Table 1. Therapies for chronic hepatitis C and genotype and serum level of HCV in patients

	Genotype 1b; <100 KIU/ml	Genotype 1b; ≥100 KIU/ml	Genotype 2a or 2b
(a) Pegylated-IFN-α2b + ribavirin	0[a]	6	0
(b) IFN-α2b + ribavirin	0	7	0
(c) IFN-α2b	1	5	4
(d) IFN-β	5	8	11
(e) IFN-β + IFN-alphacon	0	6	6
(f) IFN-β + IFN-α2b + ribavirin	1	10	7

The schedules for each therapy are outlined in the "Patients and methods" section
[a]Number of patients

et al.[6] suggested that the rapid decrease in the first phase, observed within 1 or 2 days, was due to the direct effect of IFN on HCV production or release from hepatocytes, while the slower decrease in the second phase resulted from immune-mediated elimination of hepatocytes infected with HCV.[6] Furthermore, it was shown that the slope of the decline in the second phase was high in all patients with a sustained virological response (SVR) and low in most non-responders to IFN therapies.[8] Thus, the efficacy of IFN-based therapies may be influenced not only by viral factors but also by host factors, such as the Th1 immune reaction.

Osteopontin is an extracellular matrix protein with an RGD motif, and it is physiologically expressed in the kidney and bone. Previously, we found that osteopontin was expressed in activated Kupffer cells and stellate cells, and that osteopontin contributed to the migration of macrophages into necrotic areas in injured rat liver.[9,10] On the other hand, it was shown that osteopontin also acted as a cytokine essential for the initiation of the Th1 immune response in mice,[11] and that genetic polymorphisms in the osteopontin gene (*OPN*) determined the magnitude of the immune reaction to bacterial infection in mice.[12] Recently, we found four single-nucleotide polymorphisms (SNPs) in the promoter region of human *OPN*, and suggested that SNP in *OPN* at nucleotide (nt) -443 affected hepatitis activity in patients with chronic hepatitis C.[13] From these observations, we assumed that SNPs in the promoter region of *OPN* might be markers to predict the efficacy of IFN-based therapies in HCV-infected patients.

In the present study, we analyzed SNPs in the promoter region of *OPN* in patients with chronic hepatitis C treated with IFN alone or with IFN plus ribavirin, and evaluated the significance of these SNPs as a marker predicting the efficacy of these therapies, as compared with SNPs in the myxovirus resistance protein A gene (*MxA*), the mannose-binding lectin gene (*MBL*), and the low-molecular-mass polypeptide 7 gene (*LMP7*), which had been reported to have associations with the response to IFN therapies.[14–17]

Patients and methods

Patients and interferon-based therapies

The patients were 77 Japanese with chronic hepatitis C who had medical examinations at the outpatient clinic of Saitama Medical School Hospital in August 2002, and who had finished IFN-based therapies by March 2004. All the patients were positive for HCV-RNA before the therapies and negative for hepatitis B virus surface antigen in the sera. The diagnosis of chronic hepatitis was made by histological findings in liver biopsy specimens and/or by serum biochemical tests and peripheral blood cell counts. Documented informed consent for gene analysis was obtained from all the patients.

Each patient received one of six schedules of therapy with IFN (Table 1), as follows: (a) subcutaneous injection of pegylated (Peg)-IFN-α2b, at 1.5 μg/kg body weight once a week, combined with daily oral administration of ribavirin at 600, 800, or 1000 mg/day for patients with a body weight of less than 60 kg, between 60 and 80 kg, and more than 80 kg, respectively, for 48 weeks; (b) intramuscular injection of IFN-α2b, at 6 MU daily for 2 weeks and three times a week for the following 46 weeks, combined with administration of ribavirin as in schedule (a) for 48 weeks; (c) injection of IFN-α2b at 10 MU daily for 4 weeks and three times a week for the following 20 weeks; (d) intravenous injection of IFN-β, at 3 MU, at 12-h intervals for 4 weeks and at 6 MU at 24-h intervals for the following 2 to 8 weeks; (e) injection of IFN-β at 3 MU at 12-h intervals for 4 weeks, and subcutaneous injection of IFN-alphacon at 18 MU three times a week for the following 24 weeks; or (f)

injection of IFN-β at 3MU at 12-h intervals for 4 weeks and injection of IFN-α2b at 10MU three times a week for the following 24 weeks, with administration of ribavirin at 600mg or 800mg for patients with body weights of less than 60kg or 60kg or more, respectively. The intervals between IFN-β injections, and the doses of IFN and ribavirin were changed when severe side effects occurred.

Analysis of serum HCV-RNA and determination of therapeutic efficacy of interferon alone or interferon plus ribavirin

The serum HCV-RNA level was measured using a polymerase chain reaction (PCR) Kit (Amplicor HCV Monitor; Roche Diagnostica, Tokyo, Japan). The HCV genotype was determined on the basis of the sequence of the core region, according to the method of Okamoto et al.[18]

Patients in whom serum HCV-RNA was not detected by PCR (Cobas Amplicor; Roche Diagnostica) for 6 months after the discontinuation of IFN-based therapies were classified as patients with an SVR, and the other patients were classified as those with non-response (NR).

Analysis of SNPs in the genes for osteopontin, myxovirus resistance protein A, mannose-binding lectin, and low-molecular-mass peptide 7

Genomic DNA was extracted from peripheral blood mononuclear cells. SNPs in the promoter region of *OPN*, at nt -155, -443, -616, and -1748; *MxA*, at nt -88 and -123; and *MBL*, at nt -221, and SNPs in *MBL* at G54D and *LMP7* at Q49K were determined by Invader assay.[19]

The Invader assay was done as described previously,[20] with minor modifications. Primer probes and the Invader oligonucleotides for each SNP (Tables 2 and 3) were designed with Invader Creator software (Third Wave Technologies, Madison, WI, USA) to have theoretical annealing temperatures of 63°C and 77°C, respectively. The reactions were performed using 384-well Invader assay fluorescence resonance energy transfer (FRET) detection plates (Third Wave Technologies), in which Cleavase XI enzyme, both F (FAM) dye and R (Redmond Red) dye (Epoch Biosciences, Redmond, WA, USA) FRET cassettes and reaction buffer were dried on each well. Three microliters of a mixture consisting of the appropriate primary probe, Invader oligonucleotide, and $MgCl_2$ was added to the wells, followed by the addition of 3μl of the heat-denatured genomic DNA (≧10ng/μl), and overlaid with 6μl of mineral oil (Sigma Chemical, St. Louis, MO, USA). The plates were incubated at 63°C for 4h in a DNA thermocycler (RTC-200; MJ Research, Watertown, MA, USA), and then kept at 4°C until fluorescence measurements were done. The fluorescence intensities were measured on a Cytoflour 4000 fluorescence plate reader (Applied Biosystems, Foster City, CA, USA) with excitation at 485/20nm (wavelength/bandwidth) and emission at 530/25nm for F dye detection, and excitation at 560/20nm and emission at 620/40nm for R dye detection.

Statistical analysis

The proportions of patients with an SVR were compared among patients with different alleles of the SNPs by Fisher's exact test. Also, these proportions were compared in patients with different backgrounds for viral markers. *P* values of less than 0.05 were regarded as statistically significant.

Table 2. Probes for Invader assay of SNPs in the promoter region of *OPN*

SNP	Type of Probe	Probe sequences
nt -155	Primary probe 1	acggacgcggagCCAAAAACGCACACAC
	Primary probe 2	cgcgccgaggCAAAAACGCACACACAC
	Invader probe	CCACACTTCCCCCTCTGGTTTTGTGGTTAAAACAAAAAAAAAT
nt -443	Primary probe 1	cgcgccgaggAAACTTGCCTCTGTCC
	Primary probe 2	acggacgcggagGAACTTGCCTCTGTCC
	Invader probe	GAAGGCTATTGTTCAAGCCTGCAAGGAGTTCAGAT
nt -616	Primary probe 1	acggacgcggagAAGGATGACTGCTTGAG
	Primary probe 2	cgcgccgaggCAGGATGACTGCTTGAG
	Inavder probe	GATGTTGCAGAAGTAAAGCAGTTTCTGACTGAGAGT
nt -1748	Primary probe 1	acggacgcggagGGACTTCCCTCCACTAA
	Primary probe 2	cgcgccgaggAGACTTCCCTCCACTAAA
	Invader probe	GGCACAGAGTAAACTACAGTAAATCCTGTGGAAATTTTGTTGTTTTTT AGAATTTTCT

Lower-case letters indicate the flap sequences of primary probes
nt, nucleotide

Table 3. Probes for Invader assay of SNPs in the genes for Myxovirus resistance protein A (*MxA*), mannose-binding lectin (*MBL*), and low-molecular-mass peptide 7 (*LMP7*)

SNP	Type of probe	Probe sequences
MxA nt -88	Primary probe 1	acggacgcgagGCCCGGAGCCG
	Primary probe 2	cgcgccgaggTCCCGGAGCCGC
	Invader probe	GGGGCCAGGAGCTAGGTTTCGTTTCTGCC
MxA nt -123	Primary probe 1	acggacgcggagGCAGCACTTGCCTC
	Primary probe 2	cgcgccgaggTCAGCACTTGCCTCG
	Invader probe	CCTAGCTCCTGGCCCCGCACCTC
MBL nt -221	Primary probe 1	acggacgcggagGGAAAGCATGTTTATAGTCTTC
	Primary probe 2	cgcgccgaggCGAAAGCATGTTTATAGTCTTC
	Invader probe	GCACGGTCCCATTTGTTCTCACTGCCACT
MBL G54D	Primary probe 1	acggacgcggagGCACCAAGGGAGAAAAG
	Primary probe 2	cgcgccgaggACACCAAGGGAGAAAAG
	Invader probe	GCTTCCCAGGCAAAGATGGGCGTGATGT
LMP7 Q49K	Primary probe 1	acggacgcggagCAGGTCGGGGCAG
	Primary probe 2	cgcgccgaggAAGGTCGGGGCAG
	Invader probe	CCAGAGCTCGCTTTACCCCGGGGAATGT

Lower-case letters indicate the flap sequences of primary probes

Table 4. SNPs in the genes for osteopontin (*OPN*), Myxovirus resistance protein A (*MxA*), mannose-binding lectin (*MBL*), and low-molecular-mass polypeptide 7 (*LMP7*) in patients with chronic hepatitis C treated with interferon with or without ribavirin

SNP	Number of patients (%)		
OPN nt -155	G/G Homozygotes 1 (1.3)	G/- Heterozygotes 25 (32.5)	-/- Homozygotes[a] 51 (66.2)
OPN nt -443	C/C Homozygotes 15 (19.5)	C/T Heterozygotes 40 (51.9)	T/T Homozygotes 22 (28.6)
OPN nt -616	T/T Homozygotes 1 (1.3)	T/G Heterozygotes 25 (32.5)	G/G Homozygotes 51 (66.2)
OPN nt -1748	G/G Homozygotes 1 (1.3)	G/A Heterozygotes 25 (32.5)	A/A Homozygotes 51 (66.2)
MxA nt -88	G/G Homozygotes 36 (46.8)	G/T Heterozygotes 34 (44.2)	T/T Homozygotes 7 (9.1)
MxA nt -123	C/C Homozygotes 29 (37.7)	C/A Heterozygotes 44 (57.1)	A/A Homozygotes 4 (5.2)
MBL nt -221	G/G Homozygotes 67 (87.0)	G/C Heterozygotes 10 (13.0)	C/C Homozygotes 0
MBL G54D	G/G Homozygotes 57 (74.0)	G/A Heterozygotes 13 (16.9)	A/A Homozygotes 7 (9.1)
LMP7 Q49K	C/C Homozygotes 57 (74.0)	C/A Heterozygotes 19 (24.7)	A/A Homozygotes 1 (1.3)

[a] Deletion mutation

Results

Demographic and clinical features of the patients, and therapeutic efficacy of interferon alone or interferon plus ribavirin

The patients were 45 men and 32 women, aged 53.3 ± 10.0 years (mean ± SD), with a range of 25 to 70 years. The genotype of serum HCV-RNA was 1b in 49 patients (63.6%), 2a in 23 (29.9%), and 2b in 5 (6.5%). Of the 49 patients with HCV genotype 1b, 7 patients (14.3%) had a pretreatment viral load of less than 100 KIU/ml (patients with genotype 1b and low titer) and 42 patients (85.7%) had a pretreatment viral load of 100 KIU/ml or more (patients with genotype 1b and high titer).

SVR was achieved in 45 patients (58.4%). The SVR rate was significantly higher in patients with genotype

Table 5. SNPs in the promoter region of *OPN* and response to interferon-based therapies

SNP	Total			Genotype 1b; <100 KIU/ml			Genotype 1b; ≥100 KIU/ml			Genotype 2a or 2b		
nt -155	G/G	G/–	–/–[c]	G/G	G/–	–/–	G/G	G/–	–/–	G/G	G/–	–/–
SVR[a]	1	21	23	0	3	3	1	8	8	0	10	12
NR[b]	0	4	28	0	0	1	0	3	22	0	1	5
P values[d]												
G/G and G/– vs –/–		0.018			>0.999			0.006			0.355	
nt -443	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T
SVR	7	19	19	1	4	1	4	5	8	2	10	10
NR	8	21	3	1	0	0	7	15	3	0	6	0
P values												
C/C and C/T vs T/T		0.002			>0.999			0.029			0.062	
nt -616	T/T	T/G	G/G	T/T	T/G	G/G	T/T	T/G	G/G	T/T	T/G	G/G
SVR	1	21	23	0	3	3	1	8	8	0	10	12
NR	0	4	28	0	0	1	0	3	22	0	1	5
P values												
T/T and T/G vs G/G		0.018			>0.999			0.006			0.355	
nt -1748	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A
SVR	1	21	23	0	3	3	1	8	8	0	10	12
NR	0	4	28	0	0	1	0	3	22	0	1	5
P values												
G/G and G/A vs A/A		0.018			>0.999			0.006			0.355	

[a] SVR, Sustained virological response
[b] NR, Virological non-response
[c] Deletion mutation
[d] *P* values by Fisher's exact test

1b and a low titer than in those with genotype 1b and a high titer (85.7% vs 40.5%, respectively; $P = 0.041$). Twenty-two patients with genotype 2a or 2b obtained an SVR (78.6%).

SNPs in the genes for osteopontin, myxovirus resistance protein A, mannose-binding lectin and low-molecular-mass peptide 7

As shown in Table 4, of the four SNPs in the promoter region of *OPN*, SNPs at nt -155, -616, and -1748 showed linkage disequilibrium at 100% to each others. On the other hand, there was no relationship between the prevalence of the four SNPs in *OPN* and each SNP in the *MxA*, *MBL*, and *LMP7* genes.

Table 5 shows that the response to IFN-based therapies differed depending on the alleles the three SNPs with 100% linkage disequilibrium and the SNP at nt -443. In regard to the SNP at nt -1748, the SVR rate was 84.6% (22/26) in patients with the G/G or G/A alleles, and this was significantly higher than the rate in those with the A/A allele (45.1%; 23/51). The SVR rate in patients with the T/T allele in the SNP at nt -443 (86.4%; 19/22) was also significantly higher than the SVR rate in those with the C/C or C/T alleles (47.3%; 26/55). Similar results were found in patients with genotype 1b and a high titer. Furthermore, all 14 patients showing G/G or G/A in the SNP at nt -1748 and T/T in the SNP at nt -443 obtained an SVR after IFN-based therapies.

As shown in Table 6, there was no relationship between the SVR rate and SNPs in the *MxA*, *MBL*, and *LMP7* genes, except that the rate in patients with G/G or T/T in the promoter SNP of *MxA* at nt -88 tended to be higher than the rate in those with G/T in the group of patients with genotype 1b and a high titer.

Discussion

The present study was designed to evaluate the usefulness of SNPs in the promoter region of *OPN* at nt -155, -443, -616, and -1748 as a marker to predict the therapeutic efficacy of IFN alone or combined with ribavirin in patients with chronic hepatitis C. Alleles of SNPs were determined by the Invader assay, an assay that does not include DNA amplification by polymerase chain reaction (PCR), and which is applicable to the measurement of many samples.[19] Among the four SNPs

Table 6. SNPs in the genes for myxovirus resistance protein A (*MxA*), mannose-binding lectin (*MBL*), and low-molecular-mass polypeptide 7 (*LMP7*) and response to interferon-based therapies

SNP	Total			Genotype 1b; <100 KIU/ml			Genotype 1b; ≥100 KIU/ml			Genotype 2a or 2b		
MxA	G/G	G/T	T/T	G/G	G/T	T/T	G/G	G/T	T/T	G/G	G/T	T/T
At nt -88												
SVR[a]	22	19	4	3	3	0	10	4	3	9	12	1
NR[b]	14	15	3	1	0	0	8	14	3	5	1	0
P values[c]												
G/T vs C/G and T/T		0.816			>0.999			0.057			0.173	
MxA	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A
At nt -123												
SVR	26	16	3	3	3	0	10	5	2	13	8	1
NR	18	13	1	1	0	0	12	12	1	5	1	0
P values												
C/C vs C/A and A/A		>0.999			>0.999			0.543			0.375	
MBL	G/G	G/C	C/C	G/G	G/C	C/C	G/G	G/C	C/C	G/G	G/C	C/C
At nt -221												
SVR	38	7	0	6	0	0	14	3	0	18	4	0
NR	29	3	0	1	0	0	22	3	0	6	0	0
P values												
G/G vs G/C		0.514			>0.999			0.672			0.549	
MBL G54D	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A
SVR	33	8	4	5	1	0	11	4	2	17	3	2
NR	25	5	3	1	0	0	18	4	3	5	1	0
P values												
G/G vs G/C and A/A		>0.999			>0.999			0.738			>0.999	
LMP7 Q49K	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A
SVR	33	11	1	4	2	0	13	3	1	16	6	0
NR	24	8	0	1	0	0	18	7	0	5	1	0
P values												
C/C vs C/A and A/A		>0.999			>0.999			>0.999			>0.999	

[a] SVR, Sustained virological response
[b] NR, Virological non-response
[c] *P* values by Fisher's exact test

described here, those at nt -155, -616, and -1748 had already been registered in a database of Japanese single-nucleotide polymorphisms (JSNP) and/or in the dbSNP (National Center for Biotechnology Information),[21] and we previously found that these SNPs showed linkage disequilibrium, with coefficients (D' and r^2) greater than 0.937 to each other in patients with chronic hepatitis C without any experience of IFN-based therapies.[13] The SNP at nt -443 was recently identified by our group,[13] and we found that the SNP at -443, but not the other three SNPs, had a close association with hepatitis activity in patients with chronic hepatitis C.[13] In the present study, the prevalence of the four SNPs of *OPN* (Table 4) was similar to that observed in patients without experience of IFN-based therapies,[13] and the SNPs at nt -155, -616, and -1748 showed 100% linkage disequilibrium to each other. These results suggested that there were no differences in genetic background, regarding *OPN*, between the patients with chronic hepatitis C who received IFN-based therapies and those without such experience at our hospital.

As shown in Table 5, the SVR rate differed depending on the alleles of the four SNPs in the promoter region of *OPN*. Such differences were particularly evident in patients with genotype 1b and a high titer. Moreover, all the patients with G/G or G/A at nt -1748 and T/T at nt -443 obtained an SVR after IFN-based therapies. Therefore, it was suggested that the SNP in the promoter region of *OPN* at nt -443 and the three SNPs at nt -155, -616, and -1748 with linkage disequilibrium were useful as a marker to predict the therapeutic efficacy of IFN alone or IFN plus ribavirin, especially in patients with genotype 1b and a high titer.

In this study, the SVR rate in patients with T/T in SNP of *OPN* at nt -443 was 86%. Previously, we reported that the frequency of T/T at nt -443 was about 3.5 times higher in patients with chronic hepatitis C with serum alanine aminotransferase (ALT) levels higher than 80 IU/l than in those with an ALT level lower than

30IU/l.[13] The SNP at nt -443 is located 13 base pairs (bp) upstream of the cis-acting enhancing element of human *OPN*.[22] Considering that the Th1 response is involved in the development of inflammation in chronic hepatitis C[1] and that hepatocytes infected with HCV are eradicated by Th1 response during IFN-based therapies,[6] the SNP in *OPN* at nt -443 may be crucial in provoking diverse Th1 immune reactions against HCV through the regulation of osteopontin expression in the liver. This matter should be investigated in future by carrying out promoter assays with each allele at nt -443.

Hijikata et al.[14,15] reported that G/G at nt -88 and C/C at nt -123 in the promoter region of *MxA* were observed more frequently in patients with NR than in those with an SVR after IFN therapy. *MxA* was shown to encode an IFN-inducible protein that inhibited the replication of single-stranded RNA viruses.[23,24] Matsushita et al.[16] showed that the frequencies of C/C at nt -221 in the promoter region of *MBL* and A/A at G54D in *MBL* were higher in patients with NR than in those with an SVR. Mannose-binding lectin is an acute-phase reactant protein inducing the phagocytosis of macrophages through binding to the surface of pathogens, and it is known to be essential for the innate immune system.[25,26] Sugimoto et al.[17] found that the frequency of C/A at Q49K in *LMP7* was higher in patients with an SVR than in those with NR, especially in patients with serum HCV-RNA levels less than 100KIU/ml. Low-molecular-mass polypeptides were shown to play a crucial role in human leukocyte antigen (HLA) class I-restricted antigen-presenting systems.[27] However, we found no relationship between the SVR rate after IFN-based therapies and the alleles of these SNPs in patients with chronic hepatitis C. As outlined in Table 1 and the "Patients and methods" section, most patients received IFN-ribavirin combination therapy or IFN monotherapy with 3MU of IFN-β injection given at 12-h intervals for 4 weeks as an induction therapy. This monotherapy was shown to be superior in HCV antiviral effects to 6MU IFN-β injection given at 24-h intervals.[28] IFN-ribavirin combination therapy was also reported to show a higher SVR rate than IFN monotherapy.[29] The rate of SVR in patients with genotype 1b and a high titer in the present study was 40.5%. In previous studies regarding SNPs in *MxA*, *MBL*, and *LKP7*, all the patients received IFN-α for 24 weeks or less.[14–17] After IFN-α therapy for 24 weeks, the SVR rate was reported to be only 7%–8% in patients with genotype 1b and a high titer.[30] The differences in the antiviral effects of these therapies may produce discrepancies in the results regarding SNPs in *MxA*, *MBL*, and *LMP7*. This matter should be further investigated in a large series of patients in whom standardized therapy with Peg-IFN-α2b and ribavirin is done for 48 weeks.

In conclusion, four SNPs in the promoter region of *OPN* may be useful as a marker to predict the efficacy of IFN-based therapies in patients with chronic hepatitis C.

Acknowledgments. We thank Ms. Kayoko Naiki (Division of Gastroenterology and Hepatology, Internal Medicine, Saitama Medical School) for her technical help. This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS), Scientific Research (B)(2); 14370191.

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Case Report

Unusual Endoscopic Findings of CMV Esophagitis After Liver Transplantation

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KEY WORDS:
CMV; Esophagitis; Living donor liver transplantation

ABBREVIATIONS:
Cytomegalovirus (CMV); Transcatheter Arterial Embolization (TAE); Hepatocellular Carcinoma (HCC); Cytomegalovirus Immune Globulin (CMVIG); Mycophenolate Mophetil (MMF); Proton Pump Inhibiter (PPI); Polymerase Chain Reaction (PCR)

SUMMARY

Cytomegalovirus infections are associated with a high mortality rate after liver transplantation, but they are treated successfully by administration of the combination of ganciclovir plus intravenous immunoglobulin. We herein describe cytomegalovirus esophagitis in a patient having gastrointestinal symptoms such as dysphagia, retrosternal pain and epigastralgia after liver transplantation was detected by performing the surveillance of endoscopy. At first, the findings of endoscopy that were segmental erosive areas but no ulcerative areas on the esophageal lumen were unusual in this case of cytomegalovirus infections, but cytomegalovirus esophagitis was confirmed by cytomegalovirus immunohistochemical stain using biopsies.

The patient was treated by ganciclovir at an oral dosage of 5mg/kg twice a day for 2 weeks.

Our experience suggests that cytomegalovirus esophagitis should be taken into consideration when a patient has gastrointestinal symptoms such as dysphagia, retrosternal pain and epigastralgia and has endoscopic findings such as segmental erosions on the esophageal lumen despite having no cytomegalovirus-specific endoscopic findings such as ulcerative lesions.

INTRODUCTION

Cytomegalovirus (CMV), a member of the herpes virus family, has been a cause of serious, life-threatening infections in compromised hosts such as those receiving kidney, bone marrow, and liver transplantation, and has been associated with episodes of rejection after organ transplantation (1-4).

In liver transplantation patients, CMV infections can be either a primary infection or a reactivation of a previously latent infection and can be documented in 30 to 65% of patients, and CMV infections occur and peak around the 5th week after transplantation (5). Alternatively, CMV disease or symptomatic CMV infection occurs in 18 to 40% of patients (1-4). So though the incidence of the disease appears high, a recently available antiviral agent, ganciclovir, has been shown to have antiviral efficacy in serious CMV infections (6-8), such as those occurring in patients with the acquired immune deficiency syndrome (9-11).

The present report describes unusual endoscopic findings of CMV esophagitis early after living donor liver transplantation.

CASE REPORT

The patient was a 57-year-old male who was shown to have esophageal varices and positive HCV-Ab in 1998. He underwent multiple sessions of transcatheter arterial embolization (TAE) for hepatocellular carcinoma (HCC) in 1999 and was referred to us on July in 2001 for evaluation as a living related donor liver transplantation candidate. The physical examination at admission revealed no scleral icterus and no pitting edema. An endoscopic examination of the gastrointestinal tract demonstrated no varices and no inflammation but only chronic erosive gastritis. The laboratory tests included: total bilirubin 1.7mg/dL, alkaline phosphatase 272 IU/L, AST 57 IU/L, ALT 26 IU/L, CMV IgG was positive, and IgM was negative. Since his 49-year-old brother, whose blood type was identical to the patient (O+) and preoperative laboratory tests were normal, (CMV IgG was positive and IgM was negative), he volunteered to donate his left hepatic lobe and caudate lobe, he underwent the aforementioned procedure today. The graft weight was 530g, which gave 40% of standard liver volume of the recipient.

After liver transplantation, prophylactic intravenous CMV hyper immune globulin (CMVIG) was administered at a dosage of 150mg/kg/day for 3 days. Immunosuppressive therapy, which was the triple regimen; FK506, methylprednisolone and mycophenolate mophetil (MMF), was also administered.

Fever continued from postoperative day 6 (**Figure 1**). As we suspected the catheter fever at first, we plucked out all the catheters, but fever continued. Since the patient began to have specific gastrointesti-

Hepato-Gastroenterology 2005; 52:1236-1239

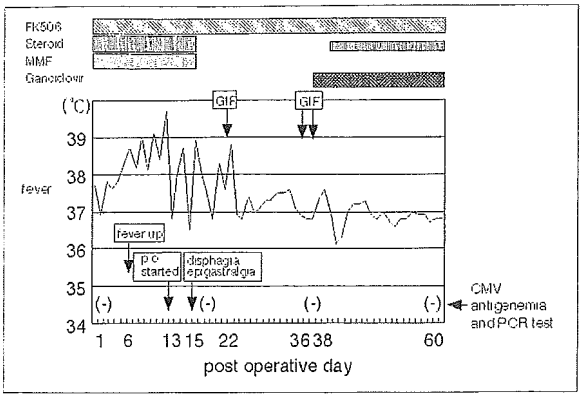

FIGURE 1 Postoperative course (from day 1 to day 60).

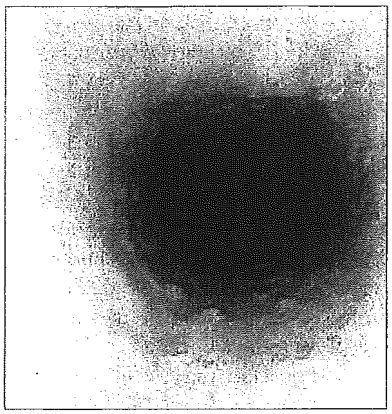

FIGURE 2
The findings of endoscopy (at about 20cm from dental arch, on post-operative day 15): the border area of normal mucosa and erosive lesions on the esophageal lumen.

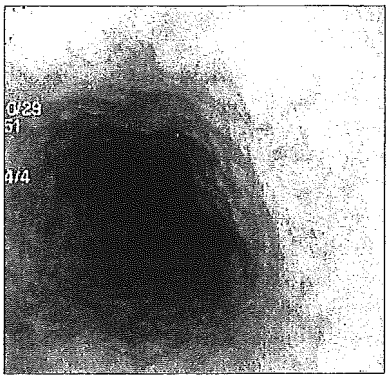

FIGURE 3
The findings of endoscopy (at about 23cm from dental arch, on post-operative day 15): diffuse, shallow, and segmental erosions, but no ulcers on the esophageal lumen.

nal symptoms like dysphagia, nausea, and epigastralgia on postoperative day 15, the surveillance of endoscopy was performed on postoperative day 22. The findings were diffuse, segmental erosions in the distal of the esophagus (at about 23cm from dental arch), but no ulcers **(Figures 2 and 3)**. Polymerase chain reaction (PCR) test and antigenemia test of CMV were negative in the peripheral blood. Suspected to have inflammatory disease of the esophagus, for example reflux esophagitis or drug-induced esophagitis, he was treated by proton pump inhibitor (PPI) and methylprednisolone and MMF were stopped. When endoscopy was performed to follow the esophageal findings before on postoperative day 36, more diffuse and shallow erosions in the distal of the esophagus were observed, and esophageal biopsies were obtained on postoperative day 38. Then in the peripheral blood PCR test and antigenemia test of CMV remained negative.

The esophageal biopsies showed inflamed granulation tissue in active inflammation and atypical cells which had inclusion bodies **(Figure 4)**. Immunohistochemical stain with cytomegalovirus antibody was positive for these cells **(Figure 5)**. The liver biopsy demonstrated no histopathological evidence of inflammation or rejection related to CMV infection.

After informed consent was obtained, ganciclovir was begun in an oral dosage of 5mg/kg twice a day. Tacrolimus was continued as before to maintain therapeutic blood levels. By the 14th day of therapy, the platelet count was 104000/μL, and hemoglobin was 8.8g/dL. During therapy, serum bilirubin decreased to 2.2mg/dL. Other hepatic enzymes did not rise transiently.

Ganciclovir was discontinued after 14 days. Repeated esophageal biopsies examined immunohistochemically were conspicuous for regression of the previously noted inflammatory changes but presence of viral inclusions. One month after ganciclovir was discontinued, the patient remained afebrile without evi-

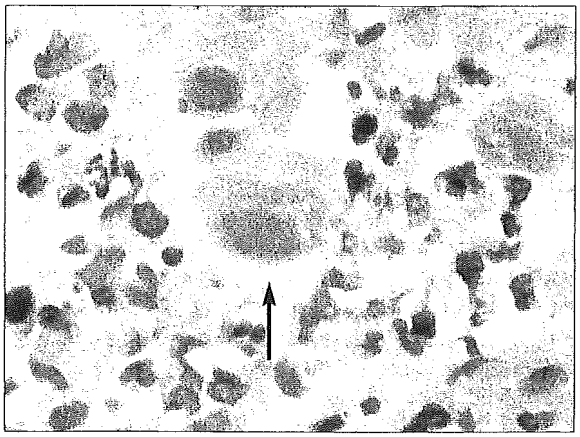

FIGURE 4 Esophageal biopsy (hematoxylin and eosin stain, x800): chronic inflammation and a single cell (center) with cytomegalic inclusions (arrow).

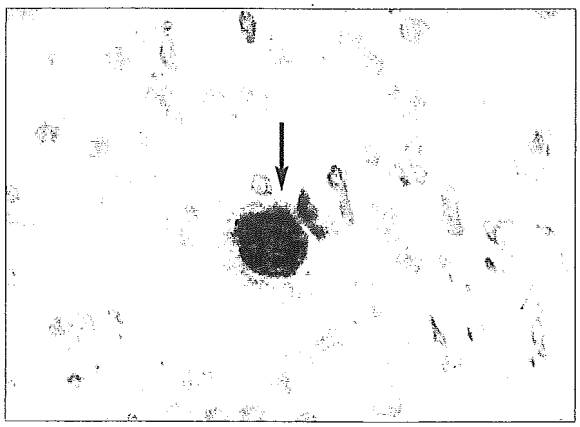

FIGURE 5 Immunohistochemical stain of esophageal biopsy (x800): a single cell (center) with a red cytomegalic inclusion (arrow).

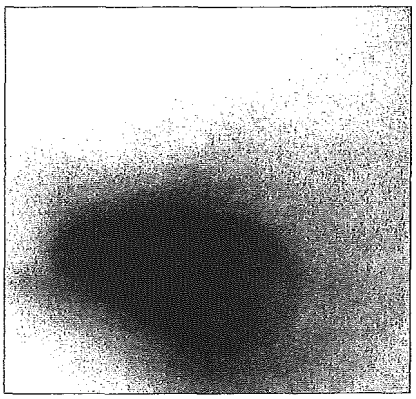

FIGURE 6
The findings of endoscopy (at about 23cm from dental arch, on postoperative day 85): almost normal.

dence of persistent active CMV infection. The surveillance of endoscopy was performed on postoperative day 85. The findings were almost normal **(Figure 6)**. Esophageal biopsies were negative.

DISCUSSION

CMV is the most common viral pathogen found after liver transplantation. The development of CMV infections or disease is linked to the CMV serological status of both the donor and recipient, the type and extent of immunosuppression, and possibly rejection. In liver transplant patients, CMV infections can be either a primary infection or a reactivation of a previously latent infection. In this case the CMV-seropositive recipient received a liver from a CMV-seropositive donor. In general the incidence of CMV disease may occur in about 5~22% when both the liver recipient and donor are CMV seropositive (8).

The clinical presentation of CMV infections in liver transplant patients is variable. Many patients have asymptomatic infection detectable only by performing routine surveillance culture or serological tests. Like this case, despite negativity in the peripheral blood PCR test and antigenemia test of CMV, CMV infections of the gastrointestinal tract occurred, and were overlooked in the patient who had fever and specific gastrointestinal symptoms like dysphagia, nausea, and epigastralgia. CMV infections of the gastrointestinal tract were detected by performing the surveillance of endoscopy and biopsy. It was reported that the esophageal findings of endoscopy of CMV infections were extensive mucosal ulceration, with marked acute and chronic inflammation. It was also reported that the findings of biopsy of CMV infections were the denser areas of inflammation at the ulcer bases around single and small nests of cells that contained intranuclear and intracytoplasmic inclusions characteristic of CMV (12,13).

In our case, at first we originally diagnosed reflux esophagitis or drug-induced esophagitis because of the findings that showed diffuse, segmental erosions in the distal of the esophagus, but no ulcers which are characteristics of esophagitis of CMV infections. But CMV infections of the segmental, erosive esophagitis could be detected by CMV immunohistochemical stain using biopsies.

CMV infections of the gastrointestinal tract can be suppressed by relatively low dose ganciclovir (the intravenous dosage of 7.5mg/kg/day for two weeks), even when the patients are maintained on immunosuppressive regimens designed to prevent graft rejection (13). This dosage has previously been shown to result in adequate serum levels of drugs while avoiding bone marrow suppression, which may occur at higher doses (1). Our patients tolerated the therapy with no apparent toxicity at the oral dose of 10mg /kg/day for two weeks, maintained on our immunosuppressive regimens. So his dosage could be shown to result in adequate serum levels of drugs and the continuation of tacrolimus in the present case did not appear to delay the patient's recovery.

On the other hand, rejection may almost double the incidence of CMV infection, from 26~49% (14). Though it was reported that association between the administration of prednisone or the blood level of cyclosporine A and esophagitis was not found (15) and that CMV infections were lower in the tacrolimus group than cyclosporin A group, but the effect was not statistically significant (16). Then we stopped treating the patient by MMF, but it is reported that MMF use does not appear to be associated with a significantly increased risk of infection like CMV infections occurring after liver transplantation (17). Modalities that reduce allograft rejection may possibly have an impact on incidence and severity of CMV disease.

So our experience suggests that CMV esophagitis should be taken into consideration when a patient has gastrointestinal symptoms such as dysphagia, retrosternal pain and epigastralgia and has endoscopic findings such as segmental erosions on the esophageal lumen despite having no CMV-specific endoscopic findings such as ulcerative lesions.

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Validity of Preoperative Volumetric Analysis of Congestion Volume in Living Donor Liver Transplantation Using Three-Dimensional Computed Tomography

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Reconstruction of middle hepatic vein (MHV) tributaries is controversial in right-lobe living donor liver transplantation (LDLT). This study aimed to evaluate the appropriateness of reconstructing MHV tributaries by volumetry using 3-dimensional computed tomography (3D-CT). Between November 2003 and January 2005, 42 donor livers (right-lobe graft, n = 25; left-lobe graft, n = 17) were evaluated using this software. The total congestion volume (CV) associated with the MHV tributaries and the inferior right hepatic vein (IRHV), and graft volume (GV) were calculated. In recipients with right-lobe grafts, CV/(right liver volume [RLV]) and (GV − CV)/(standard liver volume [SLV]) were compared between 2 groups: with reconstruction (n = 16) and without reconstruction (n = 9). To evaluate the influence of CV on the remnant right lobe in donors, total bilirubin was compared between 2 groups: high CV (CV > 20%, n = 13) or low CV (CV ≤ 20%, n = 4). The mean CV/RLV ratio was 32.3 ± 17.1% (V5, 15.2 ± 9.9%; V8, 9.2 ± 4.1%; and IRHV, 8.5 ± 11.4%) and the maximum ratio was as high as 80.8%. The mean (GV − CV)/SLV ratio before reconstruction in patients with or without reconstruction resulted in 33.5 ± 12.8% and 55.4 ± 12.9%, respectively ($P < 0.01$). In donors, total bilirubin was significantly high in the high CV group on postoperative day 1 compared with the low CV group ($P < 0.05$). In conclusion, calculation of CV using 3D-CT software proved to be very useful. We concluded that this evaluation should be an integral part of procedure planning, especially for right-lobe LDLT. (*Liver Transpl 2005;11:1556-1562.*)

Living donor liver transplantation (LDLT) was developed to overcome the shortage of suitably sized organs from cadaveric donors for children and adults with end-stage liver disease.[1] LDLT necessitates a right-lobe graft for adequate liver volume; however, a right-lobe graft without a middle hepatic vein (MHV) potentially has problems of hepatic venous congestion (HVC) caused by deprivation of drainage from the inferior right hepatic vein (IRHV) and MHV tributaries (V5 and V8).[2-4] Lee et al.[2] reported 2 cases of severe congestion of a graft without MHV; 1 resulted in sepsis due to congestive infarction and the other developed prolonged massive jaundice. We believe that impaired graft congestion venous outflow was the cause of previously unexplained graft failures during our initial experience of LDLT. Surgeons have agreed on the need of MHV reconstruction because of occasional massive congestion, and so there is an interest in criteria for MHV reconstruction.[3-5] Further, HVC has been realized only after parenchymal transection and temporary arterial clamping of the donor liver; while preoperative prediction of congestion volume (CV) has been difficult.[5] Recently, preoperative liver volumetry and the measurement of the hepatic vein diameter based on the 3-dimensional computed tomography (3D-CT) has resulted in a significantly improved outcomes, compared to the use of 2-dimensional computed tomography.[6,7] Furthermore, Kishi et al.[8] reported that CV could be calculated and predicted applying new 3D-CT software by calculating the volume from the diameter and length of intrahepatic vascular branches. This study aimed to analyze the efficacy and accuracy of predicting CV and graft volume (GV) based on the software and to evaluate the appropriateness of the reconstruction of these tributaries.

Abbreviations: LDLT, living donor liver transplantation; MHV, middle hepatic vein; IRHV, inferior right hepatic vein; CV, congestion volume; 3D-CT, 3-dimensional computed tomography; GV, graft volume; RLV, right-lobe volume; SLV, standard liver volume; HVC, hepatic venous congestion.

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Received April 13, 2005; accepted June 11, 2005.

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Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/lt.20537

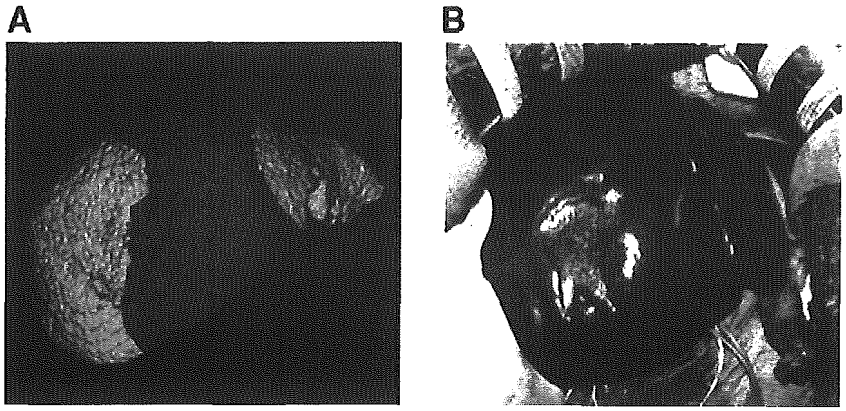

Figure 1. 3D-CT image of the congestion area and an intraoperative finding. (A) The construction of the 3-dimensional image shows the drainage area of the MHV tributaries (V5, V8; indicated by red). (B) Discoloration of the donor liver surface after clamping of the right hepatic artery and MHV tributaries, which completely matched the predicted area by 3D-CT (A,B: same donor).

Patients and Methods

Patients

From October 1996 to January 2005, 177 consecutive LDLTs were performed at Kyushu University Hospital (Fukuoka, Japan). Our preoperative volumetric evaluation for donors consisted of two-dimensional computed tomography (1996-1999) and conventional 3D-CT (2000).[6,7] In October 2003, the 3D-CT examination using new software (Region Growing Software Version 0.5a; Hitachi Medical Corporation, Chiba, Japan) was introduced for preoperative evaluation and 42 adult donors (right-lobe graft, n = 25; left-lobe graft, n = 17) have since been evaluated. The indication for 42 LDLTs included fulminant hepatic failure (n = 4), primary biliary cirrhosis (n = 7), hepatitis C-cirrhosis (n = 21), hepatitis B-cirrhosis (n = 5), alcoholic cirrhosis (n = 1), cryptogenic cirrhosis (n = 3). The donors were a father (n = 1), a mother (n = 1), husbands (n = 5), wives (n = 2), brothers (n = 2), sisters (n = 2), sons (n = 21), daughters (n = 8), and a cousin (n = 1). All patients provided written informed consent. Donors were 30 males and 12 females, mean age 35 yr (range 20-58). Donor mean height and weight was 164 cm (range 147-180) and 60 kg (range 37-83), respectively. Mean blood loss and operation time were 568 gm (range 100-725) and 417 minutes (range 320-515), respectively.

Measurement of Actual Graft Weight

Actual graft weight was measured on the back table after flushing with University of Wisconsin solution (ViaSpan®; Bristol-Myers Squibb, New York, NY) and trimming. A total of 1 cm^3 of liver was estimated as 1 gm.[7] The error ratio (%) was expressed as $|E - A|/A \times 100$, where E is the estimated GV (mL) and A is the actual graft weight (gm).[7]

3D-CT Volumetry

Preoperative multidetector helical computed tomography (MDCT) images were made using 2-mm-thick slices represented on a computed tomography machine. Enhancement was achieved by an intravenous bolus of contrast nonionic medium (Iopamion™; Schering, Erlangen, Germany) at a speed of 5 mL/second. This method allows for clear visualization of the hepatic arteries, portal veins, and hepatic veins including IRHV and MHV tributaries. Three-dimensional reconstructions of the liver and the graft were rendered by multidetector helical competed tomography using the new 3D-CT software, which was able to calculate total liver volume and the volume of each vessel's (both portal vein branches and hepatic venous branches) territories from their diameter and length. The 3-dimensional image reconstructed by this software could reflect the actual congestion area (Fig. 1). The right-lobe volume (RLV) was calculated from the right portal vein territories and the CV of each hepatic venous branch was calculated automatically (Fig. 2).

GV Excluding CV

The percentage of CV/RLV was also calculated. Further, we developed a new parameter: (GV − CV)/(standard liver volume [SLV]). The SLV was calculated using Urata's formula: SLV (mL) = 706.2 × body surface area (m^2) + 2.4.[9] In our institution, SLV calculated using this formula was found to be more suitable compared with other formulas (data not shown).[10] Recipients with right-lobe grafts (n = 25) were divided into 2 groups: with reconstruction of MHV tributaries or IRHV (n = 16), and without reconstruction of any these veins (n = 9) groups. (GV − CV)/SLV was compared between the 2 groups.

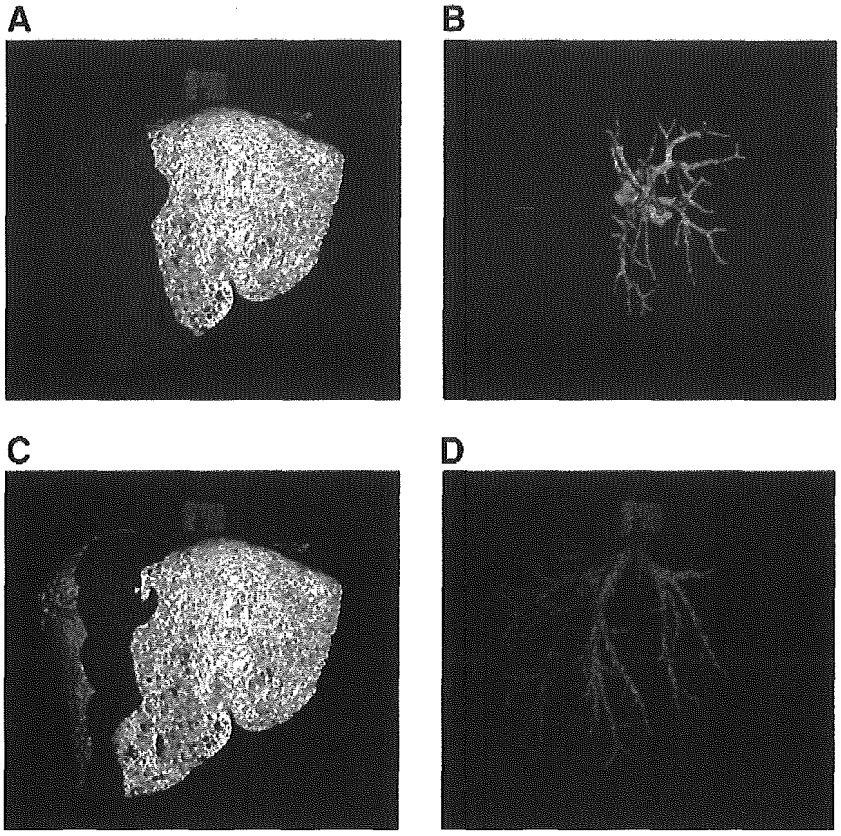

Figure 2. 3D-CT image of a liver. The volume of each vessel branch can be automatically calculated before an operation using the new software. (A,B) Construction of a 3-dimensional image shows the perfusion area (orange color) of the right portal vein. (C,D) The construction of a 3-dimensional image shows the drainage area of middle hepatic vein (MHV) tributaries (V5, V8; indicated by red).

Surgical Technique

Surgical procedures in donors have been described elsewhere.[1,11] Briefly, during mobilization of the liver, all the right accessory hepatic veins of a significant size (>5 mm in diameter) were preserved and reconstructed in the recipient. In right-lobe grafts, significant hepatic veins from segment 5 or 8 were preserved as long as was possible during parenchymal transection. The graft was procured and flushed via the portal vein on the back table using University of Wisconsin solution, and the weight of the graft measured. Interposition vein grafts from the donor or recipient (e.g., inferior mesenteric, greater saphenous, and intrahepatic portal veins of the recipient) were procured for reconstruction of V5 and V8, if their diameters were 5 mm or more.

Influence of CV in Donors' Remnant Right-Lobe

The influence of CV on the remnant right lobe in donors who underwent extended left lobectomy (n = 17) was evaluated. In cases in which CV was under 20%, the diameter of MHV tributaries and IRHV were all very small (the diameter <5 mm). Donors were divided into high CV (CV > 20%, n = 13) and low CV (CV ≤ 20%, n = 4) groups. Total bilirubin and aspartate aminotransferase level at postoperative days 1, 2, 3, 5, and 7 were compared between the 2 groups.

Statistical Analysis

Data are expressed mean ± standard deviation. Statistical analysis was performed using Student's *t*-test and the Mann-Whitney U-test. StatView™ (Version 4.11; Abacus Concepts, Berkeley, CA) software on a Macintosh computer was used for all analyses. $P < 0.05$ was considered to be significant.

Results

Relationship Between Actual Weight of Grafts and Estimated Volume of Grafts Using 3D-CT

Mean estimated total liver volume and GV were 1,185 mL (range 803-2,004) and 592 mL (range 278-1,055),

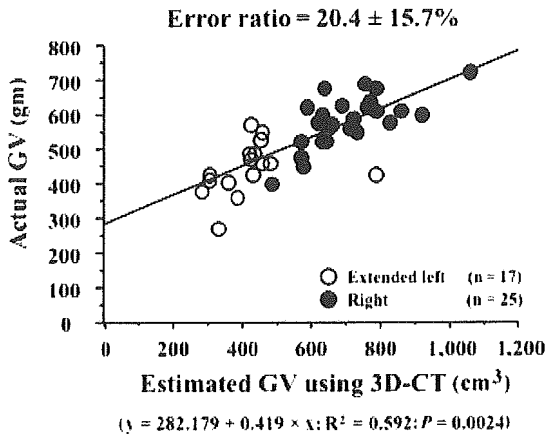

Figure 3. Relationship between actual volume of grafts and their estimated volumes using 3D-CT software: y = 282.179 + 0.419x (R^2 = 0.592, P = 0.0024). The error ratio was 20.4 ± 15.7% in this 3D-CT software. The error ratio was calculated as follows: error ratio (%) = |E − A|/A × 100, where E is the estimated graft volume (mL) and A is the actual graft volume (gm).

respectively. Mean graft weight was 531 gm (range 270-720). The relationship between estimated GV and actual graft weight using 3D-CT in 42 donors was linear: y = 282.179 + 0.419 × (R^2 = 0.592, P = 0.0024). The mean error ratio was 20.4% (range 0.0-84.0) (Fig. 3).

Ratio of CV of V5, V8, and IRHV in Right-Lobe Grafts

The mean estimated CV/RLV ratio was 32.3 ± 17.1% (V5: 17.3 ± 9.5%; V8: 8.5 ± 4.4%; IRHV: 6.6 ± 9.7%) in 25 donors (Fig. 4). Twenty-five right-lobe grafts included reconstruction with all these tributaries (n = 3), with any 2 tributaries (n = 7), with only 1 tributary (n = 6), and without any tributaries (n = 9) (Fig. 4). The mean CV/RLV ratios in patients with (n = 16) or without (n = 9) a reconstruction of MHV tributaries or IRHV were 41.7 ± 19.4% and 17.3 ± 8.4%, respectively (P < 0.01).

(GV − CV)/SLV.

The mean GV/SLV ratio in patients with (n = 16) or without (n = 9) reconstruction of MHV tributaries or IRHV was 57.6 ± 9.2% (range 44.3-78.6) and 67.0 ± 14.1% (range 47.8-90.2) (P = 0.08), respectively (Fig. 5). (GV − CV)/SLV resulted in 33.5 ± 12.8% (range 12.6-58.3) and 55.4 ± 12.9% (range 44.4-81.4), respectively, which were significantly lower than the GV/SLV ratios. However, (GV − CV)/SLV recovered to 52.4 ± 10.2% (range 38.3-78.6) after reconstruction of these tributaries (Fig. 5). There were no postoperative complications associated with graft congestion in either group.

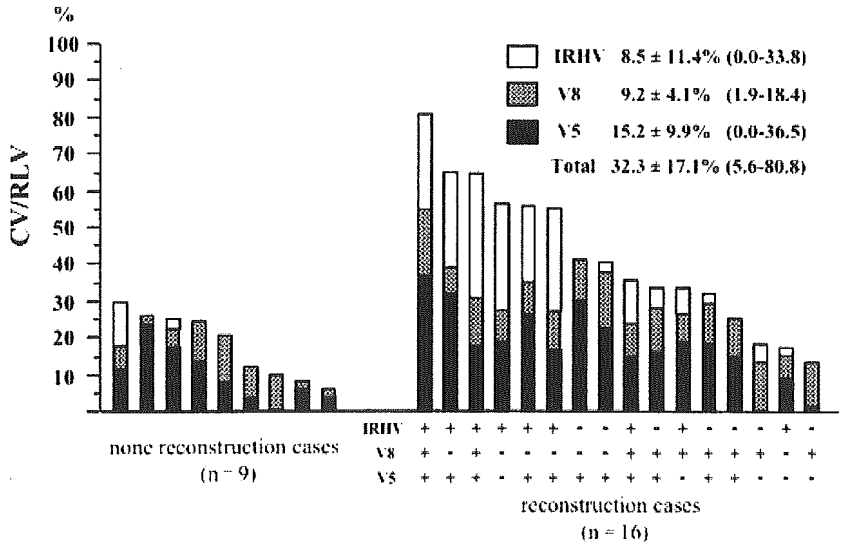

Figure 4. Estimated CV of V5, V8, and the IRHV and RLV ratios. CVs of V5, V8, and IRHV were 15.2 ± 9.9% (range 0.0-36.5), 9.2 ± 4.1% (range 1.9-18.4), and 8.5 ± 11.4% (range 0.0-33.8), respectively. Total CV of RLV was 32.3 ± 17.1% (range 5.6-80.8). A total of 16 patients were reconstructed with some branches and 9 without any branches (+, with reconstruction; −, without reconstruction).

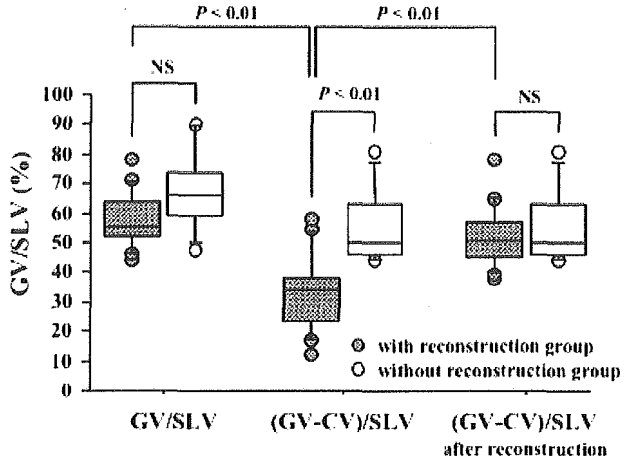

Figure 5. A new parameter (GV − CV)/SLV. Mean GV/SLV was 60.3 ± 12.9% (n = 25), but (GV − CV)/SLV was 41.8 ± 17.1%. Deducing CV from GV was significantly decreased from GV/SLV. The mean GV/SLV ratio in patients with (n = 16) or without (n = 9) reconstruction of MHV tributaries or the IRHV was 57.6 ± 9.2% (range 44.3-78.6) and 67.0 ± 14.1% (range 47.8-90.2) ($P = 0.08$), but (GV − CV)/SLV was 33.5 ± 12.8% (range 12.6-58.3) and 55.4 ± 12.9% (range 44.4-81.4), respectively ($P < 0.01$). After reconstruction, (GV − CV)/SLV in reconstruction cases increased to 52.4 ± 10.2% (range 38.3-78.6). NS, not significant.

The Outcome of CV in Donor's Remnant Liver

To estimate the effect of liver congestion, the CVs of 17 donors who underwent extended left lobectomy and their postoperative liver function were examined. Total CV/RLV ratio estimated by 3D-CT was 27.7 ± 10.6% (V5: 20.5 ± 8.1%; V8: 7.2 ± 4.7%, respectively) (Fig. 6A). We did not reconstruct any MHV tributaries in the donors, who were divided into 2 groups: high CV (CV > 20%, n = 13) and low CV (CV ≤ 20%, n = 4), to evaluate the influence of CV on the remnant right lobe. Total bilirubin was significantly higher in the high CV group on postoperative day 1 ($P < 0.05$) (Fig. 6B). Aspartate aminotransferase was not seen to have any significantly changes (Fig. 6C). No complications associated with congestion of the remnant liver have been experienced.

Discussion

LDLT using the right lobe has become a standard procedure to overcome graft size problems for adults or larger pediatric patients.[1] These grafts, especially without the MHV, can potentially lead to HVC in segments 5 and 8 due to insufficient venous drainage,[2,3] the importance of which has been recognized by many surgeons.[4] However, prediction of CV is very difficult and management of V5, V8, and IRHV in LDLT has been a controversial area. The demarcation line of HVC is evident only after parenchymal transection and performing an intraoperative hepatic arterial clamping test (Fig. 1B).[5] 3D-CT examination has been extremely helpful for us to understand the anatomy of a donor's hepatic vessels,[6,7] so that when there is an anomaly with regard to hepatic or portal veins, it is relatively easy to develop a surgical strategy. Operative simulations using these anatomical images contribute to a reduction of not only the risk for donors but also the stress involved with donor surgery. In this study, we evaluated the usefulness of a newly developed 3D-CT software that can calculate the volume of each hepatic vessel from its diameter and length. Using this, we substituted the conventional preoperative evaluation of the graft for a more substantial evaluation of the graft. Three-dimensional images of the graft and CV were well visualized (Figs. 1 and 2). Our result for the CV/RLV ratio (32.3 ± 17.1%) was compatible with that reported by Shin et al.[12] However, volumetry by this 3D-CT software produces an error ratio of approximately 20%. Some factors thus need to be considered in relation to this error. First, a mismatch exists between the cutting line in these simulations and that in the actual hepatectomy; a 2-cm discrepancy could represent a difference of as much as 200 gm.[13] Second, we need to consider the reduction of the vascular bed of the graft.[14] Total liver volume before and after fluid infusion showed an approximately 33% difference in a porcine model.[15] Third, dehydration from the osmotic pressure of the University of Wisconsin solution could result in a

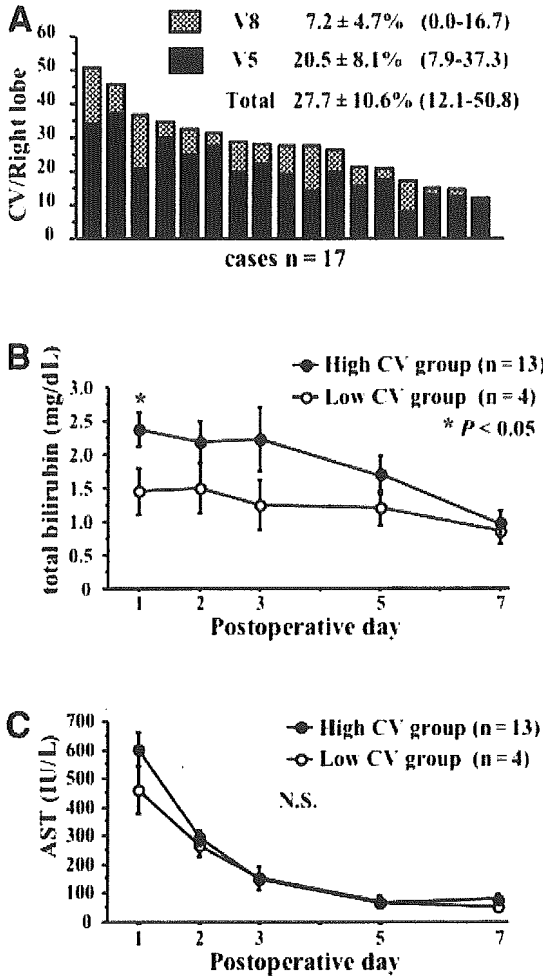

Figure 6. (A) Estimated CV of V5 and V8 in remnant right lobe of donor cases. In 17 donor cases, the CV/RLV ratio using 3D-CT was 27.7 ± 10.6% (range 12.1-50.8): V5, 20.5 ± 8.1% (range 7.9-37.3); V8, 7.2 ± 4.7% (range 0.0-16.7); and no donors had tributaries reconstructed. (B) Serial changes of postoperative total bilirubin and aspartate aminotransferase in left-lobe donors. Total bilirubin was significantly high in the high CV group ($P < 0.05$). (High CV group: CV > 20%, low CV group: CV ≤ 20%; NS, not significant.)

decrease of graft weight by approximately 4%.[7] Fourth, grafts from donors under 30 yr old were significantly overestimated as compared to grafts from those over this age (data not shown); graft compliance was seemingly the cause.

Figure 7 shows an algorithm for graft selection for LDLT as used in our institution. The left-lobe is initially considered as the graft with respect to donor safety. We have reported favorable outcomes using such grafts.[11,16,17] As left-lobe grafts are usually small, accurate assessment of graft volume before an operation is critical. Three methods of volumetry, such as formula, conventional 3D-CT, and this new 3D-CT software have been examined.[6,7,18] Moreover, to avoid graft congestion due to excessive portal flow, either a splenectomy or a splenic artery ligation has been reported.[19] Even for a right-lobe graft, graft-to-recipient weight ratio was sometimes under 1%. We selected a right-lobe graft when a left-lobe graft was insufficient for the recipient and remnant liver volume of the donor was over 35%. On the other hand, some reports have not reconstructed these tributaries if the diameter of these tributaries was under 5 mm.[2,13,20] Certainly, a branch under 5 mm is not thought to be a significant branch, but our study revealed that some branches under 5 mm had high CV/RLV (>10%). Moreover, when many branches under 5 mm existed, as in V5 and V8, CV cannot be predicted only by the simple observation of a computed tomography image. Even if tributaries are under 5 mm, there are potential risks as such the CV being high. Surprisingly, our study shows that the maximum CV/RLV was over 80%. In such cases, careful planning of the reconstruction is critical, therefore it is currently thought that every effort should be made to reconstruct all significant MHV tributaries, as much as is possible because hepatic venous outflow is sometimes unpredictable. Since we clarified the importance of the each CV of V5, V8, and IRHV (Fig. 4), the use of the new parameter (GV − CV)/SLV has become a routine

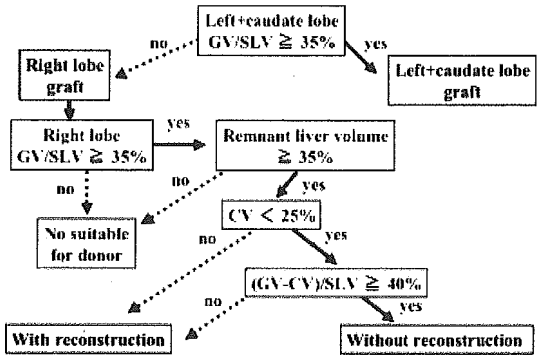

Figure 7. Algorithm for the graft selection as used in our institution. Initially the left-lobe is considered as a graft, and generally is used. The right is chosen if the estimated extended left + caudate lobe volume of the donor is less than 35% of the SLV of the recipient. If a remnant liver volume is under 35% of the total liver volume, this donor will be rejected. If CV is over 25%, or the deducted CV from the GV is under 40%, reconstruction of these tributaries is needed.

part of our graft selection algorithm (Fig. 7), and detailed simulations have become possible. Since the introduction of the criteria, we have not had any postoperative complications associated with graft congestion.

There are some reports that HVC influences graft function and regeneration in LDLT recipients.[2-4] However in healthy donors, there are few reports of how HVC influences the remnant liver.[8,21] In the donor's operation, we have not reconstructed these tributaries, except in 1 case. In this study, mean CV/RLV in the remnant right lobe was surprisingly high at 27.7 ± 10.6% and the maximum CV/RLV was as high as 50.8% (Fig. 6A). Moreover, total bilirubin was significantly high in cases with CV over 20% (Fig. 6B). These results indicate that CV is a latent risk and that estimation of CV using this software is very useful.

In conclusion, CV could be reliably predicted using this 3D-CT software. We believe that this new parameter [(GV − CV)/SLV] deserves to be an essential part of preoperative planning for hepatic vein reconstruction and graft selection.

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Clin Transplant 2005: 19: 769–772 DOI: 10.1111/j.1399-0012.2005.00419.x

Clinical Transplantation

Splenectomy and preemptive interferon therapy for hepatitis C patients after living-donor liver transplantation

Kishi Y, Sugawara Y, Akamatsu N, Kaneko J, Tamura S, Kokudo N, Makuuchi M. Splenectomy and preemptive interferon therapy for hepatitis C patients after living-donor liver transplantation.
Clin Transplant 2005: 19: 769–772. © Blackwell Munksgaard, 2005

Abstract: Recurrent hepatitis C after liver transplantation is a major cause of graft failure. We routinely perform preemptive interferon and ribavirin therapy in patients after living-donor liver transplantation indicated for hepatitis C-related cirrhosis. One of the obstacles for the therapy includes blood cytopenia. To overcome this problem, we recently performed splenectomy concurrently with liver transplantation. Thirty-five patients underwent liver transplantation and received preemptive therapy for hepatitis C. They were divided into two groups: those with splenectomy (group A, n = 21) and those without (group B, n = 14). There was no significant difference in the frequency of morbidity between the groups. Platelet counts were well maintained in group A patients during the therapy, and cytopenia led to the discontinuation of the therapy in one group B patient. The results of the preliminary study warrant a randomized control trial to examine the feasibility of splenectomy and preemptive viral therapy during liver transplantation for hepatitis C.

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Key words: hepatitis C – interferon – liver transplantation – splenectomy – thrombocytopenia

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Accepted for publication 27 May 2005

Hepatitis C virus (HCV) infection is one of the leading etiologies for liver transplantation. The main problem of the post-transplantation course is recurrent hepatitis with 11–14% of recipients redeveloping hepatitis leading to graft failure (1, 2). However, retransplantation provides poor results, with a 3-yr survival rate of only 40–56% (3, 4).

Although interferon (IFN) and ribavirin therapy is one of the standard treatments, the sustained virologic response ratio of the therapy for recurrent HCV after transplantation is limited to approximately 30% (5–7). We routinely perform preemptive IFN therapy for recipients of living-donor liver transplantation (LDLT) indicated for HCV cirrhosis (8). One of the obstacles for starting or continuing combined IFN and ribavirin therapy includes blood cytopenia. To overcome this problem, we recently performed splenectomy concurrently with liver transplantation (9). Here we analyze the results of these patients to evaluate the feasibility of simultaneous splenectomy and combined therapy against HCV.

Patients and methods

From January 1996 to September 2004, 165 adult patients underwent LDLT. Of these, 39 recipients were indicated for HCV cirrhosis and received preemptive IFN and ribavirin therapy. Of these, four were excluded from the study because two died before the start of therapy due to uncontrolled cytomegalovirus infection or resistant acute cellular rejection, and two patients were followed up at

other hospitals and detailed laboratory data could not be obtained. The remaining 35 patients were the subjects of this study. They were divided into two groups: those with splenectomy (group A, n = 21) and those without (group B, n = 14).

The protocol of the preemptive IFN and ribavirin therapy was reported previously (8). In brief, the therapy was started when the white blood cell count was >4000 mm^3, hemoglobin level >10 g/dL, and platelet count $>100\,000$/mm^3. The therapy was initiated with 3 million units of IFN-alpha2b (Intron A; Schering-Plough K.K., Osaka, Japan) three times per week and 400 mg of ribavirin per day, which was increased up to twice the initial dose according to patient tolerance. The therapy was discontinued when there was significant leucopenia (<1500/mm^3), thrompocytopenia ($<50\,000$/mm^3) despite application of granulocyte colony-stimulating factor (G-CSF), hemolytic anemia (hemoglobin level <8 g/dL), renal dysfunction (serum creatinine >2 mg/dL) or depressive psychologic status.

Preoperative blood cell count, platelet count (mm^3), leukocyte count (mm^3), and hemoglobin (g/dL) were taken just before IFN therapy, and the numbers of days from transplantation to the start of therapy were evaluated. Blood cell counts during the therapy were examined weekly for the first month, monthly for the first year, and annually later on. The frequency of discontinuation of the therapy and its cause were reviewed. Completion of the therapy was defined as the elimination of HCV (<500 copies/mL by Amplicor HCV; Roche Molecular Systems, Pleasanton, CA, USA). Here, HCV was considered to be eliminated when the serum HCV-RNA level was consistently negative for at least 6 months after cessation of combination therapy. Protocol liver biopsy was not performed.

Data are expressed as median and range. Statistical comparison was performed using Mann–Whitney test, Fisher's exact test or repeated measure analysis of variance where appropriate. p-value <0.05 was considered statistically significant.

Results

Patient profiles

In the 17 patients of group A, the duration between LDLT and starting the therapy ranged from 18 to 59 d (Table 1). In the other four patients of group A, it was longer than 2 months as we had to wait till they recovered from pneumonia, abdominal abscess, heart failure or renal failure. The number of the patients of HCV genotype 1b (HCV_{1b}) and those of the other genotypes (HCV_{non1b}) was 5 of 16 in group A and 2 of 12 in group B. There was no significant difference in preoperative blood cell counts or liver function between the groups.

Table 1. Patients profiles

	A (n = 21)		B (n = 14)		
Group	Median	Range	Median	Range	p-value
MELD score	14	4–34	10.9	2.4–25.3	0.22
Preoperative plt (x10^4/mm^3)	5.0	2.9–13.5	5.6	4.1–15.0	0.30
Preoperative WBC (x10^3/mm^3)	3.3	1.3–20.5	2.8	1.6–9.8	0.51
Preoperative Hb (g/dL)	9.0	5.5–12.7	10.5	5.6–13.3	0.24
Start day (d)	41	18–120	30	7–130	0.34
HCV-RNA before therapy (kcopies/mL)	663	186–3350	510	46–1700	0.66

MELD, model for end-stage liver disease; plt, platelet; WBC, white blood cell; Hb, hemoglobin.

Postoperative infectious diseases

In group A, six (29%) patients suffered from infectious disease: four from abdominal abscess, one from fungal pneumonia and one from bacterial pneumonia. Two of the four abdominal abscesses were related to the splenectomy because there was pancreatic juice leakage from the drainage tube in the left subphrenic space. Both of the patients responded well to surgical re-exploration. In group B, five (36%) patients had infection episode with no mortality including three abdominal abscesses, one sepsis and one osteomyelitis.

Blood cell counts after interferon and ribavirin therapy

In group A patients, platelet count significantly increased soon after LDLT and was maintained during the treatment for up to 2 yr (Fig. 1). Platelet count was kept higher in group A patients ($p = 0.008$) during the observation period. Leukocytopenia (<3000/mm^3) were observed in three patients of group A and seven in group B. All of them were well controlled by G-CSF except for one in group B who discontinued the therapy because of cytopenia.

Continuation of therapy

Six (29%) patients in group A and three (21%) in group B discontinued therapy before the HCV was eradicated (Table 2). A 40-yr-old male in group A underwent retransplantation for cholestatic