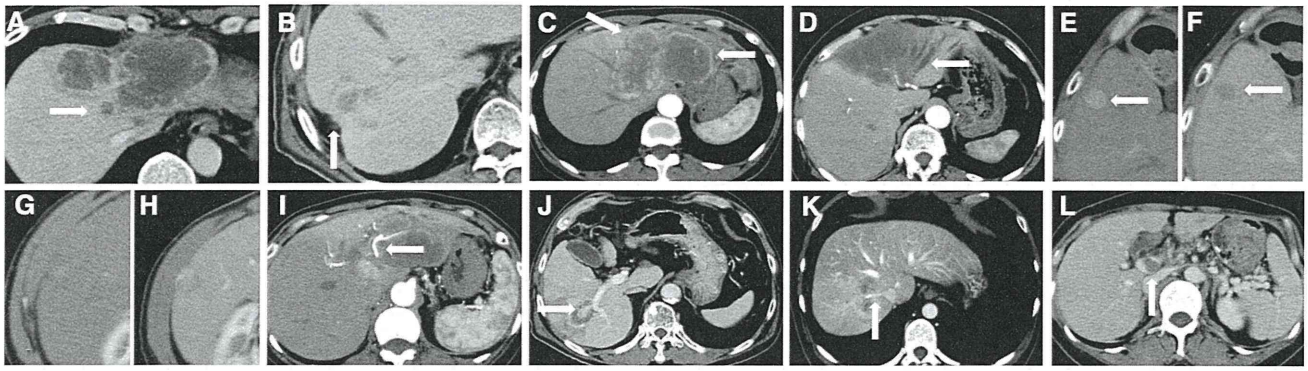

Fig. 1. Evaluated imaging features of contrast-enhanced CT for tumors. **A** CT during the arterial phase with p-HCC shows the satellite nodule (*arrows*). **B** CT during the delayed phase with ICC shows capsular retraction (*arrow*). **C, D** CT during the arterial phase with ICC shows a lobulated lesion and rim enhancement (*arrows*) (**C**) and intrahepatic bile duct dilation around the tumor (*arrow*) (**D**). **E, F** CT during the arterial phase (**E**) and delayed phase (**F**) with p-HCC shows arterial enhancement (**E**) and a washout pattern. **G, H** CT during the arterial phase (**G**) and delayed phase (**H**) with p-HCC shows delayed enhancement. **I–K** CT with ICC shows a hepatic artery running into the tumor (*arrow*) (**I**, arterial phase), a branch of the portal vein running into the tumor (*arrow*) (**J**, portal venous phase), and a hepatic vein running into the tumor (*arrow*) (**K**, delayed phase). **L** CT during the portal venous phase with p-HCC shows tumor thrombus in the portal vein (*arrow*).

Table 1. Patient characteristics of intrahepatic cholangiocarcinoma or poorly differentiated hepatocellular carcinoma

	ICC (n = 19)	p-HCC (*n* = 23)	*p* value
Gender (male/female)	13/6	19/4	0.283
Age (years)	63 (48–79)	62 (37–79)	0.535
Chronic viral hepatitis (HBV/HCV)	3 (2/1)	20 (11/9)	<0.001
Albumin (g/dL)	4.0 (3.1–4.6)	4.0 (3.1–5.2)	0.836
Total bilirubin (mg/dL)	0.8 (0.4–1.4)	0.9 (0.4–2.3)	0.389
Prothrombin time (%)	97 (79.3–135)	87 (66.1–111)	0.038
Platelet ($\times 10^4/\mu L$)	233 (122–354)	135 (42.0–305)	<0.001
AFP (10 ng/mL)	5.8 (2.3–80)	1360 (4.30–39,500)	<0.001
PIVKA-II (40 mAU/mL)	24 (7.0–1400)	208 (9.0–245,600)	0.002
CEA (5 ng/mL)	4.8 (1.0–345)	3.1 (1.4–9.1)	0.287
CA19-9 (37 U/mL)	45.1 (1.0–2590)	47.4 (1.0–237)	0.751

HBV hepatitis B virus, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *PIVKA-II* protein induced by vitamin K absence or antagonist-II, *CEA* carcinoembryonic antigen, *CA19-9* cancer-associated carbohydrate antigen 19-9

(Fig. 1D); (7) arterial enhancement (Fig. 1E); (8) washout pattern (Fig. 1E, F); (9) delayed enhancement (Fig. 1G, H); (10) intratumoral artery during arterial phases (Fig. 1I); (11) intratumoral portal vein (Fig. 1J); (12) intratumoral hepatic vein (Fig. 1k) and portal vein tumor thrombus (Fig. 1L); (13) lobar atrophy; and (14) lymphadenopathy. The washout pattern was defined as arterial enhancement (due to the presence of non-triadal neo-angiogenetic arteries) and portal/venous wash out (due to the loss of sinusoidal vascularization) on dynamic imaging.

Particularly, an intratumoral artery was defined as an artery entering the tumor and remaining inside the tumor. Intratumoral portal veins and intratumoral hepatic veins were defined in a similar way. Although minimal discrepancies were seen between readers when interpreting the shape of lesions, consensus decisions for these discrepancies were easily reached during an additional reading session.

Statistical analysis

We statistically analyzed differences in clinical characteristics and CT imaging features between ICC and p-HCC by using the Chi square test for categorical variables and the non-parametric Mann–Whitney *U* test for continuous variables. Significant variables obtained from univariate analysis were applied to multivariate stepwise binary logistic regression analysis to determine the optimal findings for differentiating ICC from p-HCC. Statistical analyses were performed by using the SPSS software package, version 20.0 (IBM, NY). For all tests, values of $p < 0.05$ were considered statistically significant.

Results

Characteristics of patients with ICC or p-HCC

Baseline characteristics of patients with ICC or p-HCC are summarized in Table 1. There were no significant

Table 2. Uni- and multivariate analysis of contrast-enhanced CT features of intrahepatic cholangiocarcinoma and poorly differentiated hepatocellular carcinoma

Pattern	ICC (n = 19)	p-HCC (n = 23)	Univariate analysis p	Multivariate analysis	
				p	Odds ratio (95% CI)
Mean diameter (mm)	64.4 (25–150)	53.7 (10–150)	0.192		
Lobulated shape	14 (73.7)	9 (39.1)	0.004	0.550	1.951 (0.218–17.445)
Satellite nodule	15 (78.9)	14 (60.1)	0.207		
Capsular retraction	6 (31.6)	5 (21.7)	0.407		
Arterial enhancement	9 (47.3)	17 (73.9)	0.078		
Bile duct dilatation	11 (57.9)	4 (17.4)	0.006	0.323	3.445 (0.296–40.070)
Rim enhancement	13 (48.4)	4 (17.4)	0.037	0.158	6.068 (0.495–74.308)
Delayed enhancement	8 (42.1)	7 (30.4)	0.432		
Washout	1 (5.3)	15 (65.2)	<0.001	0.049	0.087 (0.008–0.993)
Intratumoral artery	15 (78.9)	8 (34.8)	<0.001	0.037	10.192 (1.155–89.954)
Intratumoral portal vein	7 (36.8)	2 (8.7)	0.055		
Intratumoral vein	3 (15.8)	2 (8.7)	0.581		
Portal vein tumor thrombus	1 (5.3)	4 (17.4)	0.197		
Lobar atrophy	6 (31.6)	2 (8.7)	0.060		
Lymphadenopathy	4 (21.1)	1 (4.3)	0.094		

Values in parentheses represent percentages

differences between the 2 groups with regard to sex, age, albumin, total bilirubin, CEA, and CA19-9. However, there were differences with respect to chronic viral hepatitis, prothrombin time, platelet counts, AFP, and PIVKA-II.

Analyses of contrast-enhanced CT features of ICC and p-HCC

CT features of ICC and p-HCC and the results of univariate analysis are summarized in Table 2. Lesion diameter ranged from 2.5 to 15.0 cm (mean 64.4 mm) for ICC and from 1.0 to 15.0 cm (mean 53.7 mm) for p-HCC (p = 0.192). Lobulated lesion shape was significantly more rare in patients with p-HCC (n = 9, 39.1%) than in patients with ICC (n = 14, 73.7%) (p = 0.004). The presence of satellite nodules was not statistically significantly different between ICC (n = 15, 78.9%) and p-HCC (n = 14, 60.1%) (p = 0.207). Significant differences in arterial enhancement were not seen between ICC (n = 9, 47.3%) and p-HCC (n = 17, 73.9) (p = 0.078). Capsular retraction was present in patients with ICC (n = 6, 31.6%) or p-HCC (n = 5, 21.7%) (p = 0.407). The presence of intrahepatic bile duct dilation around the tumor differed significantly between the ICC group (n = 11, 57.9%) and the p-HCC group (n = 4, 17.4%) (p = 0.006). Peripheral rim enhancement in the arterial phase was less common in the p-HCC group (n = 4, 17.4%) than in the ICC group (n = 13, 48.4%) (p = 0.037). A washout pattern was more frequent in p-HCC (n = 15, 65.2%) than ICC (n = 1, 5.3%) (p < 0.001). There was no significant difference (p = 0.432) in the occurrence of delayed enhancement in p-HCC (n = 8, 42.1%) and in ICC (n = 7, 30.4%).

An intratumoral artery in the arterial phase was more frequently present for ICC lesions (n = 15, 78.9%) than p-HCC (n = 8, 34.8%) (p < 0.001). The ICC group more frequently showed an intratumoral portal vein (n = 7, 36.8%) than the p-HCC group (n = 2, 8.7%) (p = 0.055). An intratumoral hepatic vein was rarely exhibited in ICCs (n = 3, 15.8%) or p-HCCs (n = 2, 8.7%) (p = 0.581). Portal vein tumor thrombus was also rarely present in ICCs (n = 1, 5.3%) or p-HCCs (n = 4, 17.4%) (p = 0.197).

There was no significant difference (p = 0.060) in the presence of lobar atrophy in ICC (n = 6, 31.6%) and in p-HCC (n = 2, 8.7%). Lymphadenopathy was present in patients with ICC (n = 4, 21.1%) or p-HCC (n = 1, 4.3%) (p = 0.407).

Next, we conducted multivariate binary logistic regression analysis by using significant parameters from the univariate analysis. As shown in Table 2, the presence of an intratumoral artery was an independent CT predictor for differentiating ICC from p-HCC (p = 0.037, odds ratio = 10.192); on the contrary, washout pattern was a significant parameter favoring p-HCC (p = 0.049, odds ratio = 0.087). The presence of an intratumoral artery on CT had a sensitivity of 78.9% and a specificity of 65.2% for ICC. Furthermore, the presence of an intratumoral artery on CT had a positive predictive value of 65.2% and a negative predictive value of 78.9% for ICC.

Case presentation

Representative images from CT and histological features in patients with ICC and p-HCC are shown in Figs. 2 and 3. A 60-year-old man (Case 1) presented with a massive, advanced tumor predominantly located in the right lobe of the liver, and a hepatic artery was seen running into the tumor on CT (Fig. 2A). A right hepatic lobectomy was performed, and histological examination revealed ICC tumor cells that showed infiltrating

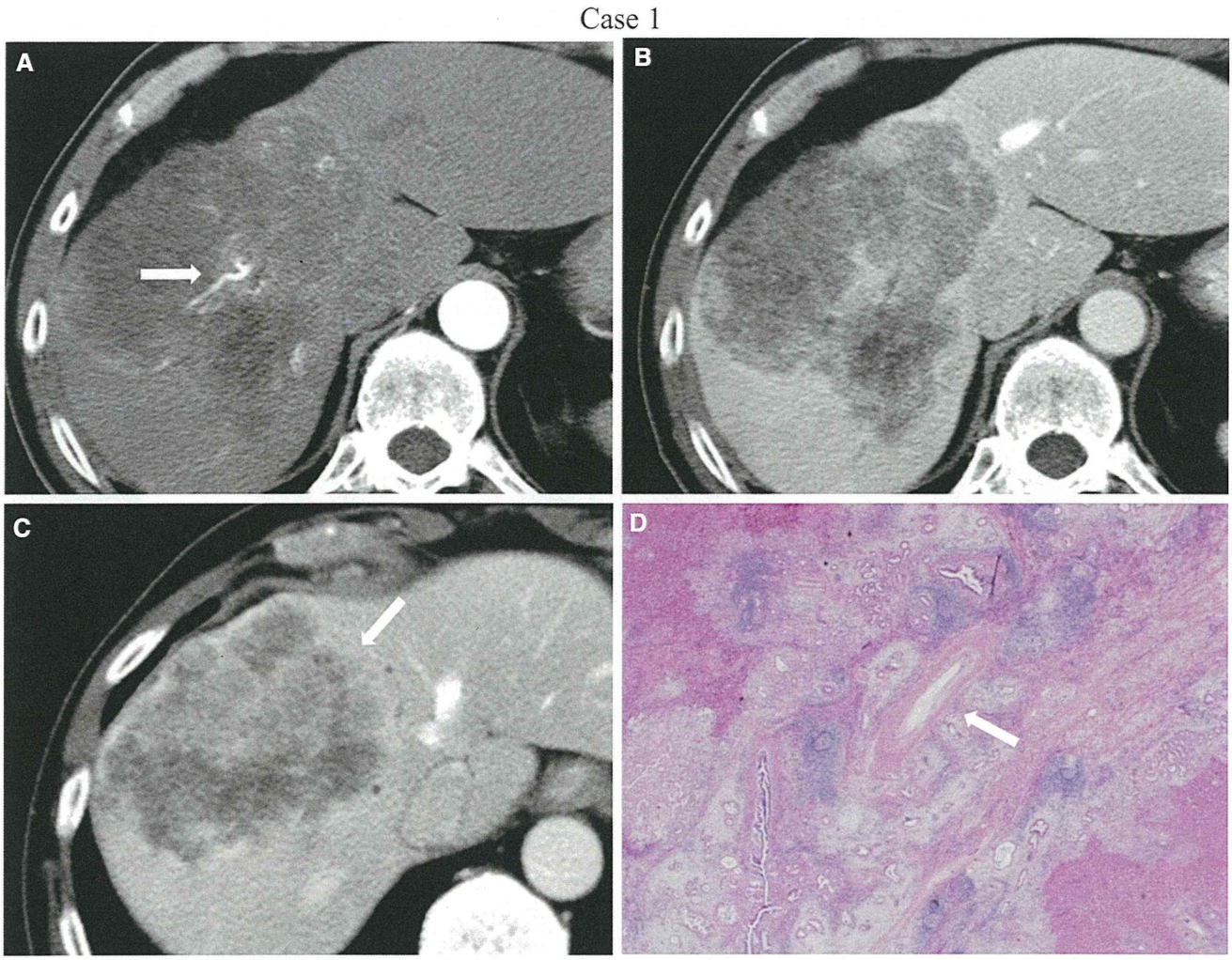

Fig. 2. Contrast-enhanced CT and histological features of ICC. **A** ICC in a 60-year-old man shows a subtle intratumoral artery on arterial phase CT (*arrow*). **B**, **C** CT on delayed phase shows the absence of delayed enhancement (**B**) and the presence of a lobulated shape (**C**). **D** ICC tumor cells show infiltrating replacement growth of the surrounding hepatic parenchyma. An intratumoral artery that has not been destroyed by tumor cells is identified (*arrow*). (Original magnification: d × 10. Hematoxylin and eosin staining).

replacement growth against the surrounding hepatic parenchyma. An intratumoral artery that had not been destroyed by tumor cells was identified (Fig. 2D). A 58-year-old woman (Case 2) presented with a massive, advanced tumor predominantly located in the left lobe of the liver, and no intratumoral artery, portal vein, or hepatic vein was identified on CT (Fig. 3A). A left hepatic lobectomy was performed, and histological examination revealed p-HCC. The tumor was compressing the surrounding liver, and compressed vessels were clearly visible (Fig. 3D).

Discussion

On contrast-enhanced CT, the typical appearance of ICC is a mass that demonstrates thin, rim-like, or thick, band-like contrast enhancement around the tumor during arterial and portal venous phases, with satellite nodules, capsular retraction, lobar atrophy, lymphadenopathy, and delayed enhancement [12–15]. The accuracy of contrast-enhanced CT in diagnosing ICC was 70% [16]. The finding of satellite nodules was associated with tendency to invade small portal vessels and along portal triads. Additionally, scirrhous stroma and biliary involvement of ICC have an influence on the imaging of capsular retraction and lobar atrophy. In our study, a lobulated shape was more closely associated with ICC than with p-HCC on univariate analysis, although satellite nodules, capsular retraction, lobar atrophy, and lymphadenopathy were not different between ICC and p-ICC. The finding of lobulated shape supported the results of previous studies [17, 18]. This trend may be

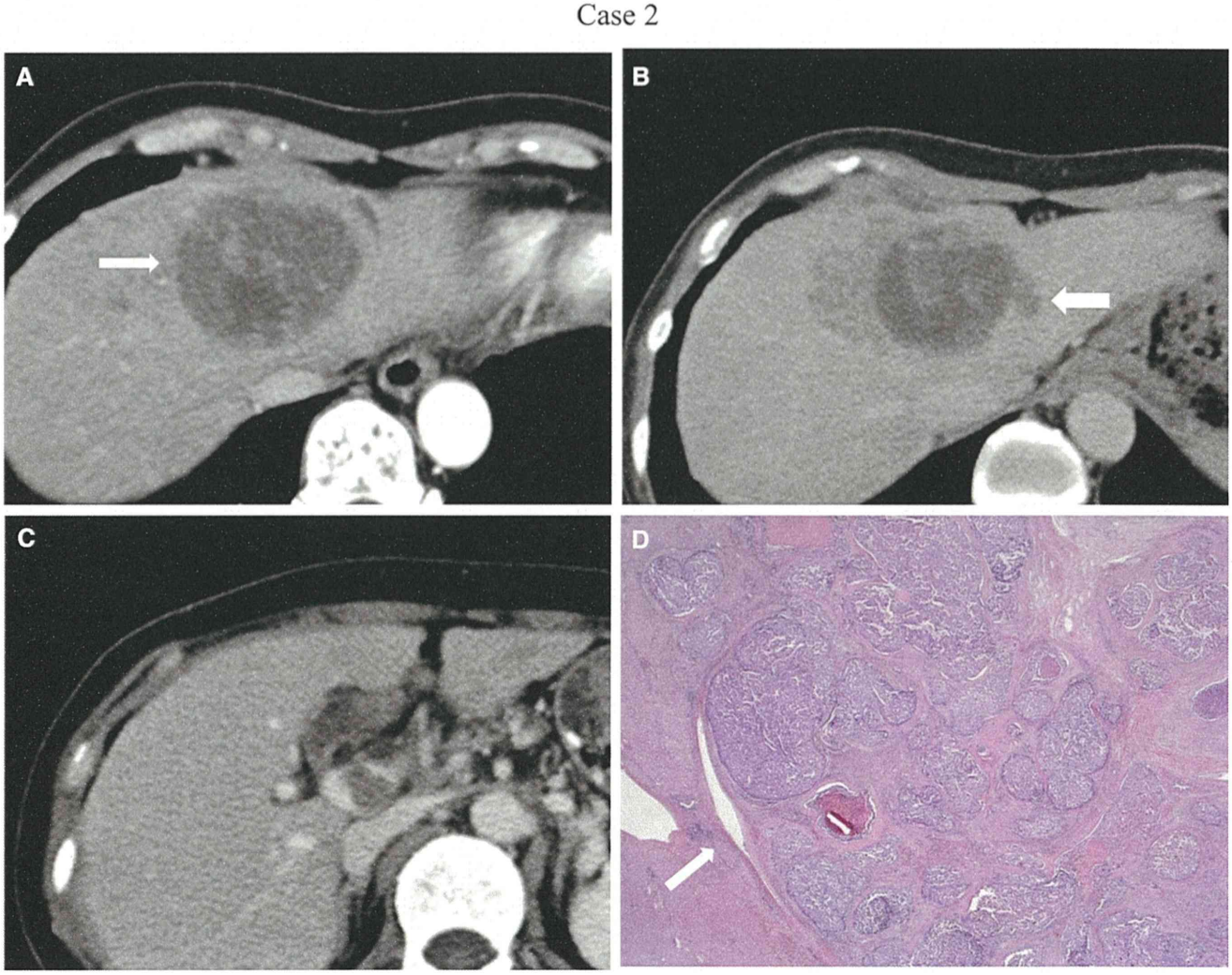

Fig. 3. Contrast-enhanced CT and histological features of p-HCC. **A** p-HCC in a 58-year-old woman shows a low attenuating tumor on arterial phase CT (*arrow*). **B** CT on delayed phase shows nodule (*arrow*) and the absence of delayed enhancement. **C** CT on portal venous phase shows portal vein tumor thrombus. **D** Compressive growth of p-HCC. Tumor compresses the surrounding liver and compressed vessels are clearly visible (*arrow*). (Original magnification: d × 10. Hematoxylin and eosin staining).

related to the fact that ICC tends to invade small portal vein branches adjacent to the main tumor, and the fusion of the primary mass and adjacent satellite tumors results in the lobulated shape [19]. Tumor shape could thus represent a differential feature between ICC and p-HCC.

The frequency of intrahepatic bile duct dilation around the tumor differed between ICC and p-HCC. Eleven of the 19 ICC tumors were accompanied by intrahepatic bile duct dilation around the tumor. The presence of intrahepatic bile duct dilation around the tumor may thus provide a useful clue for differentiation.

In our study, 13 ICC showed rim enhancement during the arterial phase. Rim enhancement patterns differing from p-HCC may relate to different pathological components in the tumor [20]. Fan et al. suggested that the degree of enhancement of ICC depends on the proportion of component fibers and tumor cells, with a tumor rich in cells resulting in strong enhancement [9]. ICC that is peripherally rich in tumor cells with fibrosis in the central portion may result in peripheral rim-like hyperenhancement.

In addition, significant differences in washout patterns were seen between ICC and p-HCC, although there were no significant differences in arterial enhancement and delayed enhancement between the two groups. According to the guidelines of the American Association for the Study of Liver Disease, nodules larger than 1 cm detected in liver cirrhosis may be confidently diagnosed as HCC only when a washout pattern is detected on contrast-enhanced CT or magnetic resonance imaging

[21]. According to our findings, the washout pattern can be useful for identifying p-HCC or ICC. However, distinguishing p-HCC from some ICCs showing diffuse hyperenhancement in the arterial phase and subsequent washout is difficult.

In our present study, 15 (78.9%) of the 19 ICCs showed an intratumoral artery in the arterial phase. Although we occasionally recognized vessels running into the tumor, to the best of our knowledge, no previous reports have described the presence of intratumoral arteries in ICC and p-HCC. In our study, ICCs were able to be differentiated from p-HCCs based on the finding of an intratumoral artery ($p = 0.037$), according to multivariate binary logistic regression analysis. Based on our results, the presence of an intratumoral artery in the arterial phase on contrast-enhanced CT could be a predictive finding for reliable differentiation of ICC from p-HCC. Few reports have described intratumoral arteries of ICC being demonstrated on contrast-enhanced CT. One study showed intratumoral arteries of the ICC identified immediately after the injection of contrast material for CT during hepatic arteriography [22]. Furthermore, that study indicated that tumor enhancement gradually spreads from each intratumoral artery [22]. Infiltrating replacement is an inherent growth feature of ICC, with the surrounding liver gradually incorporated into the tumor as it grows [23]. In this process, the blood vessel is not destroyed by tumor cells and is retained inside. By contrast, HCC shows fibrous encapsulation or compressive growth [24]. With such growth, blood vessels are pressed to the outside of the tumor. Our cases also showed these features (Fig. 2). Such differences in growth type may be related to differences in intratumoral arteries between ICC and HCC. No significant difference was seen between ICC and p-HCC in regard to intratumoral portal veins, intratumoral hepatic veins, or portal vein tumor thrombus. We supposed that intratumoral artery was retained within the ICC rather than portal or hepatic veins because of the stiffness of the arterial wall.

The results of this study have revealed features that allow ICC and p-HCC to be distinguished based on findings from contrast-enhanced CT. In clinical practice, contrast-enhanced CT is a useful diagnostic method to distinguish ICC from p-HCC, since results of tumor marker levels and tissue biopsy are difficult and often indeterminate. The optimal treatment for ICC is complete tumor resection, including lymph node removal [25–27]. In cases of HCC, the treatment modality of choice depending on the degree of cirrhosis is complete resection, topical therapy including radiofrequency ablation or liver transplantation. If the patient has advanced cirrhosis or advanced HCC, then treatments such as transarterial chemoembolization hepatic arterial infusion chemotherapy and systemic chemotherapy are applicable [28, 29]. Because misdiagnosis of ICC as HCC can lead to inadequate medical care, our identification of characteristic findings for ICC may have important practical value in attaining a correct diagnosis.

This study has several limitations that must be considered when interpreting the results. First, our study might have included some degree of selection bias, as we retrospectively analyzed only those patients with ICC or p-HCC who underwent contrast-enhanced CT and hepatic surgery. The absence of the well- and moderately differentiated subtypes of HCC in this study is an important limitation in interpreting our results. Additionally, the numbers of ICCs and p-HCCs were relatively small, because the patient group was limited to those with a pathologic diagnosis determined by surgery. Finally, most tumors were relatively large, and the findings in our results may not be observed in smaller sized tumors.

In conclusion, the presence of an intratumoral artery during arterial phase on enhanced CT is valuable in differentiating between ICC and p-HCC, as is the washout pattern. This new finding may facilitate correct diagnosis and more timely selection of appropriate treatment strategies.

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Conflict of interest. The authors declare that they have no conflicts of interest.

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INTERNAL MEDICINE

□ CASE REPORT □

Hepatosplenic Gamma-delta T-cell Lymphoma Associated with Epstein-Barr Virus

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Abstract

Hepatosplenic gamma-delta T-cell lymphoma (HSTCL) is a rare, aggressive subset of peripheral T-cell lymphoma. It has been reported that Epstein-Barr virus (EBV) infection can cause HSTCL; however, such cases are extremely rare, with only a few cases having been reported to date. We herein report an autopsy case of HSTCL associated with EBV infection. The presence of EBV infection was confirmed in serum EBV DNA and on *in-situ* hybridization, and cytotoxic molecules, such as granzyme B, perforin and T-cell intracytoplasmic antigen (TIA)-1, were all positive in lymphoma cells. These findings indicate that stimulation of persistent EBV infection may have caused HSTCL in this patient.

Key words: hepatosplenic gamma-delta T-cell lymphoma, Epstein-Barr virus, peripheral T-cell lymphoma, bronchiolitis obliterans organizing pneumonia, non-Hodgkin's lymphoma

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Introduction

Hepatosplenic gamma-delta T-cell lymphoma (HSTCL) is a rare extranodal T-cell non-Hodgkin's lymphoma characterized by hepatosplenomegaly without lymphadenopathy (1). Most HSTCL lesions are found in patients with autoimmune disease, chronic antigen stimulation, splenectomy and/or under immunosuppressive treatment, mostly after solid organ transplantation (2). HSTCL usually shows the phenotype of CD2+, CD3+, CD4-, CD5-, CD7+, CD8-, T cell receptor (TCR) gamma-delta+ (3, 4), while cytotoxic molecules, such as granzyme B and perforin, are usually negative in such lesions (3). Ohshima et al. reported a possible association between Epstein-Barr virus (EBV) infection and HSTCL; however, such HSTCL cases are extremely rare (5). We herein report an autopsy case of HSTCL associated with chronic EBV infection.

Case Report

A 71-year-old man was diagnosed with bronchiolitis obliterans organizing pneumonia (BOOP) five months prior to onset. He was treated with a corticosteroid, and the BOOP gradually improved. The dose of the corticosteroid was started at 30 mg/day, then tapered to 7.5 mg/day; however, it was subsequently increased again to 15 mg/day because the BOOP worsened. The corticosteroid treatment was continued at a dose of 15 mg/day, and the BOOP remained well-controlled. However, five months after the diagnosis of BOOP, the patient developed a fever and was treated with antibiotics, although the fever did not resolve. He subsequently developed severe weakness and jaundice and was referred to our department after one month. On admission, blood tests showed pancytopenia, severe liver dysfunction with hyperbilirubinemia and renal dysfunction. The levels of soluble interleukin-2 receptor (sIL-2R) and serum lactate de-

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Table. The Patient's Laboratory Data

Parameter, units	Patient	Reference values
Cell blood counts		
WBC, ×1,000/μL	3.4	3.5-9.3
Blood smear, %		
Segmented neutrophils	82	40-70
Banded neutrophils	1	0-5
Lymphocytes	10	30-50
Monocytes	5	0-8
Atypical lymphocytes	1	0
RBC, ×10,000/μL	401	400-557
Hb, g/dL	10.8	13.4-17.6
MCV, fl	82.5	85-101
Platelets, ×1,000/μL	71	120-400
Biochemistry		
AST, U/L	315	13-33
ALT, U/L	188	8-42
CHE, U/L	192	213-501
Total bilirubin, mg/dL	3.9	0.2-1.2
Direct bilirubin, mg/dL	3.1	0-0.3
BUN, mg/dL	20	8.0-22
Cr, mg/dL	1.81	0.6-1.1
CRP, mg/L	1.8	0-0.39
Prothrombin time, %	28.3	70-130
LDH, U/L	1,622	119-229
sIL-2R, U/mL	8,125	0-459
Serological tests		
Anti-EBV IgM, M.I	<1.0	0-0.9
Anti-EBV IgG, M.I	3.5	0-0.9
EBV DNA	6.4×10^5	1×10^2
Anti-hepatitis A IgM index	0.2	0-0.7
HBsAg, IU/mL	0.01	0-0.04
Anti-HCV, S/CO	0.07	0-0.99
Anti-CMV IgM, M.I	<1.0	0-0.9
Anti-CMV IgG, G.I	50.9	0-0.9
EB V DNA (copies/mL)#	6.4×10^5	$< 1.0 \times 10^2$

#Measured in blood samples collected after death

hydrogenase (LDH) were also markedly elevated. Serological virus markers measured while the patient was alive were all negative (Table). Ultrasound and computed tomography revealed massive hepatosplenomegaly that was not observed on the computed tomography scan performed four months before onset (Fig. 1). No lymphadenopathy was detected. As a differential diagnosis, we considered hematological malignancies, including lymphoma, drug-induced liver dysfunction and viral infectious disease. A liver biopsy and bone marrow aspiration were planned to obtain a definitive diagnosis; however, the patient's clinical course was aggressive, and he died from multiple organ failure three days after admission.

An autopsy was subsequently performed, which showed the liver and spleen to be markedly enlarged, as observed earlier on ultrasound and computed tomography; their weights were 2,823 g and 814 g, respectively. No lymphadenopathy was observed. A microscopic examination revealed massive atypical lymphocyte infiltration into the liver, spleen and bone marrow. The infiltrated lymphocytes were positive for CD3 and T cell receptor (TCR)-γ and negative for CD4, CD5, CD8, CD20, CD56 and βF1 (Fig. 2, 3). The presence of atypical cells positive for TCR-γ and negative for βF1 suggested the existence of TCR gamma-delta chains. These findings indicated the tumor cells to reflect T-cell lymphoma expressing TCR gamma-delta chains. Interestingly, *in-situ* hybridization for EBV and cytotoxic molecules, including granzyme B, perforin and T-cell intracytoplasmic antigen (TIA)-1 were all positive in the lymphoma cells (Fig. 2), indicating a persistent infection of EBV with a cytotoxic reaction. The phenotypes of the lymphoma cells in the liver, spleen and bone marrow were exactly the same. Accordingly, we later measured the EBV DNA titer in the patient's stored serum, which revealed a high level of EBV DNA (Table). The patient's condition was diagnosed as HSTCL associated with EBV infection.

Discussion

HSTCL is a rare subset of peripheral T-cell lymphoma that accounts for less than 1% of all non-Hodgkin's lymphomas (6). HSTCL has a peak incidence in adolescents and young adults. Patients with HSTCL commonly show hepatosplenomegaly in the absence of lymphadenopathy (1). Pancytopenia is also a common feature of HSTCL due to bone marrow involvement (7). The clinical course of HSTCL is aggressive, with a reported median survival time of eight months (3). No effective combination chemotherapy has been documented to date, and the only curative treatment is allogeneic stem cell transplantation (8). The patient in the present case report was relatively old; however, he exhibited the typical symptoms of HTSCL, including hepatosplenomegaly, thrombocytopenia, fever and weakness, and his clinical course was extremely aggressive.

HSTCL usually carries the phenotype of CD2+, CD3+, CD4-, CD5-, CD7+, CD8-, TCR gamma-delta+, and it is assumed that HTSCL may originate from gamma-delta TCR-expressing T-cells. Such T-cells demonstrate preferential homing to the sinusoidal areas of the liver, red pulp of the spleen and epithelial layer of the intestinal mucosa (9). The lymphoma cells of HTSCL have similar characteristics and infiltrate into the sinusoidal areas of the liver, bone marrow and red pulp of the spleen without lymph node involvement. Most HSTCL cases express only TIA-1, not other cytotoxic molecules, such as granzyme B and perforin (10). In contrast, non-hepatosplenic gamma-delta T-cell lymphomas, including nasal, cutaneous and intestinal gamma-delta T-cell lymphomas, often express all of cytotoxic molecules, and, interestingly, some are associated with EBV infection (11). Although various lymphomas, including nasal natural killer (NK)/T-cell lymphomas, are caused by EBV infection (12), only two cases of HTSCL associated with EBV have been reported thus far (5). In both cases, all cytotoxic molecules (TIA-1, granzyme B and perforin) were expressed, and the patients died within one month. In the present case, we confirmed EBV infection on *in-situ* hybridization of the EBV region, and almost all of the lymphoma cells were positive. Furthermore, the lymphoma cells expressed all of the cytotoxic molecules, including TIA-1 and granzyme B, and the

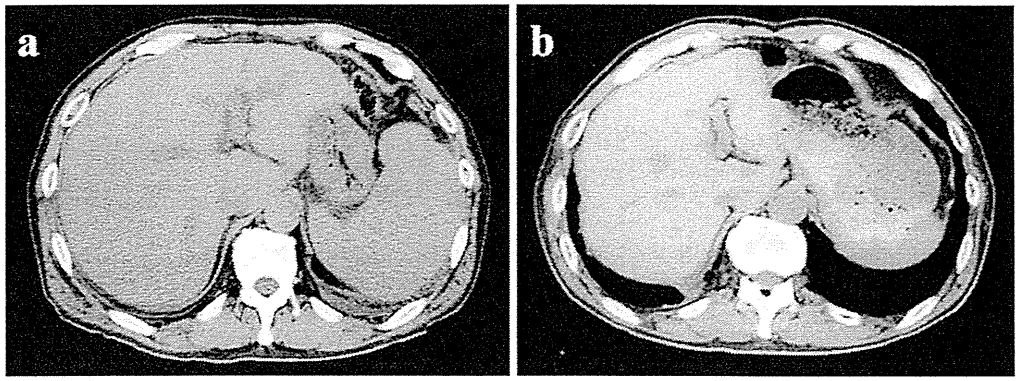

Figure 1. Computed tomography (CT) of the abdomen, transverse scan. (a) CT scan obtained at time of admission. (b) CT scan obtained five months before admission. The liver and spleen were enlarged on the last CT scan compared to that observed on the previous CT scan.

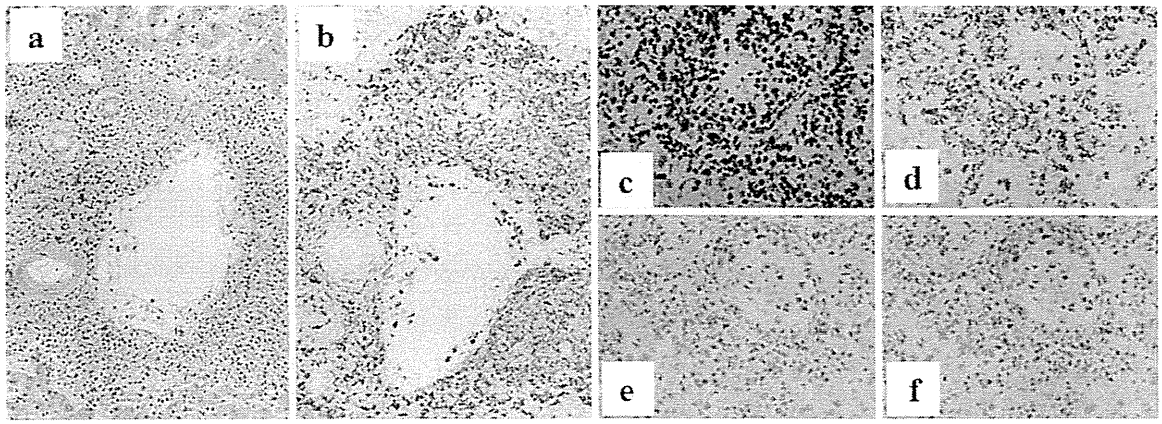

Figure 2. Histological features and immunohistochemical staining as well as *in-situ* hybridization with EBER-1 antisense oligonucleotides. The histological features of the liver showed lymphoma cell infiltration in the hepatic sinusoids with mild involvement of the portal areas (a). The atypical cells were positive for CD3 and (b) *in-situ* hybridization with EBER-1 antisense oligonucleotide (c), granzyme B (d), TIA-1 (e) and perforin (f). (Original magnification: A, B ×20; C, D, E ×40)

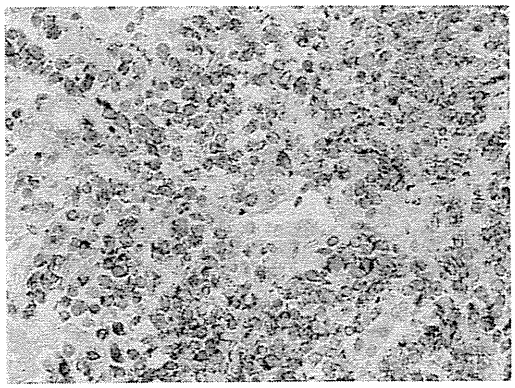

Figure 3. Immunohistochemical staining for TCR-γ. The atypical cells were positive for TCR-γ. (Original magnification: ×40)

patient's clinical course was extremely aggressive. It is possible that EBV infection may either cause or accelerate HSTCL and activate cytotoxic molecules, leading to an extremely poor prognosis.

In conclusion, we herein reported an extremely rare case of HSTCL associated with EBV infection. The present case implies a possible association between HSTCL and EBV; however, the detailed mechanism by which EBV affects the onset of HSTCL remains to be elucidated. Further studies and the accumulation of cases of EBV-associated HSTCL are necessary.

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

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(Department of Pathology, School of Medicine, Kurume University, Japan).

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Original Article

Serum granulysin levels as a predictor of serious telaprevir-induced dermatological reactions

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Aim: Telaprevir-based therapy for chronic hepatitis C patients is effective; however, the high prevalence of dermatological reactions is an outstanding issue. The mechanism and characteristics of such adverse reactions are unclear; moreover, predictive factors remain unknown. Granulysin was recently reported to be upregulated in the blisters of patients with Stevens–Johnson syndrome (SJS). Therefore, we investigated the risk factors for severe telaprevir-induced dermatological reactions as well as the association between serum granulysin levels and the severity of such reactions.

Methods: A total of 89 patients who received telaprevir-based therapy and had complete clinical information were analyzed. We analyzed the associations between dermatological reactions and clinical factors. Next, we investigated the time-dependent changes in serum granulysin levels in five and 14 patients with grade 3 and non-grade 3 dermatological reactions, respectively.

Results: Of the 89 patients, 57 patients had dermatological reactions, including nine patients with grade 3. Univariate analysis revealed that grade 3 dermatological reactions were significantly associated with male sex. Moreover, serum granulysin levels were significantly associated with the severity of dermatological reactions. Three patients with grade 3 dermatological reaction had severe systemic manifestations including SJS, drug-induced hypersensitivity syndrome, and systemic lymphoid swelling and high-grade fever; all were hospitalized. Importantly, among the three patients, two patients' serum granulysin levels exceeded 8 ng/mL at onset and symptoms deteriorated within 6 days.

Conclusion: Male patients are at high risk for severe telaprevir-induced dermatological reactions. Moreover, serum granulysin levels are significantly associated with the severity of dermatological reactions and may be a predictive factor in patients treated with telaprevir-based therapy.

Key words: drug-induced hypersensitivity syndrome, granulysin, hepatitis C virus, telaprevir, toxic epidermal necrolysis

INTRODUCTION

HEPATITIS C IS a major pathogen causing liver cirrhosis and hepatocellular carcinoma worldwide. Until recently, standard therapies for chronic hepatitis C virus (HCV) genotype 1 infection were based on the combination of pegylated interferon (PEG IFN) and ribavirin (RBV); these combination therapies yield a sustained virological response (SVR) rate of approximately 50%.[1] Several classes of novel direct-acting antivirals

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(DAA) were recently developed and tested in clinical trials. Two first-generation HCV NS3/4A protease inhibitors, boceprevir[2,3] and telaprevir,[4–6] have been approved for the treatment of genotype 1 HCV infection. The inclusion of these agents in HCV treatment regimens has led to large improvements in treatment success rates.

Telaprevir, the first DAA, is administrated in combination with PEG IFN and RBV for 24 weeks, resulting in SVR rates up to 70–80%.[4,6–8] Although the telaprevir combination regimen is highly effective, the high frequency and severity of adverse events are outstanding issues limiting its use. Dermatological reactions are particularly prevalent, developing in 56–84.6% of patients treated with telaprevir, PEG IFN and RBV combination therapy.[9,10] Moreover, the prevalence of severe dermatological reactions including Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug-induced hypersensitivity syndrome (DIHS) are substantially higher in patients treated with telaprevir-based therapy than PEG IFN and RBV combination therapy.[8,10] McHutchison *et al.* reported that 7% of patients treated with telaprevir, PEG IFN and RBV combination therapy discontinue therapy because of rash or pruritus in contrast to only 1% of patients treated with PEG IFN and RBV.[8] In some patients, serious skin reactions persist even after stopping all drugs.[10] However, the pathogenesis and clinical predictors of these adverse reactions are poorly understood.

Granulysin is a 15-kDa cationic cytolytic protein released by cytotoxic T lymphocytes and natural killer cells that induces apoptosis in target cells and has antimicrobial activities.[11] Serum levels of granulysin are elevated in primary virus infections including Epstein–Barr virus and parvovirus B19.[12] It was recently reported that serum granulysin levels are significantly elevated in patients with several types of severe dermatological lesions including SJS/TEN, which is the characteristic serious adverse event in telaprevir-containing regimens.[13,14]

Accordingly, the present study determined the risk factors for severe dermatological reactions in patients receiving telaprevir, PEG IFN and RBV combination therapy as well as the association between serum levels of granulysin and severe dermatological reactions.

METHODS

Patients and methods

IN THIS RETROSPECTIVE case–control study, at Hokkaido University Hospital and associated hospitals in the NORTE Study Group, between December 2011 and November 2013, a total of 123 patients positive for HCV genotype 1 with high serum HCV RNA titer (>5 log IU/mL) received PEG IFN, RBV and telaprevir combination therapy. Patients were excluded if they required hemodialysis or had a positive test result for serum hepatitis B surface antigen, co-infection with other HCV genotypes or HIV, evidence of autoimmune hepatitis or alcoholic hepatitis, or malignancy. Serum granulysin levels were analyzed in five healthy volunteers with no HCV, HIV or hepatitis B virus infection or any inflammatory diseases.

Written informed consent according to the process approved by the hospital's ethics committee was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of each participating hospital.

Study design and treatment regimen

Telaprevir 500 or 750 mg was typically administrated every 8 h after meals for 12 weeks. PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) 1.5 IU/kg was administrated s.c. once per week for 24 weeks. RBV (Rebetol; MSD) was administrated for 24 weeks in two divided daily doses according to bodyweight: 600, 800 and 1000 mg for patients with bodyweights of less than 60, 60–80 and more than 80 kg, respectively. The doses of PEG IFN-α-2b, RBV and telaprevir were reduced at the attending physician's discretion on the basis of hemoglobin levels, decreased white blood cell or platelet counts, or adverse events.

During treatment, patients were assessed as outpatients at weeks 1, 2, 4, 6 and 8, and then every 4 weeks thereafter for the duration of treatment. Physical examinations and blood tests were performed at all time points.

Outcomes

The primary end-point was SVR, which was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. The secondary end-points were end-of-treatment virological responses (HCV RNA undetectable in serum) and rapid virological response (RVR), which was defined as undetectable serum HCV RNA at 4 weeks after the start of treatment. Dermatological reactions were classified according to severity in the same manner as in phase III trials in Japan.[10]

Serum granulysin measurement

To evaluate serum granulysin levels in chronic hepatitis C, we first measured serum granulysin levels in five

healthy volunteers and compared them with those of 20 chronic hepatitis C patients before treatment. Serum granulysin levels were measured at the onset of dermatological reactions (within 3 days of onset); if the symptoms worsened, the time when worsening occurred was adopted. Meanwhile, in patients with no dermatological reactions, the highest serum granulysin level during treatment was adopted.

Serum granulysin levels were measured by a sandwich enzyme-linked immunosorbent assay as described previously.[12,14,15] Briefly, plates coated with 5 mg/mL mouse antibody against human granulysin, RB1 antibody, were washed with phosphate-buffered saline containing 0.1% Tween-20. Next, they were blocked with 10% fetal bovine serum in washing buffer at room temperature for 2 h. The samples and standards (Recombinant Granulysin; R&D Systems, Minneapolis, MN, USA) were incubated for 2 h at room temperature. Next, they were reacted with 0.1 mg/mL biotinylated mouse antibody against human granulysin, RC8 antibody. The plates were subsequently treated with horseradish peroxidase-conjugated streptavidin (Roche Diagnostics, Basel, Switzerland). The plates were then incubated with tetramethyl-benzidine substrate (Sigma, St Louis, MO, USA), and 1 M sulfuric acid was then added. The optical density was measured at 450 nm using a microplate reader.

Diagnosis of dermatological reactions

Dermatological reactions were investigated throughout the 24-week administration period in the telaprevir-based combination therapy. Dermatological reactions were classified according to severity as follows. Grade 1 was defined as involvement of less than 50% of the body surface and no evidence of systemic symptoms. Grade 2 was defined as involvement of less than 50% of the body surface but with multiple or diffuse lesions or rashes with characteristic mild systemic symptoms or mucous membrane involvement with no ulceration/erosion. Grade 3 was defined as a generalized rash involving 50% or more of the body surface or a rash with any new significant systemic symptoms and considered to be related to the onset and/or progression of the rash. Life-threatening reactions included SJS, TEN, drug rash with eosinophilia and systemic symptoms (DRESS)/DIHS, erythema multiforme and other life-threatening symptoms, or patients presenting with features of serious disease.

When adverse skin reactions were detected, the attending physician classified the degree of severity and referred the patients to a dermatologist as needed. In principal, when grade 3 dermatological reactions occurred, the attending physician referred the patient to a dermatologist and discontinued telaprevir. When severe dermatological reactions including SJS/TEN and DRESS/DIHS were suspected, all drugs were discontinued immediately. SJS/TEN and DIHS were diagnosed by skin biopsy and according to disease criteria, respectively.

Statistical analysis

Categorical and continuous variables were analyzed by the χ^2-test and the unpaired Mann–Whitney *U*-test, respectively. All *P*-values were two-tailed, and the level of significance was set at $P < 0.05$. Multivariate logistic regression analysis with stepwise forward selection included variables showing $P < 0.05$ in univariate analyses.

The association between dermatological reactions and serum granulysin levels were evaluated by one-way ANOVA followed by Tukey's honestly significant difference test. All statistical analyses were performed using SPSS version 21.0 (IBM Japan, Tokyo, Japan).

RESULTS

Patients

WE INCLUDED 123 chronic hepatitis C patients who received telaprevir-based triple therapy. Of these, 89 patients who had proper information of dermatological adverse events were included. The baseline characteristics of patients are shown in Table 1.

Of these 89 patients, time-dependent changes of serum granulysin concentrations were measured in 20 who had had conserved serum, at least, at the pretreatment point, 1 and 2 weeks after commencement of therapy, 1 and 2 months after commencement of therapy, the onset point of dermatological adverse reaction and the worsening point if symptoms became worse.

Among the 89 patients, 64% (57/89) developed dermatological reactions, including nine with grade 3 reactions (Table 2). The characteristics of dermatological reactions by grade are shown in Table 2. Non-grade 3 dermatological reactions tended to occur early during treatment compared to grade 3 dermatological reactions.

Association between dermatological reactions and treatment outcomes

First, we determined whether dermatological reactions were associated with final treatment outcomes.

Table 1 Baseline characteristics of the participating patients

Total number	89
HCV genotype 1b (1b/others)	89/0
Age (years)†	60.0 (19–73)
Sex (male/female)	48/41
Bodyweight (kg)†	63.0 (32–97)
Baseline white blood cell count (/μL)†	4800 (1500–9800)
Baseline hemoglobin level (g/dL)†	13.5 (9.9–16.7)
Baseline platelet count ($\times 10^3$)†	15.9 (6.6–86)
Baseline ALT level (IU/L)†	40 (15–300)
Baseline HCV RNA level ($\log^{10}$ IU/mL)†	6.5 (3.2–7.6)
Initial telaprevir dose (1500/2250 mg)	20/89
Initial PEG IFN dose (1.5/<1.5 μg/kg)	775/14
Initial RBV dose (mg/kg)†	9.8 (2.2–15.5)
IL28B gene (rs8099917) (TT/non-TT/ ND)	51/22/16
HCV 70 core mutation (wild/mutant/ND)	43/24/22
Previous treatment (naïve/relapse/NVR)	40/38/11

†Data are shown as median (range) values.
ALT, alanine transaminase; HCV, hepatitis C virus; IL28B, interleukin 28B; ND, not done; PEG IFN, pegylated interferon; RBV, ribavirin.

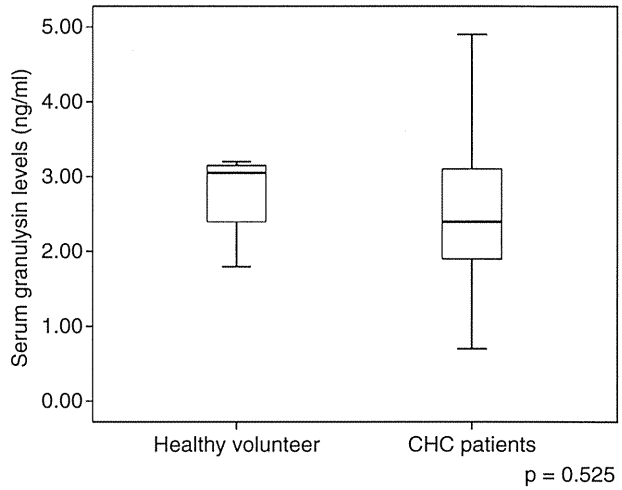

Figure 1 Serum granulysin levels of healthy volunteers and chronic hepatitis C patients. Serum granulysin levels were compared between five healthy volunteers and untreated 20 chronic hepatitis C patients. $P < 0.05$, Mann–Whitney *U*-test.

Univariate analyses identified baseline white blood cell and platelet counts, RVR, and non-grade 3 dermatological reactions significantly associated with SVR (Table 3). Among the nine patients with grade 3 dermatological reactions, three discontinued all treatment and six discontinued telaprevir administration; SVR was achieved in zero of the three (0%) and two of the six (33%), respectively.

Multivariate analysis showed that RVR and non-grade 3 dermatological reactions were significantly associated with SVR (Table 3).

Analysis of risk factors for telaprevir-induced dermatological reactions

Next, we analyzed the association between severe (i.e. grade 3) dermatological reactions and clinical parameters (Table 4). Univariate analysis showed that only sex was significantly associated with the grade 3 dermatological reactions ($P = 0.03$).

Serum granulysin levels in healthy subjects and chronic hepatitis C patients

As shown in Figure 1, serum granulysin levels did not differ significantly between healthy volunteers and chronic hepatitis C patients. Next, we evaluated the association between the severity of dermatological reactions and serum peak granulysin levels in 20 patients including five, four, five and six with grades 1, 2 and 3, and no dermatological events, respectively. One-way ANOVA showed that serum granulysin level was significantly associated with the severity of dermatological reactions ($P = 0.036$); in addition, Tukey's honestly significant difference test revealed that the serum

Table 2 Characteristics of the patients with each dermatological adverse event grade

	n	Age†	Sex (male/female)	Initial telaprevir dose (2250/1500)	Onset of DAR (days)
No DAR	32	61 (28–72)	15/17	26/6	
Grade 1	32	58 (19–73)	15/17	24/8	7 (3–50)
Grade 2	16	61 (44–73)	10/6	12/4	3.5 (1–56)
Grade 3	9	61 (48–65)	8/1	8/1	22 (1–60)

†Data are shown as median range) values.
DAR, dermatological adverse reaction

Table 3 Comparison of the clinical and laboratory characteristics of the patients with HCV infection based on therapeutic response

All patients	SVR	Non-SVR	Univariate analysis	Multivariate analysis		
$n = 89$	$n = 68$	$n = 21$	P	OR	95% CI	P
Age (years)†	60 (19–73)	62 (28–73)	0.402			
Sex (male/female)	37/31	11/10	0.870			
Bodyweight (kg)†	62 (39–97)	64 (32–87)	0.761			
Baseline white blood cells (/μL)†	5135 (1500–9800)	4200 (2490–7200)	0.048	0.492	(0.121–1.993)	0.320
Baseline hemoglobin level (g/dL)†	13.5 (10.5–16.7)	12.1 (9.9–15.4)	0.862			
Baseline platelet count ($\times 10^3$)†	16.7 (6.6–31.5)	12.8 (7.2–86)	0.025	0.388	(0.093–1.614)	0.193
Baseline ALT level (IU/L)†	37 (15–300)	53 (23–159)	0.070			
Baseline HCV RNA level ($\log^{10}$ IU/mL)†	6.7 (3.2–7.6)	6.4 (5.7–7.3)	0.812			
Baseline Cr level (mg/dL)	0.7 (0.5–1.3)	0.7 (0.5–0.9)	0.433			
Initial telaprevir dose (1500/2250 mg)	52/16	17/4	0.460			
Initial PEG IFN dose (1.5/<1.5 μg/kg)	58/10	17/4	0.430			
Initial RBV dose (mg/kg)†	9.9 (2.2–15.5)	9.5 (4.4–12.5)	0.546			
IL28B gene (rs8099917) (TT/non-TT/ND)	43/15/10	8/7/6	0.107			
Core 70 a.a. mutation (wild/mutant/ND)	36/16/16	7/8/6	0.108			
Previous treatment (naive/relapse/NVR)	34/28/6	6/10/5	0.095			
Rapid virological response (+/−)	60/8	10/11	<0.001	10.89	(2.838–41.83)	0.001
Grade 3 DAR (−/+)	66/2	14/7	<0.001	27.44	(3.718–202.5)	0.001

†Data are shown as median (range) values.
a.a., amino acid; ALT, alanine transaminase; CI, confidence interval; Cr, creatinine; DAR, dermatological adverse reaction; HCV, hepatitis C virus; IL28B, interleukin 28B; ND, not done; NVR, non-virological response; OR, odds ratio; PEG IFN, pegylated interferon; SVR, sustained virological response; RBV, ribavirin.

granulysin levels of patients with grade 3 dermatological reactions were significantly higher than those of patients with grade 1 or no dermatological reactions (both $P < 0.05$, Fig. 2).

Time-dependent changes in serum granulysin levels

We investigated the time-dependent changes in serum granulysin levels in five and 15 patients with grade 3 and non-grade 3 dermatological reactions, respectively (Fig. 3). Serum granulysin levels of patients with non-grade 3 dermatological reactions never exceeded 10 ng/ml. Of the five patients with grade 3 reactions, three had severe systemic manifestations that necessitated hospital admission: one each had SJS, DIHS, and systemic lymphoid swelling and high fever (>39 °C). All patients with grade 3 dermatological reactions with systemic manifestations had peak serum granulysin levels exceeding 10 ng/mL; importantly, the serum granulysin levels of two patients already exceeding 8 ng/mL at the onset of the reactions worsened within 6 days.

DISCUSSION

THE PRESENT STUDY demonstrates a significant association between telaprevir-induced dermatological reactions and elevated serum granulysin levels for the first time. Moreover, serum granulysin levels were significantly associated with the severity of dermatological reactions. Thus, the results indicate that serum granulysin level seems to be a useful predictor of telaprevir-induced dermatological reactions. Because the emergence of grade 3 dermatological reactions was significantly associated with non-SVR (Table 3), probably associated with high rate of treatment discontinuation, it is important to predict dermatological events in the early stage to achieve good treatment outcomes.

Table 4 Comparison of the clinical and laboratory characteristics of the patients based on the presence or absence of at least a grade 3 dermatological adverse event

All patients $n = 89$	Non-grade 3 $n = 80$	Grade ≥3 $n = 9$	Univariate analysis P
Age (years)†	60 (19–73)	61 (48–65)	0.453
Sex (male/female)	40/40	8/1	0.027
Bodyweight (kg)†	62 (32–97)	64 (51–87)	0.593
Baseline white blood cell count (/μL)†	4900 (1500–9800)	4700 (3000–7000)	0.876
Baseline hemoglobin level (g/dL)†	13.5 (9.9–16.7)	14.4 (12.1–15.4)	0.196
Baseline platelet count ($\times 10^3$)†	16.0 (6.6–86.0)	13.5 (10.4–22.5)	0.605
Baseline ALT level (IU/L)†	40(15–300)	37 (23–87)	0.765
Baseline Cr level (mg/dL)	0.7 (0.5–1.3)	0.8 (0.6–0.9)	0.123
Baseline HCV RNA level ($\log^{10}$ IU/mL)†	6.6 (3.2–7.6)	6.4 (5.7–7.1)	0.465
Initial telaprevir dose (1500/2250 mg)	62/18	7/2	0.675
Initial telaprevir/bodyweight (mg/kg)	33.7 (20–71.4)	30.0 (23.6–44.1)	0.563
Initial PEG IFN dose (1.5/<1.5 μg/kg)	66/14	9/0	0.198
Initial RBV dose (mg/kg)†	9.7 (2.2–15.5)	10.7 (7.7–12.9)	0.161
IL28B gene (rs8099917) (TT/non-TT/ND)	47/19/14	4/3/2	0.353
Core 70 a.a. mutation (wild/mutant/ND)	38/22/20	5/2/2	0.511
Previous treatment (naïve/relapse/NVR)	35/36/9	5/2/2	0.972
Onset of dermatological AE (days)	5 (1–75)	22 (1–60)	0.352

†Data are shown as median (range) values.
a.a., amino acid; AE, adverse event; ALT, alanine transaminase; Cr, creatinine; HCV, hepatitis C virus; IL28B, interleukin 28B; NVR, non-virological response; PEG IFN, pegylated interferon; RBV, ribavirin.

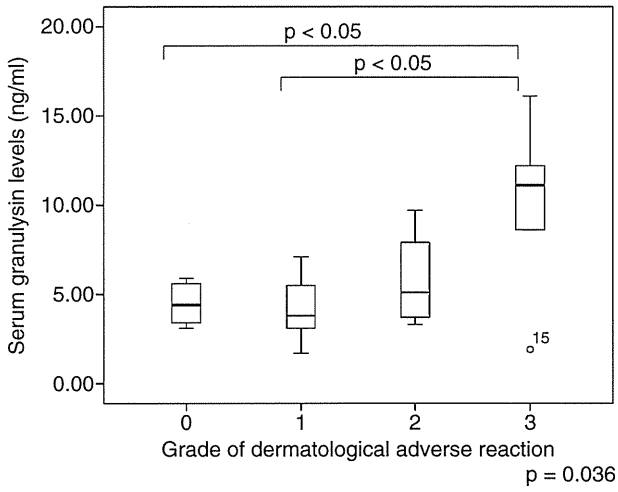

Figure 2 Association between dermatological adverse reaction severity and serum granulysin level. Serum granulysin levels were measured at the onset of dermatological reactions (i.e. within 3 days of onset); if the symptoms worsened, the time of worsening was adopted. In patients with no dermatological events, the highest serum granulysin level during treatment was adopted. $P < 0.05$, one-way ANOVA.

Recent genome-wide association studies have identified that genetic polymorphisms around the IL28B gene locus significantly associated with the outcome of PEG IFN and RBV combination therapy in HCV patients. Thus, PEG IFN and RBV combination therapy is ineffective in a subset of HCV-infected patients who have IL28B TG or GG genotypes, limiting the use of this therapy.[16] Therefore, novel drugs with different antiviral mechanisms were required. Accordingly, DAA were developed; they are mainly classified as NS3/4A protease inhibitors, or NS5B or NS5A inhibitors.[17] The NS3/4A serine protease inhibitor telaprevir, in combination with PEG IFN and RBV, has demonstrated the most promising results.[6–8] However, adverse events, especially severe dermatological reactions, develop more frequently in patients treated with telaprevir than those treated with only PEG IFN and RBV.

Little is known about the mechanisms of telaprevir-induced dermatological reactions. Reactions develop in patients treated with PEG IFN and RBV combination therapy[18,19] as well as telaprevir monotherapy.[20,21] It should be noted that the dermatological reactions in telaprevir monotherapy or PEG IFN and RBV therapy alone are generally mild.[7,8,20] However, dermatological

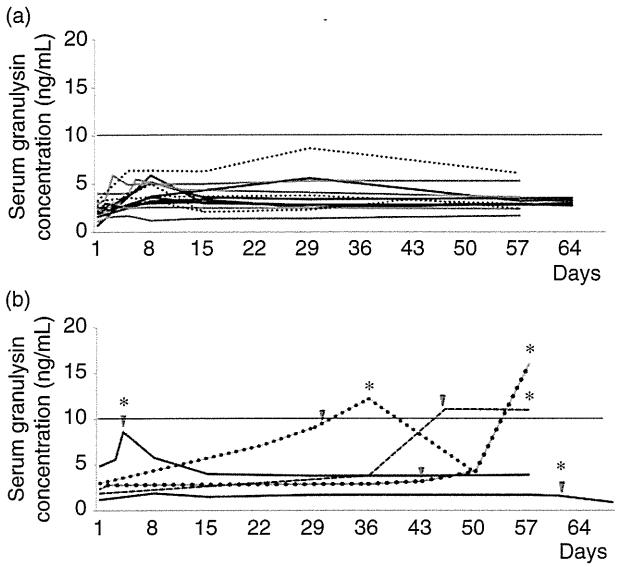

Figure 3 Association between time-dependent changes in serum granulysin levels and severe telaprevir-induced dermatological adverse reactions. (a) Time-dependent changes in serum granulysin levels patients with non-grade 3 dermatological reactions (three, five and six with grade 2, grade 1 and no reactions, respectively). The dashed line, gray line and black line indicate grade 2, grade 1 and no reaction, respectively. (b) Time-dependent changes in serum granulysin levels of five patients with grade 3 dermatological events. The dashed line indicates patients with severe systemic manifestations. Arrowheads indicate the onset of dermatological events and asterisks indicate the onset of grade 3 dermatological events.

reactions in telaprevir and PEG IFN/RBV combination therapy may be severe, indicating a synergistic effect. Severe dermatological events including SJS/TEN and DIHS have been reported in telaprevir-based triple therapy; these are life-threatening, and fatal cases have been reported.

The onset of grade 3 dermatological reactions tended to be later than non-grade 3 reactions, the same as in the study of Torii *et al.*[10] Taken together with the finding that male sex is a clinical risk factor, the results indicate that late-onset dermatological reactions in male patients treated with telaprevir-based triple therapy require more attention.

Roujeau *et al.* analyzed the risk factors for telaprevir-induced eczematous dermatitis and report that the incidence of telaprevir-related dermatitis was significantly higher age of more than 45 years, body mass index of less than 30 (kg/m^2), Caucasian ethnicity and treatment-naïve status.[9] While they analyzed the risk factors for telaprevir-induced eczematous dermatitis, the present study focused on the risk factors for severe telaprevir-induced dermatological reactions, because such reactions can affect treatment outcome (Table 2) and can be fatal. As mentioned above, male sex was significantly associated with grade 3 dermatological reactions. Sex is reported to be associated with the prevalence of some kinds of severe drug-induced dermatological events, although the underlying mechanism remains unknown.[22]

Fujita *et al.* report that serum granulysin levels are significantly elevated in SJS/TEN patients and thus may be a good predictive factor.[14] Therefore, we hypothesized that in telaprevir-based triple therapy for chronic hepatitis C patients, serum granulysin levels are associated with the severity of dermatological reactions and may thus be a predictive biomarker. However, Ogawa *et al.* report that serum granulysin levels also increase as a result of primary virus infections such as Epstein–Barr virus or parvovirus B19.[12] Thus, it remains unclear whether and how chronic viral infections, especially HCV, affect serum granulysin levels. In the present study, we compared serum granulysin levels between healthy volunteers and chronic hepatitis C patients; the results show that chronic HCV infection was not associated with serum granulysin levels (Fig. 1).

Chung *et al.* have reported that granulysin is the most highly expressed cytotoxic molecule in blisters of SJS/TEN and that massive keratinocyte death was induced by granulysin.[11] Fujita *et al.* reported that serum granulysin levels increased in the early stage of SJS/TEN caused by drugs including carbamazepine, imatinib and phenytoin.[14] Taken together with our results, we speculate that granulysin may be involved in the pathogenesis of early stage telaprevir-mediated dermatological adverse reactions possibly through induction of keratinocyte death.

Of five patients with grade 3 reactions, two patients without severe systemic manifestations did not have elevated serum granulysin of more than 10 ng/mL or did not have elevated levels before symptoms worsened. On the contrary, three patients with severe systemic manifestations had peak serum granulysin levels exceeding 10 ng/mL, and the symptoms of two patients with serum granulysin levels already exceeding 8 ng/mL at onset and within 6 days worsened. Therefore, serum granulysin tests may predict grade 3 dermatological adverse reaction with systemic manifestations. Furthermore, if serum granulysin levels elevate more than 8 ng/mL, more attention should be paid.

In Western countries, the prevalence of dermatological reactions in patients treated with telaprevir-based and

PEG IFN/RBV therapy are reported to be approximately 55% and 33%, respectively;[9,23] meanwhile, in Japanese patients, the respective rates are 74.9% and 58.7%. Moreover, approximately 4% and 9% of patients in Western and Japanese patients develop grade 3 reactions, respectively;[10] this is almost the same as that in the present study (10%). The difference may be due to genetic or ethnic variation. Therefore, genome-wide association studies may have identified a gene locus associated with telaprevir-induced severe dermatological reactions.

A limitation of this study is that the number of patients with grade 3 dermatological reactions is relatively small. However, the serum granulysin levels of patients with grade 3 dermatological reactions were significantly higher than those of other patients. Also, in two of the three patients with severe dermatological reactions, the serum granulysin level elevated before symptoms worsened, which are novel findings. Further study is required.

Triple therapy with the second-generation protease inhibitor simeprevir is reported to result in a similar prevalence of adverse reactions as PEG IFN and RBV combination therapy.[24,25] However, simeprevir is not approved worldwide. Although simeprevir-based triple therapy is effective, only 36–53% of prior non-responders achieve SVR.[24] Shimada *et al.* recently reported that by extending PEG IFN and RBV therapy from 24 to 48 weeks, telaprevir-based triple therapy improves the SVR to up to 68% in prior null responders.[26] Thus, telaprevir is a therapeutic option for prior null responders.

In conclusion, the present study suggests that male sex is a significant risk factor for severe telaprevir-induced dermatological reactions. In addition, serum granulysin levels are significantly associated with the severity of dermatological reactions and thus may be a good predictor of severe dermatological reactions with systemic manifestations in patients treated with telaprevir-based triple therapy.

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