

# Intrahepatic Hepatic Vein Stenosis After Living-Related Liver Transplantation Treated by Insertion of an Expandable Metallic Stent

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Although the incidence of stenosis and obstruction of the hepatic venous anastomosis after right hepatic living-related liver transplantation (LRLT) has been found to be higher than after orthotopic liver transplantation (OLT), to the best of our knowledge, intrahepatic stenosis of the venous trunk in the early period after right hepatic LRLT has never been reported in the literature. A 53-year-old man who underwent right hepatic LRLT, postoperatively, developed liver dysfunction and an increasing amount of ascites, and a Doppler sonogram showed a flat waveform and low-flow velocity in the hepatic vein. Based on these findings an outflow block was suspected, and a hepatic venogram and manometry revealed intrahepatic stenosis of a tortuous hepatic venous trunk and a pressure gradient of 14 mmHg at the site of the stenosis. We inserted an expandable metallic stent (EMS) at the site of intrahepatic venous stenosis, and its insertion was followed by a decrease in pressure gradient. Liver function recovered, and the volume of ascitic fluid decreased after placement of the EMS. The results of an analysis of the venogram and CT volumetric data suggested that the pathogenesis of the stenosis was twisting of the venous trunk during hypertrophy of the liver parenchyma.

**Key words:** EMS, hepatic vein stenosis, liver regeneration, LRLT

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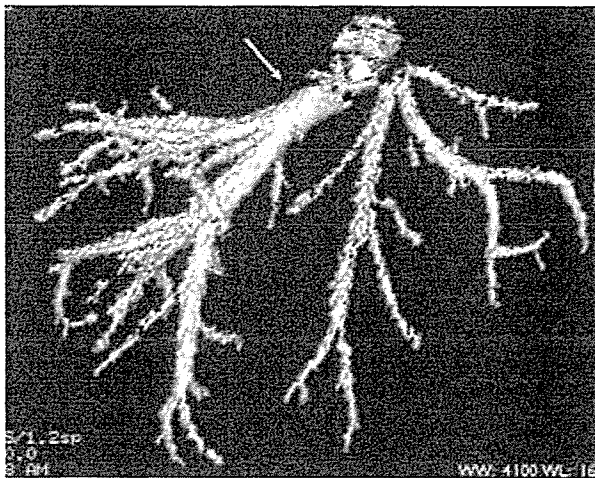
## Introduction

Right hepatic living-related liver transplantation (LRLT) is now commonly used to treat adults, and although steno-

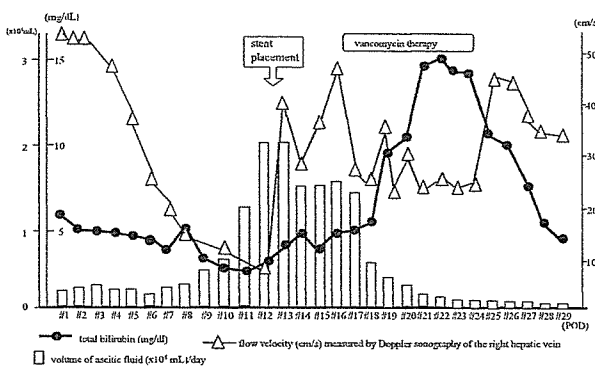
sis of the right hepatic vein anastomosis has been found to occur as a postoperative complication, to our knowledge early intrahepatic hepatic venous stenosis not at the site of the anastomosis has never been reported. We report such a case and describe the radiological findings obtained by hepatic venography and the hepatic vein manometric data in a patient treated with an expandable metallic stent (EMS). We discuss the possible pathogenetic mechanism of the intrahepatic stenosis of the hepatic vein that developed during the period of liver regeneration after right hepatic LRLT.

## Case Report

A 53-year-old man was referred to our department for LRLT. At 39 years of age he had been diagnosed with liver dysfunction secondary to hepatitis B virus (HBV) infection. Liver cirrhosis and hepatocellular carcinoma (HCC) subsequently developed, and he was treated by trans-arterial embolization at 48 years of age. The HCC recurred at 52 years of age, and he developed an umbilical hernia caused by a huge volume of ascitic fluid. Living-related liver transplantation of an ABO-compatible right hepatic lobe donated by his wife was performed. Preoperative three-dimensional dynamic enhanced computed tomography of the donor revealed that drainage veins from the middle hepatic vein had not developed in Couinaud segments V and VIII, that the main drainage route of venous return from right lobe of the liver was the right hepatic vein, and that there was no stenosis (Figure 1). The graft-to-recipient weight ratio was 0.89%. The main hepatic vein of the donor was anastomosed to the right hepatic vein of the recipient without reconstruction of the middle hepatic vein. The length of the orifice and the cuff of the right hepatic vein of the graft were 27 mm and 14 mm, respectively, and the length of the remnant right hepatic vein of the recipient was 8 mm. The detailed surgical procedure for hepatic vein reconstruction was as follows. The vascular clamp holding the stump of the right hepatic vein was positioned vertically on the inferior vena cava (IVC). The IVC was incised at the inferior end of the right hepatic vein orifice to adjust it to the length of the orifice of the hepatic vein of the graft. A portion of the anterior wall of the IVC was removed to make the orifice oval-shaped. End stitches of 5–0 polypropylene monofilament suture were placed on the superior and inferior ends. The superior stitch of



**Figure 1:** Three-dimensional dynamic-enhanced computed tomography of the donor's liver showing that the main drainage route of venous return of the right lobe was the right hepatic vein (white arrow), and absence of stenosis.



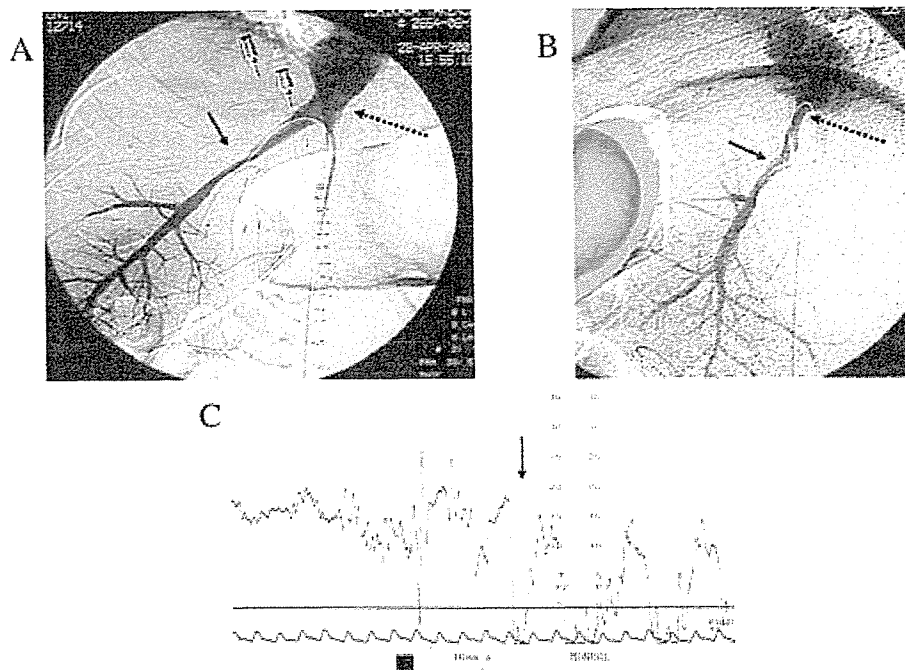
**Figure 2:** Sequential changes in the serum total bilirubin level (●), volume of ascitic fluid (□), and flow velocity (△) measured by Doppler sonography of the right hepatic vein after the operation. Flow velocity increased greatly after placement of an expandable metallic stent, and the volume of ascitic fluid decreased. The serum total bilirubin level increased to 15 mg/dL because of sepsis, but decreased in response to Vancomycin therapy.

5–0 polypropylene monofilament suture was knotted, and one arm of the knotted stitch was passed through the posterior wall of the graft vein from the outside in, close to the first knot, and continued intraluminally to the inferior end. The anterior wall closure was completed in simple running suture fashion with the same stitch. There were no areas of congestion in the graft. Cold ischemic time was 47 min, and warm ischemic time was 59 min. Intraoperative Doppler ultrasound examinations showed no evidence of either stenosis or obstruction of the hepatic venous, portal venous, or hepatic arterial blood flow after reperfusion. Portal venous pressure was monitored with

a catheter inserted into the superior mesenteric vein via a branch of the ileocolic vein. Before hepatectomy, it was 33 mmHg, but it decreased to 20 mmHg after transplantation, and ultimately to 9 mmHg on postoperative day (POD) 7. HbIg and lamivudine were given postoperatively, and immunosuppressive treatment with tacrolimus was started immediately after transplantation. On POD 10 the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-bil) values had decreased to 56 international units (IU)/mL, 41 IU/mL, and 2.8 mg/dL, respectively (Figure 2), and by POD 12 they had increased to 103 IU/mL, 81 IU/mL, and 3.6 mg/dL, respectively. The daily volume of ascitic fluid increased from less than 5000 mL to more than 10 000 mL on POD 11 (Figure 2). Ascitic fluid cultures were negative for mycobacteria and fungi, and no cytomegalovirus–antigen was detected on granulocytes in the ascitic fluid. A Doppler ultrasound examination on POD 12 revealed flat waveforms and low-flow velocity of 9.6 cm/s in the right hepatic vein (Figure 2), and a low-flow velocity of 11.7 cm/s in the right main portal vein. A hepatic venogram was performed on POD 13 because of clinical suspicion that hepatic venous obstruction had developed. The venogram showed stenosis of an intrahepatic vein, not of the anastomosis (Figure 3A), and examination of both the anterior-posterior view and lateral view (Figure 3B) hepatic venograms revealed that the stenotic intrahepatic vein was tortuous. Manometric venous pressure data obtained by withdrawing the catheter from the intrahepatic vein into the inferior vena cava showed a gradient of 14 mmHg across the intrahepatic stenosis (Figure 3C). The wedge pressure of the distal hepatic vein branch was 33 mmHg. We immediately inserted an EMS (diameter: 1.4 cm, length: 5 cm; Wallsten™, Boston Scientific, Nagoya, Japan K. K) into the right hepatic vein via the right internal jugular vein (Figure 4A), and the venous pressure gradient was found to have decreased to 4 mmHg (Figure 4B). A Doppler ultrasound examination after EMS placement showed a pulsatile waveform in the right hepatic vein and an increase in flow velocity at the same sites in the intrahepatic vein and the right main portal vein of 30.9 cm/s and 23.9 cm/s, respectively. The AST and ALT values had decreased to 31 IU/L and 30 IU/L, respectively, on POD 14. The daily volume of ascitic fluid decreased to less than 5000 mL from POD 18 onward, and continued decreasing to less than 500 mL from POD 25 onward. The patient developed to severe sepsis caused by coagulase-negative *Staphylococcus aureus* associated with increasing AST- and T-bil-values and decreasing flow velocity from POD 16 to POD 24, but treatment with Vancomycin was followed by recovery of their values. The patient was discharged on POD 55, and he has been working and enjoying a good quality of life for the past 7 months.

**Discussion**

The use of right hepatic lobe grafts is a major development in LRLT and has significantly increased the graft



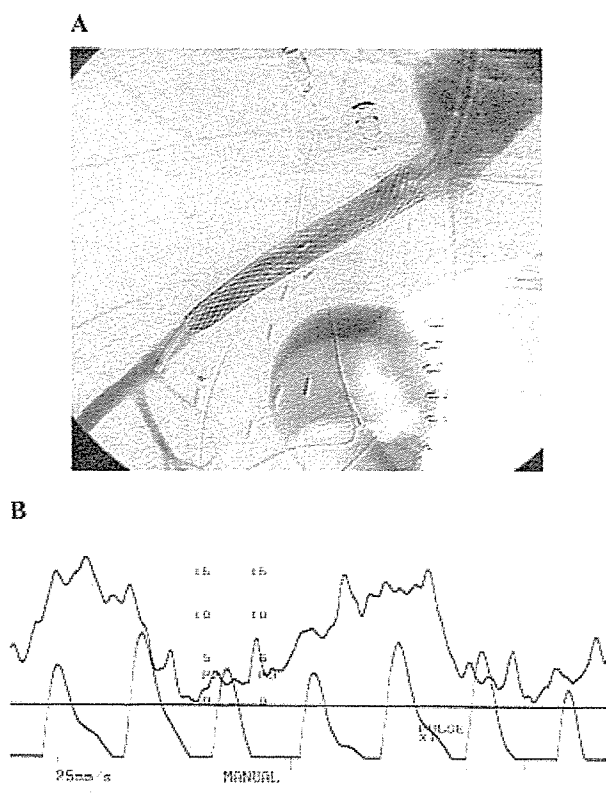
**Figure 3:** (A) Hepatic venogram (anterior-posterior view) and (B) hepatic venogram (lateral view). A tortuous stenotic intrahepatic vein (→) is seen, and the hepatic venous anastomosis is intact (dotted arrow). (C) Manometric hepatic venous pressure data. The hepatic venous pressure gradient was measured at the point indicated by the arrow.

supply. Right hepatic LRLT is a relatively new and technically challenging method. The hepatic venous anastomosis in a right-lobe graft is unique in terms of the variety of patterns of venous drainage, i.e. in some cases, such as our own, the right hepatic vein alone drains the entire graft, whereas in other cases the major anterior segment (Couinaud segments V and/or VIII) veins drain into a middle hepatic vein, or inferior hepatic veins may provide the main venous drainage of the posterior segment. While hepatic venous complications of orthotopic liver transplantations are rare, the anatomical variations may lead to hepatic venous congestion in the absence of reconstruction of drainage branches in LRLT, or to outflow obstruction owing to an insufficient anastomotic orifice. Egawa et al. reported two cases of early onset (1 week postoperatively) and six cases of late onset (2 months or more postoperatively) hepatic vein occlusion among 152 LRLTs (45 left lobe grafts, 106 lateral segment grafts, and one right lobe graft) (1). Inomata et al. reported outflow block in one case with multiple, separate hepatic vein anastomoses among 26 right hepatic LRLTs, and they suspected that a shift in the position of the graft may have led to the obstruction of the multiple hepatic venous anastomoses (2). Other surgeons and radiologists have reported incidences of outflow block after right hepatic LRLT of 0% [0/20 (3), 0/30 (4), 0/46 (5)] and 4% (1/256). Ko et al. treated 27 cases of hepatic venous outflow obstruction after LRLT by balloon angioplasty and insertion of an EMS, and analyzed the etiologies of the obstruction (7). They concluded that the causes were fibrosis or intimal hyperplasia in the perianastomotic area, kinking or buckling of a redundant hepatic vein, external compression by a hypertrophied liver graft, a tight suture

**Table 1:** CT volumetric data of the recipient

	Preoperative	Postoperative day			
		7	14	28	70
Graft volume (cm <sup>3</sup> )	860	1700	2157	2051	1636

line, and iatrogenic obstruction after stent placement in the IVC (7). In our patient, the site of stenosis was in an intrahepatic vein far from the site of the anastomosis, which provided a sufficient orifice according to the venogram. The CT volumetric data of the right hepatic lobe graft showed that graft volume had increased approximately twofold on POD 7, and approximately 2.5-fold on POD 14 (Table 1). The increase in liver volume until POD 14 may have been secondary to venous congestion of the graft. The daily volume of ascitic fluid after the operation was ranged from approximately 1000–3000 mL until POD11, however, those volumes did not indicate liver congestion because of hepatic vein stenosis caused by a technical error in the anastomosis, because daily ascitic fluid volumes of greater than 1000 mL until POD 14 have sometimes been observed in cirrhotic patients after LRLTs. As the hepatic venous obstruction appeared to have occurred around POD 13 based on the Doppler ultrasound findings, we concluded that the doubling of the preoperative graft volume on POD 7 was attributable to regeneration of liver parenchyma rather than congestion of hepatic venous return. We suspect that relatively rapid hypertrophy of the right lobe graft that was asymmetric may have led to intrahepatic twisting of the hepatic vein like a hollow tube, and resulted in outflow block. The CT volumetric data on POD 70 showed that the



**Figure 4: (A) Hepatic venogram after placement of the expandable metallic stent. (B) Manometric hepatic venous pressure data. No steep decline in pressure is seen.**

volume had decreased to approximately the same level as on POD 7, when there was no venous congestion. Inomata et al. reported that a relatively small lateral-segment or left-lobe graft in LRLTs may twist around the hepatic venous anastomosis (extrahepatic) during hepatic regeneration and cause outflow block (8). They inserted a tissue expander into the space adjacent to the graft in order to stabilize its position. Our case, in which the pathogenesis of the venous outflow blockage may have been intrahepatic twisting of the venous trunk during hypertrophy of the liver parenchyma, may be the first such case ever reported. Another possible etiology of the intrahepatic vein stenosis in our case is ischemic stricture of an intrahepatic vein, because 59 min of warm ischemic time was considered relatively long. The mean warm ischemic time in the

adult 31 cases in our LRLT series was 40.3 min (range: 21–73 min), but no intrahepatic hepatic vein stricture was found in a further seven cases in which the warm ischemic time was greater than 50 min. Interventional radiologic procedures like balloon angioplasty and EMS placement are useful treatment modalities for hepatic venous outflow obstruction after LRLT and split-liver transplantation (7,9). We inserted an EMS at a site of intrahepatic stenosis of the hepatic vein, so that the proximal portion of the EMS did not extend beyond the anastomosis. The EMS has been retained at the site and has been effective in combination with anticoagulant therapy with warfarin over the past 7 months of follow up to date.

In conclusion, we have reported a case of intrahepatic venous stenosis resembling outflow block after right hepatic LRLT in which we treated the stenosis by insertion of an EMS. We suspect that the pathogenesis of the stenosis was twisting of the venous trunk during hypertrophy of the liver parenchyma.

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## Serum vascular endothelial growth factor-receptor 1 during liver regeneration

### To the Editor:

We read with great interest the article 'Gene expression profile in the regenerating rat liver after partial hepatectomy' by Fukuhara et al. [1] in which their microarray data demonstrate the molecular events in regenerating rat liver. In this analysis, however, profiling of several potent angiogenic factors genes was not well documented. Recent studies suggest that angiogenic factors play an important role in inducing liver regeneration and molecular expression patterns for angiogenic factors has been studied in rat models [2–4].

Molecules important to angiogenesis include vascular endothelial growth factor (VEGF), vascular endothelial growth factor-receptor1 (VEGF-R1) and angiogenin (ANG). Selective activation of VEGF-R1 stimulates hepatocyte, but not endothelial, proliferation and reduces liver damage in mice exposed to a hepatotoxin [5]. Additionally, VEGF and ANG may be important stimulators of sinusoidal endothelial cell (SEC) proliferation during liver regeneration in vivo [5]. This is supported by observations in rodent models that serum VEGF levels peak at 72 h and ANG levels peak at 96 h after partial hepatectomy [2,6]. However, there is little data available regarding levels of angiogenic factors after partial hepatectomy in humans.

Therefore, we investigated serum levels of VEGF, VEGF-R1 and ANG in five donors of living liver transplantation during the first 7 days after partial hepatectomy (Fig. 1). All angiogenic factors were measured by ELISA (R&D Systems). Serum VEGF-R1 levels increased immediately, peaking at 2-fold baseline levels by 6 h after surgery and decreasing gradually. In contrast, VEGF levels rose slowly until the fifth post-operative day, and increased rapidly thereafter with a three-fold increase after the fifth post-operative day. ANG levels actually decreased below baseline levels just after surgery and increased after the third post-operative day. Thus, the expression patterns of VEGF, VEGF-R1 and ANG during liver regeneration of transplantation donors

were tightly regulated in sequence. Our findings suggest that VEGF-R1 expression is up-regulated just after liver resection, whereas VEGF and ANG expression is delayed

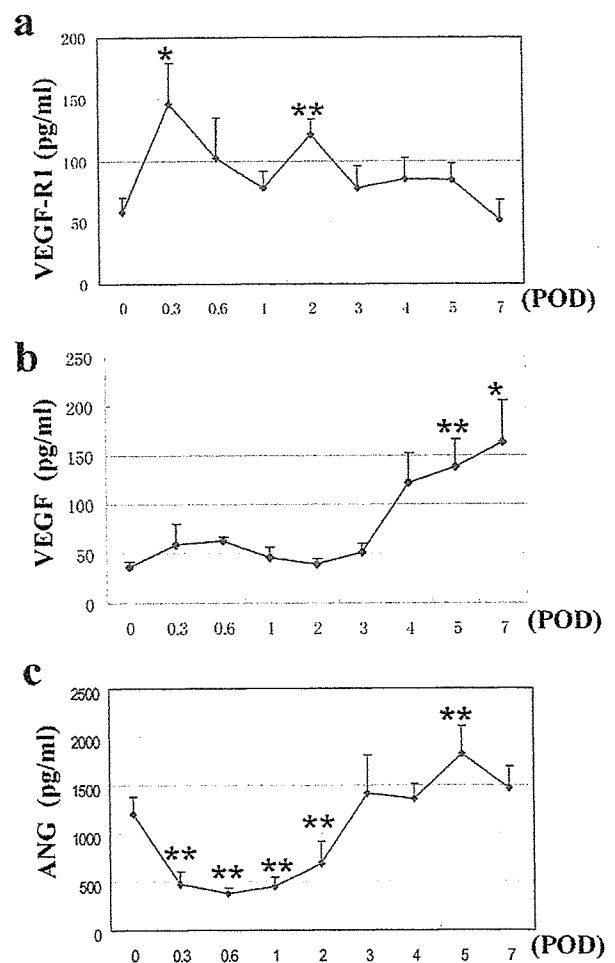


Fig. 1. (a) Serum VEGF-R1 levels (b) VEGF levels and (c) ANG levels before and after partial hepatectomy in donors. Bars indicate standard error (\*\* $P < 0.05$  vs. 0 h, \* $P < 0.1$  vs. 0 h).

by several days. Thus, in humans, VEGF-R1 may be one of the many genes up-regulated during rapid proliferation of hepatocytes after partial hepatectomy and may contribute to expression of angiogenic factors, consistent with prior studies [5]. Further evaluation of expression profiling of angiogenic factors will provide needed information about mechanisms important for liver regeneration, and future VEGF-R1 agonists may have therapeutic potential for liver regeneration.

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## Unsatisfactory quality of hepatological information on the internet

To the Editor:

Over the last decade, a continuously growing number of web sites have been dedicated to different aspects of liver disease. Physicians, patients and health operators can now easily access to a large volume of health information resources, available on the Internet. This, however, has some pitfalls, including the potential harm associated with inaccurate or misleading information, and the possibility of commercial interests influencing the contents of medical web sites. For this reason, many organisations and academic or scientific Societies have published guidelines and rating criteria for web sites evaluation [1,2].

In this study we aimed to survey and critically evaluate the information concerning three diseases of hepatological interest: chronic hepatitis (CH), hemochromatosis (HH) and Caroli's disease (CA) very different both in term of epidemiological impact, diagnostic strategies and therapeutic approaches. In accordance with a validated method [3], the three search terms were entered into four English-language search engines (Altavista [www.altavista.com], Yahoo [www.yahoo.com], Lycos [www.lycos.com] and Google [www.google.com]), and the first five links leading to relevant content were considered (accounting for a total of 60 sites). The objective items, assessed by three operators, were: the type of medical information offered (official vs. alternative medicine), the major target (physicians vs. patients vs. both), the commercial sponsorship (present vs. absent; in the case of sponsored sites, the presence of financial disclosure was also considered), the frequency of updates ( $\leq 6$  vs.  $> 6$  months), the presence of site entry restriction (present vs. absent), the statement of authorship (i.e. a clear indication of the author's name and

qualifications: present vs. absent), the attribution of the source of content (i.e. references to scientific literature: present vs. absent). The characteristics of the web sites were described and their quality evaluated by three independent reviewers who assigned a score ranging from 1 to 5 for accuracy, reliability and depth. An overall quality score was calculated for each site on the basis of the mean of the scores awarded to the three quality items of accuracy, reliability

**Table 1**  
Relationship between the characteristics of 60 hepatological websites and mean overall quality score as derived from univariate and multivariate logistic regression analysis. Odds ratios (OR) for insufficient score with 95% confidence intervals (in parentheses) are given for each site. Statistically significant results in bold

Variables	Statistical analysis	
	Univariate	Multivariate
Disease		
CH vs. HH	1.5 (0.4–5.2)	1.3 (0.2–6.8)
CH vs. CA	2.8 (0.8–10.0)	0.6 (0.1–3.5)
Type of the site		
Commercial vs. non-commercial	21.2 (2.5–177.3)	18.1 (1.7–192.5)
Update of the site		
> 6 vs. $\leq 6$ months	1.9 (0.6–6.0)	2.7 (0.5–12.5)
Attribution of source of content of the site		
Absent vs. present	2.8 (0.9–8.5)	1.9 (0.4–7.7)

CH: chronic hepatitis; HH: hereditary hemochromatosis; CA: Caroli's disease

the diagnosis of genetic  $\Delta^4$ -3-oxosteroid 5 $\beta$ -reductase deficiency and in presence of 3-oxo- $\Delta^4$  bile aciduria it should help to distinguish a genetic defect from a secondary defect.

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## Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) during liver regeneration

### To the Editor:

We read with great interest the article "Hepatitis B virus enhances tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cytotoxicity by increasing TRAIL-R1/death receptor 4 expression" by Janssen et al. [1], particularly since a role for TRAIL in HBV hepatitis is not well elucidated. Another study also suggests that TRAIL plays an important role in eliminating virus-infected cells by inducing apoptosis via caspase-dependent pathway [2].

TRAIL is also known to induce nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation as well as apoptosis in various cells, although the switching mechanism of these two different signalings is not well known. In mice model of liver regeneration, NF- $\kappa$ B activation occurs rapidly within 30 min after hepatectomy [4]. Additionally, TNF $\alpha$  is up-regulated in serum and liver tissues in the early stage of liver regeneration [5], and Fas engagement accelerates liver regeneration after partial hepatectomy [6]. Collectively, these observations suggest that TRAIL expression may have a role in liver regeneration.

Therefore, we investigated serum TRAIL concentrations during liver regeneration in nine healthy donors before and

after undergoing surgery for liver tissue resection for donation. Seven of the nine donors were for right lobe grafts, and the remaining two donors were for lateral graft. The average rate of graft volume per total liver volume was 61.3% (right) and 21.8% (lateral). Blood samples of the nine healthy patients were obtained before surgery, at 6 h post-operative and then daily to post-operative day 10. The serum TRAIL levels were measured by ELISA (BioSource International, Inc., Camarillo, CA). Before surgery, mean serum TRAIL levels were  $500 \pm 122$  pg/ml before the operation. A rapid decrease in serum TRAIL was observed after resection, reaching a nadir at 12 h post-hepatectomy, followed by a gradual increase after post-operative day 3 (POD3) (Fig. 1). There was no difference in TRAIL levels between graft types. These results suggested that TRAIL expression was down-regulated during liver regeneration, in contrast to up-regulation of other cytokines, such as TNF $\alpha$ , IL-1, IL-6 [5] or IL-18 (unpublished data) that can also activate NF- $\kappa$ B.

Although TRAIL is tough to kill selectively tumor cells, TRAIL activity can also lead to massive apoptosis of primary human hepatocytes [3]. Natural killer (NK) cells

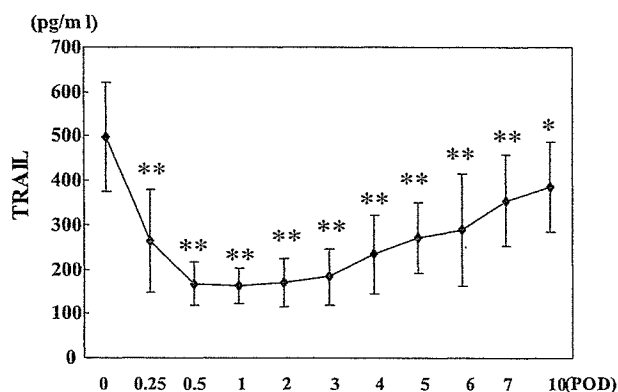


Fig. 1. Serum TRAIL concentrations before and after hepatectomy in donors. Bars indicate standard deviation (Paired Student's *t*-test; \**p* < 0.05 and \*\**p* < 0.01).

strongly and constitutively express TRAIL and these cells participate in the host defense to viral infection and tumor metastasis, indicating that the physiological TRAIL levels are not cytotoxic toward normal hepatocytes. Our findings demonstrate that serum TRAIL levels decrease during liver regeneration, and indicating a negative modulatory role for TRAIL during hepatocyte proliferation. Thus, TRAIL expression may contribute to pathways that impair cell proliferation of hepatocytes after partial hepatectomy. Further evaluation of these possible roles for TRAIL activity and regulation of TRAIL expression during liver regeneration will provide insight into pathways that control hepatocyte cell proliferation and death.

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# Significance of CT Attenuation Value in Liver Grafts Following Right Lobe Living-Donor Liver Transplantation

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In adult living-donor liver transplantation (LDLT), the assessment of the allograft functional reserve is important for adequate graft regeneration. From March 2002 to December 2003, 30 adult recipients underwent right lobe LDLT. Mean CT attenuation values (CT-AVs) in the graft were measured on unenhanced CT for 6 months after LDLT. The histological features of the graft parenchyma were evaluated with post-operative liver biopsy specimens. Mean CT-AVs after LDLT were decreased significantly from the pre-operative values, recovered to over 60 HU within 6 months. There was a positive linear correlation between the CT-AVs and the receptor index (LHL15) in technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl-human serum albumin (<sup>99m</sup>Tc-GSA) liver scintigraphy ( $r = 0.803$ ,  $p = 0.005$ ). The recipients were divided into two groups according to the CT-AV at one post-operative week (group H;  $\geq 55$  HU, group L;  $< 55$  HU). The low CT-AVs, under 55 HU, in group L were prolonged for 3 months compared with those in group H ( $p < 0.05$ ). The 1-year cumulative survival rate was 94.7% and 45.5% in groups H and L, respectively ( $p = 0.014$ ). Histological findings revealed that the parenchymal damage was severe in the grafts with low CT-AVs. The CT-AVs in the grafts may be a useful parameter for assessing the allograft functional reserve.

**Key words:** CT attenuation value, graft function, living-donor liver transplantation

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## Introduction

Living-donor liver transplantation (LDLT) and split liver transplantation (SLT) have been accepted as treatment for

end-stage liver diseases worldwide, to overcome the shortage of organs from cadavers.

Liver regeneration of a segmental liver graft is essential in adult LDLT or SLT. However, recipients often suffer from prolonged cholestasis, coagulopathy and massive ascites due to impairment of liver regeneration with small-for-size grafts (1). In addition to the assessment of the graft volume, quantitative and qualitative evaluation of the liver allograft function reserve may be necessary for proper graft regeneration.

Hemodynamics changes dramatically after liver transplantation, which influences the sinusoidal and hepatic parenchymal conditions. Therefore, several complications are possible such as the sinusoidal destruction (2) and hepatocyte ballooning, congestion (3), necrosis, and fat infiltration (4) in the graft parenchyma, especially in small-for-size grafts.

We focused on the parenchymal morphological changes in the graft, and tried to evaluate quantitatively with the mean CT attenuation value (CT-AV). The aim of the present study was to evaluate the feasibility of assessing the allograft functional reserve.

## Patients and Methods

### Patients

The subjects were 30 adult patients who underwent LDLT using right-lobe graft between March 2002 and December 2003, comprised of 23 men and 7 women ranging from 20 to 67 years old (median, 53 years). Their body weight ranged from 39.6 to 89.4 kg (median, 60.1 kg).

The etiologies of their liver disease were hepatocellular carcinoma with liver cirrhosis in 15 patients, hepatitis B or C virus (HBV/HCV)-related cirrhosis in six, acute liver failure in three, primary biliary cirrhosis in three, primary sclerosing cholangitis in two and alcoholic cirrhosis in one.

### Donors and grafts

The donors comprised 13 males and 17 females (1 parent; 12 spouses; 3 siblings; 12 children; 1 cousin and 1 grandson), ranging in age from 18 to 62 years old (median, 42.5 years). Twenty-nine grafts were ABO-compatible with the recipients, and one graft was ABO-incompatible. The graft-type comprised 28 right lobes, and 2 right lobes with the middle hepatic vein. The actual graft weight ranged from 460 to 1180 g (median, 660 g). The

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graft-to-recipient weight ratio (GRWR) ranged from 0.83% to 1.53% (median, 1.09%).

After evaluation of the MHV tributaries draining the anterior segment by pre-operative dynamic CT, we selected the graft-type. To solve the problem of potential venous congestion in the anterior segment, we selected right lobe grafts with the MHV in MHV-dominant donors, if the remnant liver volume was more than 35% of the whole liver by CT volumetry. Whenever these conditions were not satisfied, right lobe grafts without the MHV were used. All the branches of the MHV (V5, V8) of a significant size (>7 mm) were preserved with a caval cuff and reconstructed in an end-to-side fashion to the anterior wall of the inferior vena using autologous venous grafts. Twenty-four of the 30 grafts had significant accessory hepatic veins, which were reconstructed.

Mild (<30%) macrovesicular steatosis was histologically confirmed in eight grafts, and there were no moderate or severe steatotic grafts.

### Immunosuppression

The immunosuppression protocol consisted of tacrolimus and low-dose steroids. The target whole-blood trough level for tacrolimus was 10–12 ng/mL during the first 2 weeks, approximately 10 ng/mL thereafter, and 5–10 ng/mL from the second month. Steroid therapy was initiated at a dose of 10 mg/kg of methylprednisolone before reperfusion from the portal vein, then tapered from 1 mg/kg per day on day 1 to 0.3 mg/kg per day until the end of the first month. During the post-operative period, 1 mg/kg per day of the same drug was given for the first 3 days, followed by 0.5 mg/kg per day for the next 3 days. This was changed to oral prednisolone at a dose of 0.3 mg/kg per day 7 days after LDLT. Prednisolone was reduced to 0.1 mg/kg per day 1 month after LDLT, and patients were weaned off steroids at around 3–6 months post-operatively if their liver function was stable.

### Measurement of CT-AV of the graft

Helical CT studies were conducted with a CT-high speed QXI (GE Medical Systems, Tokyo, Japan). The scanning parameters were 120 kV, 200 mA, collimation at 5 mm, and a table speed of 10 mm/s, with reconstruction increments of 5 mm.

For each case, measurement of the CT-AVs (expressed in Hounsfield units: HU) of the graft parenchyma were made with 10 randomly placed, circular regions of interest (ROI) with a cursor 3 mm in diameter, on both the anterior and posterior segments on five transverse sections at different hepatic levels on non-enhanced CT (5). To avoid the partial-volume averaging effects, obvious necroses or segmental congestion, vascular and biliary systems in the graft, areas of artifact, and areas near the edge of organs were excluded from these measurements. The mean CT-AV of the ROI was calculated from measurements at 1, 2, 3 and 4 weeks, and 3 and 6 months after LDLT. Pre-operative CT-AVs were the donor's pre-operative values.

All helical CT studies were performed under same configurations in all recipients and donors.

### Technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl-human serum albumin (<sup>99m</sup>Tc-GSA) liver scintigraphy & the receptor index (LHL15)

Seven recipients (gross, 10 examinations) underwent technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl-human serum albumin (<sup>99m</sup>Tc-GSA) liver scintigraphy at 2 and 4 weeks after LDLT. <sup>99m</sup>Tc-GSA was supplied by Nihon Medi-Physics (Nishinomiya, Japan). After intravenous injection of 185Mq of <sup>99m</sup>Tc-GSA, dynamic imaging was performed with the patient supine using a large field-of-view gamma camera (GCA 7200A

gamma camera; Toshiba, Tokyo, Japan). ROIs were defined for the liver and heart using standard imaging software which was used to create time-activity data.

The receptor index (LHL15) is calculated by dividing the radioactivity of the liver ROI by the radioactivity of the whole liver (graft) plus heart ROIs 15 min after the injection, according Kudo et al. (6).

### CT volumetry

CT volumetry for recipients was performed for measurement of the graft volume (GV) at 1, 2, 3 and 4 weeks, and 3 and 6 months after LDLT. The outline of the graft in each slice was traced with ROIs. The graft area was calculated using an image-processing program (Advantage Workstation: GE Medical Systems, Tokyo, Japan), and total GV was finally calculated by integrating images from each graft region. The recipient's standard liver volume (SLV) was determined according to the Urata equation (7), and the GV/SLV ratio was calculated.

### Histological analysis

All of the histologic specimens were obtained from a needle biopsy of the right lobe grafts using a 16-gauge biopsy needle for the post-operative evaluation of liver dysfunction. Tissue specimens were fixed in 4% phosphate-buffered saline-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin by standard histological techniques. With the modified semi-quantitative scoring system for histological features of hepatic parenchyma (hepatocyte ballooning, hepatocyte necrosis, congestion, microvesicular fat, neutrophil aggregates, cholestasis) according to Neil et al. (8) (Table 1), an experienced liver pathologist reviewed the histological findings.

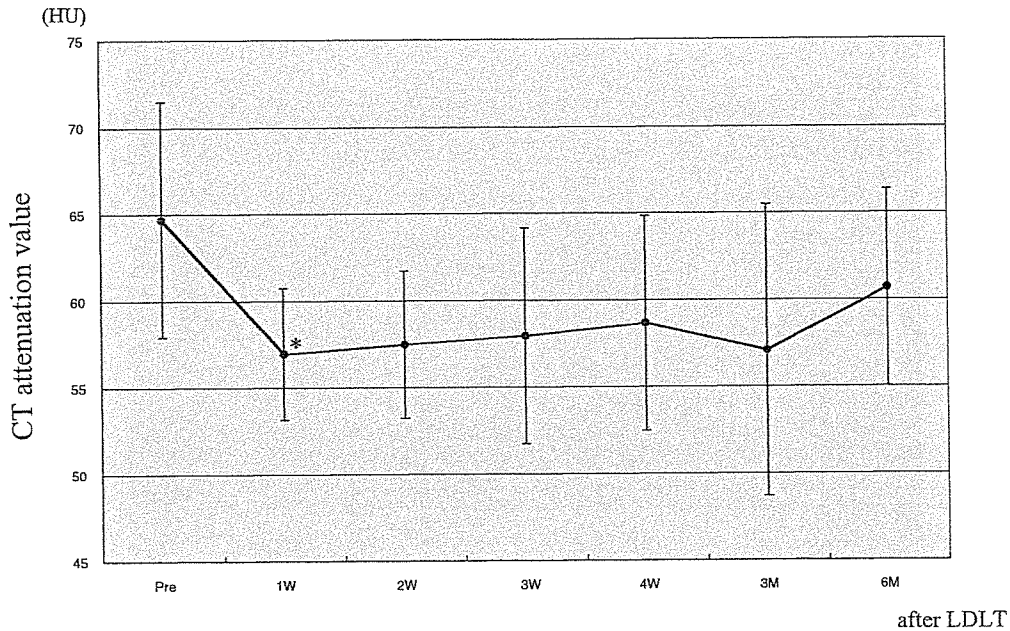
Protocol biopsy has not been performed in our institute.

### Statistical analyses

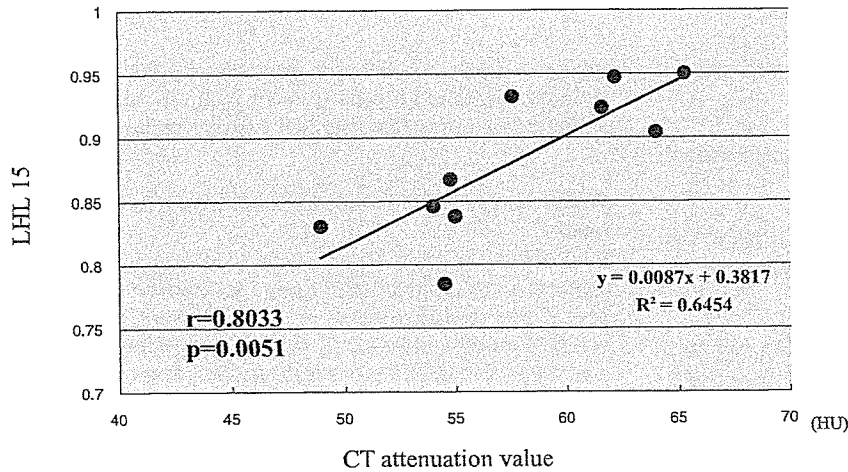
Statistical analysis was performed using Mann-Whitney for discontinuous data, Student's *t*-test for continuous data, and the Cox-Mantel test for Kaplan-Mayer survival analysis. The correlations were analyzed by Pearson test. Values of *p* < 0.05 were considered significant. Values are presented as mean ± SD.

**Table 1:** Semi-quantitative scoring system for histological features in the graft parenchyma

Ballooning	0. No 1. Yes
Hepatocyte necrosis	0. None 1. Small foci 2. Confluent areas 3. Bridging necrosis
Congestion	0. No 1. Yes
Microvesicular fat	0. None 1. <1/3 hepatocytes 2. Between 1/3 and 2/3 hepatocytes 3. >2/3 hepatocytes
Neutrophil aggregates	0. None 1. Minimal 2. Moderate 3. Extensive
Cholestasis	0. None 1. Mild 2. Moderate 3. Severe



**Figure 1: Changes in CT-AVs following LDLT.** \* $p < 0.05$  pre-operative CT-AV versus CT-AV at 1 week after LDLT. Pre-operative:  $n = 30$ , 1 week:  $n = 30$ , 2 weeks:  $n = 27$ , 3 weeks:  $n = 26$ , 4 weeks:  $n = 27$ , 3 months:  $n = 20$ , 6 months:  $n = 15$ , respectively.



**Figure 2: Correlation between CT-AV in the graft and LHL15.** Seven recipients (gross, 10 examinations) underwent  $^{99m}\text{Tc}$ -GSA liver scintigraphy at 2 and 4 weeks after LDLT. There was a significant positive linear correlation between the CT-AV and LHL15 ( $r = 0.803$ ,  $p = 0.005$ ).

**Results**

**Changes in CT-AVs following LDLT**

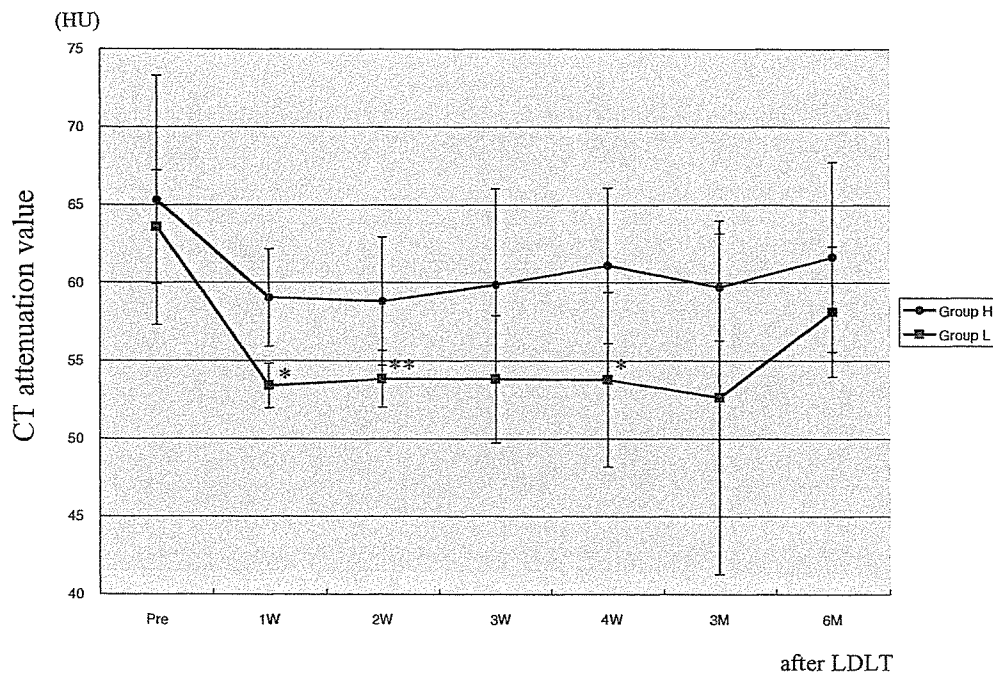
The changes in mean CT-AVs (mean  $\pm$  SD of all grafts) are shown in Figure 1. The pre-operative mean CT-AV of  $64.7 \pm 6.8$  HU decreased significantly to  $56.9 \pm 3.8$  HU ( $p < 0.05$ ) at 1 week after transplantation. Thereafter, the mean CT-AV remained under 60 HU for 3 months after LDLT. The mean CT-AV recovered to over 60 HU ( $60.7 \pm 5.7$  HU) at 6 months after LDLT.

**Correlation between CT-AV and LHL15**

Figure 2 shows the relationship between CT-AV and LHL15 in 10 examinations on the same day in 7 recipients. There was a significant positive linear correlation between the CT-AV and LHL15 ( $r = 0.803$ ,  $p = 0.005$ ).

**Changes in CT-AVs in subgroups**

The recipients were divided into two groups according to the CT-AV at 1 week after LDLT. Group H (high CT-AV) consisted of 19 recipients with a mean CT-AV of more than



**Figure 3: Changes in CT-AVs in subgroups.** \* $p < 0.05$  group H versus L, pre-operative: group H,  $n = 19$ ; group L,  $n = 11$ , 1 week: H,  $n = 19$ ; L,  $n = 11$ , 2 weeks: H,  $n = 19$ ; L,  $n = 8$ , 3 weeks: H,  $n = 17$ ; L,  $n = 9$ , 4 weeks: H,  $n = 18$ ; L,  $n = 9$ , 3 months: H,  $n = 14$ ; L,  $n = 6$ , 6 months: H,  $n = 10$ ; L,  $n = 5$ , respectively.

55 HU, and group L (low CT-AV) of 11 recipients with a mean CT-AV of less than 55 HU, respectively.

The changes in mean CT-AV in the two groups are shown in Figure 3.

There were no significant differences in the CT-AVs of the donors (pre-operative CT-AVs) between groups H and L (H,  $65.3 \pm 8.0$  HU, L,  $63.6 \pm 3.6$  HU,  $p = 0.55$ ).

CT-AVs in both groups decreased at 1 week after LDLT. The CT-AVs in group H were increased at 3 weeks after LDLT, but the CT-AVs in group L were significantly lower during the 4 weeks after LDLT than those in group H (1 week: H,  $59.0 \pm 3.1$  HU; L,  $53.4 \pm 1.4$  HU, 2 weeks: H,  $58.2 \pm 4.1$  HU; L,  $53.8 \pm 1.8$  HU, 3 weeks: H,  $59.9 \pm 6.2$  HU; L,  $53.8 \pm 4.1$  HU, 4 weeks: H,  $61.1 \pm 4.9$  HU; L,  $53.8 \pm 5.6$  HU;  $p < 0.05$ ).

The low CT-AVs in group L (3 months:  $52.6 \pm 11.3$  HU) continued to decrease for 3 months after LDLT.

The CT-AVs recovered to about 60 HU in the two groups at 6 months (H,  $61.6 \pm 6.1$  HU; L,  $58.1 \pm 4.2$  HU).

On the other hand, there were significant differences between the pre-operative CT-AVs of the 32 non-steatotic grafts and 8 steatotic grafts (non-steatotic grafts,  $68.0 \pm 4.9$  HU, steatotic grafts,  $58.5 \pm 5.3$  HU,  $p < 0.05$ ). In con-

trast, the changes in the post-operative CT-AVs in the mild steatotic grafts did not differ from those in non-steatotic grafts during 6 months after LDLT (data not shown).

#### **The post-operative course of serum total bilirubin level**

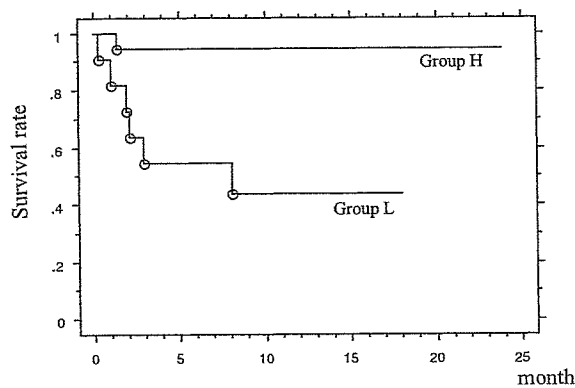
Serum total bilirubin levels in group L were significant higher than those in group H from 1 to 4 weeks after LDLT. Serum total bilirubin levels in group H were normalized within 6 months after LDLT, however, hyperbilirubinemia was prolonged until 3 months after LDLT in group L.

#### **The post-operative course of coagulation profile**

The post-operative prothrombin time international normalized ratio (PT-INR) levels in group H were restored to an almost normal level within 2 weeks after LDLT. In contrast, PT-INR levels in group L were significantly higher than those in group H for 3 months after LDLT.

#### **Changes in size of the grafts**

The GV/SVL ratio increased rapidly, to over 100%, 1 week after LDLT in all groups. The GV/SVL ratio in group L tended to be higher than that in group H for the 6 months after LDLT; in particular the GV/SVL ratio in group L was significantly higher at 4 weeks and 3 months than that in group H (4 weeks: H, 107%; L, 130%, 3 months: H, 104%; L, 133%,  $p < 0.05$ ).



**Figure 4: Actuarial survival in subgroups.** The 1-year cumulative survival rate was 94.7% and 45.5% in groups H and L, respectively ( $p = 0.014$ ).

#### Patient survival

Patient survival curves are shown in Figure 4. The 1-year cumulative survival rate was 94.7% and 45.5% in groups H and L, respectively ( $p = 0.014$ ).

The causes of death were intra-cranial bleeding ( $n = 1$ ) in group H, and graft failure ( $n = 4$ ), pneumonia ( $n = 1$ ) and cardiac failure ( $n = 1$ ) in group L.

Regarding the four recipients in group L who died of graft failure, one died due to an immunological response related to ABO incompatibility at 54 days after LDLT. The other three recipients with high model end-stage liver disease (MELD) scores had emergency LDLT, and showed hyperbilirubinemia and coagulopathy that persisted post-

operatively without size mismatch. The liver grafts did not become sufficiently functionated for the post-operative metabolic demands, which eventually led to graft failure, accompanied by deep fungal infection. The patients died at 56, 65 and 72 days after LDLT. The remaining five patients in group L are still alive. Furthermore, there were three cases in group L of stenosis of hepatic venous anastomoses of the grafts at 9, 13 and 18 days after LDLT, and these were successfully treated with stent insertions to the hepatic veins. However, hyperbilirubinemia, coagulopathy and massive ascites persisted for 2–3 weeks, and the recovery of liver function was delayed.

#### Comparison of the recipient data between the two groups

There were no significant differences between the two groups in recipient age, gender, graft-recipient weight ratio (GRWR), cold ischemic time (CIT), warm ischemic time (WIT), intra-operative blood loss or steatotic liver graft and incidence of acute cellular rejection or mean portal venous pressure during the first 3 days after LDLT. However, the MELD score, mean ascitic fluid volume during the 7 days after LDLT were significantly higher in group L than in group H (Table 2).

#### Histological findings

A gross of 41 post-operative liver biopsy specimens in 20 recipients were collected to diagnose the causes of liver dysfunction. Protocol biopsies are not performed in our institute. Therefore, liver biopsies were not performed on the nine recipients in group H who had no post-operative liver dysfunction during 6 months after LDLT, or the patient in group L who died at nine days after LDLT. Hence, liver biopsy specimens were obtained from a total of 10 recipients in group H and 10 recipients in group L.

**Table 2:** Comparison of the recipient data between the two groups

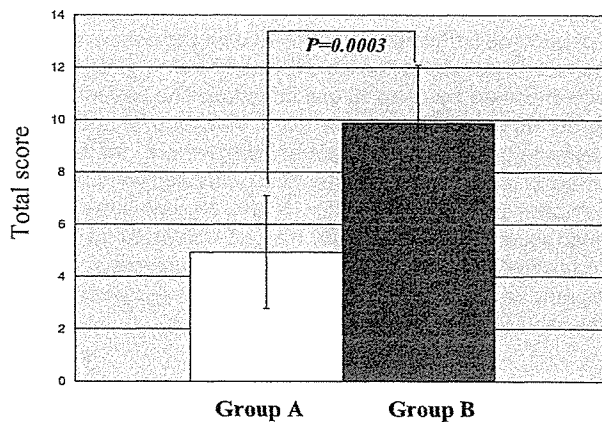
	Group H (n = 19)	Group L (n = 11)	p
Age	51.9 ± 12.6	51.3 ± 4.1	NS
Gender	M 16, F 3	M 7, F 4	NS
Etiology of liver disease	HCC 11, PBC 3, LC(B) 1, LC(C) 1, LC(alcohol) 1, ALF 1, PSC 1	HCC 4, LC(C) 4, ALF 2, PSC 1	NS
MELD score	9.9 ± 7.3	18.2 ± 12.9	0.039
Graft-to-recipient weight ratio (%)	1.07 ± 0.23	1.05 ± 0.16	NS
Cold ischemic time (min)	105.4 ± 44.5	133.5 ± 81.2	NS
Warm ischemic time (min)	45.5 ± 18.4	47.2 ± 14.6	NS
Blood loss (mL)	20249 ± 18613	18287 ± 16149	NS
Steatotic graft (mild macrovesicular steatosis)	6 cases	2 cases	NS
Incidence of acute cellular rejection	26.3% (5/19)	27.3% (3/11)	NS
Mean portal venous pressure (mmHg) <sup>†</sup>	24.8 ± 7.4	26.0 ± 6.8	NS
Mean ascitic fluid volume (mL) <sup>‡</sup>	1111.4 ± 952.8	2216.6 ± 1380.5	.02

M: Male; F: Female; MELD: Model for end-stage liver disease; HCC: Hepatocellular carcinoma; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis; LC: Liver cirrhosis; ALF: Acute liver failure.

<sup>†</sup>During first 3 days after LDLT.

<sup>‡</sup>During 7 days after LDLT.

## Significance of CT Attenuation Value in Liver Grafts



**Figure 5: Total scores of parenchymal features in the two groups.** Total scores, representing liver damage, were calculated to sum up the scores of parenchymal features. The total score in group A was  $4.94 \pm 2.15$ , and in group B was  $9.88 \pm 2.20$  ( $p = 0.0003$ ).

The specimens were divided into two groups: group A ( $n = 23$ ), in which the biopsy was performed on grafts with  $CT-AV \geq 55$  HU, and group B ( $n = 18$ ), in which the biopsy was performed on grafts with  $CT-AV < 55$  HU.

There were significant differences between the two groups for the scores of hepatocyte necrosis ( $p < 0.0001$ ), congestion ( $p = 0.0093$ ) and microvesicular fat ( $p = 0.0002$ ).

On the other hand, the scores for hepatocyte ballooning, neutrophil aggregates and cholestasis did not differ significantly between the two groups.

The total scores in group A were significantly lower than those in group B (A:  $4.94 \pm 2.15$  vs. B:  $9.88 \pm 2.20$ ,  $p = 0.0003$ ) (Figure 5).

Among all specimens, there was a significant negative linear correlation between the CT-AV and total score ( $r = -0.841$ ,  $p < 0.0001$ ) (Figure 6). The histological evaluations of the biopsy specimens revealed that the parenchymal damage was severe in the group with low CT-AV, compared to that in the group with high CT-AV.

## Discussion

Adequate allograft regeneration is important in adult partial liver transplantation. Graft dysfunction, characterized by enhanced cholestasis, coagulopathy, and massive ascites, may occur in small-for-size grafts (1). Therefore, post-operative assessment of allograft function reserve is important for regeneration after LT, in addition to simple measurement of the graft volume. Namely, increased graft

volume without functional reserve implies only the hypertrophy of the graft, not true liver regeneration. Regardless of the suitability of the liver function, the GV/SLV ratio was increased in the present study. Consequently, the GV/SLV ratio is ineligible as an indicator of liver regeneration.

CT-AV is expressed as an absolute value called the Hounsfield number in unenhanced CT. It has been well recognized since the late 1970s that unenhanced CT is useful for the detection of hepatic macrovesicular steatosis with a difference between liver and spleen CT-AV (9). In LDLT, CT-AV is used as a pre-operative evaluation of hepatic steatosis for donor selection (5,10).

Generally, low CT-AVs in the liver imply the presences of steatosis, necrosis or congestion (11). Several reports have referred to morphological changes in the graft after LT such as destruction of sinusoidal lining cells, hepatocyte ballooning (2), congestion and hemorrhagic infiltration (3), necrosis and microvesicular steatosis (4). So, we tried to evaluate these morphological changes after LDLT quantitatively using CT-AVs.

With regard to the assessment of hepatic functional reserve, the indocyanine green (ICG) clearance test is a widely accepted procedure in liver surgery. However, this test is known to accurately reflect the effective hepatic blood flow, linked to the hepatocyte volume, and infra- and extrahepatic shunts. Therefore, discrepancies between ICG R15 and histological findings of the liver are seen. Furthermore, the ICG test is not reliable for accessing the hepatic function in patients with severe jaundice.

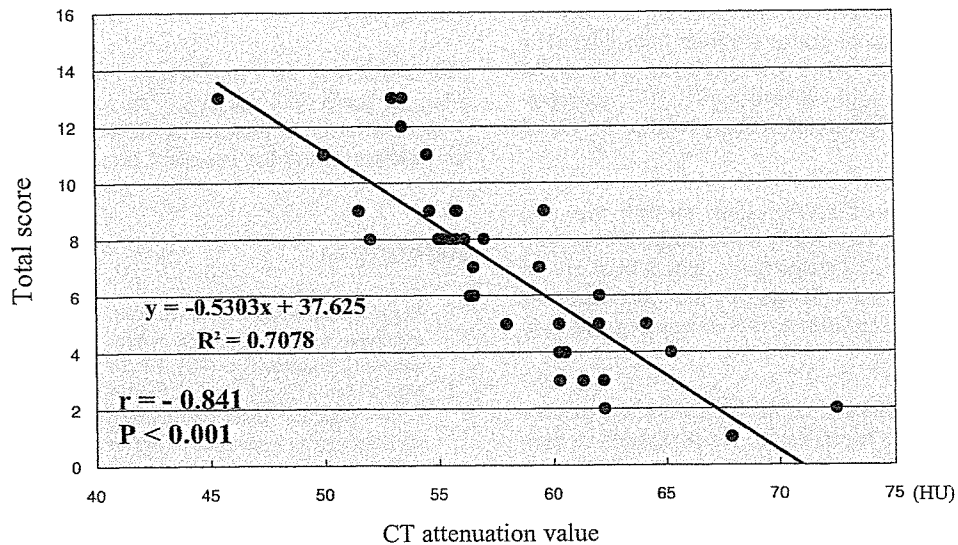
On other hand,  $^{99m}Tc$ -GSA, which binds specifically to asialoglyco-protein receptors on the hepatocellular membrane, is apparently useful for assessing hepatic function in patients with liver dysfunction under various physiological and pathological conditions, providing an important quantitative parameter of hepatic function that is totally independent of the ICG test.

Because  $^{99m}Tc$ -GSA scintigraphy can determine the functioning hepatocyte mass, this procedure is acceptable for the evaluation of remnant liver function in liver surgery (12).

Recently, the liver allograft functional reserve in liver transplant recipients has been evaluated by  $^{99m}Tc$ -GSA (13).

The receptor index LHL15 is a simple indicator calculated from three-point data in time-activity curves, and significant correlations between LHL15 and conventional liver function tests have been reported (6,14).

Although  $^{99m}Tc$ -GSA scintigraphy is a very sophisticated test for hepatic function, this examination is clinically more complicated for recipients in a serious condition, especially those in intensive care after LDLT, than unenhanced CT.



**Figure 6: Correlation between CT-AV in the graft and total score.** There was a significant negative linear correlation ( $r = -0.841$ ,  $p < 0.0001$ ). This result demonstrated that CT-AVs reflected the graft parenchymal damage.

Therefore, although we could perform unenhanced CT in all recipients,  $^{99m}\text{Tc}$ -GSA scintigraphy was only performed in seven recipients with a relatively stabilized general condition.

We managed to evaluate the translation between the CT-AVs and the objective data of  $^{99m}\text{Tc}$ -GSA such as the LHL15.

In the present study, there was a significant positive linear correlation between the CT-AV and LHL15 ( $r = 0.803$ ,  $p = 0.005$ ) in the only seven recipients.

However, due to the small number of patients who underwent  $^{99m}\text{Tc}$ -GSA scintigraphy, evaluation of the graft functional reserve by the correlation between the CT-AV and LHL15 alone was difficult. Therefore, we evaluated the graft functional reserve in the two CT-AV groups by conventional serum liver function tests, coagulation profiles and clinical findings.

Considering that group L was accompanied by hyperbilirubinemia, coagulopathy and massive ascites, this group was recognized as the graft dysfunction group.

Although we performed  $^{99m}\text{Tc}$ -GSA scintigraphy in only seven recipients, we suspected that the LHL15 deteriorated due to loss of the hepatic functioning mass in the graft, revealing a low CT-AV. It is suggested that assessment of the mean CT-AV in a graft may have the potential to be reliable for graft functional reserve.

Regardless of GV/SLV ratio, CT-AVs in liver grafts were decreased to under 60 HU in the two groups after LDLT.

CT-AVs in group H began to recover at 3 weeks after LDLT, but the low CT-AVs in group L persisted for 3 months after LDLT.

Regarding the reason for the persistent low CT-AVs in group L, six recipients died of various causes, including four graft failures, within three months after LDLT, and three recipients, who were successfully treated with stent insertions for stenosis of hepatic venous anastomoses, had persistent graft dysfunction. Therefore, we consider that the recovery of the CT-AVs in group L was delayed in comparison with group H. In fact, the CT-AVs of the remaining only two recipients in group L at 3 and 4 weeks after LDLT were  $55.7 \pm 3.3$  HU and  $57.4 \pm 1.7$  HU, respectively, and the CT-AVs began to recover at 4 weeks after LDLT.

Furthermore, 16 liver biopsy specimens in group B were obtained from 10 recipients in group L. Because we confirmed liver damage in group B by histological analysis, the CT-AVs were so low in group L.

The low CT-AVs (under 55 HU) in liver grafts at 1 week after LDLT are associated with graft dysfunction and fatal results, while CT-AVs over 60 HU may indicate adequate graft regeneration with allograft functional reserve.

The 1-year cumulative survival rate in group L was significantly lower than that in group H. Therefore, we suggest that CT-AVs at 1 week after LDLT may be predictive of the allograft functional reserve and the recipient prognosis.

Basically, this result indicated that several factors that decrease CT-AVs influence hepatocyte function, and induce the loss of actual functional mass, and graft dysfunction.

Graft dysfunction may have several causes, such as the graft size (1), the marginal grafts such as fatty liver (15), preservation-reperfusion injury, overperfusion due to excess portal flow (16) and elevated PVP (17), and pre-operative deteriorated condition closely related to MELD score (18).

With regard to recipient background, among the two groups, there were no significant differences in recipient age, GRWR, CIT, WIT, intra-operative blood loss or the presence of steatotic graft. However, the MELD scores in group L were significant higher than those in group H in our present study.

High pre-operative MELD scores have been shown to have an impact on the poor mortality of the recipients in several reports (18–20). Therefore, MELD scores can be used as predictors of the outcome after liver transplantation (21–23).

Regarding the maintenance of the initial graft function, the post-transplant metabolic and synthetic demands in recipients with severely damaged liver function (hyperbilirubinemia, coagulopathy) and a pre-operative deteriorated general condition (renal dysfunction, septic state, etc.) aggravated the graft metabolic function (1,24).

Furthermore, the liver grafts may be insufficiently functioned for the excessive metabolic and synthetic demands in high-risk recipients, including reduced metabolic and synthetic capacity (1).

Therefore, a pre-operative deteriorated condition with a high MELD score may impair the graft function, leading to graft dysfunction, graft failure and eventually multiple organ failure.

Accordingly, we consider that graft dysfunction accompanied by high MELD scores was responsible for the low CT-AVs in group L.

Our histological evaluations of biopsy specimens revealed that parenchymal damage was severe in the low CT-AV group (CT-AV < 55 HU) compared with that in the high CT-AV group ( $\geq 55$  HU) in aspects of hepatocyte necrosis, congestion, microvesicular fat and neutrophil aggregates.

Moreover, considering that there was a significant negative linear correlation between the CT-AVs and the total scores, and the scores of hepatocyte necrosis, microvesicular fat and congestion, these occurrences may cause CT-AVs to decrease in the graft. Therefore, we consider that CT-AVs are associated with the total score, low CT-AVs may represent hepatic parenchymal damage that induces the loss of actual hepatic functional mass and graft function reserve may depend on the degree of necrosis, steatosis and congestion in the graft parenchyma, histologically.

## Conclusion

The CT-AV may be a useful parameter for assessing liver allograft functional reserve. We suggest that CT-AVs at 1 week after LDLT may be predictive of the allograft functional reserve and the recipient prognosis.

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肝炎等克服緊急対策研究事業

生体肝移植後の C 型肝炎再発予防を目指した  
免疫抑制療法及びウイルス除去療法の試み  
に関する研究

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肝移植後のC型肝炎再発予防を目指した免疫抑制療法

及びウイルス除去療法の試み

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研究要旨： HCV 関連疾患に対する肝移植治療において、手術中の無肝期におけるウイルス量の減少を目的に、二重濾過血漿交換療法を行った。フィブリノゲンの監視と補充によって濾過療法は安全に施行でき、ウイルス量も一定の低下を示した。濾過治療群では移植後のウイルス血症の再燃遅延が認められた。

A. 研究目的

当該施設においては、当初予定されたステロイド剤不使用による免疫抑制療法の無作為比較対照試験に組み込める症例がなかった。一方、前年度までの結果から、肝移植時手術時に移植肝への血流が再開される時点での血中C型肝炎ウイルス(HCV)-RNA量と、移植後に血中にHCVが再出現する速度との間には正の相関が存在する傾向が認められた。そこで当該年度においては、生体肝移植の術中無肝期に焦点を当て、血中HCV-RNA量を減少させる手段の安全性と効果を検討し、さらに移植術前治療との併用の可能性について検討した。

B. 研究方法

待機移植手術として手術前に準備時間があり、また移植肝の冷阻血時間の調節が容易である生体肝移植で治療を行うHCV肝硬変症例を対象に、移

植手術中自己肝の門脈血流が遮断され、自己肝からのHCV流出が減少すると考えられる時期において、二重濾過血漿交換(DFPP)を行い(20-30 ml/分)、血中HCV粒子の低下を図る。

手術中のDFPP施行については、免疫グロブリン等とともに濾過過程で除去され、出血傾向を惹起することが懸念されるブリノゲンについて、事前に肝移植手術中の一般的な推移と出血量との関係を他の肝移植症例で解析し、体外からの補充の目安とその効果を検討して、DFPP施行に伴う安全なモニター方法と補正方法を検討した。

第1相ではDFPPの施行は肝移植手術中の1回のみとし、併用療法は行わず、免疫抑制療法はタクロリムス(Tac)またはシクロスポリン(CsA)とステロイドを基本とし、ステロイドは術後3ヶ月で減量した後0.1 mg/kgで継続した。移植後の抗HCV療法は、血

中 HCV-RNA の陰性化が持続した場合には、術後 2 週間の肝生検組織所見で拒絶を除外した上で予防的に開始することとし、この間にウイルスが再出現した場合には、通常どおり生化学的・組織学的慢性肝炎の再燃を確認してから開始することとした。

(倫理面への配慮)

前述のごとく、DFPP によって低下する血中フィブリノゲンのモニター及び補充投与による安全性を確認した上で、施設内治験審査委員会 (IRB) に計画書を提出し承認を受け、個々の患者において施行前に同意を得た。

### C. 研究結果

5 例の C 型肝硬変症例 (男性 3 例女性 2 例、年齢  $55 \pm 8$  歳、全例に肝細胞癌を合併) に対する生体肝移植において、移植術中の DFPP を行った。肝ドナー ( $34 \pm 7$  歳) は 4 例が血縁者であった。DFPP の開始時期は、自己肝門脈血流遮断後から自己肝摘出までの時期で、術野の止血が制御できた時点とし、移植肝血流再開の時点までで所定の交換量を終了した。

フィブリノゲンはプロトコールに従って補充し、有害事象は認めなかった。また、手術中の出血量についても、DFPP 非施行例との間に有意差を認めなかった。3 例は CsA、2 例は Tac にて免疫抑制導入した。

移植肝再灌流時の HCV-RNA は中央値  $10.5 (0.6-22.7)$  KIU/ml と DFPP 非施行例 ( $n=7$ ) の  $3.9 (0.5-54.3)$  と有意差を

認めなかったが、術前比では DFPP 非施行例  $0.0171 (0.0077-0.1852)$  に対し  $0.0090 (0.0006-0.0594)$  と低下傾向を示し、DFPP 施行前後でも多くの症例で良好な低下が確認された。

移植肝血流再開は血中 HCV-RNA は減少を続け、移植後再陽性化 ( $>0.5$  KIU/ml) は DFPP 群で 9 (3-29) 日と非 DFPP 群の 2 (1-21) 日よりも遅かったが、ひとたび陽性化するとウイルス量の差は消失した。DFPP 群 (追跡期間中央値 16 ヶ月) の 40% に中央値 3 ヶ月で、非 DFPP 群 (追跡期間中央値 17 ヶ月) の生存例全例に中央値 6 ヶ月で組織学的慢性肝炎 ( $>A2F1$ ) を認め、インターフェロン (IFN) + リバビリジン治療を開始しているが、抗ウイルス療法の経過については現在追跡中である。

### D. 考察

肝移植術中の血中 HCV ウイルス量に影響を与える因子としては、出血量、無肝期の長さ、移植肝の大きさや機能などが考えられ、さらに中和抗体の存在なども推定されている。したがって、DFPP の HCV-RNA 除去効果については、これらの因子を一定にしなければ評価が困難である。

今回の結果は、肝移植手術中無肝期の DFPP がそれまでの懸念に反して安全に施行できることを示すものである。HCV 粒子の除去効率の個人差は、さらに肝外組織へのウイルス分布の個人差をも反映すると思われるが、無肝期における DFPP によって移植肝へ