without HCC and 43 HCC patients), especially females, and the proportion of patients with HTLV-1 seropositivity was low [14.7 % (25/170)] compared to our study. In addition, potential confounders such as age and alcohol consumption were not fully considered. These differences may account for the conflicting results. A prospective study involving a larger number of patients is required.

Several factors, such as male sex, older age, excessive alcohol consumption, and cigarette smoking, have been reported to be associated with an increased risk for progression of chronic liver disease due to HCV infection [10–12]. Our study also found that alcohol consumption and age are independently associated with HCC, and HTLV-1 tended to be associated with HCC in the multivariate analysis involving all patients (Table 2). The prevalence of alcohol consumption in males was higher than in females. Male HTLV-1 carriers have been reported to have a higher risk of developing ATLL than female HTLV-1 carriers [15]. In addition, the prevalence of HTLV-1 infection is known to vary by age and sex, with higher rates associated with older age and female sex in Japan. The increased prevalence with age may be due to the accumulation of infections over the lifetime of surveyed individuals or an age-cohort effect due to declining HTLV-1 prevalence over the past few decades. The higher prevalence in females may be the result of more efficient male-to-female sexual transmission [33, 34], or differences in socio-demographic or behavioral factors. Therefore, we speculate that such background factors or additional undetermined ones may contribute to different effects of HTLV-1 coinfection in males and females, although age was not significantly different between patients with HTLV-1 coinfection and those with HCV infection alone regardless of the presence of HCC in our study population.

Stuver et al. [31] also reported that HTLV-1 coinfection did not have any measurable impact on liver cancer mortality in subjects positive for anti-HCV antibodies. In contrast, Boschi-Pinto et al. [32] reported that dual HCV and HTLV-1 infection has a synergistic effect on death from HCC. Although these reports used similar cohort populations, the results seem to be conflicting. In addition, although Arisawa et al. [35] have reported that HTLV-1 infection was not associated with an overall increased risk for cancer, HTLV-1 infection was associated with an increased risk for liver cancer, which may be explained by the confounding by HCV infection and the interaction between HTLV-1 and HCV. However, these studies did not confirm the presence of HCV RNA or viremia, which can affect the results. In our study, we confirmed the presence of viremia and this confirmation may provide stronger evidence that HTLV-1 infection affects liver cancer mortality in patients with persistent HCV infection than previous reports. The overall and liver-related survival rates in patients with HTLV-1 coinfection were similar to those in patients with HCV infection alone in our study. However, as a result, mortality, especially liver-related mortality, tended to be affected by HTLV-1 coinfection in female patients (Fig. 2b). This tendency might be affected by the high prevalence of HTLV-1 coinfection in HCC patients. Therefore, we speculate that in female patients with persistent HCV infection, HTLV-1 coinfection affects liver cancer mortality. The follow-up period was relatively short for many patients in our study, which may have affected the results; further long-term longitudinal studies are needed.

HTLV-1 mainly infects CD4+ T cells. Other cell types such as CD8+ T cells and dendritic cells (DCs) may also serve as reservoirs of HTLV-1 [36, 37]. HTLV-1 carriers are reported to have an impaired cellular immune response [38]. Purified plasmacytoid DCs (pDCs) from asymptomatic HTLV-1 carriers were found to have impairments in IFN-α production [39]. Soguero et al. [40] reported that the expression of HCV core protein in T cells induces immune dysregulation by increasing apoptosis of T lymphocytes and the development of liver damage results from recruitment of these apoptotic lymphocytes into the liver. pDCs stimulated by Toll-like receptor ligand obtained from subjects with chronic HCV infection have impaired the ability to activate naive CD4+ T cells [41]. Therefore, an impaired immune response in HTLV-1 carriers could exacerbate liver injury in patients with HCV infection, and these effects are thought to promote hepatocarcinogenesis. However, liver injury involving increased levels of ALT in patients with HTLV-1/HCV coinfection occurred at rates similar to those in patients with HCV alone, regardless of the presence of HCC in our study. In addition, HTLV-1 may affect HCV RNA levels or the effectiveness of IFN treatment for HCV clearance [28, 42], but these differences were not observed in our study. In contrast, Ioannou et al. [43] reported that low CD4+ cell count was independently associated with HCC, but not with cirrhosis in patients with HIV infection. They also suggested that although the increased prevalence of HCC among HIV-infected patients was driven primarily by the HCV epidemic, immune suppression by HIV infection is more directly relevant to hepatocarcinogenesis than uncontrolled viral replication or liver cirrhosis [43]. Furthermore, γ-GTP was independently associated with HCC in all patients, and serum levels of γ-GTP were significantly higher in female HTLV-1 coinfected patients with HCC in our study. γ-GTP has been reported to be associated with an increased risk of oxidative stress [44] and increased carcinogenesis in subjects with HCV infection [45]. Takahashi et al. [46] reported that the HTLV-1 Tax oncoprotein stimulates the production of reactive oxygen species via interactions with ubiquitin-specific protease 10 in HTLV-1-infected T cells, although

the association between this effect of HTLV-1 Tax oncoprotein and γ-GTP or hepatocyte is unclear. Therefore, we speculate that HTLV-1 infection is more directly associated with hepatocarcinogenesis through various mechanisms in patients with persistent HCV infection, as opposed to indirectly via exacerbating liver injury.

This work has several limitations. First, the study was retrospective in nature. There was insufficient data available on IFN-based treatment status. Recent advances in therapy to eradicate HCV, as well as in the understanding of genetic factors such as interleukin 28B, may affect the clinical course of HCV carriers [47, 48]. The natural history and treatment outcome in chronic hepatitis C patients with or without HTLV-1 coinfection should be further examined in a prospective study. Second, many patients in this study were referred to our hospital because of the occurrence of HCC. Therefore, the prevalence of HCC among our study population was higher than in cohort-based studies [49]. Therefore, selection bias should be considered, and a cohort study would be desirable. Third, the presence of HTLV-1 infection was defined as positive anti-HTLV-1 antibody based on CLEIA or ECLIA, but there is the possibility of false positive results. Confirmation of HTLV-1 infection by other methods such as western blot analysis is needed. However, Hanaoka et al. [50] reported that the seroprevalence rate of HTLV-1 using enzyme-linked immunosorbent assay (ELISA) and western blot analysis are similar in endemic areas. Among Jewish immigrants from Iran, 24 of 331 blood donors (7.2 %) were positive for anti-HTLV-1 antibodies by ELISA, and all these donors were also positive for anti-HTLV-1 antibodies by western blot analysis [51]. In Kagoshima Prefecture, Japan, the anti-HTLV-1 antibody positive rate as determined by CLEIA among pregnant women was 125/4,147 (3.01 %), and the true positive rate based on western blot analysis was 85 % (106/125). Furthermore, in stored serum samples available from 15 study patients, including six HCC patients and nine non-HCC patients among the 83 patients with HTLV-1 coinfection, anti-HTLV-1 antibodies (to p19, p24, p53, and gp46 of HTLV-1) were detected by western blot analysis in all samples. Although there is a possibility of false positive anti-HTLV-1 antibody determination in our study population, such cases might be rare.

In conclusion, HTLV-1 coinfection was independently associated with HCC in patients with chronic liver disease due to HCV infection. HTLV-1 infection may be associated with the development of HCC, with this effect being particularly important in females.

Conflict of interest H. Tsubouchi holds endowed faculty positions in research for HGF tissue repair and regenerative medicine, and has received funds from Eisai Co., Ltd. The other authors declare that they have no conflict of interest.

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Severe Veno-occlusive Disease/Sinusoidal Obstruction Syndrome After Deceased-donor and Living-donor Liver Transplantation

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ABSTRACT

Veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) occurring after liver transplantation is a relatively rare complication but it often takes a life-threatening course. However, the detailed etiology and mechanism of VOD/SOS after liver transplantation (LT) remains unclear. We report two cases with rapidly progressive VOD/SOS after ABO-identical LT resistant to various therapies. In case 1, in which the patient underwent deceased-donor LT, the first episode of acute allograft rejection was triggered VOD/SOS, and the presence of donor non-specific anti-HLA antibodies was confirmed. The recipient died with graft failure on day 46 after transplantation. Case 2, in which the patient underwent living-donor LT from the mother, had neither rejection nor mechanical venous obstruction, but condition of the patient rapidly worsened and he died on day 13 after transplantation. This recipient's direct cross-match test for the donor's B lymphocyte was strongly positive, but that for T lymphocyte was negative. In both cases, neither stenosis of hepatic vein outflow tract nor C4d deposition in post-transplantation liver biopsy specimens and autopsy specimen was found. On the other hand, in both cases, the patient was transfusion unresponsive thrombocytopenia and hyperbilirubinemia persisted postoperatively, and glycoprotein I bα was strongly stained in the neighboring centrilobular area (zone 3), especially in the space of Disse, and platelet phagocytosis was observed in Kupffer cells and hepatocytes around zone 3 such as clinical xenotransplantation of the liver in post-transplantation liver biopsy specimens. From the viewpoint of graft injury, VOD/SOS was considered that sustained sinusoidal endothelial cells injury resulted in bleeding in the space of Disse and led to around centrilobular hemorrhagic necrosis, and the fundamental cause was damage around centrilobular area including sinusoid by acute cellular rejection, antibody-mediated rejection or ischemic reperfusion injury. The extrasinusoidal platelet activation, aggregation, and phagocytosis of platelets were some of the main reasons for VOD/SOS and transfusion-resistant thrombocytopenia.

VENO-OCCLUSIVE DISEASE (VOD), also known as sinusoidal obstruction syndrome (SOS) of the liver, was first described by Bras et al [1]. The clinical diagnosis of VOD/SOS was based on the triad of jaundice, painful hepatomegaly, and ascites/weight gain. It was confirmed by histologic findings including fibrous obliteration of small hepatic veins by connective tissue and centrilobular hemorrhagic necrosis. In the transplantation setting, VOD/SOS is responsible for liver dysfunction after bone marrow [2–4] and kidney

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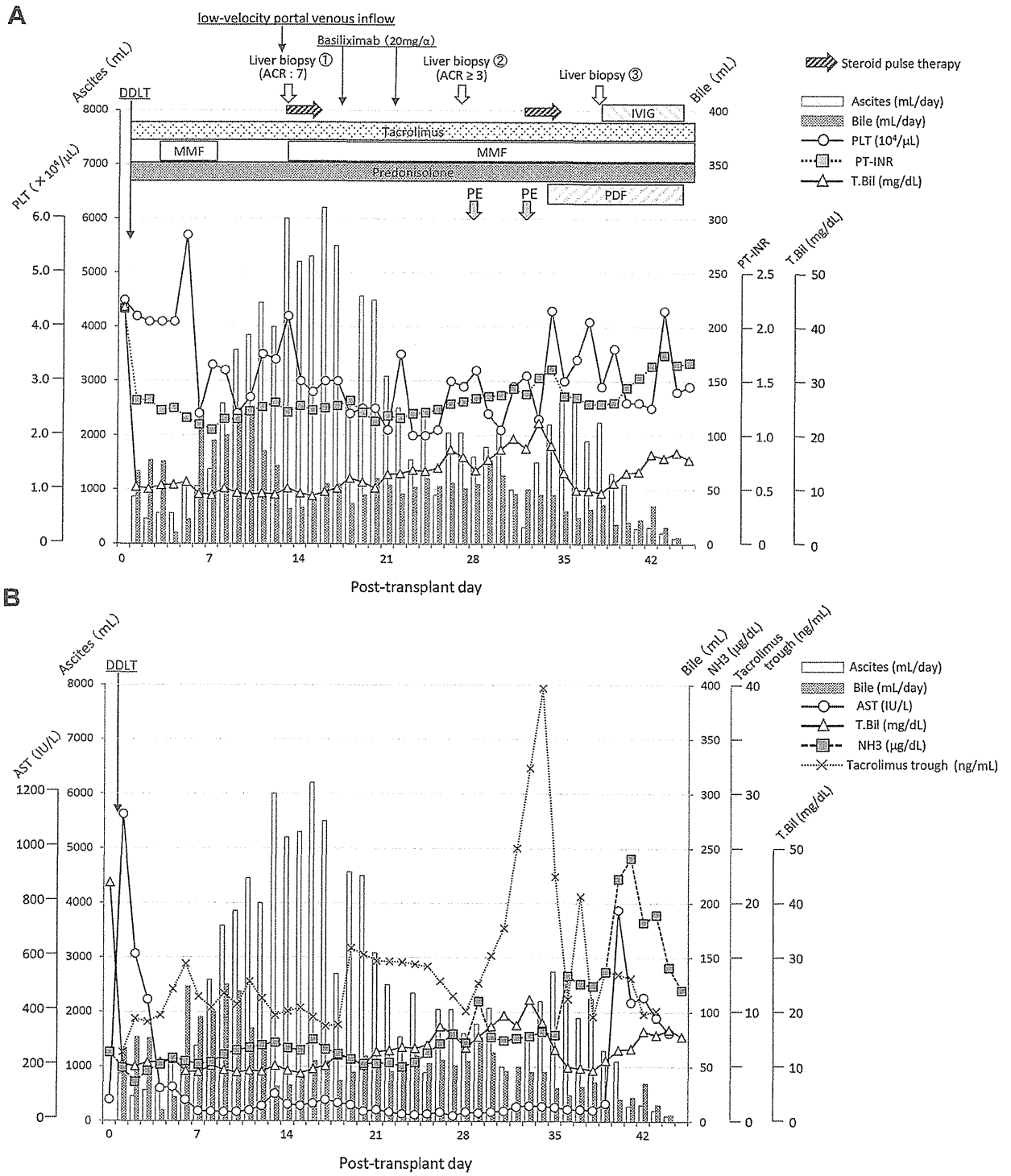
transplantation [5–10]. VOD/SOS after bone marrow transplantation is thought to be the consequence of hepatotoxicity arising mainly from pre-transplanted chemo-radiotherapy which is given for hematologic and other malignancies. On the other hand, VOD/SOS after liver transplantation (LT) is initially thought to be related to azathioprine therapy [11]. It was reported subsequently that VOD/SOS occurred at little frequency regardless of azathioprine secondary to acute cellular rejection (ACR) or antibody-mediated rejection (AMR) [12–17]. The present



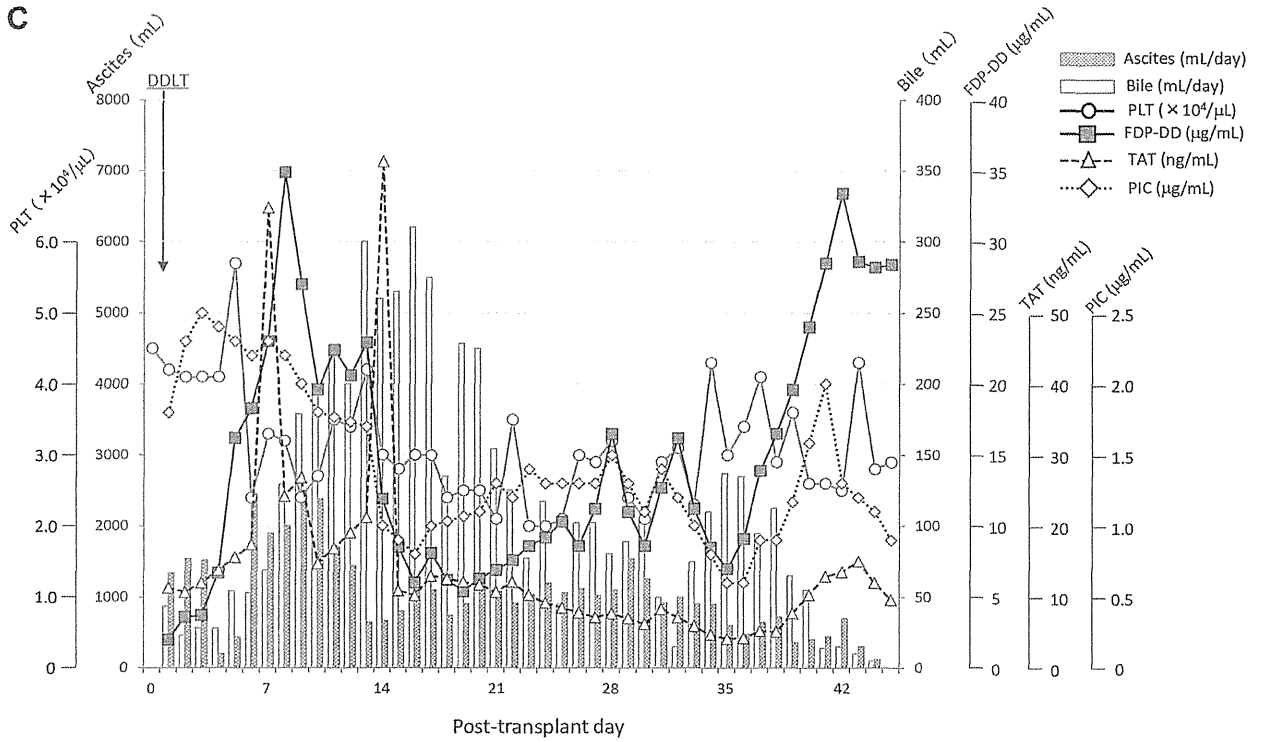


Fig 1. *(continued).*

retrospective case study was undertaken to assess the etiologic mechanism of the rapid progressive severe VOD/SOS after LT in relation to the post-transplantation course with or without ACR.

MATERIALS AND METHODS

Immunohistochemistry

Platelet activation and aggregation in the sinusoid space of Disse and parenchyma of the graft liver were evaluated by immunohistochemical staining for platelet surface receptor glycoprotein I bα (GP I bα: CD42b). The expressions of CD42b were examined immunohistochemically with respective primary antibodies using the EnVision+ System (DAKOk, Tokyo, Japan). De-waxed 4-μm sections were incubated with 1:50 with protein blocking serum for 10 minutes to block nonspecific binding; immunostaining was performed using the EnVision+ System. Briefly, the slides were incubated with each primary antibody (1:50) at 4°C overnight. After washing, the EnVision+ polymer solution was applied for 1 hour. The reaction products were visualized via a diaminobenzidine reaction. The specimens were then lightly counterstained with hematoxylin and examined under a fluorescence microscope. Primary antibodies used for immunostaining were CD42b rabbit anti-human polyclonal antibody (Atlas Antibodies. AlbaNova University Center, Stockholm, Sweden).

RESULTS

Case 1

A 44-year-old woman underwent deceased-donor LT (DDLT) from an approximately 70-year-old donor with

Fig 1. (**A–C**) The clinical course of the patient after deceased-donor liver transplantation (DDLT). These figures showed the administration of the immunosuppressants, the change of laboratory data, the amount of ascites exudate from intraabdominal drain and the amount of bile exudate from biliary drainage tube. She had transfusion unresponsive thrombocytopenia, prolonged hyperbilirubinemia, and a high level of coagulation-related markers such as fibrin/fibrinogen degradation products - D dimer (FDP-DD), thrombin - anti-thrombin complex (TAT), and plasmin - α2 plasminogen inhibitor complex (PIC). We performed liver biopsy on postoperative days 13, 27, and 38. Severe acute cellular rejection (Rejection Activity Index Score, P3V3B1 = 7), centrilobular hemorrhage, hepatocyte loss, and cholestasis were diagnosed by liver biopsy at postoperative day 13. Despite a second course of steroid pulse therapy and administration of mycophenolate mofetil (MMF), basiliximab, intravenous immunoglobulin (IVIG), anticoagulant therapy, and anti-platelet therapy for the VOD/SOS, her clinical findings (graft failure with hepatomegaly as well as graft inflow abnormalities) had not improved. Hyper-bilirubinemia and encephalopathy were worsened, so the recipients were initiated plasma-diafiltration (PDF) following plasma exchange (PE) with hydro dialysis (HD), but hepatic encephalopathy was worsened and she was dead in spite of intensive treatment on post-operative day (PTD) 46 without the chance of retransplantation. (Abbreviations: PLT, platelet; PT-INR, prothrombin time – international normalized ratio; T.Bil, total bilirubin; AST, aspartate aminotransferase; NH_3, ammonia.)

standard techniques including the piggyback technique (recipient middle and left hepatic two veins – graft inferior vena cava anastomosis) for end-stage liver disease because of autoimmune hepatitis. The patient's preoperative Model for End-stage Liver Disease (MELD) score was 29 points and the Child-Pugh score was 13 points with hepatic encephalopathy, massive ascites, and esophageal varices. The cold ischemic time was 11 hours, the warm ischemic time was 70 minutes, and total ischemic time was 12 hours 10 minutes. Her human leukocyte antigen (HLA) typing was fully mismatched with that of the donor for A, B, and DRB1, but she had anti-HLA antibody. Her postoperative clinical curse is summarized in Fig 1. The induction immunosuppression regimen consisted of triple therapy with tacrolimus (FK506), mycophenolate mofetil (MMF), and steroid (prednisolone), but MMF was stopped at postoperative day (POD) 7 because of severe watery diarrhea. Her postoperative course was almost uneventful for 1 week after the operation except for transfusion-unresponsive thrombocytopenia, prolonged hyperbilirubinemia, and a high level of coagulation-related markers such as fibrin/fibrinogen degradation products - D dimer (FDP-DD), thrombin - anti-thrombin complex (TAT), and plasmin - $\alpha 2$ plasminogen inhibitor complex (PIC). Reticulocyte and immature platelet counts in blood were within the normal range during the clinical course. Thrombocytopenia would be caused by sustained platelet activation or consumption, not reduction of platelet production from bone marrow due to the reduced thrombopoietin level. But the volume of ascites was increased and the amount of bile exudate from the bile duct drainage tube was decreased without major changes in laboratory data. Subsequently, there was development of low-velocity portal venous inflow without hepatic venous outflow block at POD 13. Although her serum hepatic transaminase levels were almost normal, we suspected ACR and potential VOD/SOS and thus performed a liver biopsy and started steroid pulse therapy (10 mg/kg for 3 days) and administration of MMF was restarted at POD 13. Initially, we diagnosed a severe ACR (Rejection Activity Index Score [RAIS]: P3V3B1 = 7; Fig 2) with centrilobular hemorrhage, hepatocyte loss and cholestasis. Furthermore, feathery degeneration, ballooning, and vacuolization of Kupffer cells were observed. Therefore, two administrations of anti-CD25 antibody (basiliximab; 20 mg $\times$ 2α) were performed for severe ACR. The C4d staining was negative. However, the patient demonstrated weight gain with tender hepatomegaly. On Doppler ultrasonography, she displayed high-velocity hepatic venous outflow but slightly decreased pulsatile hepatic venous wave forms. Discharge amount of ascites was gradually decreased. Because the hyperbilirubinemia gradually worsened, we performed a second liver biopsy on POD 27. It showed amelioration of cellular infiltration in the portal area, but the central vein (zone 3) had disappeared because of hemorrhagic degeneration (ACR; RAIS: P2V-B1 $\geq$ 3; Fig 3) and C4d staining was also negative at that time. We considered that recipient's condition could be explained as VOD/SOS followed by ACR, and we initiated a plasma exchange with hydro dialysis. But hyperbilirubinemia worsened, so we initiated a second course of steroid pulse therapy and plasma diafiltration (PDF). Hyperbilirubinemia improved by PDF, but hepatic encephalopathy worsened. We then performed a third liver biopsy on POD 38 and she resumed artificial ventilation under intubation. We diagnosed a probable acute onset chronic rejection (AMR) because centrilobular hemorrhagic hepatocellular necrosis (zone 3) and severe cholestasis had worsened with injury of the interlobular bile duct, but increment of the bile ductule was not observed (Fig 4). ACR improved clearly; C4d staining was negative at that time. Therefore, intravenous immunoglobulin (IVIG) therapy and continuous arterial infusion therapy of prostaglandin E1 and methylprednisolone were performed. Despite intensive treatment, she died on POD 46 without the chance for retransplantation. We diagnosed VOD/SOS from the pathology report of the autopsy (Fig 5). There was severe centrilobular hemorrhagic hepatocellular necrosis (zone 3) and severe cholestasis with injury of the interlobular bile duct and without increment of bile ductule, and severe portal vein and central vein venulitis without venous outflow block. However, ACR was unclear and C4d staining was also negative at that time. We performed Azan staining and silver impregnations to evaluate graft liver fibrosis by biopsy specimens and performed immunohistochemical staining for CD42b (Gp I bα) to evaluate platelets' activation and aggregation in the space of Disse and parenchyma in the graft liver. By Azan staining (Fig 4C) and silver impregnation (Fig 4D) of the liver biopsy specimen from POD 38, progressive fibrosis around the central vein (zone 3) was confirmed. According to the biopsy specimen just after the reperfusion, aggregation of activated platelets was observed in the space of Disse and hepatic parenchyma in zone 3, and platelet phagocytosis of sinusoidal endothelial cells, Kupffer cells and hepatocyte were observed in zone 3 (Fig 6A). Progression of activated platelet aggregation and platelet phagocytosis with centrilobular hemorrhagic necrosis and destruction of lobular structure around zone 3 were observed in the subsequent liver biopsy specimens (Fig 6B). Therefore, aggregation of activated platelets and platelet phagocytosis around zone 3 should be regarded as an important index of early graft failure by VOD.

Case 2

A 34-year-old man underwent living-donor LT (LDLT) with right lobe graft from his 62-year-old mother for end-stage secondary liver cirrhosis because of hepatolithiasis after the operation for congenital biliary dilatation. In this patient, preoperative MELD score was 26 points and Child-Pugh score was 12 points, and he had uncontrollable massive ascites and esophageal varices. The cold ischemic time was 90 minutes, the warm ischemic time was 47 minutes, and the total ischemic time was 2 hours 17 minutes. Graft volume versus recipient's standard liver volume [18] was 46% and graft weight versus recipient's weight was 0.85%. The size

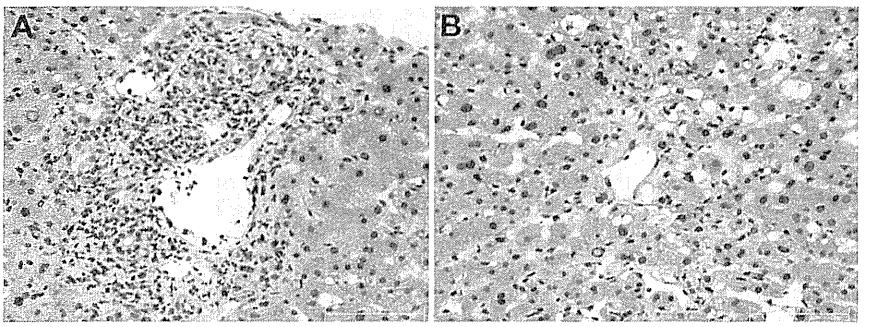


Fig 2. (**A, B**) Microscopic findings of hematoxylin eosin staining on the first liver biopsy at POD 13. Severe acute cellular rejection (ACR; RAIS: P3V3B1 = 7), centrilobular hemorrhage, hepatocyte loss and cholestasis were observed.

seemed to be the not-so-small size for graft of LDLT. His HLA typing was one mismatched with that of the donor for A, B, and DRB1. The direct cross-match test for the donor B lymphocyte was positive. For T lymphocyte it was negative before LDLT, and anti-donor specific antibody was not confirmed postoperatively. His postoperative clinical course is summarized in Fig 7. The induction immunosuppression regimen consisted of double therapy with FK506 and steroid (prednisolone). His postoperative course was almost uneventful for 5 days except transfusion-unresponsive thrombocytopenia. However, at POD 6, the volume of ascites exudate from intraabdominal drain had rapidly increased, portal venous flow had rapidly decreased without hepatic venous outflow block, and the amount of bile exudate from the biliary drainage tube was decreased. His serum hepatic transaminase levels were simultaneously increased; we suspected ACR and potential VOD/SOS and thus performed a liver biopsy and started steroid pulse therapy (10 mg/kg for 3 days). Balloon-occluded retrograde transvenous obliteration was attempted for increasing portal venous inflow after confirming no hepatic venous outflow block by angiography. We diagnosed severe and centrilobular hemorrhagic hepatocellular necrosis with neutrophilic infiltration and dilatation of sinusoid and diagnosed canalicular cholestasis without ACR by the liver biopsy specimen of POD 6 (Fig 8). The C4d staining was negative. Mild perivenular fibrosis with focal nuclear glycogen was observed in the donor's pretransplantation liver biopsy specimen. Despite intensive with steroid pulse therapy and high-dose IVIG therapy, the patient died on POD 13 without the chance of retransplantation. We diagnosed VOD/SOS based on pathology results from the autopsy (Fig 9). Severe congestion, hemorrhagic hepatocellular necrosis and cholestasis with interlobular bile duct injury were observed in the autopsy specimens, but increment of bile ductule was not observed. Also severe portal vein and central vein venulitis without venous out flow block were observed. But ACR was unclear and C4d staining was also negative at that time. We performed immunohistochemical staining for CD42b to evaluate platelet activation and aggregation in the sinusoid space of Disse and parenchyma of the graft liver. Progression of activated platelet aggregation and platelet phagocytosis with centrilobular hemorrhagic necrosis and destruction of lobular structure around zone 3 were observed in the subsequent liver biopsy specimens (Fig 10). Aggregation of activated platelets and platelet phagocytosis around zone 3 should be regarded as an important index of early graft failure by VOD.

DISCUSSION

Herein, we present two cases of VOD/SOS. The SOS was first described in a Jamaican child by Jelliffe et al in 1954 [19], replacing the previously named VOD. A considerable number of studies on VOD/SOS have since been conducted on recipients of hematopoietic stem cell transplantation. In

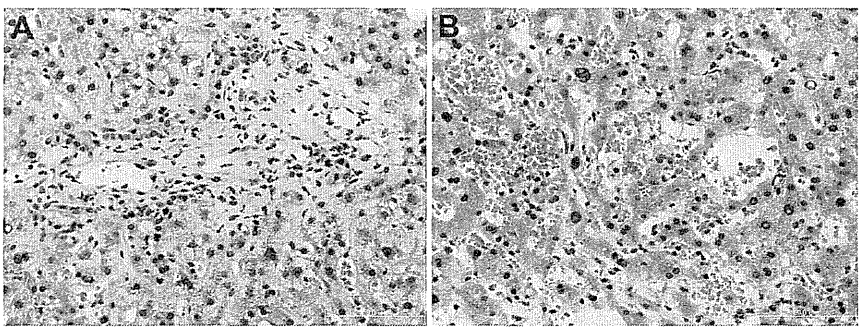


Fig 3. (**A, B**) Microscopic findings of hematoxylin eosin staining of the second liver biopsy at POD 27. These show amelioration of cellular infiltration in the portal area, but the central vein (zone 3) disappeared because of hemorrhagic degeneration (ACR; RAIS: P2V-B1 $\geq$ 3). The recipient's condition could be explained as VOD/SOS followed by ACR.

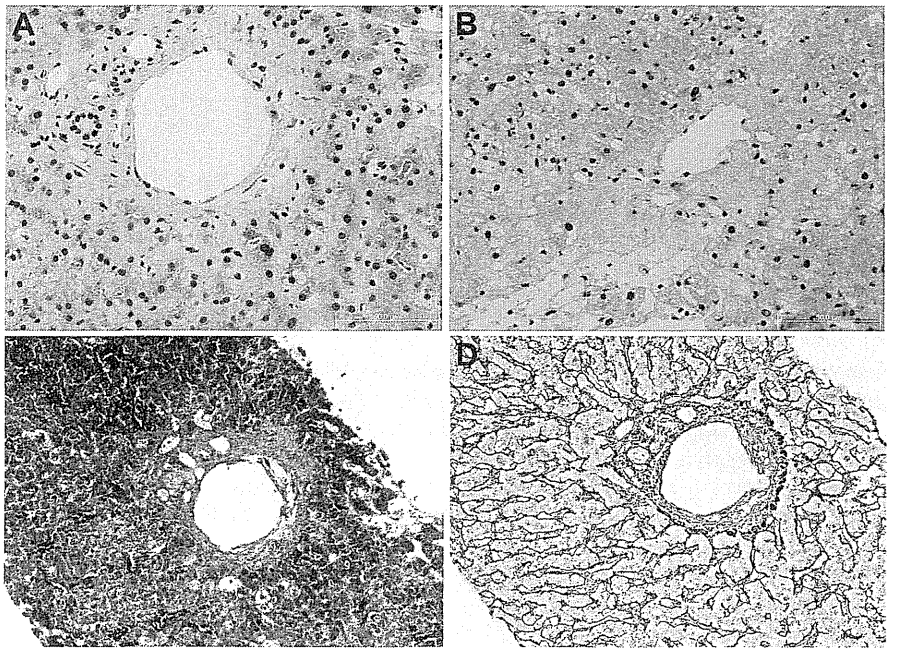


Fig 4. (**A**–**D**) Microscopic findings of hematoxyline eosin staining (**A** & **B**), Azan staining (**C**), and silver impregnation (**D**) of the third liver biopsy at POD 38. (**A**, **C**, **D**) are the histology of glisson's sheath (zone 1), and (**B**) is the histology of zone 3. The acute cellular rejection clearly improved, but centrilobular hemorrhagic hepatocellular necrosis in zone 3 and cholestasis were worsened with interlobular bile duct injury. Increment of bile ductule were not observed then either. On the other hand fibrosis and disappearance of artery in zone 1 were worsened. So we diagnosed probable acute onset chronic rejection.

the solid organ transplantation setting, SOS cases after kidney and liver transplantations were first reported as complications of azathioprine hepatotoxicity in 1982 [5] and 1991 [20], respectively. Only a few articles have been published since because SOS after LT is relatively rare at approximately 2% of LT patients according to a previous study [11].

Several authors have reported postoperative hepatic venous outlet obstruction in LT associated with venous thrombosis, kinking, and so on, to be among the major causes of massive ascites. This complication frequently results from surgical procedures such as the piggyback technique or anastomosis using orifices mismatched in size. Disturbed graft venous drainage leads to portal hypertension, finally producing over-ultrafiltration of the peritoneum [21–25]. Our two patients were confirmed to have no outflow block by angiography. Also, thrombotic obstruction and expansion of hepatic veins were not found in liver biopsy specimens. Because severe graft disorders of these patients were mainly composed of hemorrhagic necrosis in zone 3 around central veins without central venous obstruction, we diagnosed these patients with VOD/SOS.

Histopathologically, the cardinal feature of SOS is the injury of sinusoidal lining cells resulting in the disruption of

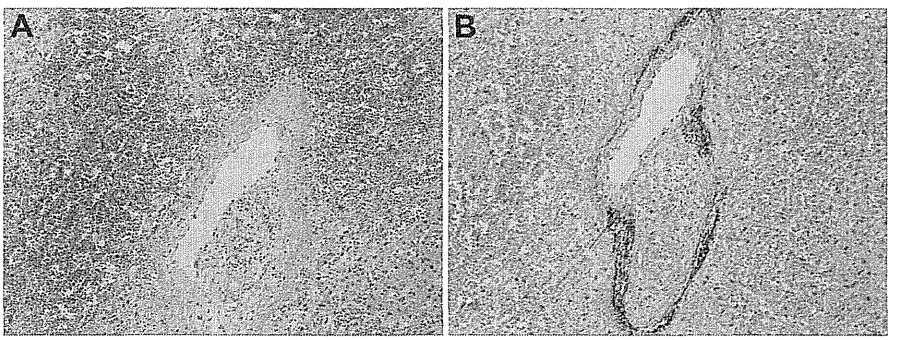


Fig 5. (**A**, **B**) Microscopic findings at autopsy of the liver on POD 46. She was diagnosed with VOD/SOS according to the pathology results. There were severe centrilobular hemorrhagic hepatocellular necrosis (zone 3) and severe cholestasis with interlobular bile duct damage, and severe portal vein and central vein venulitis without venous out flow block. ACR was unclear at the time of the autopsy.

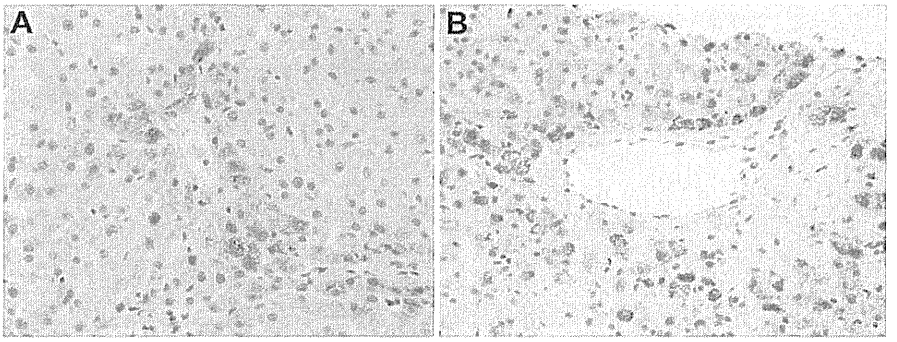


Fig 6. Representative imative imunohistochemical staining for CD42b (Gp I bα) in biopsy tissue sections of graft liver. (**A**) The biopsy specimen just after the reperfusion. Aggregation of activated platelets in the graft liver was observed in the space of Disse and parenchyma around central vein (zone 3) and platelet phagocytosis was observed in Kupffer cells and hepatocyte around zone 3. (**B**) Progression of activated platelets aggregation and platelets phagocytosis with centrilobular hemorrhagic necrosis and destruction of lobular structure around zone 3 were observed in the subsequent liver biopsy specimens at POD 13. Aggregation of activated platelets and platelet phagocytosis around zone 3 should be regarded as an important index of early graft failure by VOD.

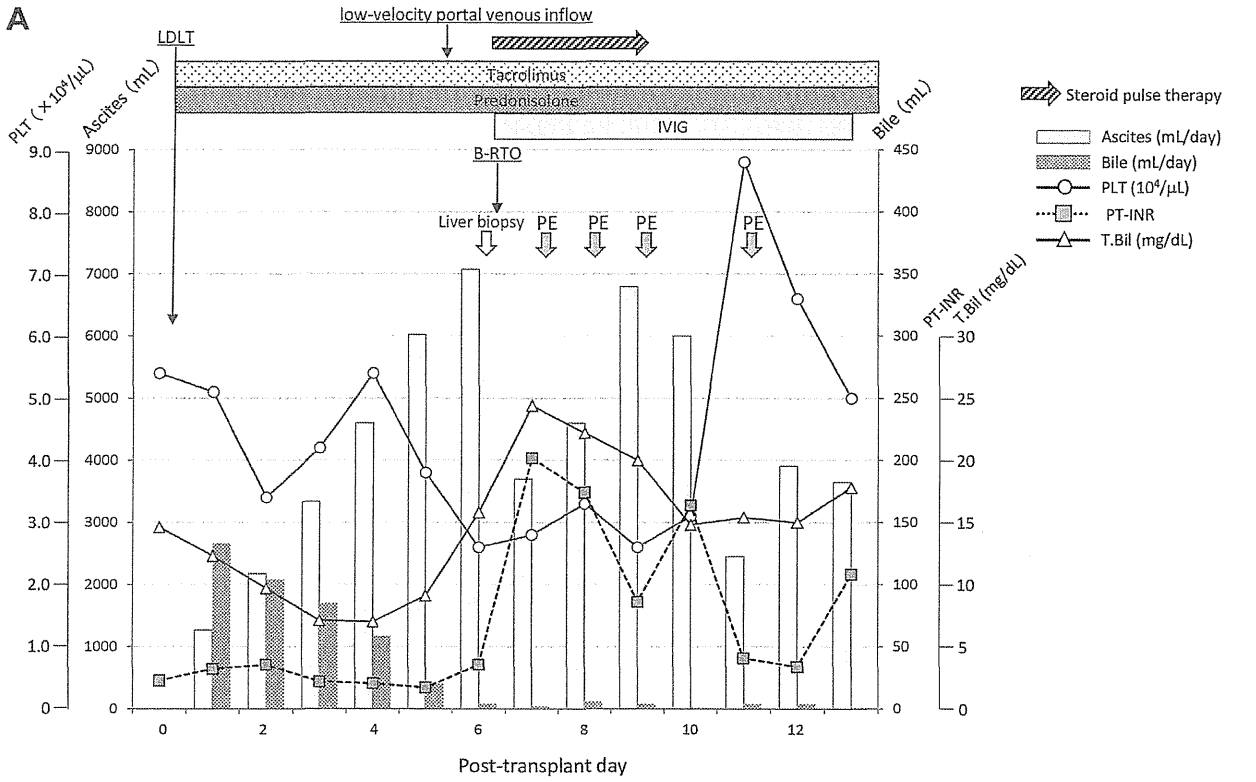
the liver circulation. The involvement of the hepatic venules is not an essential prerequisite [26,27].

However, extravasated platelet activation and aggregation would be extremely important to make up VOD/SOS as we showed by immunostaining of the liver biopsy specimens after transplantation of as well as extravasation of red blood cells to Disse's space. The platelet activation and aggregation would be caused by sinusoidal endothelial injury around central veins, and abrasion of sinusoidal endothelial cells thereby would occur. As a result, extravasated platelet aggregation in Disse's space would occur, and hepatocellular injury would aggravate, and platelet phagocytosis of hepatic cells, Kuppfer cells, and endothelial cells such as xenotransplantation [28–31] would come to be found and ultimately VOD/SOS would fall into irreversible extensive hemorrhagic graft necrosis.

It is currently recognized that the incidence of VOD/SOS after LT is frequently associated with allograft rejection [12–17]. However, not all patients with ACR and AMR after LT develop VOD/SOS, and there has been little study of the specific rejection mechanisms or factors which contribute to VOD/SOS. Accordingly, the exact cause of SOS is still obscure, due in part to various predisposing factors. We have presented herein two patients who had completely different backgrounds and clinical courses of ACR.

In case 1, the first ACR occurred in the early post-transplantation period (POD 13). She was experiencing transfusion-unresponsive thrombocytopenia and high titer anti-HLA antibodies (donor non-specific) were detected. Her HLA typing was fully mismatched with that of the donor for A, B, and DRB1. In human liver grafts, HLA compatibility is less important than with other organ transplantations in terms of rejection. Indeed, rejections in this case were neither frequent nor severe despite full-mismatch compatibility with the donor. However, major histocompatibility complex (MHC) antigens are induced on vascular endothelial cells of portal vessels and sinusoids during rejection. In particular, HLA class I antigens are major transplantation antigens, functioning as binding sites for cytotoxic T cells, because most constitutive class II–positive cells in the graft, ie, Kupffer and dendritic cells, have a limited life span and will be replaced by recipient type cells [32,33]. Furthermore, certain monoclonal antibodies are known to be reactive only with sinusoidal lining cells, ie, they do not react with endothelial cells of portal and central veins, or with arterioles [34]. Thus, endothelial cells from different anatomical compartments of the liver have different immunologic functions. Hepatic immunoreactivity is thus quite intricate, reflecting the diverse types of hepatic functional cells.

In case 2, there was no evidence of ACR during the progression of significant ascites and a severe decrease of portal venous flow without hepatic venous outflow block. The direct cross-match test for the donor B lymphocyte was positive but for T lymphocyte is negative before LDLT, and anti-donor specific antibody was not confirmed postoperatively. The impact of a lymphocytotoxic cross-match–positive liver graft on allogeneic rejection and graft survival remains controversial, both in DDLT [35,36] and in LDLT [37–39]. Some institutions have reported significantly unfavorable outcomes in LDLT recipients with a positive lymphocytotoxic cross match [38,39]. In contrast, another institution reported that lymphocytotoxic cross match test did not adversely affect the graft survival in patients without desensitization [37]. If the recipient is in T lymphocyte cold positive for donor by lymphocyte cytotoxicity test (LCT), renal transplantation would be contraindicated. However, B lymphocyte warm positive would not adversely affect the graft survival in patients without desensitization [40]. If T lymphocyte cold negative and B lymphocyte both cold and warm positive, it is necessary to perform cross-match test by flow cytometry which is more highly sensitive for detecting anti-donor specific antibody than LCT. The situation of T lymphocyte cold negative and B lymphocyte both cold and warm positive is considered that recipient would be in false-positive state or recipient would have anti-donor specific HLA class II antibody or recipient would have a


A
low-velocity portal venous inflow
LDLT
Tacrolimus
Predonisolone
IVIG
B-RTO
Liver biopsy
PE
PE
PE
PE
Steroid pulse therapy
Ascites (mL/day)
Bile (mL/day)
PLT (10⁴/μL)
PT-INR
T.Bil (mg/dL)
PLT (×10⁴/μL)
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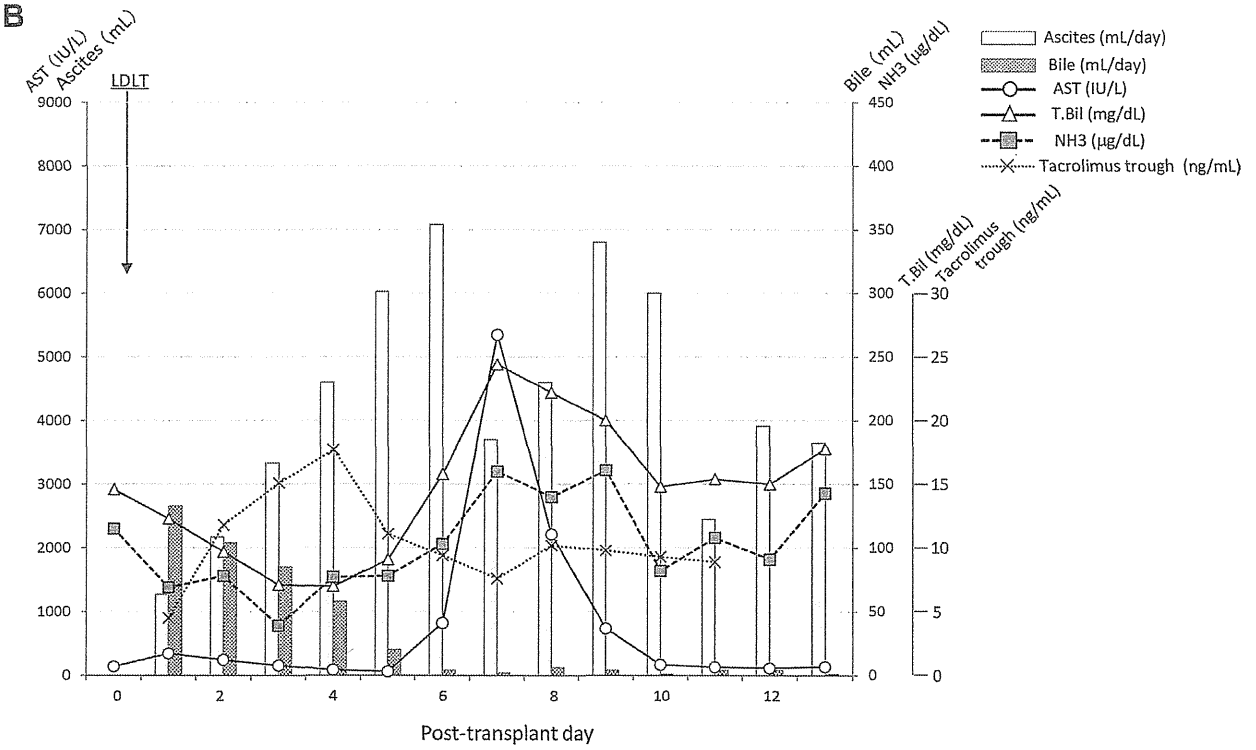
T.Bil (mg/dL)
Post-transplant day


B
LDLT
Ascites (mL/day)
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T.Bil (mg/dL)
NH3 (μg/dL)
Tacrolimus trough (ng/mL)
AST (IU/L)
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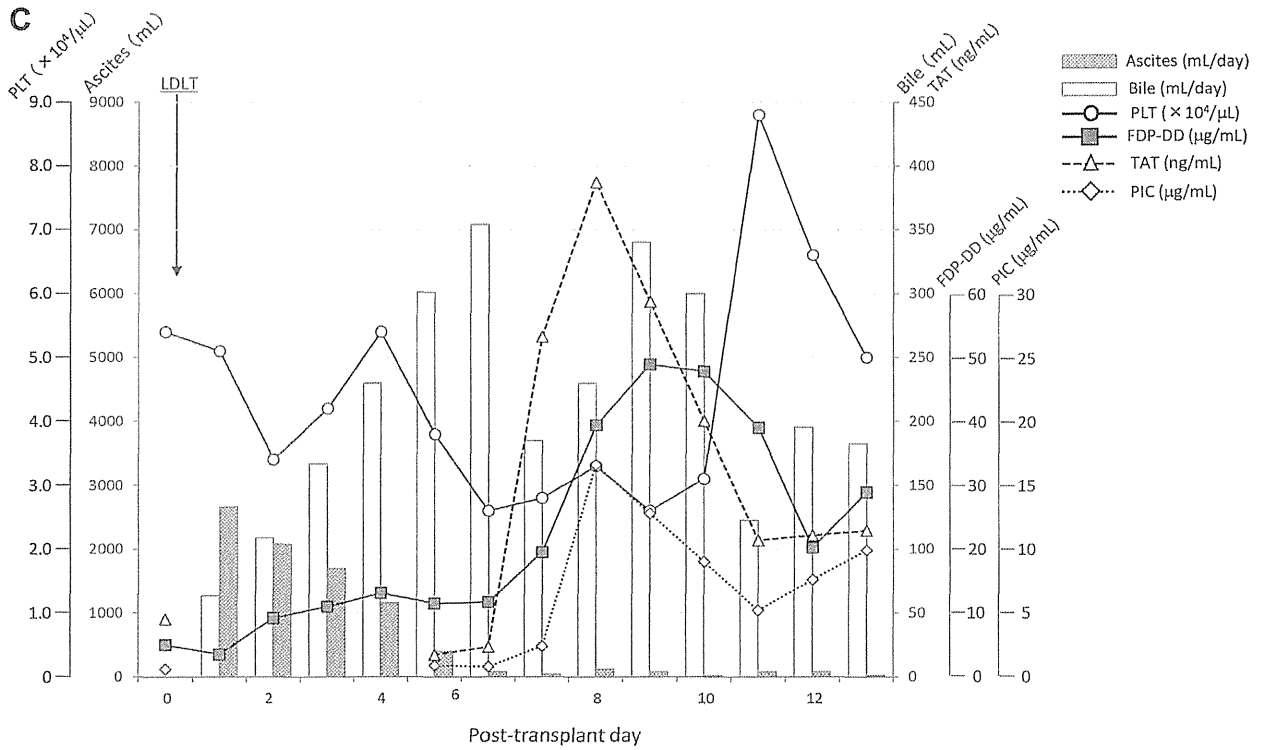

Fig 7. (*continued*).

little anti-donor specific HLA class I or anti-MHC class I chain-related gene A (anti-MICA) antibody.

Because of high risk for AMR after transplantation in the case of T lymphocyte direct cross-match strong positive, such recipient and donor pair should be thought to evade LDLT if possible. When it is unavoidable, preoperative desensitization therapy to prevent AMR such as ABO-incompatible LT would be needed. The patient in case 2 could not be evaluated for anti-donor specific alloantibody and anti-MICA antibody because preoperative serum did not remain. But B lymphocyte cold and warm were strong positive, and so we could not deny the potential severe AMR in spite of immunostaining for C4d was negative and the typical findings of AMR was not found in liver biopsy specimens after LDLT.

In consideration of the case involving an elderly donor, we speculate that the recipient rapidly developed progressive severe VOD/SOS because of ischemic and reperfusion injury overlap with AMR. It has been reported that, VOD/SOS group had a much older donor age when compared with the non-VOD/SOS group, but there was no difference in warm or cold ischemic times between the VOD/SOS group and non-VOD/SOS group [12]. The sinusoidal endothelial cell (SEC) appears to be the principal target of cold preservation injury, at least during the early phase of reperfusion. SECs remain viable until oxygenated reperfusion, but the death and the denudation of sinusoids occur rapidly after reperfusion through apoptosis [41,42]. Oxidative stress, which is the increase in oxygen free radicals with reperfusion, plays an

Fig 7. (**A–C**) The clinical course of the patient after living donor liver transplantation (LDLT). These figures showed the administration of the immunosuppressants, the change of laboratory data, the amount of ascites exudate from intraabdominal drain and the amount of bile exudate from biliary drainage tube. He had transfusion unresponsive thrombocytopenia and prolonged hyper-bilirubinemia. We performed a liver biopsy on post-operative day (POD) 6 because of low-velocity portal venous inflow without venous out-flow block, increase of ascites and decrease of bile exudate. After low-velocity portal venous inflow, the levels of coagulation related markers such as fibrin/fibrinogen degradation products - D dimer (FDP-DD), thrombin - anti-thrombin complex (TAT), and plasmin - α2 plasminogen inhibitor complex (PIC) were improved. Despite steroid pulse therapy, intravenous immunoglobulin (IVIG) and balloon-occluded retrograde trans-venous obliteration (B-RTO), his clinical findings (graft failure with hepatomegaly as well as graft inflow abnormalities) had not improved. Hyper-bilirubinemia and encephalopathy were worsened, so the we initiated plasma exchange (PE) for the recipient with hydro dialysis (HD), but hepatic encephalopathy had worsened and he was dead on POD 13. (Abbreviations: PLT, platelet; PT-INR, prothrombin time – international normalized ratio; T.Bil, total bilirubin; AST, aspartate aminotransferase; NH_3, ammonia.)

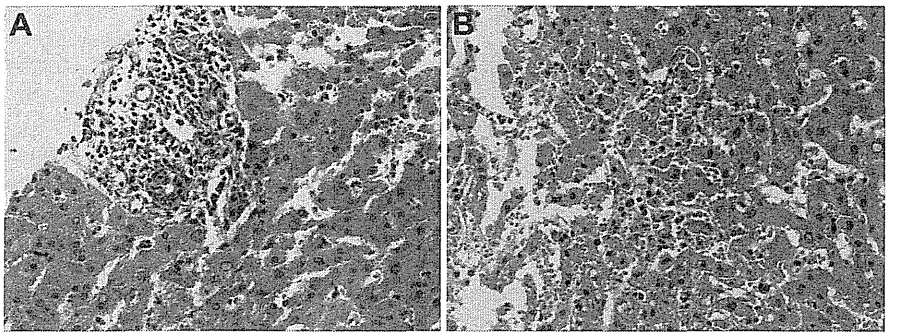

Fig 8. (A, B) Microscopic findings of hematoxylin eosin staining of the liver biopsy at POD 6. Severe centrilobular hemorrhagic hepatocellular necrosis with neutrophilic infiltration and dilatation of sinusoid, and canalicular cholestasis without acute cellular rejection were observed.

important role in this sinusoidal injury. An experimental study revealed that N-acetylcysteine prevented glutathione depletion and attenuated sinusoidal reperfusion injury. The fact that glutathione has been an important component of the preservation medium such as University-Wisconsin solution supports this result.

We administered FK506 and steroid (prednisolone) with or without MMF to the patients in our cases. A randomized study to investigate the difference in the histologic changes between cyclosporine A and FK506 with patients after orthotopic LT showed that prominent perivenular hepatocellular dropout, necrosis with sinusoidal dilatation, and red cells with activated platelets extravasation in Disse's spaces were seen in the FK506 group, even in the absence of cellular rejection, and this phenomenon targeting zone 3 has suggested the toxic effect of FK506 [43]. Recently, a lung transplantation case complicated with SOS arising from strongly suspected FK506 hepatotoxicity and a case of SOS which appeared to be due to FK506 toxicity caused by an overdose have been reported [44,45]. Experimental studies revealed that several drugs including azathioprine cause profound glutathione depletion in SECs before the onset of toxicity, leading to deterioration of drug toxicity [46]. It may be that toxic metabolites of FK506 encouraged sinusoidal injury through similar mechanism as others. In these cases, the actual cause of VOD/SOS is uncertain, although we strongly suspect that pre-existing damage in the elderly donors' livers were carried over to the recipients, and in addition ischemic and reperfusion injury of the allograft, and FK506 toxicity might have interacted to promote the development of critical SOS with ACR or AMR.

A number of challenges in the treatment of severe SOS have been made with the advance in our understanding of the pathophysiology of disease. Platelet activation and subsequent thrombosis could play a role in early genesis, and marked elevation of plasma plasminogen activator inhibitor (PAI-1) in hematopoietic stem cell transplantation (SCT) – associated SOS has been observed in several studies [47,48]. Prostaglandin E1 and tissue plasminogen activator (t-PA) with or without heparin and anti-thrombin have been therefore used as the predominant treatment agents. Although some improvement with t-PA have been reported [49,50], the use was limited because of such fatal complications as intracerebral or pulmonary hemorrhage arising from t-PA toxicity. Only defibrotide is recommended in the treatment of VOD/SOS in adults and children (1B) in the guideline of British Society for Blood and Marrow Transplantation [51]. But prostaglandin E1, pentoxifylline (non-selective phosphodiesterase inhibitor), heparin, and antithrombin were not recommended in the

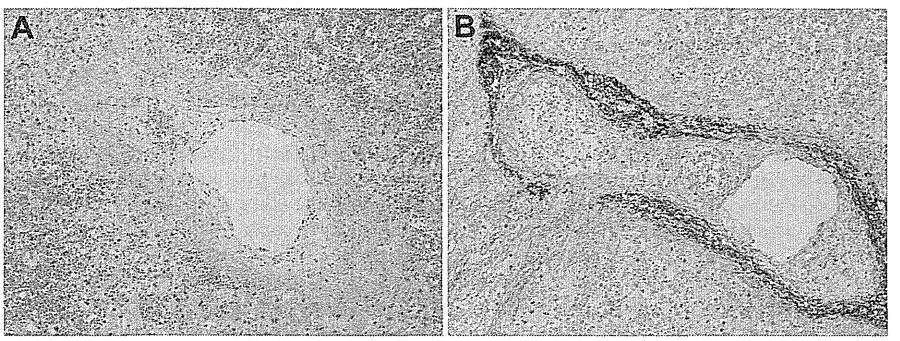

Fig 9. (A, B) Microscopic findings at autopsy of the liver on POD 13. VOD/SOS was diagnosed according to the pathology results. There were severe congestion and hemorrhagic hepatocellular necrosis, and severe cholestasis with injury of the interlobular bile duct without increment of bile ductule, and severe portal vein and central vein venulitis without venous out flow block. ACR was unclear.

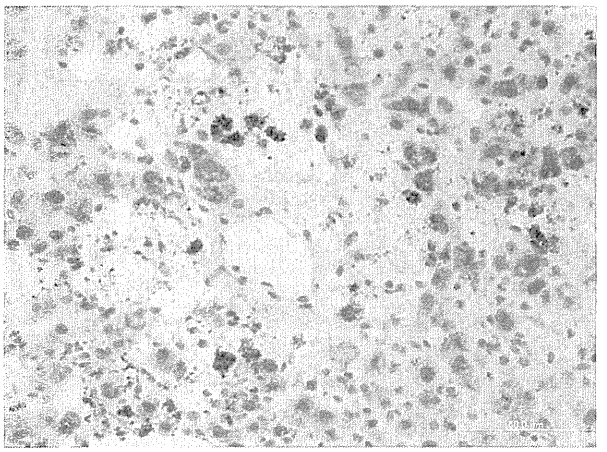

Fig 10. Representative immunohistochemical staining for CD42b (Gp I bα) in biopsy tissue sections of graft liver on POD 6. Progression of activated platelets aggregation and platelet phagocytosis with centrilobular hemorrhagic necrosis and destruction of lobular structure around zone 3 were observed. Aggregation of activated platelets and platelet phagocytosis around zone 3 should be regarded as an important index of early graft failure by VOD/SOS.

prophylaxis of VOD/SOS. Defibrotide is a single-stranded polydeoxyribonucleotide with antithrombotic, anti-ischemic, and thrombolytic effects, having originally been developed for the treatment of many vascular disorders. Defibrotide stimulates fibrinolysis through increasing endogenous t-PA activity and decreasing PAI-1 levels. The important characteristic of this agent is the absence of anticoagulant activity, per se, leading to a great advantage with the absence of serious complications such as fatal hemorrhage. The application of defibrotide for severe VOD/SOS after SCT during the past decade has achieved rather favorable results, and defibrotide was subsequently to be used in the management of VOD/SOS after solid organ transplantation. In two case reports (two patients in each) of VOD/SOS after LT treated with defibrotide, only one of four had a complete response in disappearance of ascites and decrease of bilirubin levels [52,53]. One explanation for this result could be that the timing of the diagnosis of VOD/SOS, and the interval between the onset of disease and administration of defibrotide might impact on the response to defibrotide. In this study, defibrotide was not attempted because of its unavailability for SOS after LT in Japan. The conclusive evaluation seems to be premature because of the paucity of clinical use of defibrotide in the liver transplantation setting. Although defibrotide is thus not yet available worldwide for VOD/SOS after LT, a further large, prospective study of defibrotide for VOD/SOS in LT patients will be warranted because of its promise as a therapeutic agent.

In the LT setting, the first transjugular intrahepatic portosystemic shunt (TIPS) was performed in a patient with massive ascites associated with persistent portal hypertension of unknown origin in 1998 [54]. Dramatic correction of liver dysfunction was achieved in some cases [55–57]. Some cases with not only clinical improvement but also histologic amelioration after TIPS have been reported [14,53,57]. However, little positive consideration has been given to date regarding the treatment with TIPS for severe VOD/SOS because it does not improve the outcome of disease. The TIPS should reduce fatal portal hypertension by VOD/SOS and, as bridging therapy to retransplantation, would be useful. However, it is difficult to determine the timing to perform TIPS for evading death in case 2 by VOD/SOS. Because VOD/SOS worsened rapidly in case 2, we were not able to perform TIPS. Also, we evaded invasive TIPS in case 1 because splenorenal shunt developed in addition to likelihood of the retransplantation having been extremely low.

Now we use type 3 phosphodiesterase inhibitors positively during the perioperative period of LT to ameliorate ischemic reperfusion injury of SECs in graft liver. It was identified that type 3 phosphodiesterase inhibitors would not only ameliorate ischemic reperfusion injury of SECs but also suppress PAI-1 in recipients [58–62]. Suppression of PAI-1 induces hepatocyte growth factor (HGF) activation and c-met phosphorylation, and this would lead to liver regeneration [63]. Because type 3 phosphodiesterase inhibitors do not have excessive anti-platelet aggregation, it is easy to use for recipients positively in the perioperative period of the LT. Specifically, we use type 3 phosphodiesterase inhibitors for perfusion of the graft liver and recipients were given positively from the intraoperative period. Thereby VOD/SOS has not been detected subsequently after perfusion of liver graft with type 3 phosphodiesterase inhibitor. Because the recipient should be fatal when VOD/SOS occurred just after LT, it is important to perform aggressive prevention of VOD/SOS by using type 3 phosphodiesterase inhibitors since defibrotide cannot be used.

In conclusion, we report two cases with rapidly progressive VOD/SOS after ABO identical LT resistant to all available medical therapies. The complication of VOD/SOS must always be kept in mind. While differentiating VOD/SOS on clinical backgrounds in each recipient, the diagnosis of VOD/SOS is required as early as possible, as the outcome of the disease can be considerably affected by the start of specific treatments.

In our two cases, there is difference between the DDLT and LDLT, and VOD/SOS became aggravated rapidly during the early postoperative period thus leaving no chance for retransplantation. The fundamental cause of VOD/SOS was unclear, but we speculated that organs from elderly donors may be involved in severe VOD/SOS. Severe ACR and perhaps atypical AMR would be associated with the onset of VOD/SOS in case1 because the recipient had high titer anti-HLA antibodies despite C4d negative staining. Also, AMR would be associated with the onset of VOD/SOS in case 2 because his direct cross-match test for the donor B lymphocyte was strongly positive. We speculate that the etiology of the post-LT VOD/SOS, which would be characterized with disorders of the SECs and hepatocytes with progressive venular occlusion and subsequent hemorrhagic hepatocellular necrosis, were endothelial cell

damage and dissection in sinusoid and activated platelet aggregation activation in the extravasated space of Disse by severe ischemic reperfusion injury, ACR or AMR. So we consider that sinusoidal endothelial cell protection for ischemic reperfusion injury and suppression of extravasated platelets aggregation and activation by anti-platelet drugs would be important to prevent fatal severe VOD/SOS as first step. Furthermore, it would be necessary to perform retransplantation before the patient becomes septic or experiences multiple organ failure if irreversible graft failure by VOD/SOS would be suspected.

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Original Article

Feasibility and efficacy of hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma after sorafenib

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Aim: Sorafenib is the standard treatment for advanced hepatocellular carcinoma (HCC). However, although there is no proven therapeutic procedure following the termination of sorafenib, hepatic arterial infusion chemotherapy (HAIC) may be a treatment option in advanced HCC. The aim of this study was to evaluate feasibility and efficacy of HAIC for patients with advanced HCC as subsequent therapy.

Methods: We retrospectively evaluated 27 consecutive patients with advanced HCC who were treated with HAIC following sorafenib between June 2009 and December 2012 at our hospital. Cisplatin (20 mg/m^2 per day) was administered via the hepatic artery for 10 min, prior to the continuous administration of 5-fluorouracil (330 mg/m^2 per day) over 24 h from days 1–5 and 8–12 and the s.c. administration of pegylated interferon α-2b (1 μg/kg) on days 1, 8, 15, and 22. A treatment cycle consisted of 28 days of drug administration followed by 14 days of rest.

Results: The toxicity profile showed that hematological toxicities were common, and grade 3/4 neutropenia and thrombocytopenia were observed (51.9% and 48.1%, respectively). Five patients (18.5%) experienced device-related complications. No unexpected adverse reactions and no treatment-related deaths were observed. Partial response was obtained in eight patients (29.6%), and stable disease was noted in nine patients (33.3%). Median progression-free survival and median survival time from initiation of HAIC were 4.0 and 7.6 months, respectively.

Conclusions: Because HAIC was well tolerated and exhibited moderate antitumor activity, it is a potentially useful treatment procedure in patients with advanced HCC even after failure of sorafenib.

Key words: hepatic arterial infusion chemotherapy, hepatocellular carcinoma, sorafenib

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the sixth most common cancer and the third leading cause of cancer-related mortality worldwide.[1] A variety of new techniques of imaging modalities have enabled the detection of HCC at an early stage,[2] and advances in various therapeutic procedures have improved its curability.[3,4] However, the number of patients with HCC who can be treated curatively is limited because of impaired hepatic function and frequent recurrence even after curative therapy. The prognosis of patients with advanced HCC where tumor has spread over the liver or invaded major vessels remains extremely poor.[5]

Sorafenib, an oral multikinase inhibitor that blocks tumor cell proliferation and angiogenesis, is the only systemic therapy that has shown survival benefit for patients with advanced HCC,[6,7] and it is recognized worldwide as standard first-line therapy in advanced HCC.[8,9] Alternative systemic chemotherapies using cytotoxic agents or novel targeted drugs have been attempted in patients with advanced HCC;[10,11] however, to date none have proven effective, except sorafenib. Moreover, following sorafenib therapy most patients are not suitable candidates for subsequent therapy because of the progressive nature of their disease, poor general condition, and impaired hepatic function.

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Compared with systemic chemotherapy, hepatic arterial infusion chemotherapy (HAIC) is based on theoretical advantages such as higher concentrations of drugs delivered directly to tumors[12] and first-pass effect reducing systemic toxicity.[13] Although few reports have recorded the survival benefits of HAIC, HAIC in combination with interferon (IFN) has been reported to be a useful treatment procedure in patients with advanced HCC.[14,15] Although an optimal protocol of HAIC has not been established, the clinical benefits of HAIC regimen consisting of 5-fluorouracil (5-FU) and cisplatin with IFN were reported in a randomized phase II study.[15] However, it remains unclear whether HAIC is also safe and effective in patients with advanced HCC who were previously administered sorafenib.

The aim of the present study was to evaluate the feasibility and efficacy of HAIC in patients with advanced HCC after failure of sorafenib therapy. This approach provides useful information in determining treatment strategies for sorafenib-refractory patients with HCC.

METHODS

Patients

ALL OF 68 consecutive patients with unresectable advanced HCC who had received sorafenib monotherapy at Kanazawa University Hospital and for whom this therapy was subsequently stopped because of tumor progression or/and unacceptable adverse effects between June 2009 and December 2012 were considered for enrollment. HCC was diagnosed by either histological confirmation or typical radiological findings, which showed hyperattenuation in the early phase and hypoattenuation in the late phase on dynamic computed tomography (CT).[16] All patients underwent dynamic CT to assess the extent of the cancer, and their hepatic and major organ functions were evaluated by physical examination and laboratory findings. We reviewed patients' medical records and investigated their backgrounds, treatment courses, and outcomes.

Sorafenib

The following were the inclusion criteria for sorafenib at our institution: patients with advanced HCC involving macroscopic vascular invasion, extrahepatic lesions and/or intrahepatic multiple lesions considered unsuitable for surgical resection, locoregional therapy or transarterial chemoembolization; all patients with an Eastern Cooperative Oncology Group performance status score of 2 or less and with appropriate function of major organs, such as bone marrow, kidney and heart; and patients categorized as Child–Pugh A in terms of hepatic function.

HAIC

The inclusion criteria for HAIC at our institution is nearly same as that of sorafenib. Patients with extrahepatic lesions were also considered eligible if these lesions were mild, and intrahepatic lesions were considered as prognostic factors. With regard to hepatic function, patients categorized as Child–Pugh A or B were eligible.

The reservoir system implantation technique was the same as described previously.[15] Catheters were introduced through the right femoral artery, and angiography from the celiac artery was initially performed to localize the HCC and evaluate intra- and extrahepatic vascularization. We then inserted a catheter with a side vent into the gastroduodenal artery, positioning the vent in the common hepatic artery using an image-guided procedure. The gastroduodenal artery, right gastric artery and other arteries presumed to supply the gastroduodenal region were embolized as far as possible to prevent gastrointestinal mucositis. The other end of the catheter was connected to an injection port that was subcutaneously implanted in the right lower abdomen. Finally, blood flow redistribution was confirmed.

Hepatic arterial infusion chemotherapy was initiated approximately 5 days after implantation of the reservoir, and the following protocol was then implemented: 5-FU (330 mg/m^2 per day) was continuously administrated via the hepatic artery using an infuser pump over 24 h from days 1–5 and 8–12, and cisplatin (20 mg/m^2 per day) was also administrated via the hepatic artery for 10 min prior to 5-FU administration. Pegylated IFN-α-2b (1.0 μg/kg) was s.c. administrated on days 1, 8, 15, and 22. A treatment cycle consisted of 28 days of drug administration followed by 14 days of rest. The treatment protocol was approved by the Ethics Committee of Kanazawa University, and informed consent for participation in the study was obtained from each subject. The study conformed to the guidelines of the 1975 Declaration of Helsinki.

Evaluation

Tumor staging was assessed according to the criteria of the Liver Cancer Study Group of Japan.[17,18] The efficacies of HAIC and sorafenib were assessed every 4–6 weeks by dynamic CT, and response to chemotherapy was assessed according to the Response Evaluation Criteria in Solid Tumors ver. 1.1.[19] Response rate was defined as

the sum of complete and partial response rates. Similar to an approach adopted in a recent report, the causes of progression after sorafenib therapy (progression pattern) were classified as follows: intrahepatic growth, extrahepatic growth, new intrahepatic lesion or new extrahepatic lesion and/or vascular invasion.[20] Adverse effects, including both hematological and non-hematological toxicities, were assessed by the Common Terminology Criteria for Adverse Events version 4.0.

Statistical analysis

Progression-free survival (PFS) was calculated from the first day of HAIC until either the date of radiological progression, the date of death or the last day of the follow-up period. Overall survival (OS) was calculated from the first day of HAIC until either the date of death or the last day of the follow-up period. A χ^2-test was used to analyze the predictive factor for the response to HAIC. To compare prognosis according to response to chemotherapy and the progression pattern, cumulative survival was calculated using the Kaplan–Meier method[21] and any differences were evaluated using the log-rank test. $P<0.05$ were considered to be statistically significant, and all tests were two-sided. All statistical analyses were performed using the SPSS statistical software program package (version 11.0 for Windows; SPSS, Chicago, IL, USA).

RESULTS

Patients

OF 68 PATIENTS, 41 were not treated with HAIC because of either poor general condition ($n=12$), massive extrahepatic lesions ($n=9$), inadequate major organ function ($n=8$), treatment with HAIC prior to sorafenib therapy ($n=7$) or refusal to be treated with HAIC ($n=5$). Finally, 27 patients who had been treated with HAIC were analyzed in this study, all of whom had previously received sorafenib monotherapy. The response and tumor control rates for sorafenib therapy were 7.4% and 44.4%, respectively. In 22 patients (81.5%), sorafenib therapy was terminated because of tumor progression and in five (18.5%) because of unacceptable adverse effects. The median period of sorafenib therapy was 2.4 months (range, 0.1–18.0).

Patient characteristics at commencement of treatment with HAIC are summarized in Table 1. Because hepatic function was impaired in more than half of the patients in this study, 18 patients (66.7%) were classified as Child–Pugh class B or C. Macroscopic vascular invasion and extrahepatic metastasis were observed in 25.9% and 44.4% of the patients, respectively.

Table 1 Patient characteristics

	($n=27$)
Age, years	
Median, range	68, 44–84
Sex, *n* (%)	
Male	23 (85.2)
ECOG PS†, *n* (%)	
0	24 (88.9)
1	3 (11.1)
HBs antigen‡, *n* (%)	
Positive	9 (33.3)
HCV antibody§, *n* (%)	
Positive	15 (55.6)
Child-Pugh class at start of HAIC, *n* (%)	
A	9 (33.3)
B	16 (59.3)
C‡‡	2 (7.4)
Child-Pugh class at start of sorafenib, *n* (%)	
A	21 (77.8)
B§§	6 (22.2)
Ascites, *n* (%)	
Presence	18 (66.7)
Albumin, g/dL	
Median, range	3.2, 2.1–3.9
Prothrombin consumption test, %	
Median, range	82, 37–112
LCSGJ¶ tumor stage, *n* (%)	
II, III	12 (44.4)
IVA	4 (14.8)
IVB	11 (40.7)
Macroscopic vascular invasion, *n* (%)	
Yes	7 (25.9)
Extrahepatic spread, *n* (%)	
Yes	12 (44.4)
AFP††, ng/mL	
Median, range	404, <10–175560

†ECOG PS: Eastern Cooperative Oncology Group performance status.
‡HBs antigen: hepatitis B surface antigen.
§HCV antibody: hepatitis C virus antibody.
¶LCSGJ: Liver Cancer Study Group of Japan.
††AFP: α-fetoprotein.
‡‡Child-Pugh class B at decision making of HAIC.
§§Child-Pugh class A at decision making of sorafenib.

Treatment

A total of 60 courses were administered to 27 patients, with a median number of 2 (range, 0–5). All but two patients completed at least one course of HAIC. The

median duration between cessation of sorafenib therapy and commencement of HAIC was 1.2 months (range, 0–9.0). The median observation period from commencement of HAIC was 7.0 months (range, 0.8–48.0). Treatment with HAIC was terminated in 25 patients due to radiological tumor progression (20 patients), symptomatic tumor progression (one patient) or change in the treatment procedure (four patients); however, there were no patients in whom HAIC was terminated because of adverse effects. HAIC was continued in the remaining two patients until the last day of the follow-up period.

Safety

All 27 patients were assessed for adverse effects, and the toxicity profile of HAIC is summarized in Table 2. Hematological toxicities were common, particularly grade 3/4 neutropenia and grade 3/4 thrombocytopenia, which were observed in 14 (51.9%) and 12 (48.1%) patients, respectively, even though no serious complication such as sepsis or bleeding were observed and all toxicities were tolerable and reversible. Mild and low-frequency nonhematological toxicities were observed, except in one patient who had grade 3 diarrhea. Although 5 patients (18.5%) had device-related complications (3 catheter obstruction, 1 hepatic artery occlusion, and 1 hepatic arteritis), all issues were satisfactorily resolved by either exchanging the reservoir or conservative therapy. No unexpected adverse reactions were noted, and no treatment-related deaths were observed.

Table 2 Hepatic arterial infusion chemotherapy toxicities

	All grade *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)
Hematological toxicities			
Leukocytopenia	20 (74.1)	10 (37.0)	0 (0)
Neutropenia	21 (77.8)	10 (37.0)	4 (14.8)
Anemia	12 (44.4)	1 (3.7)	1 (3.7)
Thrombocytopenia	22 (88.9)	13 (48.1)	0 (0)
Nonhematological toxicities			
Anorexia	7 (25.9)	0 (0)	0 (0)
Fever	5 (18.5)	0 (0)	0 (0)
Diarrhea	4 (14.8)	1 (3.7)	0 (0)
Fatigue	4 (14.8)	0 (0)	0 (0)
Hiccoughs	3 (11.1)	0 (0)	0 (0)
Gastric ulcer	3 (11.1)	0 (0)	0 (0)
Creatinine increased	2 (7.4)	0 (0)	0 (0)
Mucositis oral	2 (7.4)	0 (0)	0 (0)
Nausea	1 (3.7)	0 (0)	0 (0)
Ascites	1 (3.7)	0 (0)	0 (0)
Edema	1 (3.7)	0 (0)	0 (0)
Abdominal pain	1 (3.7)	0 (0)	0 (0)
Hypokalemia	1 (3.7)	0 (0)	0 (0)
Encephalopathy	1 (3.7)	0 (0)	0 (0)
Device-related complications			
Catheter obstruction	3 (11.1)	0 (0)	0 (0)
Hepatic artery occlusion	1 (3.7)	0 (0)	0 (0)
Vasculitis	1 (3.7)	0 (0)	0 (0)

Response to treatment and patient outcomes

Of the 27 patients, one died due to tumor progression and hepatic failure before radiological assessment could be performed; however, the remaining 26 were assessable for response to treatment. Tumor responses to HAIC are shown in Table 3. Although no patient achieved complete response, eight patients (29.6%) achieved partial response (PR) and nine (33.3%) achieved stable disease (SD); therefore, the response rate to HAIC was 29.6%. These results were independent of the Child–Pugh class, the response to previous sorafenib therapy and the progression pattern (Table 3), and none of the tested factors were found to be a significant predictive factor for response to HAIC (Table S1).

The median PFS of patients from commencement of HAIC was 4.0 months (Fig. 1). The median survival time (MST) of all patients was 7.6 months, with a 1-, 2-, and 3-year survival rate of 29.4%, 24.5% and 16.4%, respectively (Fig. 2a). The MST of patients who achieved PR were 36.7 months, which was significantly better than that of patients who achieved SD/progressive disease/not evaluable, namely, 6.6 months ($P < 0.01$; Fig. 2b). Patient prognosis did not differ according to the progression pattern (Fig.S1).

DISCUSSION

THE DEVELOPMENT OF a safe and effective alternative therapy is essential because sorafenib, which represented a breakthrough in the treatment of advanced HCC, had a low response rate and frequent adverse effects, often leading to a cessation of treatment.[22,23] An increasing number of emerging agents, including novel molecular targeted drugs, have been attempted in sorafenib refractory HCC. Nevertheless, their efficacy was found to be limited (response rate, 0–4.3%; time to progression, 1.6–2.7 months).[24–26]

The first aim of this study was to investigate the feasibility of HAIC in advanced HCC after the failure of sorafenib therapy. In this study, the frequency of