Discussion

The decision whether to perform liver transplantation for FHF patients is critical, but also often difficult.[19] Marker molecules in more robust prognostic models may thus have important clinical value.

Several reports have noted that macrophage-related factors may play a dominant role in determining the severity of disease in patients with FHF.[4–6,23] Expression of osteopontin is high in Kupffer cells and hepatic macrophages in rat liver after carbon tetrachloride intoxication.[25] Expression of CD163 in liver tissue is higher in patients with acute viral hepatitis than in those with chronic viral hepatitis.[26,27] Serum levels of IL-10 and tumor necrosis factor-α are high in patients with FHF and correlate with risk of fatal outcomes.[4] Serum levels of sCD163, a lineage-specific scavenger receptor regulated by IL-10 that is involved in several anti-inflammatory functions of the immune system, are significantly higher in patients with FHF compared to patients with AH[26] and again correlate with fatal outcomes.[28] *In vitro*, macrophages can take up different phenotypes dependent on the cytokine environment,[29] as reflected *in vivo* by pro- and anti-inflammatory activation states balancing the immune response. Taken together, the high levels of macrophage activation markers in FHF may represent an anti-inflammatory imbalance, particularly in patients with poor disease outcomes. The present results indicate that serum levels of ferritin and HO-1, both of which originate from activated macrophages, and are highly increased in acute liver injury.

As ferritin synthesis is stimulated by Fe2+, which is generated by HO-1-mediated heme degradation, hyperferritinemia might be caused by high HO-1 activity, irrespective of the underlying disease. Induction of HO-1 by extracellular heme has been shown to increase the free iron pool relevant for subsequent sequestration into ferritin.[14]

HO-1, an inducible heme-degrading enzyme converting heme into CO, Fe2+, and biliverdin, is a 32-kD heat-shock protein. HO-1 is expressed by macrophages and endothelial cells in response to various noxious stresses, and plays an important role against oxidative injuries.[10–12] Recent studies have shown that in MAS such as HPS and AOSD, serum HO-1 levels correlate closely with serum ferritin levels. Serum HO-1 levels could thus prove useful in differential diagnosis of hyperferritinemia and perhaps also in monitoring disease activity.[14] Although HO-1 expression was markedly increased at both transcriptional and protein levels in hepatocytes with a rat model of carbon tetrachloride-induced acute liver injury,[30] an increase in free heme concentration may upregulate HO-1 gene expression in patients with acute liver injury. In the present study, serum levels of ferritin and HO-1 were significantly higher in FHF and AHS than in AH, suggesting that activated macrophages may play a role in progression to FHF.

Serum ferritin levels are increased not only by release from hepatocytes as a result of liver damage, but also from activated macrophages.[23,24,31] Distinguishing the origin of ferritin between activated macrophages and liver cell cytolysis may be difficult. A recent report demonstrated a high ferritin level with a low percentage of glycosylated ferritin in patients with MAS, such as HPS and AOSD.[32,33] However, assaying glycosylated ferritin is not easy. We therefore determined the F/A ratio to reflect ferritin released from activated macrophages, revealing a significant difference in F/A ratio between FHF and AHS.

In clinical situations, assessing whether patients presenting with features of acute liver damage are likely to recover after an acute attack of hepatitis or will eventually develop FHF is very important. The present study found that serum levels of HO-1 and ferritin, both of which are macrophage-activation markers, were high in patients with FHF and AHS. These results suggest that activation of macrophages occurs in FHF and AHS, and that inflammatory cytokines can interact in the initiation and progression of liver cell damage. HO-1 and ferritin may be produced directly by activated macrophages in the liver of FHF patients, as macrophages play a dominant role in the pathogenesis of severe inflammation in FHF.

F/A ratio could reflect the amount of ferritin released from activated macrophages, and was significantly high in non-surviving patients with FHF in this study. In addition, the combi-

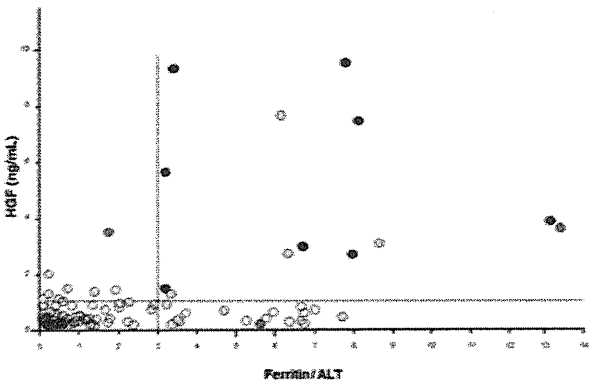

Figure 4 Ferritin/alanine aminotransferase (ALT) ratio with serum hepatocyte growth factor (HGF) level related to clinical outcomes. Horizontal dotted line indicates HGF at 1.60 ng/mL, as determined on the basis of receiver operating characteristics (ROC) curve. Vertical dotted line indicates an arbitrary cutoff value of 3.12 ng/mL for HO-1. ●, fulminant hepatic failure (FHF) non-survivor or transplantation; ●, FHF survivor; ◎, severe-form acute hepatitis (AHS); ○, acute hepatitis (AH).

Table 4 Prognostic prediction by combining HGF and F/A ratio

	Non-survivors	Survivors	Total	Sensitivity	Specificity	Likelihood ratio
HGF ≧ 1 ng/mL	8	20	28	100% (8/8)	81.1% (86/106)	5.3
HGF < 1 ng/mL	0	86	86			
HGF ≧ 1 ng/mL and F/A ratio ≧ 3	8	6	14	100% (8/8)	94.3% (100/106)	17.7
HGF < 1 ng/mL or F/A ratio < 3	0	100	100			

F/A ratio, ferritin/ALT ratio; HGF, hepatocyte growth factor.

nation of F/A ratio and serum HGF level offered a useful marker for predicting mortality from acute liver injury. However, these results may reflect both sides of the hepatocyte destruction and macrophage activation in FHF. Some limitations to the present study must be considered, such as the relatively small number of samples and the heterogeneous etiologies. Prospective studies are thus required to validate the predictive value of the markers identified.

In conclusion, our results suggest that activation of macrophages participates in the pathophysiology of acute liver injury, and that the combination of F/A ratio and serum HGF level offers a potent marker for predicting the severity and prognosis of acute liver injury.

Acknowledgments

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Letter to the Editor

Successful pegylated interferon alpha2a monotherapy for hepatitis C virus infection in a transplanted patient who relapsed after the preceding course

T. Ichikawa, N. Taura, H. Miyaaki, M. Matsuzaki, S. Eguchi, M. Takatsuki, T. Kanematsu, K. Nakao. Successful pegylated interferon alpha2a monotherapy for hepatitis C virus infection in a transplanted patient who relapsed after the preceding course.
Transpl Infect Dis 2011: **13**: 438–440. All rights reserved

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Key words: HCV; IFN; liver transplantation

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To the Editor

The combination of pegylated interferon (peg-IFN) with ribavirin is the only treatment strategy at present for the treatment for hepatitis C virus (HCV) infection after liver transplantation (LT), but its effects are still incomplete. We successfully treated a patient with peg-IFN alpha2a (peg-IFN-α2a) monotherapy who showed a relapse after treatment with peg-IFN combined with ribavirin.

A 55-year-old female with liver cirrhosis and hepatocellular carcinoma within the Milan criteria was referred to Nagasaki University Hospital in December 2005. On admission, she had no ascites or hepatic encephalopathy and was determined to have Child–Pugh score 7 and modified end-stage liver disease score 12.5. She had repeatedly ruptured esophageal varices. LT surgery was successfully performed. After living-donor LT, tacrolimus and prednisone were administered at the standard doses used in the immunosuppressive protocol. Her laboratory data on post-operative day 15 were 58 U/L of aspartate aminotransferase (AST) and 110 U/L of alanine aminotransferase (ALT). A liver biopsy revealed fibrosis stage 1 and activity grade 2. The calcineurin inhibitor was switched to cyclosporine for IFN treatment with the combination of 80 μg of peg-IFN-α2b weekly with 600 mg of ribavirin daily. Her HCV was genotype 1a and 1,570,000 IU/mL at the start of IFN treatment. The IFN treatment was introduced at postoperative day 19. The first IFN treatment continued for 52 weeks, because the HCV-RNA titer had a 2-log decrease from the baseline at 12 weeks of IFN treatment and disappeared at 18 weeks, and HCV-RNA was positive at the end point of treatment.

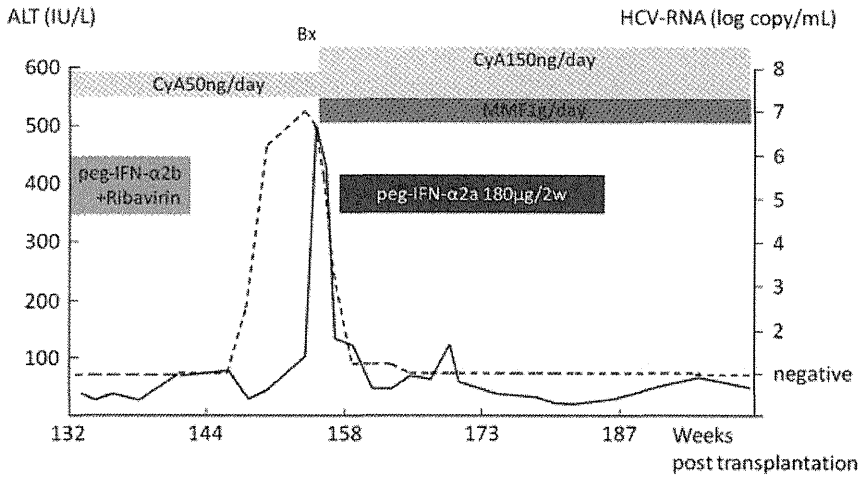

Fig. 1. Clinical course of rebound phenomenon after the second course of interferon (IFN) therapy. Solid lines are alanine aminotransferase (ALT) value (IU/L). Dotted lines are hepatitis C virus (HCV)-RNA value (log copy/mL). HCV-RNA is evaluated by the real-time polymerase chain reaction method. More than 1.2 log copy/mL of HCV-RNA is the sensitivity. Bx, liver biopsy; peg-IFN, pegylated interferon; w, weeks; CyA, cyclosporine (Neoral; Novartis Pharma, Tokyo, Japan); MMF, mycophenolate mofetil (Cellcept; Chugai, Tokyo, Japan).

After the first course, AST and ALT stabilized within the normal range and a liver biopsy revealed fibrosis stage 1 and activity score 1 at postoperative week 52. We waited for an improvement in the patient's side effects, and began the second treatment. The second course started at postoperative week 72, as her transplanted liver suffered from HCV-infected chronic hepatitis, according to the liver biopsy findings obtained at postoperative week 52. The second course was the combination of 90 μg of peg-IFN-α2a weekly with 200 mg of ribavirin daily for 9.5 g/dL of hemoglobin, because the early treatment of peg-IFN-α2b with ribavirin proved not to be effective (1).

An HCV-RNA qualitative assay, using the real-time polymerase chain reaction (PCR) method with a lower limit sensitivity of 15 IU/mL, was negative in serum at 20 weeks after IFN treatment. The HCV-RNA titer was positive in serum at 4 weeks after the end date of the second course, and reached a peak at 10 weeks after the second course. The ALT value indicated a relapse at 12 weeks. A liver biopsy revealed HCV-related chronic hepatitis. We introduced 180 μg of peg-IFN-α2a monotherapy once every 2 weeks, at 14 weeks after the second course.

HCV-RNA was negative by qualitative real-time PCR at 8 weeks after monotherapy. ALT was normalized at 4 weeks after monotherapy. The peg-IFN-α2a monotherapy was stopped at the treatment period of 6 months. HCV was negative at 6 months after the treatment end date, and she was determined to have a sustained viral response.

Some relapsing patients after IFN treatment show a flare up of HCV-RNA and ALT, the so-called 'rebound phenomenon' (2–6). ALT and HCV-RNA decrease again, after the rebound flare up. This is the best timing for induction of a second course of IFN (4, 5). The second course of IFN therapy has been reported to be IFN monotherapy for 6 months (4, 5). The second course of IFN monotherapy has a completion rate of 50% (4, 5). In our case, the third IFN monotherapy, but not the second IFN therapy, was induced at timing after rebound (Fig. 1).

The 'rebound phenomenon' is associated with immunological pressure against a rapid HCV increase (5, 7). A transplanted patient who shows relapse after the early treatment course must be treated quickly, because the HCV infection might be halted even by a single IFN treatment.

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CASE REPORT

Imaging of focal nodular hyperplastic-like nodules in alcoholic liver cirrhosis patients using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging

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Abstract We report on two patients with a history of alcohol abuse who presented with multiple hepatic nodules. Dynamic computed tomography revealed multiple nodular lesions, which were enhanced at the early contrast phase and washed out at the portal phase. In the hepatobiliary phase using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging (Gd-EOB-DTPA MRI), these tumors did not show any uptake, thus suggesting the presence of hepatocellular carcinoma. An ultrasound-guided biopsy revealed a slight increase in cell density, sinusoidal dilatation, and contained unpaired small arteries. According to immunohistochemical analyses, these arteries were positive for CD34 and alpha-smooth muscle actin. From these findings, the nodules were diagnosed to be focal nodular hyperplastic (FNH)-like nodules arising in alcoholic-cirrhosed livers. The differential diagnosis of FNH-like nodules arising in alcoholic liver cirrhosis and hepatocellular carcinoma is difficult with Gd-EOB-DTPA MRI, and therefore histological confirmation is necessary.

Keywords Focal nodular hyperplastic-like nodules · Alcoholic liver cirrhosis · Gd-EOB-DTPA MRI

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Introduction

Owing to the remarkable advances in various imaging techniques, an increased number of small nodular lesions are now being detected in the liver, and percutaneous fine-needle biopsy now makes it possible to perform a qualitative diagnosis of the detected lesions. However, Nakashima et al. [1] reported that focal nodular hyperplastic (FNH)-like nodules arising in patients with alcoholic liver cirrhosis could not be easily differentiated from well-differentiated hepatocellular carcinomas (HCCs), because the imaging results of FNH-like nodules and HCCs both reveal a high degree of hypervascularity. Recently, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) has been developed as a liver-specific magnetic resonance imaging (MRI) contrast agent [2–4]. In the hepatobiliary phase, hepatic lesions which lack normally-functioning hepatocytes are imaged, and then hepatocyte-selective uptake defects make it possible to differentiate HCCs and other benign hepatocellular nodules. However, few reports have so far examined the Gd-EOB-DTPA imaging findings of FNH-like nodules arising in patients with alcoholic liver cirrhosis. Here we outline two case reports and present the imaging findings using Gd-EOB-DTPA MRI for FNH-like nodules arising in patients with alcoholic liver cirrhosis, and show such nodules to have imaging characteristics similar to those of HCC.

Case reports

Case 1

A 47-year-old male patient was admitted to our hospital for further examination of multiple hepatic nodules. The

patient's alcohol consumption over 30 years was 120 g/day. At the time of admission, the patient's physical examination revealed a height of 160.8 cm, a weight of 37.2 kg, and a body temperature of 37.2°C. The relevant laboratory test results were as follows: platelet count 66,000/mm^3; prothrombin time 69%; serum albumin 3.6 mg/dl; total bilirubin 1.6 mg/dl; serum aspartate transaminase (AST) 115 U/l; serum alanine transferase (ALT) 56 U/l; and the hepatitis B surface (HBs) antigen and hepatitis C virus (HCV) antibody were negative. The tumor markers were elevated as follows: α-fetoprotein (AFP) 10.2 ng/ml and protein induced by vitamin K absence or antagonist (PIVKA) II 285 mAU/m. The indocyanine green clearance revealed a high degree of retention (60%). Ultrasonography revealed small nodular lesions, detected as hypoechoic or isoechoic nodules. Dynamic computed tomography (CT) revealed multiple nodular lesions which demonstrated a slightly high degree of attenuation at the early contrast phase and washed out at the portal phase. Nodular lesions in the liver S2 showed peripheral low-attenuation areas, suggesting encapsulation (Fig. 1). By MRI, the peripheral part of the nodule in S2 showed a low intensity. The inside part of this nodule showed a high intensity in the T1 in-phase images, low intensity in the T1 out-phase images, and high intensity in the T2 weighted images, thus suggesting that these nodules were steatosis. In the hepatobiliary phase using Gd-EOB-DTPA, part of the tumors show a hypointensity area, suggesting HCC component (Fig. 2). Because the possibility of HCC was not ruled out, a percutaneous liver nodule biopsy was performed on the S2 nodule for a definitive diagnosis.

On microscopic examination, in comparison to the background liver, the nodules showed a slightly increased cell density, scar-like fibrosis, sinusoidal dilatation, and steatosis. The scar-like fibrosis contained unpaired small arteries showing sinusoidal capillarization (Fig. 3). Immunohistochemically, although part of tumor did not show OATP1B3 immunostaining (Fig. 4), several CD68-positive Kupffer cells were detected in the nodule (Fig. 3). From these findings, the nodules were diagnosed as FNH-like nodules arising in a patient with alcoholic liver cirrhosis.

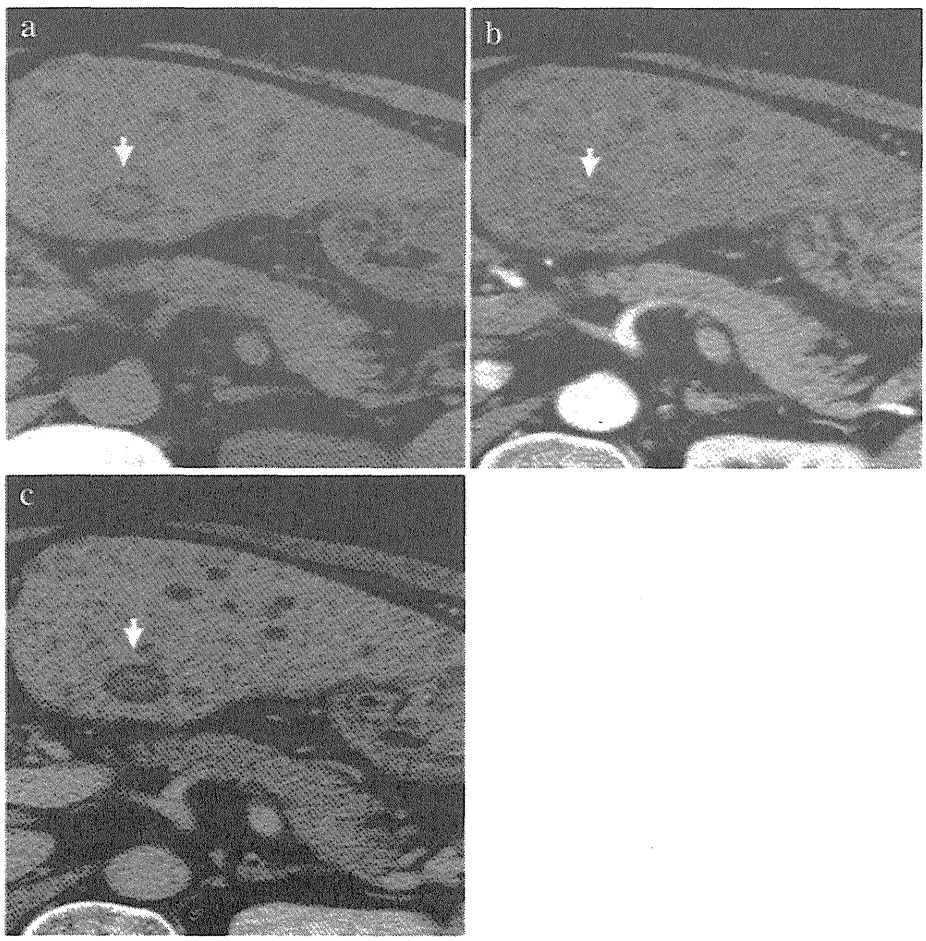

Fig. 1 Computed tomography (CT) findings in case 1. The nodular lesion in liver S2 showed slightly low attenuation on the unenhanced CT (**a**), showed a slightly high degree of attenuation at the early contrast phase (**b**), was washed out at the portal phase (**c**), and was peripherally low-attenuated on dynamic CT (*arrow*)

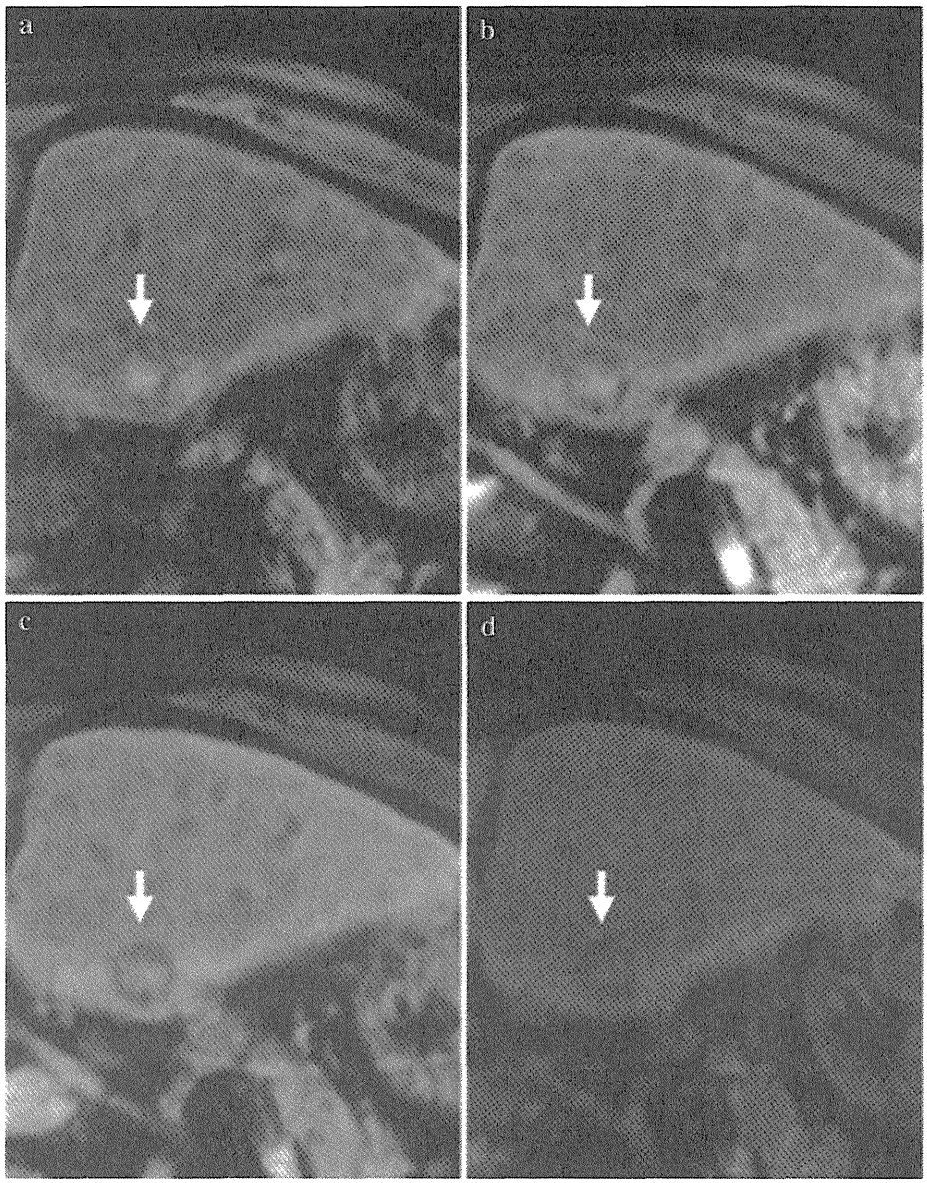

Fig. 2 Magnetic resonance imaging findings. The peripheral part of the nodule in S2 showed a low intensity. The inside part of this nodule showed a high intensity in the precontrast T1 weighted image (**a**), was slightly enhanced at the early contrast phase (**b**), and washed out at the portal phase (**c**). Part of the tumor showed defects in the hepatobiliary phase (**d**) (3D-gradient echo sequence (GRE) FS-T1W1, TR 4.30, TE 1.61, FA10)

Case 2

A 45-year-old female patient was admitted to our hospital for further examination of multiple hepatic nodules. The patient's alcohol consumption over 25 years was 180 g/day. At the time of admission, the physical examination revealed a height of 161.2 cm, a weight of 52.1 kg, and a body temperature of 36.3°C. The relevant laboratory test results were as follows: platelet count 96,000/mm^3; prothrombin time 58%; serum albumin 3.3 mg/dl; total bilirubin 0.5 mg/dl; serum AST 49 U/l; serum ALT 36 U/l; and the HBs antigen and HCV antibody were negative. The tumor markers were elevated as follows: AFP 34.8 ng/ml and PIVKA II 11 mAU/m.

Contrast CT revealed multiple nodular lesions, which had a strong contrast enhancement at the early contrast phase and washed out at the portal phase. By MRI, the nodules showed a high intensity in the precontrast T1 weighted images and a low intensity in the T2 weighted images. In the hepatobiliary phase using Gd-EOB-DTPA, these nodules did not show any uptake (Fig. 5). From these findings, the possibility of a well-differentiated HCC was not ruled out, and a percutaneous liver nodule biopsy was performed for a definitive diagnosis.

On microscopic examination, compared with the background liver, the nodules showed a moderate increase in cell density and a scar-like fibrosis (Fig. 6) which contained artery-like vessels. Immunohistochemically,

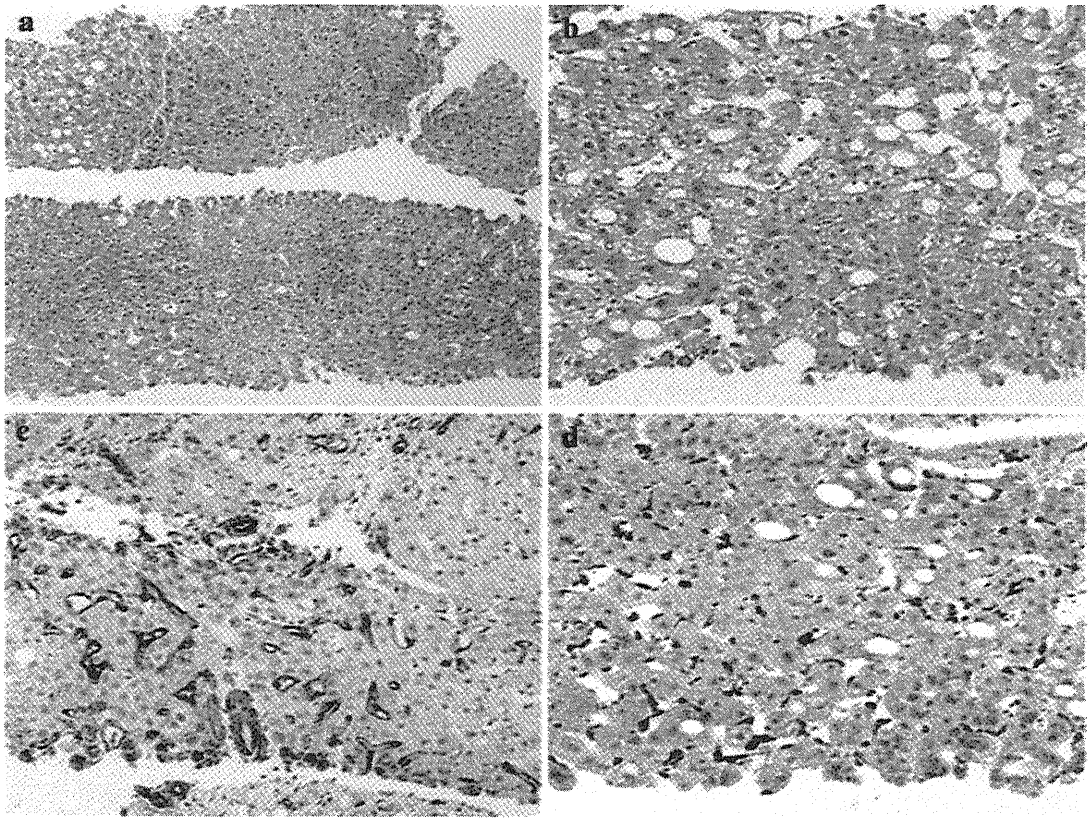

Fig. 3 Pathological findings of a liver biopsy in case 1. In comparison to the background liver tissue, the nodules showed a slightly increased cell density, scar-like fibrosis, and sinusoidal dilatation. Part of the nodule showed steatosis (**a**, **b**). The double immunostain for both CD34 and alpha-smooth muscle actin showed a diffuse staining pattern in the sinusoidal endothelial cells, suggesting sinusoidal capillarization and unpaired arteries (**c**). Several CD68-positive Kupffer cells were detected in this nodule (**d**)

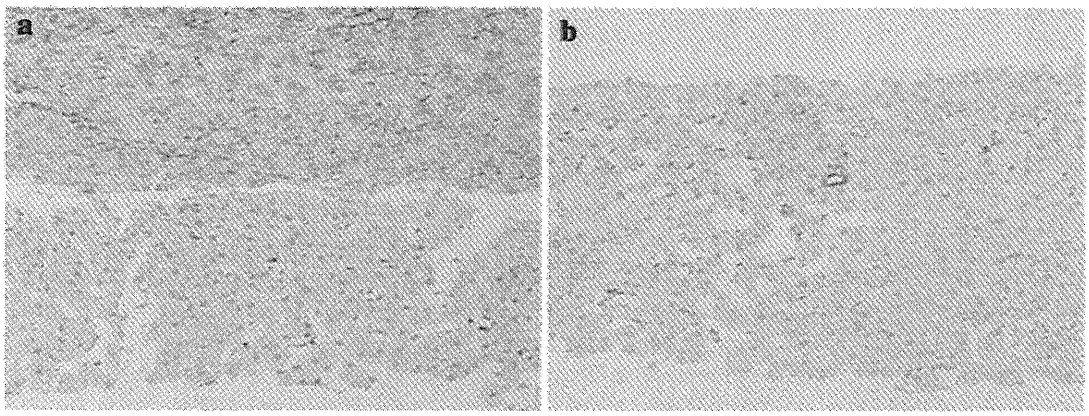

Fig. 4 The expression of OATP1B3 was observed predominantly in the cell membrane in the background liver in case 1 (**a**). However, part of this nodular lesion did not show any expression of OATB1B3 (**b**)

several CD68-positive Kupffer cells were observed in this nodule. These findings suggested a FNH-like nodule, arising from alcoholic cirrhosis, rather than a well-differentiated HCC.

Moreover, no immunostaining of OATB1B3 was observed in this nodule. These findings were consistent with the low intensity observed in the hepatobiliary phase of MRI (Fig. 7).

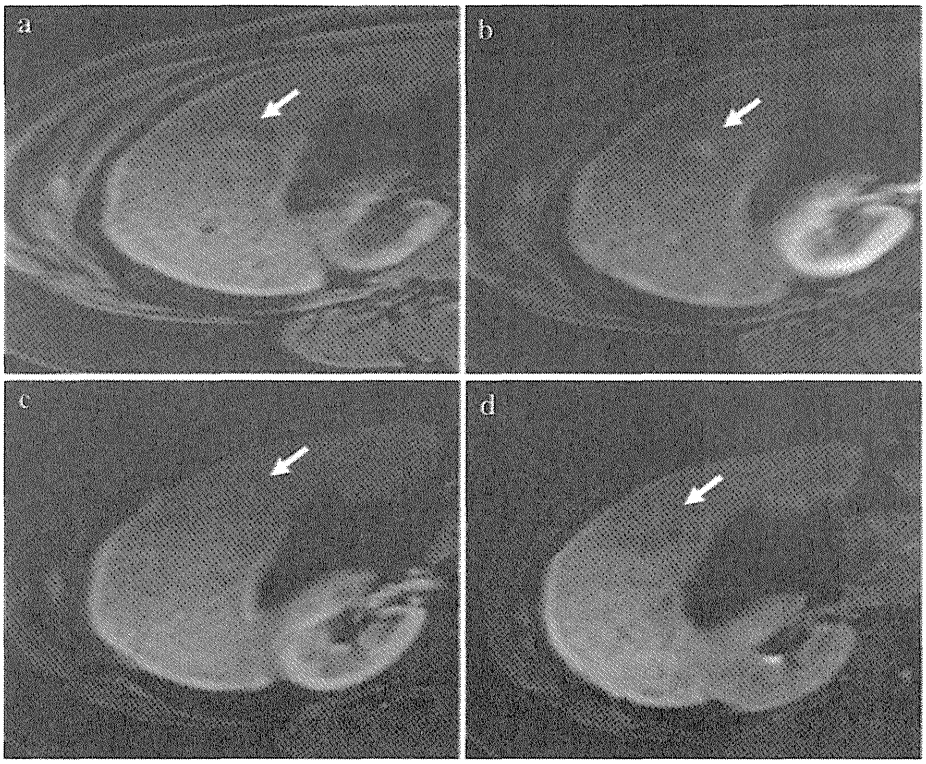

Fig. 5 Magnetic resonance imaging findings in case 2. The nodular lesion in liver S5 showed a high intensity at the precontrast T1 weighted image (**a**),was slightly enhanced at the early contrast phase of (**b**), was washed out at the portal phase (**c**), and had a defect in the hepatobiliary phase (**d**) (3D-GRE FS-T1W1, TR 4.30, TE 1.61, FA10)

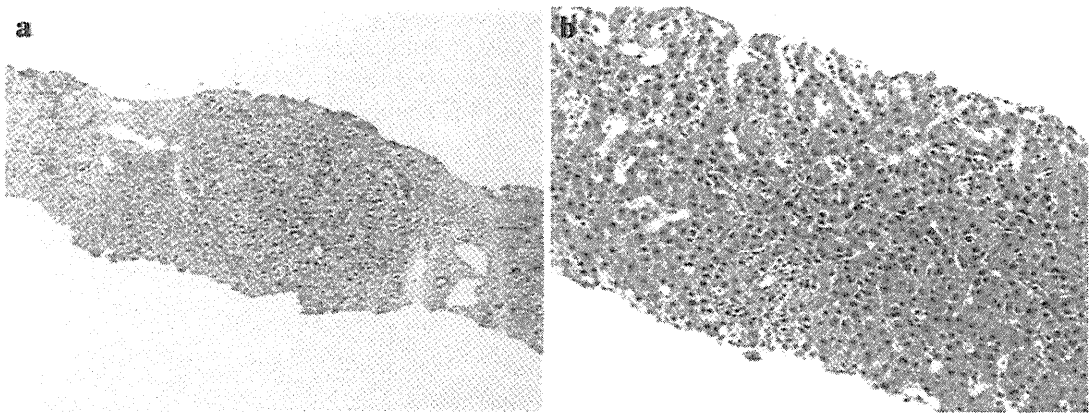

Fig. 6 Pathological findings of a liver biopsy in case 2. The background liver showed cirrhosis (**a**). The nodule showed a slightly increased cell density and sinusoidal dilatation in comparison to the background liver tissue (**b**)

Discussion

Although the International Working Party classified nodular hepatocellular lesions into several main classes, including regenerative lesions and dysplastic or neoplastic lesions [5], the most important clinical concern is the ability to distinguish between a regenerative lesion and a tumor lesion.

In the two current cases, HCC was first suspected from the imaging studies, which revealed a hypervascular tumor, although hyperplastic nodules arising from alcoholic liver cirrhosis were considered in the differential diagnoses.

Regarding the tumor markers, PIVKA II was elevated in case 1 and AFP was elevated in case 2. AFP is a well-known tumor marker of HCC, and its diagnostic efficacy has been confirmed; however, its serum levels are also observed to increase in liver diseases such as chronic hepatitis and cirrhosis. Conversely, the serum PIVKA II level is significantly elevated in alcoholic liver disease compared to viral liver disease, although the mechanism of

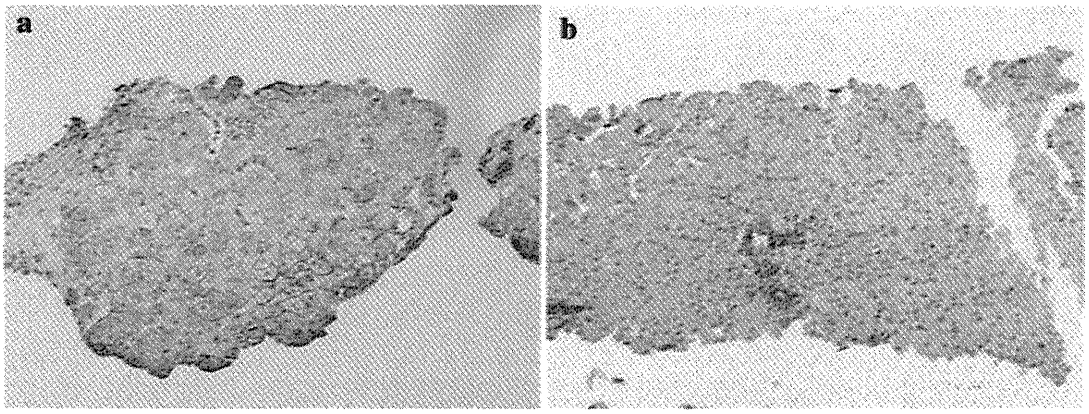

Fig. 7 The cell membrane of the background liver showed diffuse OATP1B3 in case 2 (**a**). This nodular lesion did not show any OATB1B3 expression (**b**)

the elevation of PIVKA II has not been clarified [6]. Ethanol intake and the following vitamin K deficiency may act, in part, due to an increased serum PIVKA II level in alcoholic liver disease. Taken together, tumor markers are not sufficient to differentiate between well-differentiated HCCs and FNH-like nodules arising in patients with alcoholic liver cirrhosis.

In case 1, the dynamic CT showed multiple nodular lesions with a delayed washout. In case 2, the dynamic CT showed multiple nodular lesions with arterial enhancement and a delayed washout. These findings were highly suggestive of a well-differentiated HCC. In most previous reports, these FNH-like nodules arising in patients with alcoholic liver cirrhosis lesions showed that the dynamic CT imaging resembles HCC, and furthermore, these two lesions are very difficult to differentiate from one another [1, 7–10].

Moreover, in case 1 the nodule in S2 showed encapsulation. While encapsulation is one of the typical findings in HCC, all FNH-like nodules in alcoholic liver cirrhosis are also reported to be encapsulated [1].

Recently, it has been reported that Gd-EOB-DTPA MRI has been helpful for the detection of small HCCs. In the present cases, most of the nodules showed defects in the hepatobiliary phase, suggesting HCC. Therefore, it is difficult to differentiate these hyperplastic nodules from well-differentiated HCCs using Gd-EOB-DTPA MRI alone. The difference in contrast following the administration of Gd-EOB-DTPA depends on the expression levels of the uptake transporter OATP1B3 [11] and excursion transporter (MRP2) [12]. The nodule in S2 showed partly positive expression of OATP1B3 in case 1. This finding was consistent with the MRI that showed slightly low intensity in the hepatobiliary phase. The S2 nodule in case 2 showed no OATP1B3 expression. These findings suggest that low intensity in the hepatobiliary phase depends on the lack of OATP1B3 expression in FNH-like hyperplastic nodules.

Moreover, some reports have shown the Gd-EOB-DTPA uptake to be associated with pericellular fibrosis in nonalcoholic steatohepatitis [13, 14]. Pericellular fibrosis is the one of characteristic features of alcoholic liver disease. Therefore, the poor uptake of Gd-EOB-DTPA in our cases may depend on the degree of pericellular fibrosis in the nodule.

We reported on two patients with FNH-like nodules arising from alcoholic cirrhotic livers, which revealed defects by Gd-EOB-DTPA MRI. The differential diagnosis of FNH-like nodules in patients with alcohol-induced cirrhosis and well-differentiated HCCs is very difficult with tumor marker and imaging studies alone, such as dynamic CT and Gd-EOB-DTPA MRI. Therefore, histological confirmation is required when nodules arising in patients with alcoholic cirrhosis are encountered.

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Clinical characteristics of hepatocellular carcinoma in elderly patients

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Abstract. The incidence of hepatocellular carcinoma (HCC) in elderly patients in Japan has been on the increase. The aim of the present study was to evaluate the impact of aging on the clinicopathological findings and the survival of HCC patients. A total of 624 patients with HCC were examined in this study. The patients were classified according to their age at the time of diagnosis: one group comprised younger patients (<75 years; n=544) and the second comprised elderly patients [(≥75 years; n=80, (12%)]. Results showed that there were significantly more female patients (younger:elderly, 22:36; p=0.005), normal livers (younger:elderly, 0.3:6%; p=0.0002), non-viral HCC (younger:elderly, 11:31%; p<0.001) and solitary tumors (younger:elderly, 53:76%; p=0.0008) in the elderly group. Five out of seven (71%) non-B non-C (NBNC) HCC patients who developed HCC in the normal liver were elderly patients. Survival between the younger and elderly HCC groups was not significantly different (younger:elderly, 4.38:3.45 years; p=0.665). Additionally, elderly HCC patients had fewer tumors, more mild underlying liver damage, and more frequent NBNC HCC. Their prognosis was not necessarily poorer than that of the younger HCC patients. Additionally, it appears that elderly patients develop HCC even without fibrosis. Therefore, aging may be a factor affecting hepatocarcinogenesis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers (1,2), with an estimated half a million cases annually, worldwide. Although HCC is generally diagnosed in middle-aged and elderly individuals, the age distribution of HCC varies according to etiology. The differences in age at the time of diagnosis of HCC affect the treatment strategy.

The Japanese population has one of the longest average life spans, and the size of the aged population has been increasing rapidly. As a result, the prevalence of elderly patients with HCC has increased (3-5). There is some controversy regarding whether aging plays a role in the factors and survival of patients with HCC. Previous studies reported that the long-term survival of younger HCC patients is similar to that of elderly patients (6,7). On the other hand, it has been reported that elderly HCC patients tended to have a poorer prognosis (8).

A recent increase in the number of elderly HCC patients in Japan has been reported (4,9,10). However, the impact of aging on the emergence of HCC has yet to be adequately investigated. Therefore, the aim of the present study was to investigate the effect of aging on the clinicopathological findings and the survival of HCC patients.

Patients and methods

Patients. A total of 624 patients presenting with HCC at the Department of Gastroenterology and Hepatology, Nagasaki University School of Medicine, Japan, were recruited for this study, between October 1981 and October 2007. The diagnosis of HCC was based on α-fetoprotein (AFP) levels, des-γ-carboxy prothrombin (DCP) levels, imaging studies including ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI), hepatic angiography (HAG) and/or liver biopsy. The diagnosis of chronic liver disease and liver cirrhosis was based on the level of platelets and imaging studies and/or liver histology. The patients were classified into two groups according to their age at the time of diagnosis: a younger group (<75 years; n=544) and an elderly group (≥75 years; n=80).

Etiology of HCC. A diagnosis of chronic hepatitis C virus (HCV) infection was based on the presence of HCV antibodies (microparticle enzyme immunoassay; Abbott Laboratories, Tokyo, Japan) and HCV-RNA detected by polymerase chain reaction (PCR), whereas the diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBs/Ag) (enzyme-linked immunosorbent assay; Abbot

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Key words: hepatocellular carcinoma, aging, non-viral hepatocellular carcinoma

Table I. Patient characteristics.

Characteristics	
Age (years)	63.9±9.8
Gender, male : female	478:146
BMI	22.6±3.2
Normal : CH : LC	7:120:497
Child-Pugh grade	6.3±1.6
NBNC : HBV : HCV	74:139:430:19
Tumor diameter (cm)	4.3±3.5
No. of tumors	2.8±3.1

BMI, body mass index; CH, chronic hepatitis; LC, liver cirrhosis, NBNC, non-B non-C; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table II. Comparison of the patient backgrounds.

	<75 Years old	≥75 Years old	p-value
	544 Cases	80 Cases	
Gender (female)	117 (22%)	29 (36%)	0.0050
Normal liver	2 (0.3%)	5 (6%)	0.0002
Liver cirrhosis	440 (80%)	57 (71%)	0.0450
Child-Pugh grade	6.3±1.7	6.0±2.2	0.1650
Prothrombin time (%)	77±19	79±24	0.4600
Bilirubin (mg/dl)	1.5±2.4	1.0±0.7	0.1080
Albumin (g/dl)	3.8±3.2	3.6±0.5	0.7380

Table III. Comparison of risk factors for hepatocellular carcinoma.

	<75 Years old	≥75 Years old	p-value
	544 Cases	80 Cases	
HBsAg-positive	131 (24%)	8 (10%)	0.004
HCVAb-positive	381 (70%)	49 (61%)	0.112
NBNC	59 (11%)	25 (31%)	0.001
Diabetes mellitus	152 (28%)	22 (28%)	0.934
Alcohol consumption	117 (22%)	10 (12%)	0.085

HBsAg, hepatitis B surface antigen; HCVAb, Hepatitis C antibody; NBNC, non-B non-C.

Laboratories). The history of alcohol intake was noted from medical records. Habitual drinking was defined as an average daily consumption of an amount equivalent to 80 g of pure ethanol over a period of >10 years.

Statistical analysis. The SPSS 9.0 for Windows statistical software program was used to assess correlations among multiple variables. When appropriate, clinical and laboratory data were compared with a χ^2 analysis, the Student's t-test or the Mann-Whitney test. The survival from time of diagnosis of HCC was analyzed using the Kaplan-Meier method and compared using the log-rank method. P<0.05 was considered to be statistically significant.

Table IV. Comparison of tumor characteristics and therapy for hepatocellular carcinoma.

	<75 Years old	≥75 Years old	p-value
	544 Cases	80 Cases	
Diameter (cm)	4.2±3.4	4.3±3.9	0.8250
No. of tumors	4.4±5.2	1.9±2.3	0.0060
Solitary cases	293 (53%)	56 (76%)	0.0008
TNM, stage I or II	338 (62%)	59 (73%)	0.0430
Surgical resection	68 (12.5%)	7 (9%)	0.3350
Local ablative therapy	144 (26%)	27 (33%)	0.1780
TACE	260 (47%)	40 (50%)	0.7130

TACE, transarterial chemoembolization.

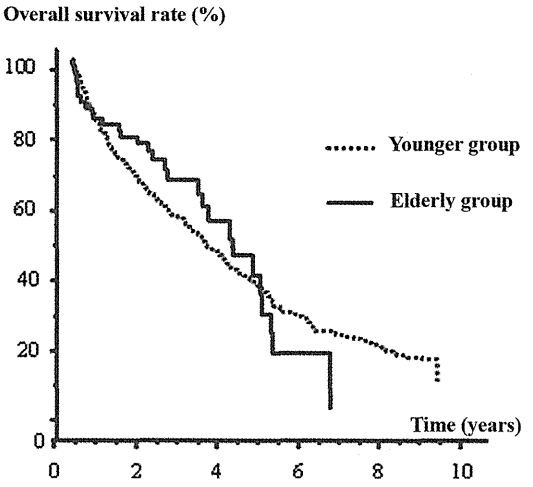

Figure 1. Kaplan-Meier model of the overall survival rate for the younger and elderly groups (younger HCC, 544 cases; elderly HCC, 80 cases). The overall survival between the younger and elderly HCC groups was not significantly different (p=0.665).

Results

Of the 624 patients, 80 (12%) patients were aged 75 years or older. The mean age of these older patients was 78.7±3.6. The clinical characteristics of the patients are shown in Table I. Significantly more patients in the elderly group were female (22:36%; p=0.005). The incidence of patients with liver cirrhosis was significantly higher, and the presence of a normal liver was significantly lower in the younger group than in the elderly group (80:71%; p=0.045; 0.3:6%, p=0.0002). No significant differences were observed in the protrobin time, total bilirubin, albumin or liver function as expressed by the Child-Pugh grade between the two groups (Table II).

Regarding viral status, the number of patients positive for HBsAg was significantly lower in the elderly group (24:10%, p=0.004), and the number of patients who were HBsAg and HCV antibody-negative [non-B non-C (NBNC)] was higher in the elderly group than in the younger group (11:31%, p=0.001).

In the NBNC HCC patients in the elderly group, 6 of 23 patients showed chronic hepatitis and 5 of 25 showed normal livers. No significant differences were found between the younger and elderly HCC groups with regards to alcoholism and diabetes mellitus (Table III).

No significant differences were noted in the tumor diameter between the younger and elderly groups. The number of HCC nodules was significantly lower in the elderly group than that in the younger group (4.4±5.2:1.9±2.3, p=0.006). The incidence of solitary cases and TNM stage I or II disease was significantly higher in the elderly group compared to that of the younger group (53:76%, p=0.0008; 62:73%, p=0.043). No significant differences were found between the younger and elderly HCC groups with regards to surgery, ablation therapy and transarterial chemoembolization (TACE) (Table IV).

The overall survival rate between the younger and elderly HCC groups was not significantly different (p=0.665). The overall median survival for the younger group was 4.38 years, compared with 3.45 years for the elderly group (Fig. 1).

Discussion

Age at diagnosis has been shown to have significant prognostic value in certain types of cancer. Although the number of elderly patients with HCC is on the increase in Japan (3,4), the characteristics and prognosis of HCC in elderly patients has yet to be elucidated. In this study, patients with HCC aged 75 years or older were examined, and their clinicopathological characteristics were identified and compared to those of the younger patients.

There were more male patients presenting with HCC in the younger group as compared to the elderly patients; one of the reasons for this being the difference in viral status. In this study, HBV infection, which is more common in males (11,12), was lower in the elderly group than in the younger group. Moreover, males were more likely to be heavy drinkers.

The prevalence of a normal liver was higher, whereas that of liver cirrhosis was lower in the elderly group. Of note is that 5 of 23 (21%) patients with NBNC HCC in the elderly group had normal livers. Additionally, 5 of 7 patients whose HCC developed in a normal liver were in the elderly group.

Chronic inflammation and viral infection are considered to be significant risk factors for HCC, but the elderly patients recruited in this study had neither factor. A previous study reported that the telomere length in the liver is shortened, not only with the progression of fibrosis staging, but also with aging (13). Moreover, the reduction of telomere length has been reported to increase the risk of HCC (14). Thus, elderly patients may have shorter telomeres, predisposing them to develop HCC, even if chronic liver disease was not prevalent. Findings of various studies have suggested that aberrant DNA methylation is a crucial epigenetic alteration in HCC (15-17). Some of the aberrant methylation observed in human cancer may be a consequence of chronic viral inflammation (18,19). On the other hand, aberrant methylation is also observed in the normal aging process (20), and may contribute to the occurrence of HCC in elderly patients with normal livers.

In this study, the HBV infection rate was lower, while the NBNC rate was higher in the elderly group than that in the younger group. Previous reports have shown that the average age of diagnosis of HBV-related HCC is approximately 55 years of age, whereas that of HCV-related HCC is approximately 65 years of age, and that of NBNC HCC is approximately 70 years of age (3,4). In Japan, the predominant time of transmission of the hepatitis B virus is during the prenatal period. The subsequent genomic long interreactions from an early age may lead to hepatocarcinogenesis at a younger age in the infected individuals.

On the other hand, patients with non-alcoholic steatohepatitis (NASH)-related HCC are older at diagnosis than those with HCC related to HBV and HCV (21,22). These results suggest that some of the NBNC HCC are NASH-related HCC.

The number of HCC nodules was lower, and the prevalence of single nodule HCC was higher in the elderly group than that in the younger group. Two main types of HCC occurrence exist, the first of which occurs at the time of the initial diagnosis with multicenter occurrence, which is associated with the degree of underlying liver damage. In this study, the prevalence of liver cirrhosis in the elderly group was lower than that in the younger group. The mild underlying liver damage in the elderly group may be associated with the smaller number of tumors observed in these patients.

Since elderly patients had fewer tumors and milder underlying liver damage at the time of the initial diagnosis, a more favorable prognosis in the elderly group may be expected. In this study, the overall survival rate was not significantly different between the two HCC groups. Overall, the majority of the elderly patients experienced various comorbidities, including cardiovascular disease, respiratory disease and diabetes mellitus. Taken together, the causes of death unrelated to HCC may have affected the survival rate in the elderly group.

In conclusion, elderly HCC patients had fewer tumors, milder underlying liver damage, and more frequent NBNC HCC. Additionally, it appears that elderly patients develop HCC even without fibrosis. Aging may therefore be a factor affecting hepatocarcinogenesis.

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Original Article

Suppressor of cytokine signal 3 and IL28 genetic variation predict the viral response to peginterferon and ribavirin

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Aim: The aim of this study was to investigate the relationship among the expression of suppressor of cytokine signaling 3 (SOCS 3) in the liver, the SNPs in the IL28B locus, and the outcome of interferon therapy.

Methods: Prior to interferon treatment, we immunostained 67 liver specimens from chronic hepatitis C (CHC) patients who were receiving peginterferon alpha-2b/ribavirin therapy for suppressor of cytokine signaling 3 (SOCS3), and compared the expression of SOCS3, IL28 polymorphisms and other clinical factors between the patients and compared their eventual outcomes.

Results: Significant differences between the low SOCS3 group and high SOCS3 group were found in age, as well as in the platelet, transaminase, gamma-glutamyl transpeptidase levels. The incidence of high SOCS3 was not significantly different between subjects with the TT genotype and the TG genotype (TT : TG = 71%:29%, $P = 0.250$). In a multivariate analysis, age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), IL28B gene (genotype TT) (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) were significant predictors of the interferon response. In patients with the TT genotype, those with low SOCS3 immunostaining showed a high sustained virological response (69%), while the sustained virological rate was low (27%) in the patients with high SOCS3 immunostaining.

Conclusions: Using a combination of the SOCS3 immunostained area in the liver and the expression of IL28B single nucleotide polymorphisms might be a useful predictor of hepatitis C virus clearance by interferon therapy.

Key words: hepatitis C virus, IL28B, interferon, suppressor of cytokine signaling 3

INTRODUCTION

APPROXIMATELY 200 MISSION people worldwide are infected with hepatitis C virus (HCV). In Japan, about 2 million people are chronically infected, and HCV is the leading cause of hepatocellular carcinoma (HCC). The current standard care for chronic hepatitis C (CHC) is a combination of peginterferon-α (PEG-IFN) and ribavirin. This treatment is effective in approximately 40–50% of CHC patients with a high viral load of genotype 1.[1–5] This therapy is costly and frequently associated with side effects. Therefore, predicting the outcome of interferon therapy is important.

Several factors, such as gender, body mass index, the presence of steatosis and liver fibrosis, drug adherence and viral factors including the serum quantity of HCV RNA and HCV genotype have been reported to be significantly associated with the treatment outcome.[2,6–11] Among viral factors, Akuta *et al.* recently reported that the substitution of the HCV core amino acid was a predictor for the effect of interferon and ribavirin combination therapy.[2,12] Among the host factors, recent reports showed that genetic variations near the IL28 gene (rs8099917, rs1297860) on chromosome 19 were predictors of the virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals

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with HCV, and also affected the clinical outcome, including spontaneous clearance of HCV.[13–15]

We previously reported that the expression of suppressor of cytokine signaling 3 (SOCS3), which is related to insulin resistance, impairs the response to interferon treatment and might be a useful predictor of HCV clearance by interferon therapy.[16]

In this study, we examined the relationship among the expression of SOCS 3 in the liver, single nucleotide polymorphisms (SNPs) in the IL28B locus, and the outcome of interferon therapy.

METHODS

NEEDLE BIOPSIES OF the liver were obtained from 67 patients with positive HCV antibodies prior to interferon treatment at Nagasaki University Hospital and National Hospital Organization (NHO) Nagasaki Medical Center. Twenty of 67 cases were also examined in a previous study.[16] All patients with genotype 1b received weekly injections of PEG-IFN. The clinical data of the patients are summarized in Table 1. Liver biopsy was performed by needle puncture for diagnostic purposes. The diagnosis of each case was independently confirmed histologically by liver pathologists according to the Japanese chronic hepatitis classification criteria (New Inuyama classification). According to these criteria, mild activity was defined as A0 or A1, severe activity as A2 or A3, mild fibrosis as F0 or F1, and severe fibrosis as F2, F3, or F4. Fatty changes in >5% of all areas were defined as steatosis.

Table 1 Clinical backgrounds of the patients

Age	56.8 ± 9.3
Gender	Male : Female = 37:30
BMI (kg/m^2)	23.5 ± 2.9
Viral load (KIU/mL)	2320 ± 1519
White blood cell (/uL)	5074 ± 1713
Hemoglobin (mg/dL)	14.1 ± 1.3
Platelet (×10^3/uL)	167.3 ± 75.6
AST (IU/L)	77.1 ± 45.2
ALT (IU/L)	101.2 ± 56.3
γGTP (IU/L)	70.6 ± 65.5
HCV core 70 wild	40 cases
HCV core 91 wild	50 cases
Steatosis (>5%)	37 cases
A (0–1:2–3)	36:31
F (0–1:2–4)	22:45

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

All patients received PEG-IFN (Schering-Plough, Tokyo, Japan) + ribavirin (Schering-Plough, Tokyo, Japan) therapy for 48 weeks. The patients who were treated with a dose of PEG-IFN or ribavirin reduced by more than 20% were excluded from the study. PEG-IFN (1.5 μg/kg) was administered once per week, and the ribavirin dose was titrated according to body weight. A sustained virological response (SVR) was defined as undetectable HCV RNA at 6 months after the end of interferon treatment.

Of 38 patients who could not achieve an end-of-treatment response, 28 patients required a re-elevation of their viral loads regardless of the fact that the HCV-RNA levels were temporarily negative, and 10 patients did not achieve an HCV negative result during the entire treatment period.

SOCS3 immunohistochemistry

All tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin, and 4 μm thick serial sections were cut from each paraffin block. In the immunohistochemical study, an anti-SOCS3 antibody (dilution 1:100, Affinity BioRegents, Golden, CO, USA) was used for SOCS3. Immunohistochemistry was performed with the labeled streptavidin biotinylate antibody (LSAB) method and a commercially available kit (Histofine, SAB-PO(R); Nichirei Corporation, Tokyo, Japan). The area immunostained for SOCS 3 was divided according to the number of immunoreactive cells per unit area. Immunoreactive cases were classified as those with less than 30% of the hepatocellular cells stained (low SOCS3 group) and those with 30% or more of the cells stained (high SOCS3 group), because our previous study showed that staining of more than 30% of the area was a significant predictor of viral clearance.[16]

Genetic variation near the IL28B gene

Genotyping for replication was performed by use of the Invader assay or direct sequencing. In this study, genetic variation near the IL28B gene (rs8099917), which was previously reported to be a predictor of the virological response was investigated.[13]

Statistical analysis

The SPSS 9.0 for Windows statistical software program was used to assess correlations among multiple variables. When appropriate, clinical and laboratory data

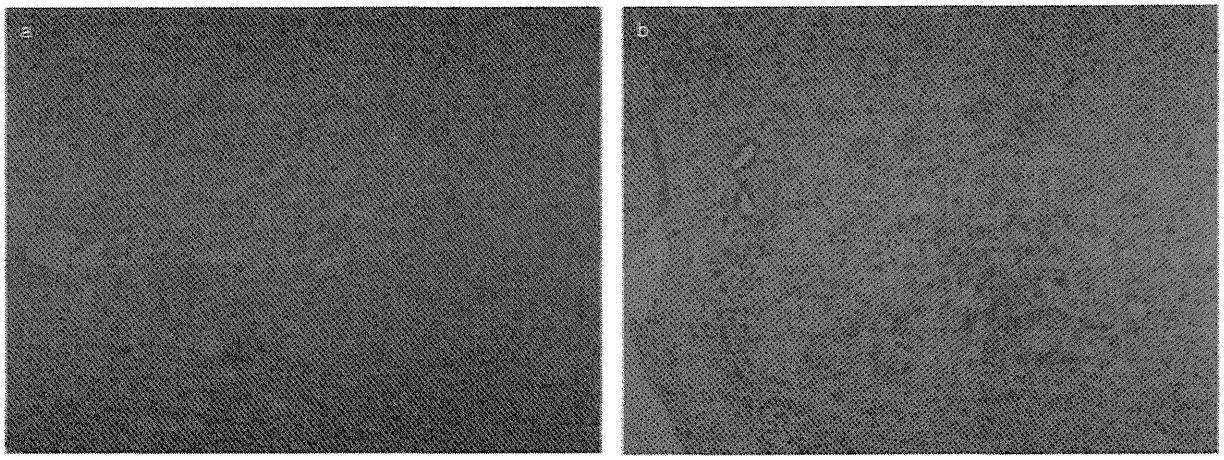

Figure 1 (a) This case showed less than 5% suppressor of cytokine signaling 3 (SOCS3) immunostained areas (low immunostaining). (b) This cases showed about 50% SOCS3 immunostaining areas (high immunostaining).

were compared with the Student's *t*-test or the Mann–Whitney test. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Immunostaining of SOCS3 in the liver (Figs 1,2)

IMMUNOSTAINING FOR SOCS3 was mainly seen in the periportal area. Less than 30% SOCS3 immunostained areas were found in 36 cases (54%) and areas with 30% or more immunostaining for SOCS3 were found in 31 cases (46%).

The frequency and distribution of the SOCS3 expression are shown in (Fig. 2)

Correlation between SOCS3 immunostaining and clinicopathlogical factors

A significant difference between low and high SOCS3 groups was found in age (low : high = 54.5 ± 9.8:59.5 ± 8.1, $P = 0.028$), the levels of platelets (low : high = 189.5 ± 90.0:141.6 ± 41.3, $P = 0.009$), aspartate aminotransferase (AST) (low : high = 94.5. ± 56.0:62.1 ± 33.5, $P = 0.003$), alanine aminotransferase; (ALT) (low : high = 85.8 ± 52.4:119.0. ± 56.3, $P = 0.015$), gamma-glutamyl transpeptidase (γGTP) (low : high = 48.8 ± 53.5:94.7 ± 70.6, $P = 0.004$). The incidence of steatosis (low : high = 33%: 81%, $P = 0.001$), severe activity (low : high = 27%: 67%, $P = 0.001$) and sever fibrosis (low : high = 52%: 84%, $P = 0.006$) was significantly higher in the SOCS3 high

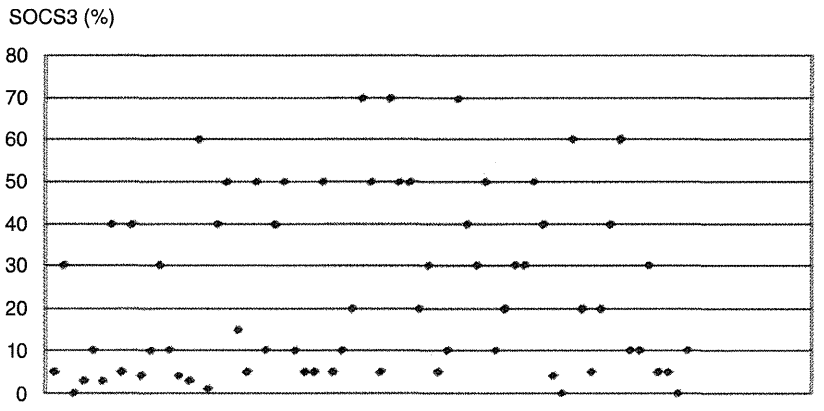

Figure 2 The distribution of the SOCS3 immunostaining area is shown.

Table 2 Comparison of the suppressor of cytokine signaling 3 (SOCS3) immunostaining groups

	SOCS3 high 31 cases	SOCS3 low 36cases	*P*-value
Age	59.5 ± 8.1	54.5 ± 9.8	0.028
Gender (male)	16 (53%)	21 (58%)	0.581
BMI (kg/m^2)	23.3 ± 2.2	23.6 ± 3.5	0.719
Viral load (KIU/mL)	2139 ± 1367	2475 ± 1950	0.427
White blood cell (/uL)	4935 ± 1386	5039 ± 1384	0.765
Hemoglobin (mg/dL)	14.1 ± 1.1	14.0 ± 1.3	0.570
Platelet (×10^3/uL)	141.6 ± 41.3	189.5 ± 90.0	0.009
AST (IU/L)	94.5. ± 56.0	62.1 ± 33.5	0.003
ALT (IU/L)	119.0 ± 56.3	85.8 ± 52.4	0.015
γGTP (IU/L)	94.7 ± 70.6	48.8 ± 53.5	0.004
Core 70 wild	17 (55%)	23 (63%)	0.451
Core 91 wild	23 (74%)	27 (75%)	0.939
Steatosis	25 (81%)	12 (33%)	0.001
Activity (severe)†	21 (67%)	10 (27%)	0.001
Fibrosis (severe)‡	26 (84%)	19 (52%)	0.006
IL28 TT rs8099917	22 (71%)	29 (80%)	0.358

†Severe activity was defined as A2 or A3.
‡Severe fibrosis was defined as F2, F3, or F4.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

immunostaining group than in the SOCS3 low immunostaining group. No significant difference was observed between the SOCS3 low and high groups in any of the other clinical factors (age, body mass index [BMI], viral load, white blood cell count, hemoglobin, substitution of the core 70, 91) (Table 2).

Comparison of SOCS3 expression and the genetic variation of IL28B gene

No significant difference in the genetic variation of the IL28 TT genotype was observed between the SOCS3 low and high immunostaining groups (low : high = 80%: 71%, $P = 0.250$).

Assessment of SOCS3 expression and genetic variation in IL28 as predictors of a sustained virological response

The age of patients in the non responder (NR) group was significantly higher than that in sustained virological response (SVR) group (SVR : NR = 52.3 ± 11.5: 59.6 ± 6.1, $P = 0.003$).

The incidence of the IL28 TT genotype was significantly lower, and that of SOCS3 high immunostaining group was significantly higher in the NR group than in the SVR group (Table 3).

As determined by a logistic regression analysis, the significant predictor of an SVR was high age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), the IL28 TT genotype (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) (Table 4).We found that two of nine (22%) patients with the IL28 TG genotype and SOCS3 high immunostaining showed a SVR, while one of seven (14%) patients with the IL28 TG genotype and SOCS3 low immunostaining, six of 22 (27%) patients with the IL28 TT genotype and SOCS3 high immunostaining, and 20 of 29 (69%) patients with the IL28 TG genotype and SOCS3 low immunostaining showed a SVR (Fig. 3).

DISCUSSION

RECENT IMPROVEMENTS IN the efficiency of antiviral therapy have led to approximately 50% of patients with HCV genotype 1 achieving sustained viral clearance.[1–5] However, some patients are refractory to interferon therapy. A recent study reported that the presence of genetic variation near the IL28B gene (rs8099917, rs1297860) can be used as a pretreatment predictor of virlogical response to a 48-week PEG-IFN plus combination therapy in patients with HCV geno-