. 4).

Transforming growth factor- $\beta$  levels were higher in PB samples from patients with HCC than in those without. TGF- $\beta$ 1 levels slightly increased in PB samples 1 month after splenectomy, then decreased, and subsequently returned to the level measured before splenectomy in 1 year.

### Relationship of the CD4<sup>+</sup>/CD8<sup>+</sup> ratio between PB and the spleen or liver

In the splenectomy group, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in PB had a significant positive correlation with the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the spleen ( $P < 0.05$ ), and was also positively associated with the liver ( $P = 0.07$ ). As a result, a

significant positive correlation was observed between the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the spleen and that in the liver ( $P < 0.05$ ) (Fig. 5).

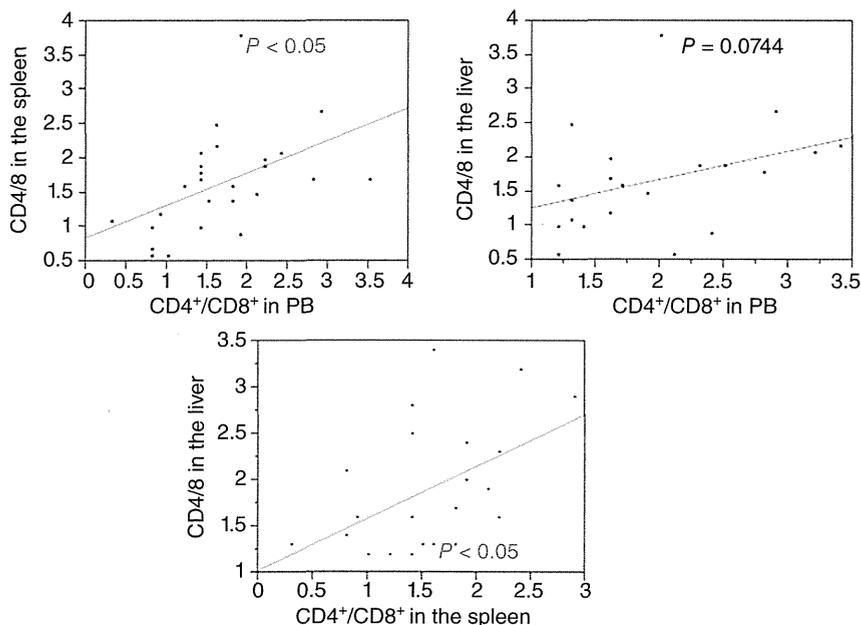
### Correlation between the CD4<sup>+</sup>/CD8<sup>+</sup> ratio and clinical prognosis

We compared the CD4<sup>+</sup>/CD8<sup>+</sup> ratio between PB obtained pre-splenectomy and 1 month after splenectomy ( $n = 19$ ). The median of differences between pre-splenectomy and 1 month after splenectomy was 0.5. The occurrence of HCC was significantly lower in cases in which the difference in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio between the perioperative period and 1 month later was over 0.5 ( $\geq 0.5$  vs  $< 0.5$ ,  $P < 0.05$ ) (Fig. 6a).

A positive correlation in PB was observed between the CD4<sup>+</sup>/CD8<sup>+</sup> ratio before splenectomy and differences in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio between pre-splenectomy and 1 month after splenectomy ( $P < 0.001$ ). As the median of the preoperative CD4<sup>+</sup>/CD8<sup>+</sup> ratio was 1.7, the post-operative (1 month after splenectomy) CD4<sup>+</sup>/CD8<sup>+</sup> ratio significantly decreased in groups in which the preoperative value was larger than 1.7 (Fig. 6b,c).

## DISCUSSION

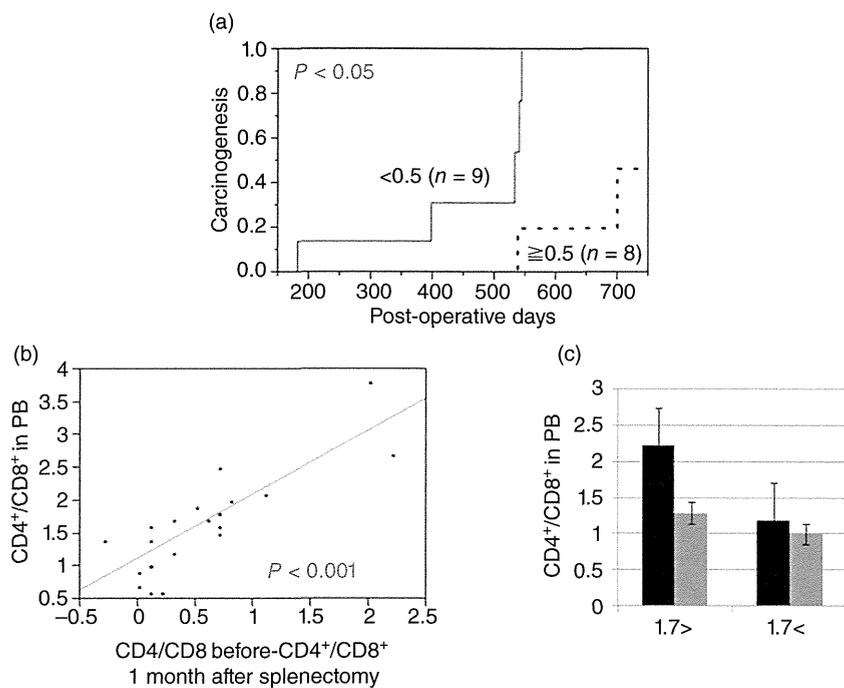
PREVIOUS STUDIES HAVE shown that splenectomy was effective in improving pancytopenia, the decompression of portal hyperpressure and liver function.<sup>1,2,27,28</sup> Morinaga *et al.* reported that splenectomy



**Figure 5** Correlations between the CD4<sup>+</sup>/CD8<sup>+</sup> ratios in the spleen, liver and peripheral blood (PB). A significant positive correlation was observed between the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the spleen and that in the liver.

significantly improved liver fibrosis with a reduction in plasma TGF-β1 levels in the rat. However, all these reports of hepatic fibrosis were conducted in animal models<sup>1,16,29,30</sup> whereas the present study described improvements in liver fibrosis after splenectomy in

humans. Interestingly, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio changed after splenectomy without other treatment. However, many confounding factors may be implicated in this change. It is likely that patients with a high fibrotic area in their liver specimens had a high CD4<sup>+</sup>/CD8<sup>+</sup> ratio;



**Figure 6** (a) Correlation between carcinogenesis, the perioperative period and 1 month later. The occurrence of hepatocellular carcinoma was significantly lower in cases in which the difference in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio between the perioperative period and 1 month later was over 0.5. (b,c) Correlation in peripheral blood (PB) between the CD4<sup>+</sup>/CD8<sup>+</sup> ratio before surgery and differences in the CD4<sup>+</sup>/CD8<sup>+</sup> ratios before splenectomy and 1 month after splenectomy. (b) A positive correlation in PB was observed between the CD4<sup>+</sup>/CD8<sup>+</sup> ratio before splenectomy and differences in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio between pre-splenectomy and 1 month after splenectomy. (c) The postoperative (1 month after splenectomy) CD4<sup>+</sup>/CD8<sup>+</sup> ratio significantly decreased in groups in which the preoperative value was larger than 1.7. ■, pre; ▒, post.

therefore, we may expect a decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio after splenectomy. A decrease in Treg cells that stimulate TGF-β1 may lead to alleviation of fibrosis.

Because the immune function of CD4<sup>+</sup> CTL, CD8<sup>+</sup> CTL and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio is affected by a wide variety of factors including recent exercise, poor nutrition and coincident acute viral infections, it is difficult to evaluate immune function using only CD4<sup>+</sup> CTL, CD8<sup>+</sup> CTL and the CD4<sup>+</sup>/CD8<sup>+</sup> ratio. However, in our study, the ratio of CD4<sup>+</sup> T cells to all lymphocytes in PB was significantly decreased in cirrhotic patients after splenectomy, while the ratio of CD8<sup>+</sup> T cells to all lymphocytes slightly increased, resulting in a significant decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio. The CD4<sup>+</sup>/CD8<sup>+</sup> ratios in PB, spleens and livers were significantly higher in patients with hypersplenism and in those in whom liver fibrosis had progressed than in the controls. As a positive correlation was observed between the CD4<sup>+</sup>/CD8<sup>+</sup> ratios in the spleens, livers and PB, it is possible to expect to predict the immunological state of the liver and spleen from the immunological state of PB. In addition, carcinogenesis was significantly lower in groups in which a large difference in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio was observed between before and after splenectomy or in those with a high CD4<sup>+</sup>/CD8<sup>+</sup> ratio before splenectomy though there were few cases that we could observe. The CD4<sup>+</sup>/CD8<sup>+</sup> ratio is likely to be a key parameter for appropriate tumor-infiltrating lymphocyte function, and was shown to be different in different types of cancer.<sup>2,31–35</sup> Host immune responses to cancer were reported to depend on T lymphocytes, particularly CD8<sup>+</sup> lymphocytes.<sup>18,19,24,36–39</sup> An increase in their ratio after splenectomy and the consequent decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio observed in this study may be a positive change in terms of immunology against HCC. Such a change was particularly marked in patients with a high CD4<sup>+</sup>/CD8<sup>+</sup> ratio before splenectomy.

In our study, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio also significantly increased as the fibrosis of non-tumor areas in the liver tissue progressed. These significant differences were observed regardless of the HCC status. Although the cause of these differences is unknown, it appears to depend on the background of histological factors in the liver such as fibrosis. Many studies have investigated the relationship between tumors, Treg and TGF-β.<sup>20–22,25,40</sup> Guo-He *et al.* showed that the expression of TGF-β appeared to be positively correlated with Treg in HCC tissue. The 5-year survival rate was significantly lower in patients with HCC tissues with high Treg cell infiltration than in those with low infiltration.<sup>20,22,36,41</sup> Our study also revealed that Treg cells were positively correlated

with TGF-β1 positive cells even in “non-tumor areas” of liver tissue, and that TGF-β1 positive cells were positively correlated with liver fibrosis. There were no significant differences of TGF-β1 before and after splenectomy. The reason for the chronological changes in TGF-β1 levels after splenectomy is unknown because various factors including platelets may be involved in the production of TGF-β1. We also found a slightly higher number of TGF-β1 positive cells in non-tumor areas in the liver tissue of patients with HCC than in those without. Furthermore, the number of TGF-β1 positive cells significantly increased with the progression of liver fibrosis.<sup>4,21,26,42</sup>

In conclusion, splenectomy in cirrhotic patients with hepatitis may be able to improve liver fibrosis, cause beneficial immunological changes and lower the risk of carcinogenesis. It seems necessary to accumulate further cases to establish a convincing conclusion.

## ACKNOWLEDGMENTS

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# Hepatic resection for hepatocellular carcinoma in super-elderly patients aged 80 years and older in the first decade of the 21st century

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## Abstract

**Purpose** We evaluated the preoperative and postoperative characteristics and prognosis of super-elderly patients with hepatocellular carcinoma (HCC).

**Methods** Four hundred and thirty-one patients who underwent hepatic resection for HCC were classified into three groups according to their age at the time of surgery: super-elderly ( $\geq 80$  years;  $n = 20$ ), elderly (70–80 years;  $n = 172$ ) and younger ( $< 70$  years;  $n = 239$ ). We compared the clinical characteristics, preoperative and postoperative factors and prognosis among the groups to evaluate whether liver resection is appropriate for super-elderly patients.

**Results** The liver function was not significantly different among the groups. The proportion of patients with preoperative cardiovascular and respiratory disease and hypertension was higher in the super-elderly group compared to the other groups. The super-elderly group had shorter operations and reduced hemorrhage rates compared to the other groups. Postoperative cardiovascular complications and delirium were more frequently observed in the super-elderly group. The overall and tumor-free survival rates were not significantly different among the groups. Super-elderly patients had a lower rate of liver or HCC-related death and a higher rate of death due to other causes than the other groups.

**Conclusions** Super-elderly HCC patients who are appropriately evaluated and selected might have a favorable prognosis after undergoing hepatic resection.

**Keywords** Hepatectomy · Super-elderly · Hepatocellular carcinoma · Delirium

## Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide [1] and the fourth most common cancer in Japan [2]. In recent years, the prognosis of HCC patients has improved because of new developments in surgical techniques and perioperative management. Japan has the highest national life expectancy in the world, with life expectancies at birth of 79.55 years for males and 86.30 years for females [3]. Therefore, the rates of hepatic resection for HCC are increasing in elderly patients aged  $\geq 70$  years. Recently, and particularly in the 21st century, liver resection has been performed at increasing rates to treat HCC in Japanese patients aged  $\geq 80$  years.

Many reports on the outcomes of hepatic resection in elderly HCC patients have been published [4–25, 27]. Previous reports from the 1980 s defined elderly patients as those aged  $\geq 65$  years [4–6], whereas reports from 1990 to 2009 defined this group as patients aged  $\geq 70$  years and  $> 75$  years [12–27]. In the present study with a follow-up period during the most recent decade (2000–2010), the age distribution of HCC patients was as follows:  $\sim 40$  % were elderly patients aged  $> 70$  years and  $\sim 5$  % were octogenarian patients. Therefore, at our institution, hepatic resection was commonly performed on elderly HCC patients, and hepatectomy had also become relatively common for octogenarian patients with HCC.

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The proportion of octogenarian HCC patients is expected to increase further in the near future. In a search of the PubMed database, only a few reports were found that described the outcomes of hepatic resection in HCC patients aged  $\geq 80$  years [7–11]. Wu et al. [9] initially reported the surgical outcomes of octogenarian patients with HCC in Taiwan. In Japan, Sirabe et al. [10], Yamada et al. [7] and Tsujita et al. [8] reported on hepatic resection for HCC in patients aged  $\geq 80$  years. Nanashima et al. [11] reported that the number of patients in the  $>80$ -year age group had increased during the period from 2000 to 2009, and significant differences were not observed among the age groups with regard to long-term prognosis.

In the present study, we evaluated the preoperative and postoperative characteristics and prognosis of super-elderly patients (aged  $\geq 80$  years) with HCC to evaluate whether liver resection is appropriate for octogenarian patients.

## Methods

The study subjects were 431 patients who underwent initial hepatic resection for HCC between January 2000 and December 2010. The patients were classified into three groups according to their age at the time of surgery: a super-elderly group ( $n = 20$ ),  $\geq 80$  years old; an elderly group ( $n = 172$ ), 70–80 years old and a younger group ( $n = 239$ ),  $<70$  years. We compared the patient's clinical characteristics (background factors, hepatitis viral infection status, preoperative comorbidity and liver function data), surgical factors (hepatic resection type, length of operation, blood loss and blood transfusion status), pathological factors, postoperative complications (including delirium [28]), hospital stay and prognosis among the three groups to evaluate whether liver resection is appropriate for super-elderly patients.

At our institution, the general condition of super-elderly patients is evaluated to determine their ability to tolerate surgery. Most of the patients in the super-elderly group consulted with a specialist who assessed the adequacy of their preoperative cardiovascular and pulmonary condition through electrocardiography, echocardiography, myocardial scintigraphy, respiratory function testing and chest computed tomography. Furthermore, the super-elderly patients also consulted with a psychiatrist who assessed their psychological condition (including the risk of postoperative delirium). Super-elderly patients concerned about postoperative delirium were prescribed antipsychotic treatments by a psychiatrist.

The terminology for the hepatic anatomy and type of liver resection were classified according to the Brisbane 2000 Terminology of Liver Anatomy and Resections [29]. Liver resection was performed according to the criteria proposed by Makuuchi et al. [30]. In the super-elderly group, if more than one type of liver resection was allowed, the less

invasive method (shorter operation and less tissue removal) was selected.

The postoperative complications analyzed were those that corresponded to a Clavien-Dindo grade above IIIa. Postoperative delirium was diagnosed by consulting with a psychiatrist. In addition, professional psychiatric treatment was recommended for patients with a Clavien–Dindo grade above II [31]. Postoperative bile leakage [32] and postoperative liver failure [33] were defined and graded according to the guidelines of the International Study Group of Liver Surgery (ISGLS).

This study was conducted in accordance with the declaration of Helsinki and the guidelines of the ethics committee of our institution. Written informed consent was obtained from each patient.

## Statistical analysis

The data are presented as medians with the 10th and 90th percentiles. The Tukey–Kramer honest significant difference test or  $\chi^2$  test was used to compare clinicopathological variables among the three groups. The survival rates were estimated according to the Kaplan–Meier method, and differences in the survival rates among the groups were compared using the log-rank test. All statistical analyses were performed using a statistical software program (JMP 9.0.0.; SAS, Cary, NC, USA). A  $p$  value  $<0.05$  was considered to be significant.

## Results

Clinical characteristics of the three groups (Table 1)

The super-elderly group comprised 14 males and six females, with a mean age of  $81.5 \pm 1.5$  years. The elderly group comprised 125 males and 47 females, with a mean age of  $73.9 \pm 2.6$  years. The younger group comprised 199 males and 43 females, with a mean age of  $61.6 \pm 7.0$  years. The percentage of patients positive for the hepatitis B surface antigen (HBs Ag) was higher in the younger group. The percentage of patients positive for the hepatitis C virus antibody (HCV Ab) was higher in the elderly group and the percentage of patients negative for both HBs Ag and HCV Ab (non-B non-C HCC) was higher in the super-elderly group. There were no significant differences among the three groups with regard to alcohol abuse or body mass index. The prevalence of diabetes was lower in the super-elderly group than in the other groups, but the prevalence of hypertension was higher in the super-elderly group compared to the other groups.

The percentages of patients with histories of preoperative cardiac disorders and pulmonary disorders were higher in the

**Table 1** The preoperative patient characteristics according to age

Characteristics	Super-elderly group <i>n</i> = 20 (4.6 %)	Elderly group <i>n</i> = 172 (39.9 %)	Younger group <i>n</i> = 239 (55.4 %)	<i>p</i> value
Age (years, mean ± SD)	81.5 ± 1.5	73.9 ± 2.6	61.6 ± 7	<0.001
Gender (male/female)	14/6	125/47	197/42	0.043
HBs Ag	0 (0 %)	5 (3 %)	67 (28 %)	<0.001
HCV Ab	11 (55%)	121 (70 %)	115 (48 %)	<0.001
Non-B non-C	9 (45 %)	46 (26 %)	59 (24 %)	0.140
Alcohol abuse I	(5%)	30 (17%)	52 (21 %)	0.139
BMI (kg/m <sup>2</sup> )	22.6 (18.6–25.9)	22.5 (18.4–27.4)	23.1 (19.4–27.7)	0.120
Preoperative complication				
Diabetes mellitus	3 (15 %)	65 (38 %)	66 (27 %)	0.023
Hypertension	13 (65 %)	77 (45 %)	81 (34 %)	0.005
Cardiovascular disease	5 (25 %)	13 (8 %)	9 (4 %)	<0.001
Respiratory disease	3 (15 %)	13 (8 %)	9 (4 %)	0.049
Renal failure	1 (5 %)	5 (3 %)	4 (2 %)	0.512
Brain disease	3 (15 %)	8 (5 %)	11 (5 %)	0.120
ASA-PS(1–2/3)	16/4	141/31	210/29	0.185
Cancer in other organs	4 (20 %)	22 (13 %)	14 (6 %)	0.014
History of abdominal surgery	7 (35 %)	43 (25 %)	34 (14 %)	0.005
Liver function data				
Total bilirubin (mg/dL)	0.65 (0.4–1.5)	0.8 (0.4–1.3)	0.8 (0.4–1.3)	0.500
Albumin (g/dL)	3.85 (3.1–4.5)	3.9 (3.3–4.4)	3.9 (3.3–4.4)	0.236
Prothrombin time (%)	94 (78–105)	94 (76–112)	92 (73–113)	0.860
Platelet count (10 <sup>4</sup> /μL)	17.6 (9.3–33)	14.7 (8.3–23)	14.6 (8.6–22)	0.396
AST (IU/L)	40 (25–69)	42 (24–79)	44 (26–87)	0.304
ALT (IU/L)	30 (15–70)	37 (19–85)	46 (23–98)	0.002
Child-Pugh (A/B)	19/1	145/26	203/36	0.545
ICG R15 (%)	18 (8.2–26.7)	14.7 (7.9–28)	14.6 (6.6–27.8)	0.805
α-FP (ng/mL)	18.3 (2.1–1510)	12.8 (3.9–880)	21 (3.7–1177)	0.530
PIVKA-II (AU/mL)	171 (14.8–26933)	151 (15–6435)	105 (14–5574)	0.208

The results of the laboratory tests are expressed as the medians with 10th and 90th percentiles

*HBs Ag* hepatitis B surface antigen, *HCV Ab* hepatitis C virus antibody, *BMI* body mass index, *ASA-PS* American Society of Anesthesiologists Performance Status, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ICG R15* indocyanine green dye retention rate at 15 min, *α-FP* a-fetoprotein, *PIVKA-II* protein induced by vitamin K absence or antagonists-II

super-elderly group than in the other groups, but there were no significant differences in the preoperative complications in other organs among the three groups. All three groups had similar American Society of Anesthesiologist (ASA) scores. The percentage of patients with a history of cancer in other organs and abdominal surgery increased with age. There were no significant differences in the liver function among the three groups, with the exception of the alanine aminotransferase level. No differences were found in the Child-Pugh classifications, alpha-fetoprotein levels or levels of protein induced by vitamin K absence or antagonists-II among the three groups. No differences in the oncological aspects of the disease were found among the three groups.

Intraoperative and postoperative factors of the three groups (Table 2)

Anatomical resection was performed for nine patients in the super-elderly group. However, segmentectomy was

selected more often in the super-elderly group than in the other groups. Right lobectomy and trisectionectomy were not performed in the super-elderly group.

The super-elderly group had shorter operations and reduced hemorrhage rates compared to the other groups. Intraoperative transfusions, thoracotomy and laparoscopic hepatectomy were performed at equivalent rates among the three groups. The pathological factors were not significantly different among the three groups. The percentage of patients with a solitary tumor was higher in the super-elderly group.

The percentages of patients with postoperative cardiac disorders and delirium were higher in the super-elderly group than in the other groups, but there were no differences in the postoperative complications associated with other organs among the three groups. The median postoperative hospital stay was 11 days in the super-elderly group, 15 days in the elderly group and 17 days in the younger group.

**Table 2** The postoperative patient characteristics according to age

Characteristics	Super-elderly group <i>n</i> = 20 (4.6 %)	Elderly group <i>n</i> = 172 (39.9 %)	Younger group <i>n</i> = 239 (55.4 %)	<i>p</i> value
Operation-related factors				
Resection (anatomical/limited)	9/11	59/113	114/125	0.024
Limited resection	11 (55 %)	113 (66 %)	125 (52 %)	0.024
Segmentectomy	5 (25 %)	14 (8 %)	35 (15 %)	0.033
Sectionectomy	3 (15 %)	29 (12 %)	50 (21 %)	0.525
Two or more sectionectomies	1 (5 %)	16 (9 %)	29 (12 %)	0.461
Resected liver weight (g)	90 (13–435)	70 (15–442)	105 (20–509)	0.066
Length of operation (min)	205 (90–316)	274 (148–434)	270 (131–463)	0.027
Hemorrhage (mL)	300 (14–1159)	585 (90–2567)	510 (80–2107)	0.041
Transfusion	4 (20 %)	45 (26 %)	66 (28 %)	0.746
Thoracotomy	0 (0 %)	3 (1.2 %)	1 (0.6 %)	0.715
Laparoscopic surgery	2 (10 %)	20 (8.3 %)	13 (7.6 %)	0.917
Pathological factors				
Tumor size (cm)	4.1 (2.6–9.4)	3.2 (1.5–7.2)	3 (1.6–6.7)	0.162
Tumor number (solitary)	18 (90 %)	123 (71.5 %)	155 (64.8 %)	0.038
Differentiation (well/mod/poor)	0/10/8	12/103/53	14/128/88	0.475
Pathological vascular invasion	5 (26 %)	50 (29 %)	88 (37 %)	0.186
Degree of hepatic fibrosis(0–3/4)	10/6	94/62	131/95	0.868
Grade of active hepatitis (0–2/3)	16/0	145/6	211/10	0.675
Postoperative complications				
Surgical site infection	0 (0 %)	6 (3.5 %)	9 (3.7 %)	0.682
Abdominal infection	1 (5 %)	12 (7.0 %)	10 (4.1 %)	0.444
Bile leakage	1 (5 %)	10 (5.8 %)	10 (4.1 %)	0.734
Pleural effusion/ascites	0 (0 %)	28 (16 %)	31 (13 %)	0.115
Liver failure	0 (0 %)	1 (0.6 %)	5 (2.1 %)	0.383
Cardiovascular disease	2 (10 %)	2 (1.2 %)	2 (0.8 %)	0.003
Respiratory disorder	0 (0 %)	0 (0 %)	3 (1.2 %)	0.302
Delirium	6 (30 %)	34 (20 %)	23 (9.5 %)	0.002
Sepsis	0 (0 %)	1 (0.6 %)	3 (1.2 %)	0.715
Hospital stay (days)	11 (7–20)	15 (9–41)	17 (8–40)	0.107
Hospital death	0 (0 %)	0 (0 %)	1 (0.4 %)	0.668
Long-term prognosis				
Liver- or HCC-related death	2 (10 %)	36 (21 %)	71 (30 %)	0.040
Other causes of death*	1 (5 %)	13 (7.5 %)	8 (3 %)	0.040

The results of the laboratory tests are expressed as the medians with 10th and 90th percentiles

\*Other causes of death include pneumonia, cardiovascular disease, cerebrovascular disease and other malignancy excluding hepatocellular carcinoma (HCC)

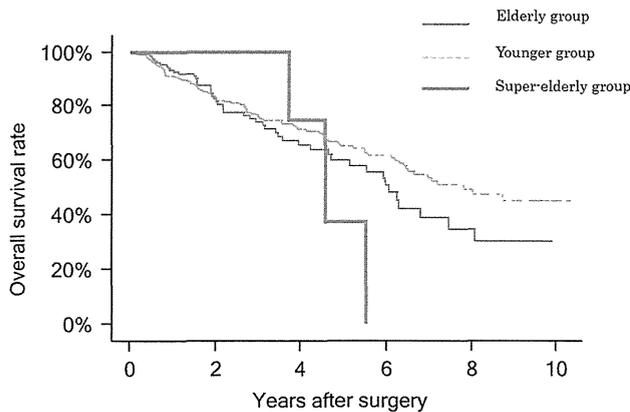
The long-term prognosis in the three groups

The overall survival rates of the three groups are shown in Fig. 1, and the tumor-free survival rates are shown in Fig. 2. There were no significant differences in the overall survival and tumor-free survival rates among the three groups. The 5-year overall survival rates in the super-elderly, elderly and younger groups were 66.9, 59.5 and 65.3 %, respectively ( $p = 0.44$ ). The 3-year tumor-free survival rates in the super-elderly, elderly and younger groups were 33.7, 41.2 and 46.4 %, respectively ( $p = 0.78$ ). The super-elderly group had a lower rate of liver or HCC-related deaths, and a higher rate of death due to other causes (pneumonia,

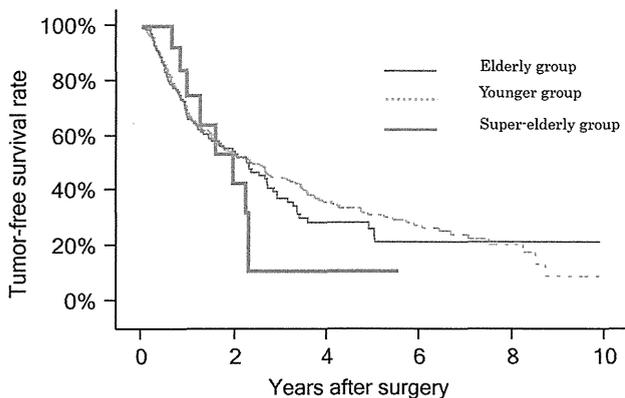
cardiovascular disease, cerebrovascular disease and other malignancy excluding HCC) than the other groups (Table 2).

## Discussion

There have been many reports about the outcomes of hepatic resection in elderly HCC patients. Over the past few decades, the definition of “elderly patients” has changed. In the 1980s [4–6], elderly patients were defined as those aged  $\geq 65$  years, whereas the same term was used for those aged  $\geq 70$  years or those  $>75$  years during the period from 1990



**Fig. 1** A comparison of the overall survival curves after primary hepatectomy for the 1 super-elderly ( $\geq 80$  years), elderly ( $\geq 70$  years and  $< 80$  years) and younger ( $< 70$  years) two groups. *Thin line* is elderly group, *dotted line* is younger group, and *thick line* is three super-elderly group



**Fig. 2** A comparison of the tumor-free survival curves after primary hepatectomy for the super-elderly ( $\geq 80$  years), elderly ( $\geq 70$  years and  $< 80$  years) and younger ( $< 70$  years) groups. *Thin line* is elderly group, *dotted line* is younger group, and *thick line* is three super-elderly group

to 2009 [12–27]. In the present study, the follow-up period was during the most recent decade (2000–2010), and ~40 % of the elderly HCC patients were aged  $> 70$  years. Therefore, hepatic resection for HCC was commonly performed on elderly HCC patients at our institution, and liver resection for octogenarian HCC patients has become relatively common. The proportion of octogenarian patients with HCC is expected to increase in the near future.

Wu [9] stated that octogenarians had a lower incidence of HBs Ag positivity. In the present study, no patient in the super-elderly group was positive for HBs Ag, and only a small proportion of patients were positive for HCV Ab in the elderly group. The proportion of patients negative for both HBs Ag and HCV Ab was higher in the super-elderly group than in the other groups. One reason for this may be

that, in Japan, recent HCC screenings for viral hepatitis patients have led to the early detection and rapid treatment of HCC. Additionally, the HBs Ab titers might decrease with age, which may explain why few elderly patients in this study were positive for HBs Ag. In fact, at least three patients in the super-elderly group were positive for the HBs Ab and/or hepatitis B core antibody (data not shown). Some authors have reported that a higher proportion of elderly HCC patients had HCV Ab positivity [15–17]. Therefore, the number of HCV patients aged  $\geq 80$  years might increase in the future.

The incidence of preoperative comorbidities among elderly patients is controversial [7–12, 16, 19, 23, 25, 27]. In this study, there were no significant differences among the three groups with respect to the incidence of preoperative systemic complications, with the exception of cardiovascular and respiratory complications and hypertension, which were more common in the super-elderly patients. Kaibori et al. [12] reported that elderly HCC patients were more likely to have a history of alcohol abuse. However, in our study, the super-elderly patients did not have any remarkable history of alcohol consumption. The ASA scores were similar in the three groups in our study. However, most patients in the super-elderly group consulted with specialists, who confirmed the adequacy of their preoperative cardiovascular, pulmonary and psychological condition, including a favorable risk for postoperative delirium. Therefore, in the super-elderly group, the patients had already been pre-selected so that they had a good physical status before they would be considered as candidates for liver resection.

The mean tumor size tended to be larger in the super-elderly group than in the other groups. This might be because of the higher incidence of non-B non-C HCC in this group; as such patients might not have been followed up or screened until the diagnosis of HCC. Although anatomical resection was selected more often for the super-elderly group than for the elderly group, the super-elderly patients tended to undergo less invasive resections (i.e., segmentectomy) than the other groups. Therefore, although the liver resection volume was similar among the three groups, the length of the operation and intraoperative hemorrhage rates were lower in the super-elderly group than in the other groups. Furthermore, the postoperative complication rates did not differ among the three groups. Although the prevalence of cardiovascular disease was higher in the super-elderly group than in the other groups, this difference was not significant.

Delirium is a major complication in elderly patients undergoing liver resection [28]. In the present study, elderly patients, particularly the super-elderly patients, consulted with a psychiatrist to evaluate their preoperative psychiatric condition. The psychiatrist provided advice or premedication for the patients. Therefore, the postoperative delirium

rate was relatively low in our patients. Nevertheless, in the present study, the incidence of postoperative delirium was higher in the super-elderly group than in the other groups. Therefore, postoperative delirium needs to be appropriately managed, and meticulous nursing care should be provided to super-elderly patients, especially those who exhibit risk factors for delirium, such as hypoalbuminemia and lengthy operations [28].

The postoperative hospital stay tended to be shorter in the super-elderly group than in the other groups. This was likely because we tended to perform less invasive surgeries on super-elderly patients. The increasing use of laparoscopic surgery has permitted super-elderly HCC patients to undergo less invasive surgeries. Furthermore, the shorter postoperative hospital stays might have led to a reduced risk of delirium in the super-elderly patients. After selection, the outcome of hepatic resection for HCC was not significantly different between the super-elderly group and the other groups.

Nearly all of the previous studies have reported a lack of significant differences between younger and elderly patients with regard to the resection type, length of operation and hemorrhage. Yamamoto et al. [25] reported that, in elderly patients (>70 years), right hepatic lobectomy was associated with an increased risk of postoperative hepatic failure and in-hospital death. They also reported that the regeneration rate of the residual left lobe at 1-month after a right hepatectomy tended to be lower in the elderly group than in the younger groups. Kaibori et al. [12] reported that the liver weight and hepatic blood flow were decreased in elderly patients, whereas Schmucker [26] noted that aging led to decreased hepatic regeneration. In our study, right lobectomy was not performed in the super-elderly group, and therefore, we cannot discuss the outcome of more invasive hepatectomies in super-elderly HCC patients.

In conclusion, super-elderly HCC patients ( $\geq 80$  years) might have favorable prognoses after hepatic resection if they are appropriately selected and evaluated not only according to their risk of liver function-related complications, but also according to their general condition, including their cardiovascular and pulmonary condition and psychological state.

**Conflict of interest** A. Nozawa and other co-authors have no conflict of interest.

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